N.G. Ravichandra

Horticultural Nematology

Springer

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Dedicated to My Mother

Foreword

Horticulture is an integral and important component in an agrarian economy like India. Horticultural crops constitute a significant segment of the total agricultural production of the nation. The importance of horticulture in improving the productivity of land, generating employment, improving economic conditions of the farmers and entrepreneurs, enhancing exports, and, above all, providing nutritional security to the people is widely acknowledged. Horticulture has and will always exist as a matrix of interrelated areas with overlapping and complex relationships. Describing its importance in the physiological, psychological, and social activities of people is the key to expanding understanding of horticulture.

Controlling phytonematodes is of great economic importance, when it comes to successful cultivation of horticultural crops cultivated either in open fields or polyhouse conditions. Worldwide, they cause significant damage and yield loss. The paradox is that much of the loss can be prevented with a little care. There is clearly a lot that needs to be done to expand our knowledge on plant-parasitic nematodes and our ability to deal with them.

These "hidden enemies" are difficult to manage as they are soilborne and their populations vary mainly with soil type, soil moisture/temperature, and genotype. Complex diseases involving nematodes and other soilborne fungi and bacteria complicate the problem. Most insidiously, phytonematodes evolve, often overcoming the resistance that is many a time hard-earned achievement of the plant breeder. Major horticultural crops worldwide are subject to nematode attack in the nursery, field, protected cultivation, or even during postharvest stage, which affects both the quantity and quality of the produce, resulting in loss to the grower.

The book entitled *Horticultural Nematology* authored by Dr. N.G. Ravichandra is an attempt to provide a detailed information on nematodes of important horticultural crops and their management. The book is written with the objective of providing a comprehensive and latest information on phytonematodes. An exclusive chapter on novel methods of managing phytonematodes in horticulture without dealing with usual nematode management practices provides a new dimension to nematode management using latest, novel molecules. The book also throws light on the potential and future novel nematode management strategies.

I congratulate the author Dr. N.G. Ravichandra, Nematologist, AICRP (Nematodes), Department of Plant Pathology, Agricultural College,

University of Agricultural Sciences, GKVK campus, Bangalore, Karnataka, India, for providing details of essential topics pertaining to phytonematodes associated with horticultural crops. I am sure that its readers will find it very useful and informative.

Bangalore
12 Jan 2014

Director of Research

Preface

Recent advances have shown that horticulture could become an environmentally benign technology. Horticulture causes much less degradation of the natural resources than agriculture. It is well known that phytonematodes are of major concern to a wide range of horticultural crops grown worldwide, which cause sufficient crop damage and severe loss in the yield. Plant-parasitic nematodes pose an enormous problem for global horticultural security. In the first instance, there is a lack of complete knowledge on precise identity/diagnosis of strains of few damaging nematode pathogens that currently exist. Potentially most dangerous phytonematodes are genetically variable, which stands as a warning that genetic uniformity of even a small part of the genome of a widely grown crop risks the hazard of a major epidemic of a nematode with the ability to exploit the trait(s) that it governs.

However, detailed and latest information on major aspects of phytonematodes associated exclusively with horticultural crops is lacking. Hence, it was thought to present a comprehensive book that covers some major nematode topics of relevance to horticulture. The purpose of this book is to highlight the significance of phytonematodes in horticulture. It also provides basic information on plant-parasitic nematodes since it is required for a better understanding of advanced topics. Several popular topics, information on which is already available in plenty, have been avoided. Thus, this book explicates both the essential fundamental and advanced aspects pertaining to nematodes associated with horticultural crops.

The book is conveniently divided into 13 chapters, which cover the latest information on the major fundamental and advanced aspects related to phytonematodes including the role of phytonematodes in horticultural industry, phylogenetic and evolutionary concepts in nematodes, major phytonematodes associated with horticultural crops and their diagnostic keys, symptoms caused by phytonematodes and disease diagnosis, nematode population threshold levels, crop loss assessment, nematode diseases of horticultural crops and their management, nematode disease complexes, genetics of nematode parasitism, important nematological techniques, and nematodes of quarantine importance. One chapter in particular can be singled out for special comment. An exclusive chapter on novel methods of nematode management has been included mainly to provide information on the latest molecules and novel modes of managing nematodes attacking horticultural crops. Routine nematode management aspects, information on which is already available,

have not been discussed; instead, this topic reflects the changing scenario of future nematode management.

This comprehensive book can serve as a friendly guide to meet the requirements of the students, teachers, and researchers interested in these "hidden enemies" of the grower, apart from the research and extension personnel working under public organizations, officials of state departments of horticulture and forestry, field workers, and all those concerned and working with plant-parasitic nematodes. Appropriate diagrams, convincing tables, and suitable graphs/illustrations have been furnished at the right places. A bibliography providing the list of references cited has also been included at the end.

The author appreciates receiving suggestions and constructive criticism that would improve the quality of the book (e-mail: ravichandrang_3@yahoo.co.in).

Bangalore, Karnataka, India N.G. Ravichandra
2014

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I acknowledge with thanks the assistance rendered by Prof. B. M. R. Reddy, Professor and University Head; Dr. T. Narendrappa, Professor and Head; the faculty of the Department of Plant Pathology, UAS, GKVK, Bangalore; and Mr. B. R. Nagendra, AICRP (Nematodes).

I would like to express my deep sense of gratitude to my beloved parents Sri. N. Gurushankaraiah and Smt. Parvathamma to whom I affectionately dedicate this book.

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1 Horticulture and Its Role in the National Economies

Horticulture forms an integral and important component in the economy of a nation. Horticultural crops constitute a significant segment of the total agricultural production of a country. The importance of horticulture can be substantiated by its benefits like high export value, high per unit area yield, high returns per unit area, best utilization of wasteland, provision of raw materials for industries, whole engagement by a grower/laborer, production of more food energy per unit area than that of field crops, better use of undulating lands, and stabilization of women's empowerment by providing employment opportunities through processing, floriculture, seed production, mushroom cultivation, nursery preparation, etc. In addition, fruits and vegetables constitute the important energy-giving material to the human body. It also improves the economic condition of many farmers, and it has become a means of improving livelihood for many unprivileged classes too. Flower harvesting, nursery maintenance, hybrid seed production and tissue culture, propagation of fruits and flowers, and food processing are highly remunerative employment options for women in rural areas.

Horticulture is one of the main agricultural practices in a nation. It is basically the science of cultivating gardens or orchards, that is, it refers to the process of cultivation of fruits, vegetables, flowers, and ornamental plants. It involves increasing the area and productivity of farming lands, bringing technological aspect in agriculture, raising the farmers' incomes and their standard of living, being a source of employment opportunities, etc. Horticultural crops play a unique role in a country's economy by improving the income of the rural people. They are the important exportable commodities in many countries. Cultivation of these crops is labor intensive, and as such they generate a lot of employment opportunities for the rural population. The horticulture sector has emerged as an economically rewarding and most viable option in diversification of agriculture. It refers to a wide variety of crops suitable for cultivation under different agroclimatic conditions and terrain with the possibility of multi-tier cropping, thus enhancing the returns per unit of land, generating employment, and providing food and nutritional security. The shift in the dietary patterns with preference to horticulture products has resulted in increasing demand.

Various horticultural products contribute to national wealth. They are the important exportable commodities in many countries. Horticulture is the mother of many auxiliary industries like canning industries and processing industries. Several agro-industries, based on horticultural products, are being established, thereby solving the unemployment problem to some extent, for example, rubber, coir (coconut), and sago (tapioca) industries. Horticultural crops provide gainful employment for small farmers and agricultural labor throughout the year. One hectare of fruit production generates 860 man-days per annum as against 143 man-days for cereal crops. Some industrial attribute crops and cultural intensive crops, like grape, banana, and pineapple, generate

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much large employment ranging from 1,000 to 2,500 man-days per hectare.

Horticulture actually is the high-value crop alternative. Horticultural crops for which most developing nations' topography and agroclimates are well suited are an ideal method of achieving sustainability of small holdings, increasing employment, improving environment, providing an enormous export potential, and above all achieving nutritional security. These crops form a significant part of the total agricultural produce in the country comprising of fruits, vegetables, root and tuber crops, flowers, ornamental plants, medicinal and aromatic plants, spices, condiments, plantation crops, and mushrooms. Fruits and vegetables are recognized as protective foods as they are necessary for the maintenance of human health, which are a rich source of vitamins, minerals, proteins, carbohydrates, etc., which are essential in human nutrition. Hence, these are referred to as protective foods and assumed great importance as nutritional security of the people. Thus, cultivation of horticultural crops plays a vital role in the prosperity of a nation and is directly linked with the health and happiness of the people. Horticulture also deals with raising of trees for shade, ornamental, and avenue purposes, planning and raising of ornamental gardens and parks, and raising of seed and planting material apart from the utilization of horticulture produce and improvement of horticulture crops.

Over the years, horticulture has emerged as one of the potential agricultural enterprise in accelerating the growth of economy. Its role in the country's nutritional security, poverty alleviation, and employment generation programs is becoming increasingly important. It not only offers a wide range of options to the farmers for crop diversification but also provides ample scope for sustaining a large number of agro-industries which generate huge employment opportunities. Fruits and vegetables are not only used for domestic consumption and processing into various products (pickles; preserves such as sauces, jam, jelly squares; etc.), but also substantial quantities are exported in fresh and processed form, bringing much needed foreign exchange for the country. These groups of crops also provide ample scope for achieving biodiversity and diversification to maintain ecological balance and to create sustainable agriculture and can make an impact on the national economy in the years to come.

Horticulture today is not merely a means of diversification but forms an integral part of food and nutritional security and also an essential ingredient of economic security. Adoption of horticulture, both by small and marginal farmers, has brought prosperity in many regions of the country. Recent areas of visions in horticulture include new developments in postharvest management, hi-tech plant protection, protected cultivation, hi-tech production of horticultural crops, and opportunities for new biology for germplasm and crop improvement. It is high time that policy-makers recognize that horticulture is often the most appropriate use of small landholdings in many parts of the world. Horticulture enterprise can provide work for whole families. Horticultural industry spawns value-added opportunities that can provide employment for entire communities. Horticulture makes more efficient use of scarce or costly inputs like water and fertilizer, and fruits and vegetables are needed to avoid nutrient deficiencies prevalent in many parts of the world. All of this adds up to wealth-creation potential far exceeding that of traditional staple crop agriculture.

The importance of horticulture in improving the productivity of land, generating employment, improving economic conditions of the farmers and entrepreneurs, enhancing exports, and, above all, providing nutritional security to the people is widely acknowledged. Initiating and supporting high-value horticultural crops is an excellent long-term approach to addressing the multiple challenges of ending poverty, improving nutrition, and sustaining rural communities in the developing world. Several decades of targeting staple crops with internationally supported research and development has not succeeded in reducing rural poverty. It is time for some fresh thinking. When smallhold farmers are properly prepared to engage in horticulture, they quickly change their mindset from

one of subsistence or survival to one of entrepreneurship, entrepreneurship because the production of perishable plant products must be time linked to a market, whether it is local, regional, or even international, and because horticultural products of good quality command a higher price, a reality that motivates producers to maximize the proportion of the crop that meets basic quality standards, reduce postharvest losses, and embrace new knowledge about producing safe food. Horticulture has and will always exist as a matrix of interrelating areas with overlapping and complex relationships. Describing its impact on the physiological, psychological, and social activities of people is the key to expanding an understanding of horticulture.

2 Phytonematodes: Threat to Horticulture

Nematodes are second only to insects in the number of species in the animal kingdom. However, only about 3 % of all nematode species have been studied and identified. One cubic foot of soil may contain millions of individual nematodes belonging to several different taxonomic groups. Plant-parasitic nematodes are nearly microscopic, worm-shaped animals virtually invisible to the naked eye when in the soil. Phytonematodes can cause significant plant damage ranging from negligible injury to total destruction of plant material. The severity of plant injury resulting from nematode activity depends on several factors such as the combination of plant and nematode species and prevailing environmental factors including rainfall, soil types, land contour, and culture practices.

Although a few nematode species feed on aboveground plant parts, such as leaves, stems, flowers, and seeds, the majority of these parasites feed on underground parts of plants, including roots, bulbs, and tubers. Because of this belowground, "hidden" feeding activity, nematode damage to plants cannot always be diagnosed readily. Nematodes are microscopic roundworms that live in many habitats. At least 2,500 species of plant-parasitic nematodes have been described, characterized by the presence of a stylet, which is used for penetration of host plant tissue. Most attack roots and underground parts of plants, but some are able to feed on leaves and flowers.

Phytonematodes are of great economic importance. However, because most of them live in the soil, they represent one of the most difficult pest problems to identify, demonstrate, and manage. Often, plant damage caused by nematodes is overlooked because the resulting nonspecific symptoms, such as slow growth, stunting, and yellowing, can also be attributed to nutritional and water-associated disorders (Stirling et al. 1998). Their effects are commonly underestimated by growers, horticulturists, and nematode management consultants, but it has been estimated that around 10 % of world crop production is lost as a result of plant nematode damage (Whitehead 1998). Most nematodes feed on bacteria, fungi, or other microscopic creatures. As such, they are a major component of soil and sediment ecosystems.

Of late, nematodes are being considered as a very successful class of animals. Four out of five multicellular animals on earth are nematodes. They are present almost everywhere: in cultivated fields, in sand dunes, in the sediments beneath the ocean floor, in groundwater, in plants, in animals, and even in humans. Some of the best-known nematodes are animal parasites such as heartworms, pinworms, and hookworms. Another important group of nematodes parasitize plants, which results in an estimated $8 billion a year loss to US agriculture/horticulture and nearly $78 billion loss worldwide.

The importance of nematodes especially in horticultural productivity was realized during the Second World War. The famous quote by Thorne, a renowned nematologist, "each year phytonematodes exact an ever increasing toll from almost every cultivated acre in the world-a bag of potatoes

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in England, a box of apples in New York or a crate of oranges in California…" clearly depicts the significance of plant-parasitic nematodes in horticulture. In developing countries like India, the 1960s was considered as the most active phase of nematology. In spite of significant contributions being made to manage phytonematodes during the past few decades, new and serious nematode problems warrant considerably increased effort and support than they are currently receiving. The problems are real and the challenge is great.

There are numerous estimates of the economic importance of nematodes in crop production on a worldwide and individual country basis, but precise values cannot be determined. For many countries, few or no studies have been made to determine the prevalence and extent of damage caused by parasitic nematode. Extensive research in developed countries and in more than 70 developing countries leaves little doubt concerning the destructive nature of plant-parasitic nematodes and the importance of their management for successful crop production. However, the overall average annual yield loss in important horticultural crops worldwide accounts to 13.54 % (Reddy 2008). Sasser and Freckman (1987) summarized the estimated annual losses in the yields of life-sustaining and economically important horticultural crops (Table 2.1).

Table 2.1 Estimated annual yield losses in horticultural crops due to phytonematodes

Life-sustaining crops	Loss (%)	Economically important crops	Loss (%)
Banana	19.7	Brinjal	16.9
Cassava	8.4	Cacao	10.5
Coconut	17.1	Citrus	14.2
Field bean	10.9	Coffee	15.0
Potato	12.2	Cowpea	15.1
Sugar beet	10.9	Grape	12.5
Sweet potato	10.2	Guava	10.8
		Melons	13.8
		Okra	20.4
		Ornamentals	11.1
		Papaya	15.1
		Pepper	12.2
		Pineapple	14.9
		Tea	8.2
		Tomato	20.6
		Yam	17.7
Average	**12.77 %**	**Average**	**14.31 %**
Overall average: 13.54 %			

In general, root symptoms vary widely but can include galling, lesions, cysts, stunting, and decay. Roots infected by parasitic nematodes are often darker than healthy roots. In addition, infected roots are often more susceptible to secondary infection by opportunistic bacteria and fungi. Often, new growth is stunted and infected plants are smaller than their healthy counterparts.

Nematodes continue to threaten horticultural crops throughout the world, particularly in tropical and subtropical regions. For centuries, crop plants have been plagued by these microscopic organisms. The degree of damage to a particular crop is influenced by the crop and cultivar nematode species, level of soil infestation, and environment. Severe damage may result if high infestation levels occur in soil where susceptible crops are planted. These deleterious effects on plant growth result in low yields and poor quality. Worldwide, major horticultural crops such as vegetables, fruit crops, flower crops and ornamentals, plantation crops, spices and condiments, medicinal and aromatic crops, crops grown under protected conditions/polyhouse, and tuber crops are severely affected by phytonematodes. Nematodes often cause decline or death of several of these highly prized crops including turf.

Table 2.2 presents the avoidable losses in the yield of some horticultural crops due to phytonematodes (Reddy 2008).

Although over 4,100 species of plant-parasitic nematodes have been identified (Decraemer and Hunt 2006), new species are continually being described while others, earlier considered as minor or non-damaging, are becoming pests as cropping patterns change (Nicol 2002). However, the phytonematodes of economic importance can be grouped into relatively restricted specialized groups that either cause direct damage to their host or act as virus vectors (Table 2.2). Most nematodes affect crops through feeding on or in plant roots, while a minority are aerial feeders. It is a known fact that in addition to direct feeding and migration damage, nematode feeding facilitates

Table 2.2 Major horticultural crops and the avoidable yield losses caused by phytonematodes

Crop	Phytonematode	Yield loss (%)
Banana	*Radopholus similis*	32–41
Betel vine	*Meloidogyne incognita*	21.1–38
Bitter gourd	*M. incognita*	36.72
Black pepper	*R. similis*	59
	M. incognita	46
Brinjal	*M. incognita*	27.30–48.55
Cardamom	*M. incognita*	32–47
Carnation	*M. incognita*	27
Carrot	*M. javanica*	56.64
Chilli	*M. javanica*	24.54–28
Coconut	*R. similis*	30
Coleus forskohlii	*M. incognita*	70.2
Colocasia	*M. incognita*	24
Coriander	*M. incognita*	51
Cowpea	*M. incognita*	28.60
	Rotylenchulus reniformis	13.2–32.0
Crossandra	*M. incognita*	21.64
Cumin	*M. incognita*	34
Cymbopogon	*M. incognita*	20
Davana	*M. incognita*	50
Fennel	*M. incognita*	39
French bean	*M. incognita*	19.38–43.48
Gerbera	*M. incognita*	31
Ginger	*M. incognita*	29.60–74.10
Grapevine	*M. javanica*	53–55
Lemon	*Tylenchulus semipenetrans*	29.0
Menthol mint	*M. incognita*	30
Mushroom	*Aphelenchoides sacchari*	40.6–100
	A. composticola	35–60
	A. avenae	25.8–53.5
Okra	*M. incognita*	28.08–90.90
Papaya	*R. reniformis*	28
Patchouli	*M. incognita*	47
Peach	*Macroposthonia xenoplax*	33
Peas	*M. incognita*	20–50.61
Plum	*M. xenoplax*	10
Pointed gourd	*M. incognita*	30–40
Pomegranate	*M. incognita*	27.0
Potato	*M. incognita*	42.50
	Globodera rostochiensis	99.50
Sweet lime	*T. semipenetrans*	19.0
Sweet orange	*T. semipenetrans*	69.0
Tomato	*M. incognita*	30.57–46.92
	M. javanica	77.50
	R. reniformis	42.25–49.02
Tuberose	*M. incognita*	13.78
Turmeric	*M. incognita*	18.6–25.0
	R. similis	46.76
Watermelon	*M. incognita*	18–33

subsequent infestation by secondary pathogens, including soil fungi and bacteria (Powell 1971), that complicates the condition of the host.

On a global scale, the distribution of nematode species varies greatly. Some are cosmopolitan, such as certain *Meloidogyne* spp., while others are particularly restricted geographically as *Nacobbus* spp. or are highly host specific, such as *Heterodera carotae* which attacks only carrots. Some crops may have very few nematode pests, while others have a particularly wide range of genera and species associated with them leading to difficulties for nematode management strategies. Distribution maps and host range data are available and updated regularly as a useful source for determining nematode damage potential. One difficulty with assessing nematode impact is that damage resulting from nematode infection is often less obvious than that caused by many other pests or diseases. Losses that result from nematode attack may not necessarily be as a consequence of direct cell death, necrosis, or “diseased” tissue, but may derive from other more insidious aspects, such as interference with the root system, reducing their efficiency in terms of access and uptake of nutrients and water; to the unaware, nematode-affected plants present typical drought and nutrient stress symptoms, which are easily and often misdiagnosed.

In the USA, a survey of 35 states on various crops indicated nematode-derived losses of up to 25 % (Koenning et al. 1999). Handoo (1998) estimated global crop losses due to nematode attack in the region of $80 billion, which, given the more subtle effects of low infestation levels, is probably a vast underestimate. Globally, a wide range of crops are produced, with some grown in specific areas,

Table 2.3 Phytonematodes of economic importance

Genus	Common name	Type of parasitism	Plant tissue affected
Anguina	Seed gall	Migratory endoparasite	Seeds, stems, leaves
Bursaphelenchus	Red ring/wilt	Migratory ectoparasite	Seeds, stems, leaves
Criconemella	Ring	Sedentary ectoparasite	Roots
Ditylenchus	Stem and bulb	Migratory ectoparasite	Stems, leaves
Globodera	Golden cyst	Sedentary endoparasite	Roots
Helicotylenchus	Spiral	Migratory ecto-/endoparasite	Roots
Heterodera	Cyst	Sedentary endoparasite	Roots
Hirschmanniella	Rice root	Migratory endoparasite	Roots, tubers
Hoplolaimus	Lance	Migratory ectoparasite	Roots
Meloidogyne	Root knot	Sedentary endoparasite	Roots
Pratylenchus	Lesion	Migratory ecto-/endoparasite	Roots
Radopholus	Burrowing	Migratory endoparasite	Roots, tubers
Rotylenchulus	Reniform	Sedentary semi-endoparasite	Roots
Scutellonema	Yam	Migratory ecto-/endoparasite	Roots, tubers
Tylenchulus	Citrus	Sedentary semi-endoparasite	Roots
Virus vectors			
Xiphinema	Dagger	Migratory ectoparasites	Roots
Longidorus, Paralongidorus	Needle	Migratory ectoparasites	Roots
Trichodorus, Paratrichodorus	Stubby root	Migratory ectoparasites	Roots

Table 2.4 Estimated global annual monetary losses due to nematodes for major horticultural crops

Crop	No. of estimates/crop	FAO production estimates (‘000MT)	Estimated price/MT (US$)	Estimated yield loss due to nematodes (%)	Estimated monetary loss due to nematodes (US$)
Banana	78	2,097	431	19.7	178,049,979
Cassava	25	129,020	90	8.4	975,391,200
Citrus	102	56,100	505	14.2	4,022,931,000
Cocoa	13	1,660	2,584	10.5	450,391,200
Coffee	36	5,210	3,175	15.0	2,481,262,500
Field bean	70	19,508	544	10.9	1,156,746,300
Potato	141	312,209	152	12.2	5,789,403,696
Sugar beet	51	293,478	37	10.9	1,183,596,774
Sweet potato	67	117,337	219	10.2	2,621,073,906
Tea	16	2,218	2,807	8.2	510,562,300
				Total	**19,369,408,865**

which are infested by one or the other phytonematodes. Some phytonematodes, apart from inciting diseases, also carry viral pathogens (Table 2.3).

Nematode attack can also predispose plants to attack by other pathogens through mechanical damage but also on a genetic basis. Monetary annual losses due to phytonematodes on major crops including “life-sustaining crops” have been estimated at $77 billion (Sasser and Freckman 1987) (Table 2.4). The loss in the USA alone accounts to $5.8 billion. However, when all crops are taken into consideration, the loss may exceed up to $100 billion. Due to the higher percentage of crop losses, the dollar value losses are higher in developing countries as compared to developed ones.

2.1 Ten Economically Important Genera of Phytonematodes Associated with Horticultural Crops

The list of economically important ten genera of phytonematodes associated with various horticultural crops around the globe comprises of *Meloidogyne* (root-knot nematode), *Heterodera* (cyst nematode), *Ditylenchus* (stem and bulb nematode), *Globodera* (golden cyst nematode), *Tylenchulus* (citrus nematode), *Xiphinema* (dagger nematode), *Radopholus* (burrowing nematode), *Rotylenchulus* (reniform nematode), and *Helicotylenchus* (spiral nematode). However, this order of genera may vary depending upon a specific location, with some more added genera. For example, in Europe, the top ten most destructive phytonematodes include *Heterodera, Globodera, Meloidogyne, Ditylenchus, Pratylenchus, Aphelenchoides, Xiphinema, Trichodorus, Longidorus*, and *Tylenchulus* (Sasser and Freckman 1987).

The importance of nematodes in world horticulture can be judged by whether or not their damage is catastrophic to major crops. Several phytonematodes are responsible for this kind of damage. Some of the important ones affecting major horticultural crops include the following.

1. **Root-Knot Nematode** (*Meloidogyne* spp.): It is the most common sedentary endoparasite on horticultural crops and can damage and cause severe economic loss. It has worldwide distribution though more common in temperate, subtropical, and tropical areas. Female embedded in root tissue, melon shaped, induces characteristic knots/galls on the roots of affected plants. This genus includes more than 60 species, with some species having several races. However, four species, *M. incognita, M. javanica, M. arenaria*, and *M. hapla*, are major pests worldwide. They modify normal cells into giant cells before feeding. Female produces a large number of eggs embedded in a gelatinous egg mass attached to the knot on the root, which is produced by six rectal glands and secreted before and during egg laying. Normally, 500–1,000 eggs are laid per female. Gall formation is a visible sign of root-knot nematode infection resulting from the hypertrophy and hyperplasia indicative of feeding site formation within the roots. The matrix initially forms a canal through the outer layers of root tissue and later surrounds the eggs, providing a barrier to water loss by maintaining a high moisture level around the eggs. As the gelatinous matrix ages, it becomes tanned, turning from a sticky, colorless jelly to an orange-brown substance which appears layered. This egg mass serves as a dormant structure for this group of nematodes. Males are vermiform and free living in soil. Juveniles are slender and vermiform, about 450 μm long. These nematodes are more destructive when they associate with other soilborne fungal and bacterial pathogens to form complex diseases. The major hosts of root-knot nematodes include most horticultural crops, viz., vegetables, fruits, ornamentals, polyhouse-grown crops, plantation crops, and medicinal, aromatic, and spice crops.
2. **Burrowing Nematode** (*Radopholus* spp.): It constitutes an important group of endoparasitic nematodes of plant roots and tubers, which is migratory. The major species is *R. similis* with two host races that differ in parasitism of citrus. It completes its entire life cycle within the root tissue. Infected plants experience malnutrition. It induces lesions and burrows in the roots, which later turn into tunnels and cavities causing destructive damage. Infection by burrowing nematode causes toppling-over disease in banana, yellows disease in pepper and spreading decline in citrus. Because of the damage it causes worldwide, burrowing nematode is considered as one of the most regulated nematode pests. Burrowing nematode, although native to Australasia, is found worldwide in tropical and subtropical regions of Africa, Asia, Australia, North and South America, and many island regions. The widespread range of this nematode is due to its dissemination with propagative plant material, especially infected banana

corms/rhizomes/suckers. This devastating nematode has been observed infecting more than 300 plant species including banana, coconut, areca nut, tea, ginger, pepper, betel vine, turmeric, avocado, coffee, ornamentals, sugarcane, and citrus; *R. similis* infects both banana and citrus, while *R. citrophilus* infects only citrus. However, these two species are categorized as the "banana race" and "citrus race," respectively, within *Radopholus similis*.

3. **Cyst Nematode** (*Heterodera* species): This sedentary endoparasite is a potential pathogen on several crop plants. Different species of this genus attack several crops, often causing great economic damage. The genus is unique among nematode genera because of the ability of the female to transform into a tough, brown cyst. Cysts are either partially enclosed in the root tissue or in the soil. It is called a cyst nematode because the greatly swollen, egg-filled adult female is referred to as the "cyst stage." Female appears swollen or obese and lemon shaped with a distinct neck. Females produce several hundred eggs, and after death, the female cuticle forms a protective cyst. Eggs are retained within the cyst. Males are vermiform which are present in soil. The genus has worldwide distribution with a wide host range including horticultural crops. Major species include *H. avenae, H. glycines, H. cajani, H. schachtii, H. trifolii, H. gottingiana, H. carotae, H. cruciferae, H. ciceri, H. oryzae, H. oryzicola, H. sacchari,* and *H. polygoni.*
4. **Potato Cyst Nematodes** (*Globodera rostochiensis* and *G. pallida*): *Globodera* is similar to *Heterodera* but the cyst is globose in shape. Golden cyst nematode, *Globodera rostochiensis*, and pale cyst nematode, *G. pallida*, are major pests on potato. Altogether 90 species of the genus *Solanum* are known to be hosts for this group of nematodes. Both species are confined to the cooler places. Apart from potato, they are also pests on tomato, eggplant, members of the Solanaceae plant family (such as nightshade), and other root crops. Both species feed on and produce cysts on potato roots, thus causing substantial damage to potato crops. The cysts are typically yellow-brown/golden yellow in color. Both the golden and pale cyst nematodes are regulated federally. Major species include *G. rostochiensis, G. pallida*, and *G. tabacum.*
5. **Lesion Nematode** (*Pratylenchus* spp.): It is an important group of migratory endoparasites on roots of several crops. It causes serious damage to many economic plants worldwide including coffee. Root lesion nematodes infect a great variety of hosts. *Pratylenchus penetrans* alone has over 350 host plants. Major hosts include coffee, potato, ornamentals, mint, plantation crops, corn, banana, turf, peanut, and wheat. They induce lesions on root surface. The major species are *P. penetrans, P. brachyurus, P. coffeae, P. pratensis, P. zeae, P. teres, P. goodeyi, P. thornei*, and *P. vulnus.*
6. **Reniform Nematode** (*Rotylenchulus reniformis*): This is a common pest associated with the rhizosphere of several horticultural crops. *R. reniformis*, a semi-endoparasite, exists practically everywhere in tropical and subtropical soils. The term "reniform" refers to the kidney-shaped body of the mature female. The female penetrates the root and remains in one position at a permanent feeding site with its posterior end projecting from the root. The immature female is the infective agent, attacking the root and growing to maturity at its feeding site. Males are vermiform. Eggs are laid in gelatinous matrix. At least 314 plant species are hosts to reniform nematode including cotton, castor, cowpea, pigeon pea, various fruit trees, tea, tobacco, soybean, banana, pineapple, cucumber, radish, and eggplant. The major species is *R. reniformis*, which is found in both tropical and warm temperate soils.
7. **Stem and Bulb Nematode** (*Ditylenchus dipsaci*): It is a slender, vermiform, migratory endoparasite of plant stems and leaves. *D. dipsaci* is one of the most devastating phytonematodes in the world. Its races are very diverse and found in most temperate areas of the world. In this nematode, in the

fourth stage, juveniles are infective but not in the second stage, as in most other phytonematodes. It produces a dormant structure known as "nematode wool," which is a bundle of juveniles to survive under adverse climatic conditions. This pathogen infects 400–500 plant species worldwide including onion, narcissus, potato, garlic and other bulb crops, alfalfa, strawberry, carrot, tobacco, tulips, and faba beans. Potato rot nematode (*D. destructor*) is another destructive pest on potato.

8. **Citrus Nematode** (*Tylenchulus semipenetrans*): It is a major pest on all members of *Citrus* group fruits. Their feeding strategy is semi-endoparasitic and has a very narrow host range among commonly grown crops. Apart from *Citrus* group, they can also attack olive, grape, persimmon, and lilac. It is the causal agent of slow decline in citrus. Mature females are non-vermiform; the anterior part is embedded in root tissues, while the slender posterior part protrudes from roots and is swollen. Males and juveniles are vermiform and slender.
9. **Spiral Nematode** (*Helicotylenchus* spp.): This nematode attains usually spiral shape and is distributed worldwide, and endoparasitic nematodes on roots of several crops, viz., banana, maize, ornamentals, plantation crops, fruit trees, turf, etc. They are also ectoparasites of several plant roots, which insert their stylets into root epidermis to feed. Some species live half-buried in the root tissue, while others penetrate the root and live inside. Out of 200, the most damaging species is *H. multicinctus*, apart from *H. mucronatus, H. dihystera, H. pseudorobustus*, and *H. vulgaris.*
10. **Dagger Nematode** (*Xiphinema* spp.), **Needle Nematode** (*Longidorus* spp. and *Paralongidorus* spp.), and **Stubby-Root Nematode** (*Trichodorus* spp. and *Paratrichodorus* spp.): All these are slender pests on crop plants and also are involved in transmitting plant viruses. They are ectoparasites on roots of perennial and woody plants with a worldwide distribution.

    Dagger nematode is of economic importance on grape, strawberry, hops, and a wide range of crops, viz., nectarine, oak, rose, grapevine, raspberry, carrot, cherry, peach, woody plants, fruit trees, soybean, corn, and some cereals. *Xiphinema* has around 163 species, the major ones being *X. americanum, X. diversicaudatum, X. index, X. italiae*, and *X. pachtaicum*. They have a very long life cycle, several months to 2 years. Nematodes induce "giant cells and galls" and necrosis; giant cells are caused by karyokinesis without cytokinesis yielding multinucleated cells. They are easily recognizable, due to a long body length and a long stylet capable of reaching vascular tissue. Major species include *X. americanum, X. elongatum, L. africanus*, and *P. minor.*

    Needle nematodes are very large, ranging in length from 2 to 8 mm. Odontostyle in these nematodes is long. They have a worldwide distribution, usually moist environments. At least seven species of *Longidorus* are reported from grape vineyards throughout the world, viz., *L. attenuatus*, *L. diadecturus, L. iranicus, L. macrosoma*, *L. protae*, *L. sylphus, Paralongidorus maximus, P. bullatus, P. iberis, P. lutensis*, etc. Lettuce, corn, mint, bean, pea, radish, tomato, spinach, rose, avocado, broccoli, watermelon, carrot, and fig are some other hosts.

    Stubby-root nematodes are short, with a cigar-shaped body. Feeding activity stops root growth resulting in stubby roots. Common species are *T. christiei, T. nanus, T. obscures, T. pachydermis*, *T. porosus*, *T. primitives*, *Paratrichodorus minor, P. divergence, P. teres, etc. Trichodorus* species are worldwide in distribution on a wide range of crops including apple, onion, turf, forest trees, vegetables, ornamentals, rose, tomato, sugarcane, mulberry, grape, avocado, fig, banana, pear, walnut, citrus, beet, date palm, and spinach.

2.2 Less Widely Distributed Phytonematodes

In addition to the above worldwide distributed phytonematodes, some pose problems on horticultural crop plants in several regions (Ravichandra 2008). They are as follows.

1. **Lance nematode** (*Hoplolaimus* spp.): This is a major migratory ectoparasite, which feeds mostly on roots of various crops, viz., pepper, tomato, citrus, pines, banana, pine, oak, and grasses. Major species include *H. columbus, H. seinhorsti*, and *H. indicus.*
2. **Ring nematode** (*Criconemella, Criconemoides*): It is a migratory ectoparasite associated with rhizosphere of several horticultural crops, viz., all *Prunus* species, including peach, almond, apricot, cherry and plum, apart from lettuce, carnation, pine, turf, walnut, and grapes. Major species include *C. xenoplax, C. axestis*, and *C. spharocephalum.*
3. **Bud and leaf nematode** (*Aphelenchoides* spp.): It is a foliar nematode and has a worldwide distribution. *A. fragariae*, *A. ritzemabosi*, and *A. besseyi* are major pests on strawberry, chrysanthemum, and rice, respectively. The strawberry crimp nematode/fern nematode *A. fragariae* also infects carnation, fern, African violet, Easter lily, etc. The chrysanthemum foliar nematode *A. ritzemabosi* causes necrosis on leaves of chrysanthemums and other ornamentals.
4. **Pine wood nematode** (*Bursaphelenchus xylophilus*): It has been implicated in a serious disease of pine trees, "pine wilt disease," which has devastated pine forests in Japan and occurs in North America. In 1997, white pine trees in Maryland were devastated due to the heavy infestation of this nematode. This foliar nematode is a serious quarantine pest, and all pine wood chips or wood products for import and export purposes need to be checked for this nematode. When the beetle feeds on a susceptible host pine, the pine wilt nematode enters the tree and feeds on the epithelial cells which line the resin ducts, which results in pine wilt disease. Water transport in the tissues of the infested tree is disrupted and the disease can manifest within a few weeks. Major signs include browning of the needles or yellowing of the leaves, and the tree may die within 2–3 months. Susceptible pine species include Scotch, slash, Japanese red, and Japanese black pines.
5. **Red ring nematode of coconut** (*Bursaphelenchus*/*Rhadinaphelenchus cocophilus*): Red ring nematode causes red ring disease of palms. It parasitizes the palm weevil *Rhynchophorus palmarum* L., which is attracted to fresh trunk wounds and acts as a vector for *B. cocophilus* to uninfected trees. The major internal symptom of red ring infection is the "red ring" for which the disease is named. A crosscut through the trunk of an infected palm 1–7 ft above the soil line usually exhibits a circular, colored band around 3–5 cm wide. External symptoms include shortening and deformation of already established leaves that might turn yellow-bronze and later deep reddish-brown. The discoloration usually begins at the tip of each leaf and starts in the older leaves before moving to the younger ones. As the leaves change color and dry up, they wilt and die. It has not been reported to be present in some regions including continental USA, Hawaii, Puerto Rico or the Virgin Islands, and India.

2.3 General Characteristics of Phytonematodes

The nematodes are a very successful group of animals that can destroy up to 10 % of all arable crops, a figure that is comparable with the destructive effects of insects. Despite the tremendous economic importance of nematodes, the great majority of them are free living and they occur in every conceivable environment. It has been claimed that "if all matter was destroyed except nematodes, one would still be able to see the world outlined by nematodes." Nematodes are "hidden and unseen enemies" of crop plants as they are microscopic and present in soil, particularly in rhizosphere. They are microscopic animals, which are vermiform, bilaterally symmetrical, unsegmented, pseudocoelomatic, and triploblastic. Their body is not segmented as in earthworms. There are no respiratory or circulatory organs present in nematodes. All phytonematodes possess a structure called "stylet" or spear,

which is a unique feature. The stylet is the major feeding organ present in the mouth of phytonematodes that penetrates into the host tissue. Nonplant parasitic and other forms do not possess the stylet.

2.3.1 Body Size

There is a great variability in the size of nematodes. Free-living nematodes in soil and freshwater vary in length from about 150 μm to 10 mm. Marine species vary from 83 μm to about 50 mm in length. The animal parasites range from about 1 mm to 7 m in length, the largest being nematodes parasitic in whales. However, most plant parasites are microscopic and range from 0.25 mm to approximately 12 mm in length with a body width from 0.01 to 0.5 mm. The longest phytonematode is *Paralongidorus* and the smallest is *Paratylenchus.*

2.3.2 Body Shape

Nematodes generally are elongate, cylindrical, unsegmented worms. They are bilaterally symmetrical. Though most of them are vermiform, in some genera the body of the adult female may be greatly modified (*Meloidogyne, Heterodera, Rotylenchulus*, *Tylenchulus*, etc.). In these genera, the body may be melon shaped, spindle shaped (fusiform), pear shaped, kidney shaped, lemon shaped, or variations of saccate. The entire nematode body may be divided into three regions: the outer body tube or body wall, the inner body tube, and the body cavity of pseudocoelom.

The outer body tube constitutes cuticle (exoskeleton), hypodermis, and muscle layer. The body wall is composed of a thick cuticle, which may be considered to be an exoskeleton, a syncytial hypodermis, and the mucus layer composed of a single layer of longitudinal muscle only. Pseudocoelom is the false body cavity (the area between the body wall and the digestive tract), which is not lined with epithelial tissue. It is filled with pseudocoelomic fluid, which bathes the muscles, the digestive system, the reproductive system, and some or all of the excretory system. The fluid in the pseudocoelom serves as a transport system for oxygen, food materials, and products of metabolism. The gut of nematodes consists of a terminal mouth, buccal cavity with six lips, pharynx, intestine, rectum, and subterminal anus. In the male, there is cloaca, with the rectum and vas deferens opening into the same cavity. The inner body tube consists of the digestive system, esophagus, intestine, rectum, and cloaca. The nervous system and reproductive system are well developed, while the excretory system is not very well developed in phytonematodes. The nervous system is well equipped with a range of sophisticated sense organs. Reproduction in nematodes is well developed and of diverse types, which plays a major role in the pathogenicity of a phytonematode.

2.3.3 Reproduction in Nematodes

The reproductive systems are major organs of the nematodes and can occupy a large portion of the body cavity in males and females. There are many morphological and physiological differences between the species and so they are separated here.

2.3.4 Males

Males are either monorchic (most Secernentea) or diorchic (most Adenophera), with regard to the number of testes present. The testes are tubular structures lined with epithelium and glandular tissue; sperms are produced at the end and mature as they migrate toward the shared opening of the cloaca. Many males have paired chitinous protrusion from the cloaca known as spicules, which are main copulatory organs and are used for attaching to a female during copulation. The spicules are easily seen under a microscope due to their chitinous structure and their position, and shape may be used as an identifying feature. Sperms are ejaculated from the cloaca around

the spicule, rather than through it. The sperm produced by nematodes is amoeboid and is very motile, employing the same locomotion mechanism as seen in amoeboid species.

One of the most distinctive features of some male nematodes is the presence of a copulatory bursa, seen in nematodes of the order Strongylida. Nematodes with a copulatory bursa are known as bursae, while those without are non-bursae. This bursa is at the posterior end of the nematode and is formed from alae with lateral rays that are used for grasping onto the body of the female during copulation.

2.3.5 Females

Female nematodes usually have a single genital pore through which sperm may enter the uterus and oviduct; this pore is also referred to as a vulva and may be covered by a vulval flap. The uterus may take many forms from being short and straight, long with a single bend, or a coiled form. Eggs produced in the ovaries populate the oviducts and uterus and may be released as embryonated or nonembryonated eggs once fertilization has occurred. Nematodes may have either one (monodelphic) or two ovaries (didelphic) with various types of orientation in case of two ovaries. A small muscular organ exists at the vulval opening of some species known as the "ovijector," which aids in the expulsion of eggs from the vulva. Unlike in males, the end of the female is usually blunt ended with the anus being positioned proximally on the body wall.

Male and female nematodes are usually similar in appearance except for the reproductive systems. It mainly includes one or two tubular gonads which may vary in length and may be straight, reflexed, or coiled back and forth. In males and females, the genital tubes are lined with a single layer of epithelium which covers the germ cells and forms the ducts. In majority of nematodes, the germ cells are proliferated only in the distal end of the gonad, i.e., telogonic. In a few nematodes, the germ cells are formed along the entire length of the gonad, i.e., hologonic. Most phytonematodes are characterized by an increase in the size of the entire reproductive system. The uteri and the growth zone of the ovary are lengthened that leads to more egg production. However, pronounced dimorphism occurs in some species. Females become swollen and males remain slender and cylindrical.

2.3.6 Major Types of Reproduction

1. *Bisexual* (*amphimixis*): Male and female reproductive structures are present on two different individuals (e.g., burrowing nematodes, root-knot nematodes).
2. *Parthenogenesis*: Fertilization of eggs occurs without the help of males in this type. Mating and genetic recombination do not occur (e.g., root-knot nematodes, citrus nematodes, cyst nematodes).
3. *Hermaphroditism*: Both male and female reproductive structures are present on the same individual (e.g., root-knot nematodes, stem and bulb nematodes).

Intersexes are also common in some nematodes like root-knot nematodes. It is a variation in sex characteristics including chromosomes, gonads, and/or genitals that do not allow an individual to be distinctly identified as male or female. These intersexes have normal male head and tail structures and exhibit some mating behavior, but possess hermaphrodite-like gonads which produce no sperm and usually contain a few oocytes.

All nematodes pass through an embryonic stage, four larval/juvenile stages, and an adult stage. Juveniles hatch from eggs as vermiform, second-stage juveniles, the first molt having occurred within the egg. Newly hatched juveniles may reinvade the host plants of their parent or migrate through the soil to find a new host root. In most nematode genera, second-stage juveniles and adult females only are plant parasitic. The duration of life cycle depends on important factors like nematode species, soil temperature, soil moisture, soil structure, soil texture, and type of host.

Most phytonematodes attack roots/underground parts (bulbs, suckers, rhizomes, corms,

etc.) of host plants, while some attack aerial plant parts too. General aboveground symptoms of nematode infestation on horticultural crops include stunted growth, wilting, and yellowing in patches. However, characteristic symptoms are expressed in belowground, which may comprise the presence of knots/galls, cysts, lesions, and cavities/tunnels on the roots. Excessive branching of roots, deformed/abnormal roots/rootlets, rotting and discoloration of roots, and decreased/complete destruction of feeder roots are some of the symptoms of nematode damage, which contribute to overall reduction in the growth and development of plants. This affects the quality and quantity of the produce that ultimately affects the grower. Each phytonematode possesses a common and a scientific name as listed below.

2.4 Common Names of Major Phytonematodes of Horticultural Crops

Anguina spp.: Seed and leaf gall nematode
A. agrostis: Bent grass nematode
Aphelenchoides spp.: Bud and leaf nematode, foliar nematode
A. besseyi: Rice white-tip nematode, strawberry bud nematode, summer crimp, summer dwarf nematode
A. fragariae: Spring crimp nematode, spring dwarf nematode, strawberry bud nematode
A. ritzemabosi: Chrysanthemum foliar nematode
Bursaphelenchus cocophilus: Coconut palm nematode, red ring nematode
Bursaphelenchus xylophilus: Pinewood nematode
Cacopaurus pestis: Walnut nematode
C. cacti: Cactus cyst nematode
Criconema/*Criconemoides*: Ring nematode
D. destructor: Potato rot nematode
D. dipsaci: Stem and bulb nematode, alfalfa stem nematode
Dolichodorus spp.: Awl nematode
G. pallida: Pale/white potato cyst nematode
G. rostochiensis: Golden nematode, golden potato cyst nematode
H. multicinctus: Banana spiral nematode, spiral nematode
Hemicriconemoides spp.: False sheath nematode
Hemicycliophora spp.: Sheath nematode
Heterodera carotae: Carrot cyst nematode
H. cruciferae: Cabbage cyst nematode
H. cyperi: Nutgrass cyst nematode
H. fici: Fig cyst nematode
H. goettingiana: Pea cyst nematode
H. schachtii: Sugar beet cyst nematode
Hoplolaimus spp.: Lance nematode
Longidorus spp.: Needle nematode
Meloidodera spp.: Cystoid nematode
Meloidogyne spp.: Root-knot nematode
M. carolinensis: Blueberry root-knot nematode
M. exigua: Coffee root-knot nematode
M. graminis: Grass root-knot nematode
M. hapla: Northern root-knot nematode
M. incognita: Southern root-knot nematode
M. javanica: Javanese root-knot nematode
M. konaensis: Kona coffee root-knot nematode
M. lusitanica: Olive root-knot nematode
M. megatyla: Pine root-knot nematode
M. nataliei: Michigan grape root-knot nematode
M. pini: Sand pine root-knot nematode
Nacobbus spp.: False root-knot nematode
Paratrichodorus spp.: Stubby-root nematode
Paralongidorus spp.: Needle nematode
Pratylenchus spp.: Pin nematode
Pratylenchoides spp.: False-burrowing nematode
Pratylenchus spp.: Lesion nematode
P. coffeae: Coffee lesion nematode
P. penetrans: Meadow nematode
P. vulnus: Boxwood lesion nematode, walnut lesion nematode
Punctodera punctata: Grass cyst nematode
R. similis: Banana burrowing nematode, burrowing nematode
Rotylenchulus reniformis: Reniform nematode
Rotylenchus spp.: Spiral nematode
Scutellonema bradys: Yam nematode
Subanguina radicicola: Grass root-gall nematode
Trichodorus spp.: Stubby-root nematode
Tylenchorhynchus spp.: Stunt nematodes
Tylenchulus semipenetrans: Citrus nematode
Xiphinema spp.: Dagger nematode
X. americanum: American dagger nematode

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3 Phylogenetic and Evolutionary Concepts in Nematodes

Keeping the medical, ecological, and economical importance of nematode phylum in mind, it is remarkable to see that nematode systematics is far from established. It has a long history of constant revision, and there may be as many classifications as there are nematode taxonomists. Ferris and Ferris (1987) anticipated about the growing sense of excitement pervading systematics as new techniques make it possible a depth of understanding of phylogenetic relationships and affinities never before thought possible. They further stated that Darwin's "genealogical taxonomy," based on the concepts of descent with modification, is linked directly with two approaches to phylogenetic inference, viz., phenetics and cladistics. In both of these, patterns of descent take precedence over processes, and in classifications based on these procedures, "grades" and "gaps" beloved by the evolutionary systematics are ignored and categories are usually of lesser importance (Dupuis 1884). The phenetic approach deals with "natural classification" based on overall similarity and the belief that the more characters a classification is based on, the more reliable it will be.

A phylogeny allows the reconstruction of the historical changes that have led to current variation and provides a way to test how often convergent changes have occurred. Species phylogenies are also crucial for bioinformatic analyses of genomes; they provide a basis for selecting species for comparative genomic sequencing and for testing orthologous and paralogous relationships in gene phylogenies, an important foundation for genome annotation and prediction of gene function (Eisen 1998; Eisen and Fraser 2003). Although molecular biologists have long appreciated the value of sequence comparisons to identify conserved regions as indicators of function, arguably the most interesting aspects of evolution are changes in molecular functions, domains of expression, and developmental roles. Elucidating how such changes have shaped functional diversity at a variety of levels also has potential for augmenting our understanding of genome function and developmental mechanisms. Reciprocally, information from this model system facilitates studies of evolutionary pattern and process. The success of such comparative approaches to enhance the understanding depends upon the availability of material and information from multiple related species, as well as different wild populations of *C. elegans*. Knowing the phylogenetic relationships between *C. elegans* and other nematodes or animals is important for comparative analyses of behavior, morphology, development, molecular mechanisms, and genomics.

A quick tour of nematode diversity and the backbone of nematode phylogeny provide a view of general nematode diversity and phylogeny. The phylogenetic relationship of *C. elegans* and other rhabditids reviews what is known so far about the closer relationships within the rhabditids and within genus *Caenorhabditis* in particular. There is substantial variation among rhabditids at genetic and developmental levels.

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Reconstructing how this variation arose is likely to illuminate how developmentally robust systems can nevertheless be modified by evolutionary change, one of the most intriguing and fundamental questions in evolutionary biology today. Nematode genome evolution reviews work on the evolution of genome organization and chromosome architecture. Evolution of development in nematodes related to *C. elegans* provides an overview of comparative developmental biology using *C. elegans* and other satellite model organisms, such as *Pristionchus pacificus*.

3.1 Nematode Relationships to Other Animals

Nematodes were once classified with a very large and heterogeneous cluster of animals grouped together on the basis of their overall wormlike appearance, simple structure of an internal body cavity called a pseudocoelom, and the lack of features such as cilia and a well-defined head that are found in most animals. This group, variously known as Aschelminthes or Pseudocoelomata, is today no longer recognized as a natural one. It is quite likely that the simple body plan of these organisms has resulted from reduction and simplification from more than one group of ancestral organisms and so the pseudocoelom is neither a uniquely derived nor useful character (Wallace et al. 1996) The simplicity is thus a result of secondary simplification from a more complex body design and not necessarily an indication of primitive or simple origins. Current studies indicate that nematodes are actually related to the arthropods and priapulids in a newly recognized group, the Ecdysozoa. Nematode fossils are hard to find because the organisms are microscopic and lack hard structures. However, fossils have been found dating from the Cambrian period, and it is very likely that nematodes have been around since then (Waggoner and Brain 2004). As rather small and primitive organisms, nematodes display mostly simple evolutionary developments. The important steps in evolution follow a pattern similar to this:

- No symmetry (e.g., unicellular organisms) to radial symmetry (e.g., jellyfish) to bilateral symmetry (e.g., vertebrates, worms, crustaceans)
- Segmentation (e.g., earthworms)
- No coelom or body cavity (e.g., unicellular organisms) to with coelom (e.g., vertebrates, annelids)
- Vertebrae (e.g., mammals, fish, birds)

The following animal phylogeny illustrates many of the important relationships between nematodes and other phyla (Fig. 3.1).

The phylum Nematoda or roundworms obviously do not contain vertebrae. They are bilaterally symmetrical but lack segmentation. This characteristic distinguishes nematodes from other common segmented worms such as those in phylum Annelida. The difference between other bilaterally symmetrical organisms and worms lies in the presence of an internal body cavity or coelom in those organisms. The pseudocoelomates represent the first organisms to have an internal body cavity. This is significant in that it promotes more sophisticated and efficient mobility (Raven and Johnson 1985). Again, the insufficiency of nematode study makes comprehensive classification very difficult. Because only a small percentage of the different species of nematodes have been classified, constructing true phylogenetic relationships is hard. Similarly, because nematodes are so uniform in structure, classifying them is tough. It is widely believed that the shared ancestor of present-day nematodes had the same basic characteristics that we see in all species of roundworms. Thus, the differences between the most primitive and the most evolved nematode species are fairly small. Even where evolution is seen from primitive to advanced specimens, it is almost uniformly present in every branch (Malakhov 1994). This idea of parallelism presents further difficulty in classifying nematodes. Nonetheless, nematodes are all classified as pseudocoelomates because they have a primitive body cavity.

The division of nematodes into two classes in effect distinguishes between the more advanced in Secernentea and the more primitive in

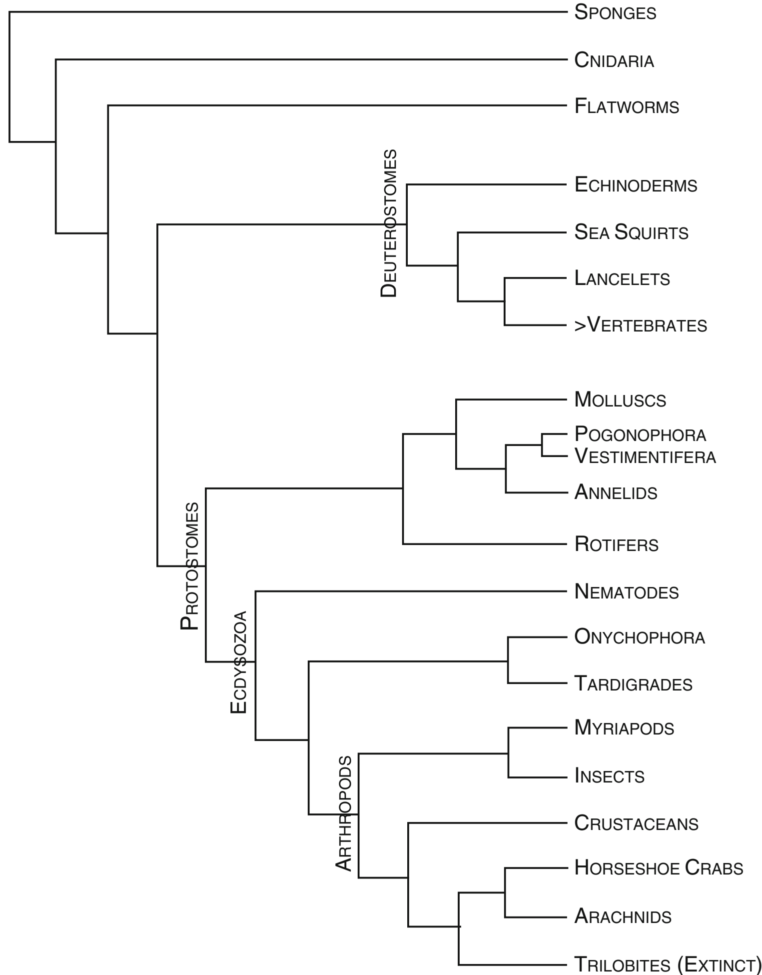

Fig. 3.1 Phylogeny depicting relationships between nematodes and other phyla

Adenophorea (Poinar 1983). As technology and taxonomy have become more advanced, the classification of nematodes has changed significantly. However, when considering the phylogenetic tree for nematodes, it is imperative to keep in mind that nematologists have not reached a consensus. There is no single comprehensive tree that all scientists agree on for nematodes.

3.2 Phylogenetic Concept

Molecular phylogenetic methods allow comparison of disparate taxa using the same metric, the evolution of a single conserved molecule. This approach sidesteps some of the problems of the definition of homology and is synergistically compatible with morphological systematics. The use of molecular markers certainly brings its own problems, but in general, the mode of evolution of DNA sequences is better understood than that of morphological traits and can be modeled with some confidence. This allows alternative analytical tools to be used and permits calculation of statistical support for the phylogenies produced. An important consideration is that the rates of phylesis (the generation of taxa; speciation) and fixation of molecular change must be of the same order. Thus, a rapidly evolving DNA segment should be used to examine the relationships

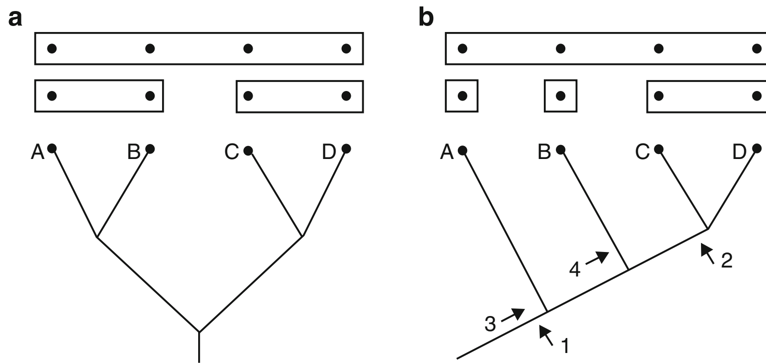

Fig. 3.2 Phylogenetic tree of a monophyletic species, (**a**) is a lumper which unites species in a genera and (**b**) is a splitter that divides into genera

between species in a genus and a very conserved segment for interordinal or interphylum relationships. It should be borne in mind that the phylogeny derived from a single molecule might not faithfully reflect the history of all species studied and that information from multiple unlinked genetic loci will give more robust estimates (Mortiz and Brown 1986).

The basic principle of phylogeny is that all are related and evolved from a common ancestor; phylogenetic systematics differs from the traditional approach as it is based on genealogy (common ancestry) and the diagram obtained is a cladogram (Dorris et al. 1999; Subbotin et al. 2004). Some of the basic principles to be assumed for phylogeny include that evolution has occurred; new taxa can be characterized by new features and these characters are derived (apomorphies) from previously existing ones (plesimorphies). The monophyletic group with four species (a) with two subgroups and (b) with three subgroups has been depicted in Fig. 3.2.

3.3 Criteria for Inferring Phylogenetic Tree

Different criteria can be used to infer phylogenetic trees from morphological or molecular data (Stone 1983). All methods are based on two processes: an algorithm for finding trees and a criterion for selecting the best ones. It is expedient to apply all the criteria to each data set and to test the derived trees for consistency and statistical support. The three major criteria used are as follows:

(a) *Neighbor Joining*: Neighbor joining analysis yields a point estimate of a minimum evolution tree based on data transformed into a pairwise distance matrix. In this method, the algorithm for finding the tree and the criteria for assessing its quality are combined.
(b) *Maximum Likelihood*: Maximum likelihood analysis uses an explicit model of evolution (direction and probability of character change) to derive the tree most likely to have occurred given the data. Many different trees can be built and tested.
(c) *Maximum Parsimony*: Maximum parsimony is a criterion for selecting an optimal tree based on the principle that the tree requiring the least number of changes in character states is more favored. Many different trees can be built and tested. Among the methods for testing the internal statistical support for the inferred tree is the bootstrap. Bootstrap resampling rebuilds a number (usually >100) of model data sets based on the test set (by sampling with replacement) and reanalyzes them with the chosen criteria. The percent retention of nodes in the set of bootstrap trees is a strong indication of their robustness. Bootstrap values >65 % are considered robust.

The 18S rDNA of 19 populations of *Meloidogyne* spp. has been amplified and directly sequenced. The region of mitochondrial

DNA, located in the 3′ portion of the gene that codes for cytochrome c oxidase subunit II (*COII*) through a portion of the 16S rRNA (*lRNA*) gene, from 16 of these populations was cloned and sequenced. Heteroplasmic sequences were identified from both rDNA and mtDNA regions for several taxa. Several sequences sampled from nominal taxa differed from previously published accounts. Phylogenetic trees based on alignments of these sequences were constructed using distance, parsimony, and likelihood optimality criteria. For 18S rDNA data, three main clades were identified. One well-supported clade (86–91 % bootstrap) included the most common and widely disseminated species, e.g., *M. arenaria*, *M. javanica*, and *M. incognita*, and some recently described or redescribed species (*M. floridensis*, *M. paranaensis*, and *M. ethiopica*) plus numerous unidentified isolates. All mitotic parthenogenetic species, except for *M. oryzae*, were included in this clade. Other, less well-supported clades included the amphimictic and facultative meiotic species *M. hapla*, *M. microtyla*, *M. maritima*, and *M. duytsi*. One such clade comprised three meiotic parthenogens (*M. exigua*, *M. graminicola*, and *M. chitwoodi*) and *M. oryzae*. This clade was moderately supported (77 % bootstrap) but the relationships within this clade were poor. For mitochondrial DNA data, only the species in clade 1 from rDNA analysis and *M. hapla* were analyzed. These species formed a well-supported clade (100 % bootstrap) to the exclusion of *M. mayaguensis* and *M. hapla*. The addition of taxa and mtDNA data to publicly available records improved the discrimination sensitivity of species and atypical, non-identified isolates.

Current accepted classification of the phylum Nematoda is based on morphological and ecological traits, primarily in the context of free-living or parasitic phenotypes (Dorris et al. 1999). The deceptively uniform basic anatomy of nematodes masks a complex pattern of diversity, and estimates of species number within the phylum range from 40,000 to 100 million. The reliability of nematode morphology in producing a coherent phylogeny has been called into question for several reasons. Not least is the disagreement in resolution at the highest levels evident in systematic studies based on morphology. In five major phylogenetic representations of the phylum, two classes are recognized: Adenophorea and Secernentea. In two of these analyses, 2 and 3, both classes are monophyletic, arising from the same ancestor. The other three phylogenies 4–6 suggest that the Adenophorea are paraphyletic and give rise to the Secernentea. The broad ecology of nematodes within each class supports the latter view. Adenophorea include a wide range of marine, freshwater, and soil nematodes but relatively few parasites of animals and plants, whereas Secernentea occur mostly in terrestrial habitats and include a plethora of parasitic and free-living groups. This sort of disagreement is echoed by competing analyses at all taxonomic levels within the phylum.

Current taxonomy relies largely on the nematode morphological traits. Traits most commonly used are buccal and pharyngeal structure, but other anatomical features such as the cuticle, lip region, intestine, reproductive system, sense organs, and tail are also used, as well as life history traits such as parasitic host. Problems can arise when using morphological traits for phylogeny inference, and these become crucial when the paucity of applicable nematode characters is considered. In addition to observational bias and error, nematodes provide a limited number of characters that can be observed across taxa in relation to the known diversity of species.

The first major classification to incorporate both morphological and molecular phylogenetic information is that of De Ley and Blaxter (2002) (Fig. 3.3a, b). Till 1963 the double division was accepted by all the taxonomists with altering the name of two divisions, i.e., Phasmida (= Secernentea) and Aphasmidia (= Adenophorea). In 1963, Goodey rejected this double division as he found a connecting link (Teratocephalidae) between these two classes showing intermediate characters between the two. Later, that system was thoroughly reviewed by Kaestner (1965). Since nematodes form an incomparably more uniform group of Secernentea than Adenophorea

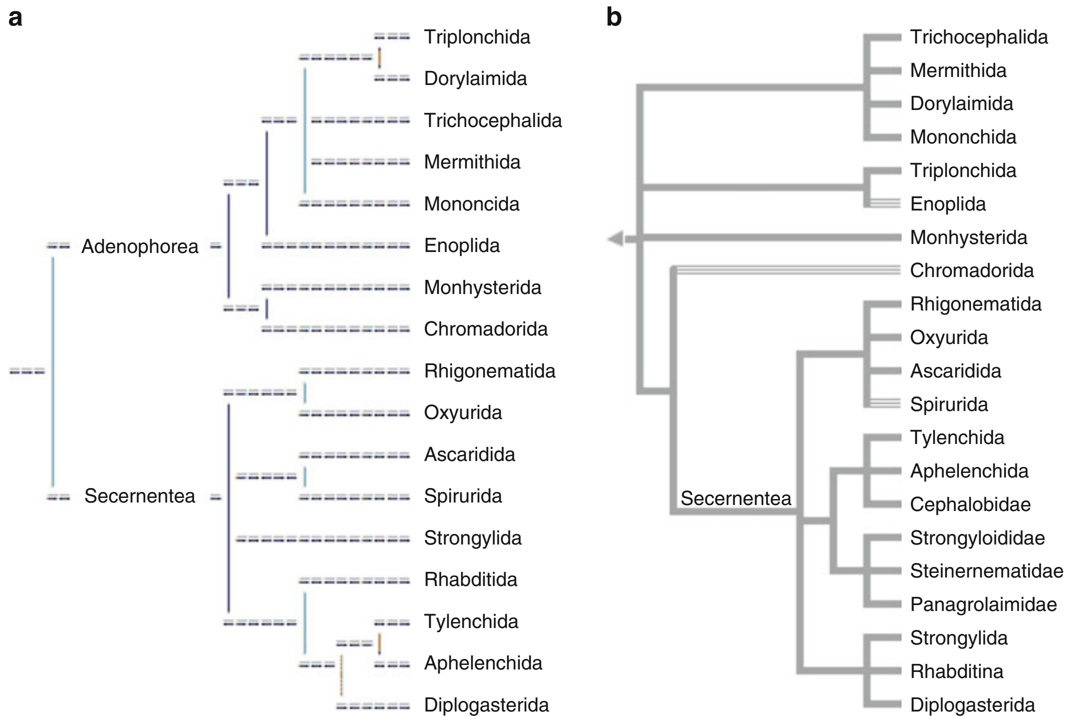

Fig. 3.3 (**a**, **b**) Relationship based on SSU rDNA sequences

as the proportion of uniform characters is 17:4 (Secernentea/Adenophorea), a three-line hypothesis was proposed by keeping Secernentea unaltered and splitting Adenophorea into Chromadorida and Enoplida.

The proportion of differential/identical characters emerged after the pairwise analysis, viz., Secernentea–Chromadorida, 10/6; Secernentea–Enoplida, 12/1; Chromadorida–Enoplida, 7/4; and Secernentea–Chromadorida–Enoplida, 10/7/3; it was thought that this could be the order or trend of evolution. Kaestner gave three nomenclatures as Chromadorida (spiral amphids) (= Torquentia), Enoplida (pocket-like) (=Penetrentia), and Secernentea (to separate or secrete off) based on the amphid arrangements and morphology.

Molecular data have also clarified the position of Nematoda in relation to other animals. Before the late 1990s, nematodes, along with a ragbag of other soft-bodied, "wormy" phyla, had been placed in a group termed the Pseudocoelomata (describing the nature of the body cavity in these taxa). However, the morphological arguments supporting this superphylum were never strong, and despite the absolute certainty expressed in textbook treatments of the phylogeny of the animals, leaders in the field, such as Libby Hyman, always expressed grave doubts as to the biological reality of this grouping (Hyman 1951). Analysis of ribosomal RNA sequence data from a range of nematodes, however, suggested instead a radical rearrangement of the animal part of the tree of life (Aguinaldo et al. 1997) (Fig. 3.4). In this new model, which has strong support from several genes and some support from morphological data, Nematoda is part of a superphylum of molting animals, the Ecdysozoa, that includes arthropods (and thus *D. melanogaster*, the other major non-vertebrate model), Nematomorpha (horsehair *worms*), Onychophora (velvet *worms*), Tardigrada (water

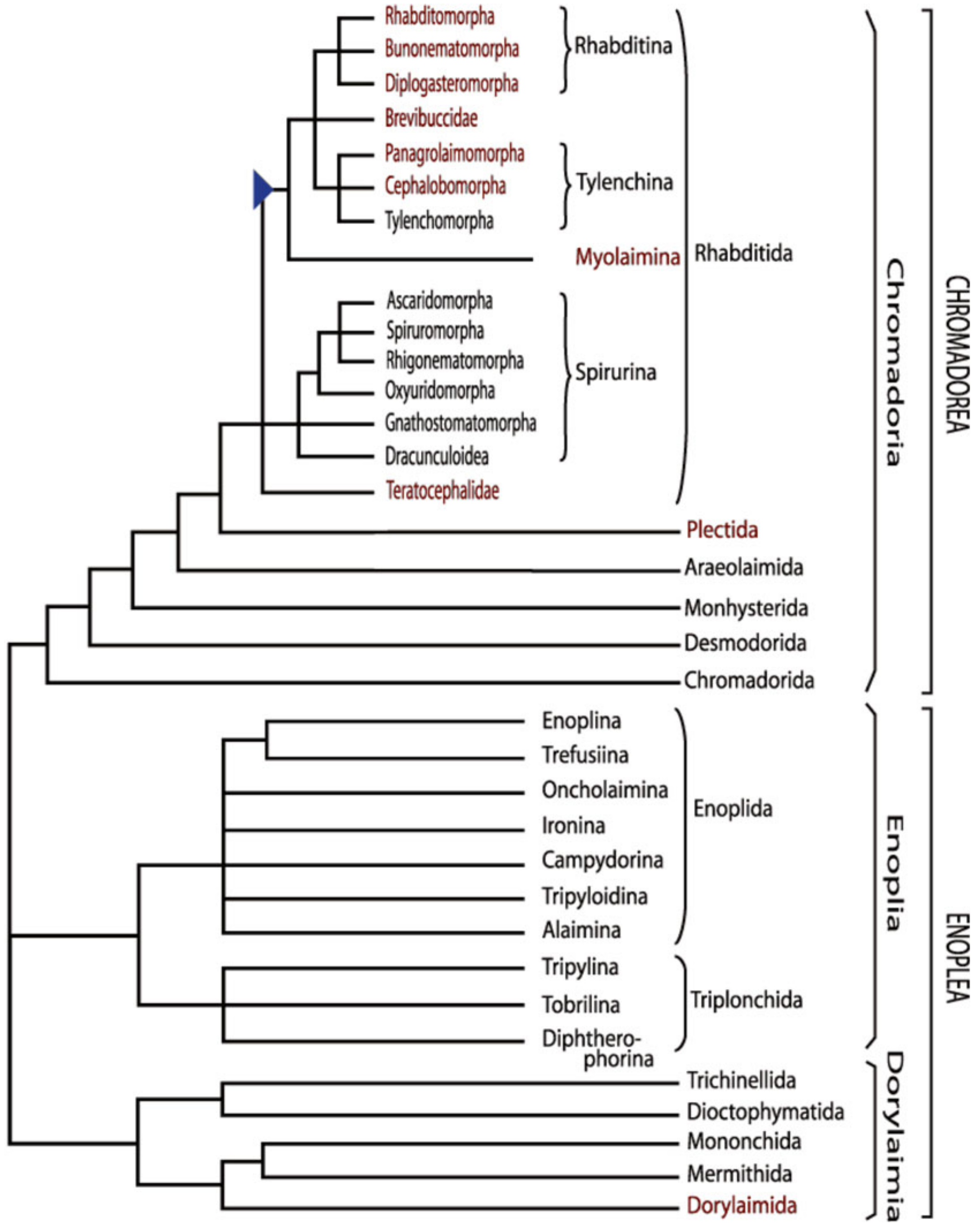

Fig. 3.4 Phylogenetic outline of Nematoda, derived primarily from small-subunit rDNA sequence analysis

bears), Priapulida (penis *worms*), and other minor phyla. The rest of the "pseudocoelomates" are now placed in the Lophotrochozoa (Halanych 1995; Philip et al. 2005), a group that includes Mollusca (snails and clams), Annelida (rag *worms* and earth*worms*), and Platyhelminthes (flat*worms*), among others.

3.4 Common Terminologies Used

Apomorphy: A derived character state.
Branch: The segment linking one node with another, or a node with a terminal taxon.
Characters: Variable features that can assume one of a number of different states.
Clade: A (monophyletic) group of organisms related by descent from a common ancestor.
Cladistics: A phylogenetic approach that only admits to bifurcations in lineages (no polytomies or reticulate evolution) and has explicit rules for their derivation.
Cladogram: A cladistic representation of a phylogeny, whereby only the branching order is displayed.
Homology: Common ancestry of two genes (characters, genes, positions).
Homoplasy, parallelism, or convergence: Independent derivation of a character state in two lineages.
Ingroup: The taxa under analysis.
Monophyletic: A clade where all the taxa derive from a single common ancestor and which includes all the descendents of that ancestor.
Node: A branch point in a tree (a presumed ancestral taxon).
Orthology (true homologues): Homologues that have arisen through speciation of their host genomes rather than by gene duplication within a genome.
Outgroup: A group of taxa assumed a priori to lie outside the monophyly of the taxa under analysis; used to give direction to determination of character change.
Paralogy: Homology having arisen through gene duplication.
Paraphyletic: A taxonomic group which does not include all the descendants of an ancestral taxon.
Phylogram: A representation of a phylogeny where evolutionary relatedness is shown by both branching order and a distance measure.
Phylogeny: A hypothesis of the relationships of organisms.
Plesiomorphy: The ancestral character state.
Polyphyletic: A taxonomic group which derives from >1 ancestral taxon.
Polytomy (unresolved node): A node that gives rise to >2 descendent taxa.
Resolved phylogeny: A phylogeny in which all relationships are represented by bifurcations.
Reversal: Change of a character from an apomorphic to a plesiomorphic state.
Rooted phylogeny: A phylogeny in which, by use of an outgroup, the last common ancestor of the clade of taxa under consideration can be placed.
Synapomorphy: A shared derived character state (in reference to a phylogenetic hypothesis).
Unrooted phylogeny: A phylogeny where no outgroup is specified.

3.5 Features Shared by Nematoda with Related Groups

Nematodes share a basic wormlike appearance with most other worms of different phyla. Segmentation is what differentiates Annelida from Nematoda. The difference between phylum Platyhelminthes and Nematoda is evident in their respective names – flatworms and roundworms. Nematodes share the pseudocoelom with rotifers and horsehair worms of the phyla Rotifera and Nematomorpha, respectively. Horsehair worms and rotifers are very common aquatic animals distinguishable by their cilia-crowned head and “wheel-like” appearance during cilia motion (Raven and Johnson 1985). The feature of a pseudocoelom is important in that it represents an intermediate step between total absence of a body cavity in unicellular organisms and a true coelom in more complex organisms. Recently, however, features like the pseudocoelom have been questioned when used to group organisms together. The nematode had previously been placed in the group Aschelminthes, which included the phyla Rotifera, Gastrotricha, Kinorhyncha, Priapulida, and Nematomorpha (Poinar 1983).

Similarly, the cuticle is a structure that is present in arthropods and other ecdysozoans, a group in which nematodes are now generally placed. The cuticle of the nematode is a rigid structure

that must be shed before further growth can occur. This process of molting is considered to be a link between nematodes and arthropods (Waggoner and Brain 2004). Moreover, the cuticle is often used to resolve phylogenetic issues within the phylum Nematoda. However, the cuticle may have arisen independently within the phylum and cannot necessarily be used to determine relationships between very closely linked nematodes (Decraemer et al. 2003). Finally, along with rotifers and tardigrades, nematodes are able to undergo a process known as cryptobiosis where normal life processes and functions are suspended during periods of environmental instability and inhospitality (Waggoner and Brain 2004).

Phylum Nematoda is found across the globe almost anywhere there is organic matter. Roundworm habitats include but are not limited to seas, freshwater, soil, and almost every species of plant and animal. Around 20,000 species of roundworms are known and have been classified (Malakhov 1994). Because there are very few scientists looking for new roundworm species, the discovery of new species can be rather slow, especially in regard to free-living nematodes. Moreover, nematodes share basic morphologies and are difficult to distinguish between each other. Roundworms are either parasitic or free living. Parasitic roundworms are much more likely to be discovered and classified because they are of more concern to humans. In fact, for some time only parasitic roundworms were known, and today they are much more likely to be studied. This provides skewed knowledge of nematodes because there are more free-living species than parasitic ones; around 65 % of classified nematode species are free living.

All nematodes, however, show incredible ability to reproduce. There are certain species that can carry more than 27 million eggs at once. These species can lay up to 200,000 eggs in one day (Waggoner and Brain 2004). So, needless to say, nematodes are extremely abundant in the world. Unfortunately, the amount of classified nematode species can be no more than 20 % of the total number of existing nematode species. Some scientists, taking into account the problems with finding and classifying roundworms and their relative abundance, have estimated the amount of undiscovered nematodes to be anywhere from 100,000 to 1 million (Malakhov 1994). If this statement has any merit, then nematodes would be second only to arthropods as the most diverse group of animals (Waggoner and Brain 2004).

3.6 Unique Features to Nematoda

Nematodes are often confused with other closely related types of worms. These are often part of the phylum Platyhelminthes and are known as flatworms because they lack a body cavity. Similarly, annelids can sometimes be confused with nematodes but are distinguishable because they have a true coelom. Generally, nematodes are cylindrical, unsegmented, bilaterally symmetrical pseudocoelomates (Raven and Johnson 1985). Roundworms have a thick cuticle that covers their bodies and is shed in order to allow growth. Located between the cuticle and the pseudocoel are muscles that run the length of the nematode. These muscles push on both the cuticle and the pseudocoel and create a kind of hydrostatic skeleton. In contrast with most animals whose nerve cells branch out to each individual muscle cell, nematode's muscle cells branch toward the nerve cells. Nematoda is the only phylum of pseudocoelomates that includes a large amount of species. The function of this pseudocoel is very important in that it allows nematodes to gain or lose rigidity by way of fluid pressure. This rigidity allows resistance to muscle contraction, which in turn provides for more efficient motion. Nematodes do not possess a defined circulatory system as their pseudocoel fluids accomplish circulation (Raven and Johnson 1985). The nervous system of roundworms is comprised of anterior nervous tissue surrounding the pharynx that forms dorsal and ventral nerve cords that go from end to end (Waggoner and Brain 2004).

All nematodes do have a simple but defined digestive tract. A roundworm's mouth usually has 16 protruding sensory organs, and phytoparasites display piercing structures, "stylets." Food goes straight through the conveyor belt-like tract and is

broken down, diluted with water, absorbed, and excreted. Unlike most animals, nematodes do not depend on cilia or flagella for excretion. Rather they utilize cells that work as glands or systems of canals in order to get rid of waste (Raven and Johnson 1985).

Nematodes are sexual animals and the male is generally slightly smaller than the female, which usually displays a bent tail. Nematode reproduction in free-living specimens is a very interesting process involving six stages including an egg stage, four juvenile stages, and an adult stage. Males are dioecious in that they can have one or two testes and can have a variety of accessory sex organs depending on the species. Females give rise to eggs that are then fertilized and laid. Once the embryos in these eggs are mature, they will hatch into the first juvenile stage. The juvenile will then undergo four molts before it becomes an adult and is capable of reproduction. During molting, a nematode will shed its skin in order to facilitate growth. The third juvenile stage is normally the infectious stage for parasitic nematodes. Parasitic nematode life cycles vary more than those of free-living specimens. Often parasitic roundworms will have multiple stages and alternate between hosts and regions in their hosts' bodies. Finally, nematodes have much less cell multiplication than most other organisms as they achieve growth mainly through cell enlargement. The juvenile specimens, for the most part, have the same number of cells as adults (Poinar 1983).

In *C. elegans*, three possible hypotheses were outlined for relationships between three major model systems, the arthropod *Drosophila melanogaster*, the vertebrate *Mus musculus*, and the nematode *C. elegans* (Fitch and Thomas 1997). They emphasized that elucidating these relationships was important for making inferences and predictions about which components, mechanisms, and functions might be unique and derived or ancestrally shared by these or other related model and non-model species, such as humans. Of course, additional representatives of animals (including other nematodes) are needed in the phylogenetic framework for greater accuracy of such predictions. After we wrote that review, several interesting studies addressed relationships among the major animal phyla and particularly the relationship of nematodes to other animals. There is still considerable (even polemical) debate, but additional data and increased analytical sophistication may provide answers in the not-distant future.

On the basis of complete 18S ribosomal RNA (rRNA) sequences, Aguinaldo et al. (1997) proposed that nematodes were related to arthropods in a clade of molting animals they called "Ecdysozoa," to the exclusion of deuterostomes (represented by an echinoderm in their study), and some other protostome groups, such as mollusks and annelids. This hypothesis differed substantially from the more traditional "Coelomata" hypothesis that placed nematodes on a branch diverging before coelomates diverged from one another (i.e., before the divergence of lineages leading to mice and flies). Support for Ecdysozoa depended on excluding all but one nematode, *Trichinella spiralis* (which unfortunately possesses an rRNA sequence with an odd nucleotide composition compared to other nematodes characterized so far).

However, when other nematodes were included, the nematodes clustered together near the bottom of the tree, consistent with Coelomata and consistent with data from RNA polymerase II (Sidow and Thomas 1994). Use of *Trichinella* as a representative nematode was justified on the basis that its rRNA sequence evolved more slowly than that of other nematodes, such as *C. elegans*. A phenomenon called "long-branch attraction" (LBA) can cause taxa with long branches (representing many evolutionary changes) to artifactually group with other long branches, particularly those of the outgroup taxa near the root of the tree (Felsenstein 1978). Of course, the other possible reason that nematodes have long branches is simply that they diverged early from the other taxa, as predicted by the Coelomata hypothesis. The putative effect of LBA to provide artifactual support to Coelomata has been central to the debate, along with issues of taxon and character sampling. Thus, in all of the studies described above, inappropriate use of a potential ingroup taxon to measure relative rates could have mistakenly biased the conclusions in favor of Ecdysozoa. It has been claimed that a

phenomenon called "long-branch attraction" (LBA) results in an artifactual placement of nematodes near the base of the bilaterian phylogeny, thus appearing to be consistent with the "Coelomata" hypothesis and obscuring the phylogenetic signal for "Ecdysozoa." Recent studies using genome-scale numbers of genes have generally supported Coelomata and rejected Ecdysozoa (e.g., Brown et al. 2001; Blair et al. 2002; Wolf et al. 2004). Some of these studies tested for effects of LBA and found no significant effect.

A major criticism of these studies is that the sampling of taxa is small, as might be expected for whole-genome comparisons. To determine the effect of both taxon and character sampling, Philip et al. (2005) reviewed by Jones and Blaxter (2005) used data from 146 genes and a fairly diverse taxonomic sample of 35 species. In this case, the authors identified the fastest-evolving genes by appropriate comparison to the outgroup species and found strong support for Ecdysozoa when these genes were excluded. By including or removing taxa, the authors also demonstrated a clear effect of taxon selection. For example, adding hydra to the outgroup, which otherwise had only fungi and choanoflagellates, caused *C. elegans* to jump from a position consistent with Coelomata to one consistent with Ecdysozoa. The authors conclude that both accounting for LBA effects and including a denser sampling of taxa are required to uncover the phylogenetic signal for Ecdysozoa. This effect of taxon addition is explained by the ability of added taxa to "break" long branches and apportion changes more appropriately into different lineages, thus providing better phylogenetic information about which states are primitive and which are derived (Kim 1998).

Even when only one or a few genes are employed (such as for 18S rRNA), including more taxa has apparently aided resolution, generally resulting support for Ecdysozoa (Giribet and Ribera 1998; Giribet and Wheeler 1999; Peterson and Eernisse 2001). Adding more taxa, however, means that statistically testing the robustness of relationships becomes computationally much more time-consuming and most of these taxon-dense analyses do not have such tests.

Balavoine et al. (2002) focused on contributions from recent work using multigene data along with the insight provided by a few molecular characters which nevertheless have phylogenetic signatures complex enough to have arisen only once, such as insertions, deletions, and organization of gene clusters. For example, Hox gene organization may be one such complex and therefore informative feature. The *Abd-B* gene appears to be specific for Ecdysozoa. However, orthology of the *C. elegans php-3* to *Abd-B* is only very weakly supported (de Rosa et al. 1999). One problem with *C. elegans* as a representative nematode is that it clearly has a highly derived organization of the *Hox* gene cluster relative to other nematodes; genes have been lost and rearrangements have occurred in lineages leading to *C. elegans* (Aboobaker and Blaxter 2003).

3.7 Evolutionary Trends of Nematoda

Nematodes are suitable objects to study evolution of development because species from all branches of the phylogenetic tree can be analyzed; embryos develop outside the mothers and most of them are transparent enough to perform cellular analysis in vivo. Nematodes can be subdivided into basal Enoplea (clades 1 and 2) and more derived Chromadorea (clades 3–12). Embryogenesis of *Caenorhabditis elegans* (clade 9) has been analyzed in most detail. Their establishment of polarity and asymmetric cleavage requires the differential localization of PAR proteins. Earlier studies on selected other nematodes revealed that embryonic development of nematodes is more diverse than the essentially invariant development of *C. elegans* and the classic study object *Ascaris* had suggested. Studies conducted by Schulze and Schierenberg (2011) revealed that early embryogenesis varied considerably among species indicated that constraints are high on the preservation of crucial developmental steps but not on cellular behavior leading to these. The direction of evolution went from indeterminate

early cleavage without initial polarity to invariant development with establishment of polarity before division of the zygote. The observed action of primary polarity organizing centers gave a clue how polarity in certain nematodes and other related taxa like tardigrades can be established in a way that differs from *C. elegans*, that is, independent of the sperm entry point.

Nematodes are wholly unsuitable for fossilization, and as a result, the study of their evolution is a difficult task as there is no proof of when, where, and how they evolved, their primitive forms and advanced forms. So dependence is on the indirect means of evaluating most important morphological and anatomical characters and evaluating them phylogenetically, by studying primary (which are present in ancient nematodes) and secondary (advanced characters or derived characters in advanced forms) characters. Chitwood was the first to make efforts that these animals be grouped in such natural unit as would reflect the trends of phylogeny. He was the first to recognize (Maggenti 1983). Nematoda phylogenetically and morphologically do not comprise a uniform class as was thought but that they represent two well-delimited evolutionary trends. The two groups were named after the presence or absence of a peculiar little organ, the phasmids, as Phasmidia and Aphasmidia in 1933, containing two subclasses, Rhabditida and Spirurida and Chromadorida and Enoplida, respectively. Of course he did not mean it as an important organ of the group but he simply chose them from among many to provide a name for the two classes.

3.8 Morphological Characters of Nematode in the Light of Evolution

Several important morphological and anatomical characters were used for arriving the trend of nematode phylogeny and evolution, which are as follows:

- *Appearance*: Spindle or filiform shape represents primitive character and all other diverging characters are specialized.
- *Symmetry*: Radial symmetry is primitive and any deviation from this could be due to evolution.
- *Head*: Most of the species even today possess 3 or 6 lips, though evolved forms may show reduced lateral lips, still reduced to 2 lips in highly parasitic forms indicating they are evolved.
- *Setae*: Presence of six setae is the primitive character; out of 6, 1–1 lateral and 2–2 subventral and four or three setae is a derived character. The presence of setae is the most primitive character as advanced forms are without setae.
- *Amphids*: These are particular characteristic of nematode that are always present on each side of the proximal end, and their position and form are an indication of evolution as shape and structure vary in three subclasses indicating three routes of evolution, and they are evolved out of lateral lips (papillae origin).
- *Esophagus*: It is a muscular tube which is either cylindrical or may bear one or more swellings (bulb); a simple tube-like structure is a primitive character and bulb-like is an advanced character. The presence or absence of a bulb is of evolutionary importance (Secernentea have a bulb while Penetrentia do not have).
- *Esophageal glands*: The number of esophageal glands of evolutionary importance is 3, 5, or more, and as far as tri-radial symmetry is concerned, 3 is primitive and 5 gland systems may be an evolved or derived character.
- *Female genital organ*: It exists in two forms as paired or unpaired and branched or unbranched. Unbranched or single organ is a primitive form and paired and branched organs are evolved characters similar to male genital organs.
- *Caudal glands*: Three unicellular glands situated in the hollow of the tail (meant for hold fast) seen in aquatic nematodes are the primary characters as they are absent in evolved forms (Secernentea).

In the light of above characters, it was concluded that nematode development might have taken place in the phase of geohistory. As most primitive forms are found in marine species, subclass Torquentia (Chromadorida) comprising the highest number of marine species is the only evolved form among the three subclasses excluding parasitic forms and the parasites of plants and animals.

3.9 Evolutionary Concepts

The complete genomes of three animals have been sequenced by global research efforts: a nematode worm (*Caenorhabditis elegans*), an insect (*Drosophila melanogaster*), and a vertebrate (*Homo sapiens*) (Blair et al. 2002). Remarkably, their relationships have yet to be clarified. The authors feel that the confusion concerns the enigmatic position of nematodes. Traditionally, nematodes have occupied a basal position, in part because they lack a true body cavity. However, the leading hypothesis now joins nematodes with arthropods in a molting clade, Ecdysozoa, based on data from several genes.

Traditionally, the animal body cavity has played a major role in interpretations of metazoan evolution, from groups (e.g., flatworms) lacking a coelom to those (e.g., nematodes) with a false coelom and finally to the bulk of animal phyla having a true coelom (Coelomata) (Jenner 2000). There has never been complete agreement on animal phylogeny and classification, but most researchers have divided living coelomate animals into deuterostomes (echinoderms, hemichordates, urochordates, cephalochordates, and vertebrates) and protostomes (arthropods, annelids, mollusks, and other phyla) based on differences in early embryonic development. An analysis of small-subunit ribosomal RNA (18S rRNA) sequences challenged this arrangement by placing acoelomate and pseudocoelomate phyla in more derived positions among the protostomes and in further defining a clade (Ecdysozoa) of molting animals that includes arthropods and nematodes. This "Ecdysozoa" hypothesis has influenced diverse fields and interpretations of developmental evolution in animals (Carroll et al. 2001). Since its publication, evidence has appeared both for and against this hypothesis. Knowing the branching order of the major animal lineages, especially those three with fully sequenced genomes, is of importance to diverse fields such as medical genetics, physiology, neurobiology, paleontology, and astrobiology. With a genealogy of animals, it will be easier to determine the origins and inheritance of mutations, genes, gene functions, and structures.

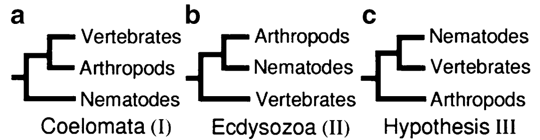

Fig. 3.5 The three possible relationships of vertebrates, arthropods, and nematodes

The three possible relationships of these animal phyla are as follows: (I) arthropods + vertebrates, (II) arthropods + nematodes, and (III) nematodes + vertebrates. The first hypothesis corresponds to the traditional grouping Coelomata and the second corresponds to Ecdysozoa (Aguinaldo, et al. 1997). For convenience, we will use these names in reference to the two hypotheses while recognizing that this study, by necessity, involves only a subset of all animal phyla. The third hypothesis will be referred to as "hypothesis III" (Fig. 3.5). To test each hypothesis, sequence alignments of more than 100 nuclear proteins were assembled and subjected to a series of analyses designed to reveal biases that could result in an incorrect phylogeny.

Blair et al. (2002) proposed that although it is possible that a basal position of nematodes is the result of some unknown and widespread bias not yet identified, a simpler explanation is that the grouping of nematodes with arthropods is an artifact that arose from the analysis of a single gene, 18S rRNA. The results obtained by them suggested caution in revising animal phylogeny from analyses of one or a few genes or sequence signatures. Although many other aspects of animal phylogeny remain unresolved, their findings indicated that insects (arthropods) are genetically and evolutionarily closer to humans (vertebrates) than to nematodes. Given the task of recovering and representing evolutionary history, nematode taxonomists can choose from among several species concepts (Adams 1998). All species concepts have theoretical and/or operational inconsistencies that can result in failure to accurately recover and represent species. This failure not only obfuscates nematode taxonomy but hinders other research programs in hematology that are dependent upon a phylogenetically correct taxonomy, such as biodiversity, biogeography, cospeciation, coevolution, and

adaptation. Three types of systematic errors inherent in different species concepts and their potential effects on these research programs are presented. These errors include overestimating and underestimating the number of species (type I and II error, respectively) and misrepresenting their phylogenetic relationships (type III error). For research programs in hematology that utilize recovered evolutionary history, type II and III errors are the most serious. Linnean, biological, evolutionary, and phylogenetic species concepts are evaluated based on their sensitivity to systematic error. Linnean and biological species concepts are more prone to serious systematic error than evolutionary or phylogenetic concepts. As an alternative to the current paradigm, an amalgamation of evolutionary and phylogenetic species concepts is advocated, along with a set of discovery operations designed to minimize the risk of making systematic errors.

Tahera Sultana et al. (2013) reported that among tylenchomorph plant parasites, members of the superfamily Tylenchoidea, such as root-knot nematodes, have great impact on agriculture. Of the five superfamilies within Tylenchomorpha, one (Aphelenchoidea) includes mainly fungal-feeding species but also some damaging plant pathogens, including certain *Bursaphelenchus* spp. The evolutionary relationships of tylenchoid and aphelenchoid nematodes have been disputed based on classical morphological features and molecular data. For example, similarities in the structure of the stomatostylet suggested a common evolutionary origin. In contrast, phylogenetic hypotheses based on nuclear SSU ribosomal DNA sequences have revealed paraphyly of Aphelenchoidea, with, for example, fungal-feeding *Aphelenchus* spp. within Tylenchomorpha, but *Bursaphelenchus* and *Aphelenchoides* spp. more closely related to infraorder Panagrolaimomorpha. They investigated phylogenetic relationships of plant-parasitic tylenchoid and aphelenchoid species in the context of other chromadorean nematodes based on comparative analysis of complete mitochondrial genome data, including two newly sequenced genomes from *Bursaphelenchus xylophilus* (Aphelenchoidea) and *Pratylenchus vulnus* (Tylenchoidea).

3.10 Evolutionary Relationships of Root-Knot Nematodes

To elucidate the biological relationships and to suggest positive pathways of evolution of one of the potential phytonematode group, root-knot nematodes, cytogenetic information has been very useful (Triantaphyllou 1985). The obligatory amphimictic species, viz., *M. megatyla, M. carolinensis*, and *M. microtyla*, are the closest relatives of the assumed ancestral root-knot nematode. Facultatively parthenogenetic species like *M. exigua, M. naasi*, and *M. graminis,* with $n = 18$, have evolved from an amphimictic ancestor with the same chromosome number, following evolution toward meiotic parthenogenesis. Some forms within this group, including *M. hapla* (race A) and *M. chitwoodi,* have evolved further by additional modifications of their chromosomal complement that resulted in the reduction of the haploid chromosome number from 18 to 17, 16, 14, and 13. Triantaphyllou (1985) opines that all the mitotic parthenogenetic forms are evolved from meiotic parthenogenetic ancestors or less likely from amphimictic ones during maturation of the oocytes. The variation in chromosome numbers noticed among the mitotic parthenogenetic forms indicates the existence of many pathways of derivation. Species with about 26 chromosomes apparently are diploid and may have evolved from diploid meiotic forms without any change in the degree of ploidy. Species with about 54 chromosomes could be considered as triploids and most likely they have been derived following hybridization of meiotic parthenogenetic forms involving, for example, fertilization of an unrelated egg with 36 (18 + 18) chromosomes. However, species with intermediate numbers of chromosomes, i.e., hypotriploid, may have been derived from the triploid forms through actual loss or fusions of a number of chromosomes. They may have derived from meiotic diploid forms with reduced chromosome numbers following fusion of unequal gametes. Thus, a hypotriploid form with 45 chromosomes may have been derived from the fusion of an unreduced egg with 30 chromosomes with a normal sperm with 15 chromosomes.

However, Triantaphyllou (1984), after analyzing the behavior of tetraploid forms of *M. hapla* and further consideration of the chromosomal complement of nematodes in general, offered alternative explanations about the possible pathways of evolution of root-knot nematodes. Since all nematodes, with the exception of some ascarids and a few other polyploidy forms, possess small chromosomal numbers ranging from 5 to 9 (n), the $n=18$ chromosomes of the genus *Meloidogyne* may represent a state of polyploidy/tetraploidy. The existence of *Hypsoperine spartinae*, a species very closely related to root-knot nematodes, with only seven chromosomes as the haploid number, provides additional support to this assumption.

Hampering in the inference of evolutionary relationships between nematodes was observed by Martijn Holterman et al. (2006) by their conserved morphology, the high frequency of homoplasy, and the scarcity of phylum-wide molecular data. To study the origin of nematode radiation and to unravel the phylogenetic relationships between distantly related species, they analyzed 339 nearly full-length small-subunit rDNA sequences from a diverse range of nematodes. Bayesian inference revealed a backbone comprising 12 consecutive dichotomies that subdivided the phylum Nematoda into 12 clades. The most basal clade is dominated by the subclass Enoplia, and members of the order Triplonchida occupy positions most close to the common ancestor of the nematodes. Crown clades 8–12, a group formerly indicated as "Secernentea" that includes *C. elegans* and virtually all major plant and animal parasites, show significantly higher nucleotide substitution rates than the more basal clades 1–7. Accelerated substitution rates are associated with parasitic lifestyles (clades 8 and 12) or short generation times (clades 9–11). The relatively high substitution rates in the distal clades resulted in numerous autapomorphies that allow in most cases DNA barcode-based species identification. *Teratocephalus*, a genus comprising terrestrial bacterivores, was shown to be most close to the starting point of Secernentean radiation. Notably, fungal-feeding nematodes were exclusively found basal to or as sister taxon next to the three groups of plant-parasitic nematodes, namely, Trichodoridae, Longidoridae, and Tylenchomorpha. The exclusive common presence of fungivorous and plant-parasitic nematodes supports a long-standing hypothesis that states that plant-parasitic nematodes arose from fungivorous ancestors.

Philippe Castagnone-Sereno et al. (2013) studied the diversity and evolution of root-knot nematodes (*Meloidogyne*) and observed that these worms exhibited a wide continuum of variation in their reproductive strategies, ranging from amphimixis to obligatory mitotic parthenogenesis. Molecular phylogenetic studies highlighted the divergence between mitotic and meiotic parthenogenetic root-knot nematode species and probable interspecific hybridization as critical steps in their speciation and diversification process. The recent completion of the genomes of *Meloidogyne hapla* and *M. incognita* that exhibit striking differences in their mode of reproduction (with and without sex, respectively), their geographical distribution, and their host range has opened the way for deciphering the evolutionary significance of (a)sexual reproduction in these parasites. Further, the accumulating evidence suggested that whole-genome duplication (in *M. incognita*) and horizontal gene transfers (HGTs) represent major forces that have shaped the genome of current root-knot nematode species and may account for the extreme adaptive capacities and parasitic success of these nematodes.

Root-knot nematodes are known to reproduce either by cross-fertilization (amphimixis), facultative meiotic parthenogenesis, or obligatory mitotic parthenogenesis (Castagnone-Sereno et al. 1993). Among them, *M. incognita, M. arenaria*, and *M. javanica* are obligatory mitotic parthenogenetic species, while *M. hapla* can reproduce by both cross-fertilization and meiotic parthenogenesis. Phylogenetic relationships in this genus have been investigated by hybridization of *Bam*HI-digested genomic DNAs of 18 geographical isolates belonging to six species with three homologous repeated DNA probes cloned at random from a genomic library of one population of *M. incognita*. Due to the repetitive

nature of the probes, the autoradiograms exhibited extensive restriction fragment length polymorphisms (RFLPs) both between and within nematode species. Genetic distance values estimated from hybridization patterns were analyzed by two phylogenetic tree-building distance methods, respectively, based on constant (UPGMA) and varying (FITCH) rates of nucleotide substitution, and the resulting dendrograms showed a very similar clustering of species and populations. Comparison of these results with the other sources of phylogenetic data available for this genus, i.e., cytogenetic, isoenzymatic, and mitochondrial DNA (mtDNA) data, revealed consistency with all but the mtDNA phylogeny. Due to the maternal inheritance of mtDNA and the parthenogenetic reproductive mode of these organisms, which excludes any possibility of horizontal transfer, they concluded that nuclear DNA phylogeny should represent a more likely evolutionary history of this particular genus and that interspecific hybridizations between sexual ancestors may account for the results with mtDNA. Thus, the early split-off of the mitotically parthenogenetic species cluster and *M. hapla* confirms the amphimictic ancestral mode of reproduction of root-knot nematodes. The authors also discussed the existence of polymorphism within each species at the repeated DNA level in relation to the adaptive evolution of these parthenogenetic species.

3.11 Nematode Genome Evolution

Nematodes are the largest animal phylum. But, out of the estimated number of 1–10 million species, only approximately 25,000 are formally described (Lambshead 1993). Next to their species richness, their ecological omnipresence in virtually all terrestrial and aquatic habitats and also their high number of individuals contribute to the importance of nematodes (Floyd et al. 2002).

One of the best studied model organisms, the free-living worm *Caenorhabditis elegans* belongs to the nematode community (Rödelsperger et al. 2013). With the extensive knowledge about *C. elegans* as an excellent baseline, nematodes are becoming increasingly popular for evolutionary studies. *C. elegans* was the first multicellular organism that had its genome sequenced in 1998. It is important to note that until today, *C. elegans* is the only metazoan with a fully sequenced genome in the sense that there are no sequence gaps left. Recently, draft genome sequences of multiple other free-living and parasitic nematodes were published and their number is increasing rapidly. These genome sequences are a yielding source for the investigation of the structure and evolution of genomes. Among nematodes, examples of phylogenetically very closely related species that have completely different ecologies and species with very similar ecologies that are, however, only very distantly related are found. This makes nematodes an attractive system to study how genomes are shaped by the environment and evolutionary descent.

In terms of the numbers of individuals, nematodes are the most abundant type of animal on earth. So far 25,000 species have been classified, and there could be 100 million species (Blaxter 2003). This abundance results from their ability to adapt, as well as their small size, resistant cuticle, and simple body plan. Small changes to their body plan have allowed invasion of many different habitats. Nematodes live in hot springs, polar ice, soil, and fresh- and saltwater and as parasites of plants, vertebrates, insects, and other nematodes. This evolutionary plasticity, which hints at an underlying genetic plasticity, has long fascinated biologists. In 1965, the German zoologist Alfred Kaestner wrote, “our knowledge concerning the evolution of nematodes is next to nothing.” Happily, with the genome sequences of the nematodes *Caenorhabditis elegans* and *C. briggsae* in hand and those of *C. remanei*, *C. japonica*, *C.* sp. PB2801, *Pristionchus pacificus*, *Haemonchus contortus*, *Meloidogyne hapla*, *Brugia malayi*, and *Trichinella spiralis* soon to follow, our knowledge is now growing fast (Avril Coghlan 2005).

In comparison with genomes of many other metazoans, in particular vertebrates which have genome sizes between 300 Mb and 3.3 Gb

(Rödelsperger and Dieterich 2010), all published nematode genomes are very small and compact. Variation in the gene composition of nematode genomes is attributed to extensive gain and loss of genes. Nematodes acquire their genes through the processes of de novo formation, gene duplication, and horizontal gene transfer, among others. The process of horizontal gene transfer allows nematodes to acquire new physiological features. In other words, after the transfer of genes, the nematodes appear different from what they were originally. Nematodes lose their genes through the processes of gene deletion and evolutionary changes. The genes are lost to a point where they cannot be recognized as homologous to genes in other species. Only a few nematode genomes have been sequenced so far. The sequenced nematodes contain multigene transcription units and operons, which give rise to a single pre-mRNA. The pre-mRNA is then broken up into single protein-coding mRNAs through the processes of trans-splicing and polyadenylation.

Rapid evolution, in particular after gene duplication events, seems to be a plausible explanation for the apparent lack of homologues of some genes. Duplications have been proposed to allow for the generation of novel protein functions in one of the two copies, whereas the original function is still retained by the other duplicate (Katju and Lynch 2006). Indeed, many orphan genes belong to larger gene families of which other members do have homologues in other nematode species. This suggests that evolution within gene families is highly dynamic and some members might have diverged to the extent that they are classified as orphan genes, whereas other members have recognizable homologues in other species.

In *C. elegans*, in a process called trans-splicing, a 22-nucleotide-long ribonucleic acid (RNA) fragment (spliced leader, SL) is added posttranscriptionally to the 5′ ends of the messenger RNAs (mRNAs) of approximately 70 % of all genes (Blumenthal 2005). Trans-splicing, along with polyadenylation, is also used to break up polycistronic pre-mRNAs into multiple mRNAs coding for a single protein each. In *C. elegans*, approximately 25 % of all genes are organized in such polycistronic transcription units called "operons." Although the same term is used, operons in nematodes are neither evolutionarily related nor functionally equivalent to bacterial operons, which combine multiple functionally related genes and give rise to a single polycistronic mRNA (Rödelsperger et al. 2013). Trans-splicing and operons were shown to exist in all nematode species with a sequenced genome, and the process appears widespread among nematodes of clades 3–5.

Nematode genomes emerge as an excellent test case for the study of the evolutionary dynamics of genomes (Rödelsperger et al. 2013). Although the genomes currently available are only able to detect the most obvious features of nematode genomes, the small size and low abundance of repetitive sequences will facilitate the sequencing of many more species and different isolates of the same species with manageable effort. In the future, within- and cross-species comparisons over the full range of evolutionary distances will facilitate dating the formation of novel genes and detecting signatures of selective constraints or rapid evolution.

3.12 The Range of Genome Size Across the Nematoda

Most nematodes have genomes ranging from 50 to 250 Mb. Among the nematodes being sequenced, sizes vary from 53 Mb for *Haemonchus contortus* to 240 Mb for *Trichinella spiralis* (Avril Coghlan 2005). A few nematodes even have genomes as large as those of mammals, such as the ~2,100 Mb genome of *Parascaris univalens*. Other nematode genomes are tiny, such as the ~30 Mb *Bursaphelenchus mucronatus* genome. The varying size in the genomes of the nematodes has only been estimated for about 50 species of nematodes, which is a small number as compared to the number or nematode species that exist today. Also, notable about nematode genomes is that they are compact and, therefore, make for a good study of the structure and evolution of genomes. Research has shown that the size of nematode genomes is

similar to that of flatworms, insects, and annelids. However, the genomes are smaller than those of invertebrates like echinoderms and mollusks. The causes for the variations in size of the nematode genomes are not known, but they have been linked to spontaneous deletions.

Most nematodes contain the haploid chromosome numbers of $n=4$–12. Over 300 species of nematodes have been studied and studies indicate that nematodes display a lot of karyotypic variations. Additionally, it has been found that a third of the genes in the sequenced nematode genomes have no recognizable homologues outside their genus. Also noticeable is the fact that there are high rates of gene losses and gains among the nematode genomes. There are numerous examples of gene acquisitions that have been observed through gene transfers.

3.12.1 Genome Size and Gene Count

Nematode genomes are similar in size to those of flatworms, annelids, and insects (~60–100 Mb upward) but are smaller than those of some invertebrates such as mollusks and echinoderms (~400–500 Mb upward). The compact nature of nematode genomes may be due to a high rate of large, spontaneous deletions and perhaps to selection for deletions (Denver et al. 2004). The *C. briggsae* genome is slightly (~4 Mb) larger than the *C. elegans* genome, due to a larger amount of repetitive DNA in the *C. briggsae* genome (Stein et al. 2003). This must be due to proliferation of repeat families in the *C. briggsae* genome or loss of repetitive DNA from *C. elegans*. Comparison of the *C. elegans* and *C. briggsae* genomes to those of closely related nematodes will shed light on the relative importance of deletions (which will decrease the genome size) versus insertions and proliferation of repeats (which will both increase the genome size).

Species with smaller effective population sizes (a smaller number of individuals that contribute different alleles to the next generation) have larger genomes, because they tend to accumulate repetitive DNA and genomic duplications. In a study of two nuclear genes, the diversity in *C. elegans* and *C. briggsae* was just 6–13 % of the diversity seen in *C. remanei*. The effective population sizes of parasitic nematodes probably depend on those of their hosts, so parasites of herbivores may have larger effective population sizes than parasites of carnivores or omnivores. Thus, one could speculate that this explains why the sheep parasite *Haemonchus contortus* has such a small genome (53 Mb) compared to the human parasite *Brugia malayi* (85–95 Mb) or the pig parasite *Trichinella spiralis* (240 Mb). Since the size difference between the 104 Mb *C. briggsae* and 100 Mb *C. elegans* genomes is due to repetitive DNA, they both have ~19,500 genes. The *Brugia malayi* genome has a similar size to the *Caenorhabditis* genomes, ~85–95 Mb, and a similar number of genes, ~18,500 genes. The *Haemonchus contortus* genome is just 53 Mb, but it is not yet clear whether it contains half as many genes as *C. elegans* or rather has the same number of genes but half as much noncoding DNA (Whitton et al. 2004).

Meloidogyne hapla was established as a tractable model phytonematode amenable to forward and reverse genetics and presented a complete genome sequence (Opperman et al. 2008). It was observed that at 54 Mbp, *M. hapla* represented not only the smallest nematode genome yet completed but also the smallest metazoan and defined a platform to elucidate mechanisms of parasitism by what is known as the largest uncontrolled group of plant pathogens worldwide. The *M. hapla* genome encoded significantly fewer genes than *C. elegans,* most notably through a reduction of odorant receptors and other gene families, yet it acquired horizontally from other kingdoms numerous genes suspected to be involved in adaptations to parasitism. In some cases, amplification and tandem duplication had occurred with genes suspected of being acquired horizontally and involved in parasitism of plants. Although *M. hapla* and *C. elegans* diverged >500 million years ago, many developmental and biochemical pathways, including those for dauer formation and RNAi, were conserved. They concluded that although overall genome organization is not conserved, there are areas of microsynteny that may

suggest a primary biological function in nematodes for those genes in these areas.

Most nematodes have haploid chromosome numbers of $n=4$–12. The karyotypes of just ~300 species have been studied, but nematodes display a lot of karyotypic variation. The lowest haploid number is $n=1$ in *Parascaris univalens*, but very high counts are seen in polyploid species in the Tylenchomorpha. For example, the race of *Meloidogyne hapla* being sequenced is diploid and has $n=14$, but another race of *M. hapla* is polyploid with $2n=45$–48. Many tylenchomorphs including *M. hapla* are parthenogens, in which unfertilized eggs develop into new individuals. Animal species that reproduce in this way seem to be susceptible to polyploidization. The *M. hapla* race being sequenced has twice as many chromosomes as most rhabditines, so it could reveal traces of an ancient genome duplication in the Tylenchomorpha. In contrast to the tylenchomorphs, most rhabditines have $n=5$–6 (Blaxter et al. 2000). Indeed, *C. elegans* and *C. briggsae* both have $n=6$, even though their chromosomes have undergone ~4,000 rearrangements since they diverged. The lack of fissions or fusions suggests that there could be selection for a stable chromosome number in the Rhabditina.

3.13 Ancient Linkage Groups

The genome of *C. elegans* was compared to that of *C. briggsae*, and ~4,800 conserved segments, with an average size of 37 kb, were observed (Stein et al. 2003). They estimated that there have been 3.6 interchromosomal rearrangements per Mb in the *C. briggsae* genome. Thus, an average *C. briggsae* chromosome of ~10–20 Mb consists of a mosaic of ~35–70 chunks that match several *C. elegans* chromosomes. However, some of these segments are very small, so it may be possible to detect ancient *Caenorhabditis* linkage groups by considering just the largest conserved segments. A genetic map for *C. briggsae* is currently underway and should allow us to match each *C. briggsae* chromosome to the *C. elegans* chromosome(s) with which it shares common ancestry. Sequencing of random regions of the *Pristionchus pacificus* and *Brugia malayi* genomes suggests that despite the frequent occurrence of reciprocal translocations, ancient Secernentean linkage groups may still be detectable.

In an 11-gene region sequenced from *P. pacificus* chromosome III, 10/11 genes had orthologs on *C. elegans* chromosome III. This led Lee et al. to suggest that *P. pacificus* chromosome III and *C. elegans* chromosome III shared a common ancestor. If this is true, there must have been a lot of intrachromosomal rearrangement since just three pairs of the *P. pacificus* genes are closely linked in *C. elegans*, but these pairs are scattered over 12 Mb. An evidence was found suggesting that *B. malayi* chromosomes can be matched to their *C. elegans* homologues. They sequenced BAC ends containing 8 Mb of *Brugia malayi* sequence and found that 60 % of the BACs matched the same *C. elegans* chromosome at both ends. However, large rearrangements seem to have occurred within chromosomes, because the average distance between two matches was 4 Mb.

With respect to the evolutionary patterns in the arms and centers of nematode chromosome, each of *Caenorhabditis elegans*' chromosomes is divided into a repeat-poor "central cluster" that rarely undergoes meiotic exchange and two repeat-rich "arms" that have a ~7-fold higher recombination rate (*C. elegans* Sequencing Consortium 1998). Intriguingly, the arms are evolving far more rapidly than the centers of chromosomes, in terms of both substitutions and chromosomal rearrangements such as translocations, inversions, and duplications (*C. elegans* Sequencing Consortium 1998). This may reflect a lower tolerance to mutation in the central clusters, which contain most of the essential genes and operons. Alternatively, the arms may simply have a higher mutation rate, since the high recombination rate may provoke substitutions, while the abundance of repeats probably triggers chromosomal rearrangements (Coghlan and Wolfe 2002).

There are ~1,000 operons in the *C. elegans* genome, of which 96 % are conserved in *C. briggsae*, far more than expected if selection did not act to preserve operons (Stein et al. 2003).

Gene order in ~15 % of the genome is stabilized by selection against rearrangements of operons, since 15 % of *C. elegans* genes are part of operons. In fact, operons are concentrated in the central clusters of *C. elegans* chromosomes, so they probably contribute to the lower rearrangement rate in the centers compared to the arms. One *C. elegans* operon is conserved in the closely related rhabditine *Oscheius*, but at least one *C. elegans* operon has been broken in *Pristionchus pacificus.*

Operons probably exist in the Rhabditina, Tylenchina, and Spirurina, since *trans*-splicing has been observed in *Haemonchus contortus*, *Panagrellus redivivus*, *Ascaris suum*, *Anisakis* spp., and *Brugia malayi*. Two unresolved questions are whether *Trichinella spiralis* has *trans-splicing* and operons and whether nematode operons are related to those in flatworms. *C. elegans* chromosomes also contain small clusters of ~2–5 genes that are co-expressed in the muscle, even though they do not belong to operons, as well as clusters co-expressed in the germ line, intestine, and neurons (intestine = Mountain 08 and neurons = Mountain 06).

3.14 The Nematode HOX Gene Cluster

Hox genes are of much significance and their central role in anterior–posterior patterning provides a framework for molecular comparison of animal body plan evolution (Aboobaker and Blaxter 2003). The nematode *Caenorhabditis elegans* stands out as having a greatly reduced Hox gene complement. To address this, orthologs of *C. elegans* Hox genes were identified in six species from across the Nematoda, and they show that rapid homeodomain sequence evolution is a general feature of nematode Hox genes. Some nematodes express additional Hox genes belonging to orthology groups that are absent from *C. elegans* but present in other bilaterian animals. Analysis of the genomic environment of a newly identified *Brugia malayi* Hox6-8 ortholog (*Bm-ant-1*) revealed that it lay downstream of the *Bm-egl-5* Hox gene and that their homeodomain exons are alternately *cis*-spliced to the same 5′ exon. This organization may represent an intermediate state in Hox gene loss via redundancy. The Hox clusters of nematodes are the product of a dynamic mix of gene loss and rapid sequence evolution, with the most derived state observed in the model *C. elegans*.

Hox proteins have been intensively studied in insects and vertebrates, but little is known about how Hox proteins provide specificity to their many specific roles during nematode development (Gutierrez et al. 2003). Nematodes provide an interesting example, as studies in *C. elegans* have indicated that several aspects of Hox genes, including their organization in the cluster and their function and sequence, differ strongly from Hox genes in other phyla. Nematodes are renowned for sharing a conservative body plan. The model *C. elegans* has a strongly determinative, lineage-driven mode of development resulting in an invariant cell lineage and eutely (Voronov and Panchin 1998). Hox gene functions in *C. elegans* have been evolving within this deterministic developmental mode, and their expression is now cell lineage, and not cell position, dependent. They suggested a three-step process in which a lineage-dependent mechanism of development was first adopted, ultimately releasing some Hox genes from a core role in positional identity pathways, followed by recruitment of these genes to new function in the context of lineage. Once a gene is released from its essential role, it is free to be lost, possibly through the exon-sharing mechanism observed for *B. malayi ant-1* and *egl-5*, or to move rapidly through sequence space to assume novel functions. Current models of the modes of evolution of Hox gene function involve gene duplications, micro and macro changes in expression pattern, and changes in sequences outside the 60-amino-acid homeodomain (Averof and Patel 1997). In general, the homeodomains evolve slowly, but, when Hox genes are divorced from homeotic function, as has happened with *Hox3* and *ftz* genes in the Diptera, their homeodomains are observed to evolve more rapidly. The independently duplicated posterior-group Hox genes of deuterostomes also have elevated rates of substitution.

Aboobaker and Blaxter (2003) observed that all of the nematode ortholog groups show elevated substitution rates across the phylum when compared to genes from arthropods and other bilaterians. By analogy to other systems, the functions of all the nematode Hox genes may have changed rapidly across the phylum, as constraint on all the Hox homeodomains has been lost.

HOX genes are transcription factors that are closely clustered in the genomes of most animals (Bürglin 1994). They control the expression of anterior–posterior patterning along the body axis during early embryogenesis collinearly with their arrangement on the chromosome. The HOX cluster has been conserved in most animal phyla over hundreds of millions of years of evolution, but the nematode HOX cluster is surprisingly poorly preserved. The ancestral bilaterian probably had a cluster of nine HOX genes (nine ortholog groups), but all nematodes have lost at least three ortholog groups (Aboobaker and Blaxter 2003). A further two ortholog groups were lost in the lineage leading to *C. elegans*, after the Spirurina–Rhabditina–Tylenchina clade diverged from other nematodes. Aboobaker and Blaxter (2003) pointed out that these two HOX ortholog groups were lost around the time when *C. elegans*' ancestor switched from a regulative mode of development to a deterministic lineage-driven mode. They suggest that perhaps the transition freed the two HOX ortholog groups from their role in anterior–posterior patterning, making their loss tolerable. Interestingly, the HOX cluster has been broken up in *C. elegans*: its six HOX genes (belonging to four ortholog groups) are arranged in three pairs scattered over 5 Mb of chromosome III. *Trichinella spiralis* probably has a regulative mode of development, but it is not yet known whether its HOX genes are clustered. However, even though *Brugia malayi* has lineage-driven development, most of its HOX cluster seems to have been preserved intact.

Hox genes encode evolutionarily conserved transcription factors involved in morphological specification along the anterior–posterior body axis of animals (Arturo Gutierrez et al. 2003). The two most striking features of Hox genes are colinearity and the strong sequence conservation. Among all animals studied so far, the nematode Caenorhabditis elegans contains one of the most divergent Hox clusters. The core cluster contains only four members, which in part deviate from the colinearity rule. In addition, orthologous and paralogous nematode Hox sequences diverged substantially. They investigated the role of MAB-5 during ray formation and established an in vivo assay using Cel-mab-5 regulatory elements to express orthologous, paralogous, and chimeric cDNAs in a Cel-mab-5 mutant background. It was shown that the MAB-5 ortholog from *Pristionchus pacificus* but not the *C. elegans* paralogous Hox proteins can rescue Cel-mab-5. Experiments with chimeric, truncated, and mutagenized Hox proteins suggest the specificity to be conferred by the *N*-terminal arm and helix I, but not helix II of the homeodomain.

3.15 Evolution of X and Y Chromosomes in Nematodes

Nematodes were one of the first animals chosen for cytological studies which ultimately led to the discovery of the correlation between chromosomal makeup of an embryo and its future sexual development, male or female (McClung 1902). The study of the mechanisms and evolution of sex determination intersects several fundamental questions in biology such as why and how sex is maintained, what forces govern the evolution of genome structure, how ecological factors constrain or favor reproductive mode, and how genetic networks evolve.

Most nematodes have chromosomal sex determination, in which the female is XX and the male is heterogametic sex (XY) or XO (the O indicates the absence of Y) (Pires-daSilva 2007). It is likely that the XX:XO sex determination system is ancestral because it is found in most of the nematode clades so far studied. The few XY systems occur among parasitic nematodes of clades 1, 3, and 4. It is thus possible that the XY system is derived from the fusion of an autosome to the old X chromosome, although clear cytogenetic evidence is still lacking. In *C. elegans*, sex determination acts through an X-chromosome dosage

mechanism: animals with two X chromosomes develop as hermaphrodites, whereas XO animals develop as males. XX/XO sex determination is very common across the Nematoda, suggesting that the first nematode possibly had XX/XO sex determination. Even if the *C. elegans* and *Trichinella spiralis* XX/XO systems did share common ancestry, the traces will be hard to find, since sex determination pathways and genes are evolving very quickly both in terms of sequence change and gene regulation (Haag and Doty 2005). However, at least one key gene is conserved in the XX/XO sex determination pathways of *C. elegans* and *Pristionchus pacificus*, so it should be possible to determine whether the *P. pacificus*, *C. elegans*, and *Haemonchus contortus* XX/XO systems are orthologous.

It is well established that in other phyla the molecular and genetic mechanisms underlying sex determination can be completely distinct, even when comparisons of closely related species are undertaken (Pires-daSilva 2007). The most striking differences are in the more upstream events of the sex determination pathway. Intriguingly, the ortholog of the *C. elegans* X signal element fox- 1 is also in the X chromosome of the distantly related nematode *P. pacificus.* Functional analysis of this gene and other signal element gene orthologs will be necessary to test if the counting mechanism is the same between these nematodes. Nothing is known about the molecular switch mechanism present in XX:XY species. One possibility is that it is based on a genic balance mechanism, which depends on a balance between female-determining genes in the X chromosome and male-determining genes in autosomes, as with *C. elegans.* Another option is that the Y chromosome plays a dominant role in the sex determination.

Nematodes with environmental sex determination have a high rate of intersexes (Triantaphyllou 1960). Intersexes are usually functional females (i.e., individuals with well-developed reproductive system that produces oocytes) with some male somatic characters such as spicules. In *M. javanica* and *M. incognita,* female larvae can undergo sex reversal relatively late in development, which causes the appearance of males with two gonadal arms (typical of females) instead of one. Sex reversal and intersexes can be induced experimentally in crosses between two different species such as in the rhabditids *C. remanei* and *C. briggsae*. In this case, the effect has been attributed to dysgenic interactions among fast-evolving sex determination genes.

Phylogenetic analyses suggest that hermaphroditism in *C. elegans* and *C. briggsae* has occurred due to an evolutionary convergence rather than being homologous (Pires-daSilva 2007). This conclusion is supported by genetic data, which indicate that there are fundamental differences in spermatogenesis between these two species. fog-2, the most upstream germ line-specific regulator of spermatogenesis in *C. elegans* hermaphrodites, is not present in the *C. briggsae* genome. Other genes involved in *C. elegans* hermaphroditic spermatogenesis (e.g., gld-1, fem-2, and fem-3) do not have a function in sperm formation in *C. briggsae* hermaphrodites. Traditional forward genetic approaches will be necessary to unravel the genes involved in germ line sex determination in *C. briggsae.*

Sex is determined in *C. elegans* by an X-chromosome-counting mechanism that reliably distinguishes the twofold difference in X-chromosome dose between males (1X) and hermaphrodites (2X) (Nicoll et al. 1997). This small quantitative difference is translated into the "on/off" response of the target gene, xol-1, a switch that specifies the male fate when active and the hermaphrodite fate when inactive. Specific regions of X contain counted signal elements whose combined dose sets the activity of xol-1. They ascribed the dose effects of one region to a discrete, protein-encoding gene, fox-1, and demonstrated that the dose-sensitive signal elements on the X chromosome control xol-1 through two different molecular mechanisms. One involves the transcriptional repression of xol-1 in XX animals. The other uses the putative RNA-binding protein encoded by fox-1 to reduce the level of xol-1 protein. These two mechanisms of repression act together to ensure the fidelity of the X-chromosome counting process.

Random amplification of polymorphic DNA was used to analyze genomic DNA from virgin females and males of *Brugia malayi,* with a view to identifying sex-specific differences predicted by an XX/XY system of chromosomal sex determination (Underwood and Bianco 1999). A product of 2,338 bp, amplified with the arbitrary primer 5′ GTTGCGATCC 3′, was obtained exclusively from males. Primers based on the sequence of this product amplified a DNA fragment of the expected size from each of two independent isolates of *B. malayi* (from Malaysia and Indonesia) by PCR. No reaction product was obtained from the closely related species *Brugia pahangi.* In a genetic cross between *B. malayi* males and *B. pahangi* females, F1 hybrid microfilariae were PCR positive, indicating that the locus is paternally inherited. Southern blotting demonstrated that the target sequence resides in the high molecular weight fraction of genomic DNA, confirming that it is of chromosomal, rather than mitochondrial, origin. Sequencing of the locus revealed significant similarity with members of a family of reverse transcriptase-like genes in *Caenorhabditis elegans.* In-frame stops indicate that the gene is nonfunctional, but multiple bands of hybridization in Southern blots suggest that the RT sequence may be the relic of a transposable element. Multiple repeats of the dinucleotide AT occurred in another region of the sequence. These varied in number between the two isolates of *B. malayi* in the manner of a microsatellite, surprisingly the first to be described from the *B. malayi* genome. Because of its association with the Y chromosome, we have given the locus the acronym TOY (tag on Y). Identification of this chromosome-specific marker confirms the XX/XY heterogametic karyotype in *B. malayi* and opens the way to elucidation of the role of Y in sex determination.

To achieve X-chromosome dosage compensation, organisms must distinguish X chromosomes from autosomes (Csankovszki et al. 2004). They identified multiple, cis-acting regions that recruit the *C. elegans* dosage compensation complex (DCC) through a search for regions of X that bind the complex when detached from X. The DCC normally assembles along the entire X chromosome, but not all detached regions recruit the complex, despite having genes known to be dosage compensated on the native X. Thus, the DCC bound first to recruitment sites and then spread to neighboring X regions to accomplish chromosome-wide gene repression. From a large chromosomal domain, a 793-base-pair fragment was defined that functioned in vivo as an X-recognition element to recruit the DCC. Only a handful of nematodes have Y chromosomes: *Brugia malayi*, *Onchocerca volvulus, Baylisascaris transfuga*, *Contracaecum incurvum*, and *Trichuris muris*. Since Ys are only known in these few distantly related nematodes, it was suggested that they probably emerged recently. In papaya the sequence of the Y chromosome betrays its recent origin from autosomes, and it will be interesting to see if the *Brugia malayi* Y arose in a similar way.

The involvement of the *C. elegans* X chromosome in sex determination may have restrained its pace of structural evolution. Since *C. elegans* diverged from *C. briggsae*, its X chromosome has undergone about half as many rearrangements as its autosomes. Indeed, two of the three largest conserved segments between the two genomes are on *C. elegans* X. Furthermore, a genetic linkage map of *Pristionchus pacificus* suggests that the X chromosome may have been preserved largely intact since the divergence of *P. pacificus* from *C. elegans.*

Nematodes are very diverse in reproductive modes and in sex determination mechanisms. It has been suggested that gonochoristic nematodes evolved into hermaphrodites and that some of those became parthenogenetic. Autotokous reproduction seems to have evolved relatively often in nematodes, which makes an interesting case for studying which factors lead to the evolution of this reproductive mode (Castagnone-Sereno 2006). Although rare in other animals, nematodes that reproduce mainly asexually are one of the most ubiquitous plant parasites in the world. To understand the factors that are driving the evolution of sex determination and asexual reproduction, integration of many diverse fields such as ecology, genetics, and cell

biology will be required. Basic questions such as whether there is the occurrence of hermaphroditism in non-rhabditid nematodes are still unanswered.

The most common reproductive strategy among nematodes is sexual reproduction between males and females or amphimixis, which is seen in *Caenorhabditis remanei*, *Caenorhabditis* sp. PB2801, *Caenorhabditis japonica*, *Haemonchus contortus*, *Brugia malayi*, and *Trichinella spiralis* apart from several phytopathogens. However, alternative reproductive strategies have arisen in some nematode groups, including hermaphroditism, parthenogenesis, and haplodiploidy. For example, *C. elegans*, *C. briggsae*, and *Pristionchus pacificus* are hermaphroditic. The strain of *Meloidogyne hapla* sequenced is a facultative meiotic parthenogen. Because hermaphroditic species (and perhaps parthenogenetic species) have a smaller effective population size than amphimictic species, they will tend to accumulate deleterious mutations, resulting in a faster substitution rate and rate of chromosomal rearrangement (Archetti 2004). This may explain why substitution rates in *C. elegans* and *Meloidogyne* seem to be high compared to most nematodes (Blaxter et al. 1998).

Studies have been conducted on the effect of kinetochore organization on genome stability. Since *C. elegans* and *C. briggsae* diverged, their chromosomes have been splintered by ~250 reciprocal translocations, ~1,400 inversions, and ~2,700 transpositions. Intrachromosomal rearrangement is about four times more frequent than interchromosomal rearrangement. Even so, translocations are surprisingly common in *Caenorhabditis* compared to flies, in which translocations are extremely rare (Sharakhov et al. 2002). This may be because almost all dipterans have "monocentric" chromosomes, in which the kinetochores assemble on a localized region in each chromosome. In contrast, "holocentric" species such as *C. elegans* and *C. briggsae* have diffuse kinetochores that form along the length of their chromosomes during mitosis. Since the kinetochores are the primary chromosomal attachment site for spindle microtubules, they play a key role in ensuring high-fidelity chromosome transmission in both monocentric and holocentric species.

However, little is known of the relationship between the distribution of kinetic activity along chromosomes and the pattern of chromosomal rearrangement. In species with monocentric chromosomes, many translocations will be lethal because they will give rise to acentric or dicentric chromosomes, while species with holocentric chromosomes may be more tolerant of translocations. Most nematodes have holocentric chromosomes, but *Trichinella spiralis* and some other trichinellids have monocentric chromosomes. Thus, comparison of the *T. spiralis* genome to that of *C. elegans* may provide clues as to whether holocentric chromosomes are more susceptible to rearrangement and whether the first nematode had holocentric chromosomes.

3.16 Evolution of Gene Content

The Nematoda is one of the oldest among the animal phyla. Subclass divergence within the Secernentea is thought to have occurred over 550 million years ago, with separation of the class Adenophorea predating that event. It may be predicted that mtDNA sequence comparisons between nematode classes and subclasses would generate low similarity scores, as observed with any alignment involving *R. culicivorax* and *M. incognita* (Hyman and Beck Azevedol 1995). In their study, ten of 12 mitochondrial protein-coding genes and the large (16s) mitochondrial rRNA gene were identified and mapped within the *Romanomermis culicivorax* mitochondrial genome. This transcriptional map differs from other nematode mitochondrial DNAs (mtDNAs) with respect to gene order and transcriptional orientation of some genes. Several of these coding regions are components of a 3.0-kilobase mtDNA repeating unit, allowing a direct comparison of nucleotide and amino acid sequence composition for repeated and single-copy genes. Analysis of protein-coding regions representing repeated (ND3, ND6) and single-copy genes (ATPase 6, cyt.b, COI, COIII, NDl, ND4, ND5) and four repeat-associated open reading frames (ORFs)

with unassigned function have revealed striking similarities in nucleotide composition, amino acid frequencies, and codon biases. Although they anticipated that reiterated protein-coding regions might be evolving under relaxed selection, results indicated that both repeated and unique mitochondrial genes appear subject to similar functional constraints.

Mitochondrial genomes of metazoa are typically composed of a single, circular molecule that varies in size from approximately 14–39 kilobases (kb) (Brown 1985; Moritz et al. 1987; Snyder et al. 1987). These molecules reveal a nearly identical coding potential consisting of structural genes for an organelle-specific translation system (2 rRNAs and 22 tRNAs) and 12 or 13 protein-coding genes. The encoded polypeptides are components of the mitochondrial electron transport and oxidative phosphorylation systems: apo-cytochrome b (cyt.b); F-ATPase subunits 6 and 8 (ATPases 6 and 8); cytochrome c oxidase subunits I, II, and III (COI–COIII); and subunits L-6 and 4 L of the respiratory chain NADH dehydrogenase (NDl-ND6 and ND4L) (Chomyn and Attardi 1987). Therefore, the large size variation observed among animal mitochondrial DNAs (mtDNAs) is not usually a consequence of differential gene content. Rather, size polymorphism most frequently results from copy number variation of tandem repeats within noncoding mtDNA sequences, often residing in the vicinity of the control region.

Parkinson et al. (2004) sequenced ESTs from 30 different nematode species across the phylum and defined ~94,000 genes from ~60,000 families. Surprisingly, only about 15,000 (15 %) of the ~94,000 genes are found in all four clades of nematodes studied (Rhabditina, Tylenchina, Spirurina, Dorylaimia). These 15,000 genes are probably involved in core metabolic or structural pathways, since most of them (91 %) have sequence matches outside the Nematoda. In addition, they identified ~1,300 genes that are nematode specific but that are found in most nematodes. These ~1,300 genes probably have roles that are important for nematode body plan and life history and so may shed light on the early evolution of the phylum.

3.17 Proliferation and Loss of Gene Families

Since *C. elegans* has diverged from *C. briggsae*, chemoreceptors have proliferated in the *C. elegans* genome so that it now has almost twice as many as *C. briggsae* (718 versus 429) (Stein et al. 2003). Duplication and divergence of extra chemoreceptors may have allowed *C. elegans* to adopt a slightly different ecological niche than *C. briggsae*, since it uses chemoreceptors to find food and to avoid predators, pathogens, and toxins. On the other hand, *C. elegans* seems to have lost several genes (~30 genes) that are found in both *Pristionchus pacificus* and *Haemonchus contortus*. For example, *C. elegans* has lost a DNA methyltransferase gene that is found in *P. pacificus,* a loss that probably led to the abolition of DNA methylation in *C. elegans* (Gutierrez and Sommer 2004). Contrasting the gene families that have been duplicated or lost in each of the ten nematode genomes may reveal selection for different gene contents in different species.

The seven transmembrane receptor (*str*) and *srj* (renamed from *stl*) families of chemoreceptors have been updated, and the genes were formally named following completion of the *C. elegans* genome sequencing project (Hugh J. Robertson 2001). Analysis of gene locations revealed that 84 % of the 320 genes and pseudogenes in these two families reside on the large chromosome V. Movements to other chromosomes, especially chromosome IV, have nevertheless been relatively common, but only one has led to further gene family diversification. Comparisons with homologues in *C. briggsae* indicated that 22.5 % of these genes have been newly formed by gene duplication since the species split while also showing that four have been lost by large deletions. These patterns of gene evolution are similar to those revealed by analysis of the equally large *srh* family of chemoreceptors and are likely to reflect general features of nematode genome dynamics. Thus, large random deletions presumably balance the rapid proliferation of genes and their degeneration into pseudogenes, while gene movement within and between chromosomes keeps these nematode genomes in flux.

With completion of the sequencing of the *C. elegans* genome, it is now possible to provide a complete description and formal naming of the *str* family, as well as the related family previously called *stl* but here renamed *srj*. Phylogenetic analysis of these two gene families confirms several of the genome dynamics inferred from the *srh* family, including loss of *C. briggsae* orthologs, recent formation of many genes within *C. elegans*, and the frequent occurrence of movements of genes between chromosomes. In addition, preliminary analysis of gene location within chromosome V revealed frequent gene movement within it.

3.18 Species-Specific Genes

C. elegans has ~1,000 genes that are not found in *C. briggsae* and that lack any match in sequence databases (Coghlan 2005). Of these, ~200 have been confirmed by EST or cDNA data, so they are definitely not gene prediction errors. These genes may have diverged so rapidly that their *C. briggsae* homologue is unrecognizable or may have been assembled de novo via chromosomal rearrangements in the *C. elegans* genome. Duplications, chromosomal rearrangements, and transposable elements are known to play a role in the birth of novel genes. Thus, the abundance of species-specific genes in the arms of *C. elegans* chromosomes probably results from the arms' high rate of rearrangement. *C. elegans* is not alone in having so many species-specific genes.

Taxonomically restricted genes (TRGs) are genes that are restricted to a limited subset of phylogenetically related organisms and may be important in adaptation. In parasitic organisms, TRG-encoded proteins are possible determinants of the specificity of host–parasite interactions (Tomalova et al. 2012). In the root-knot nematode (RKN) *Meloidogyne incognita*, the *map-1* gene family encodes expansin-like proteins that are secreted into plant tissues during parasitism, thought to act as effectors to promote successful root infection. MAP-1 proteins exhibit a modular architecture, with variable number and arrangement of 58 and 13-aa domains in their central part. The evolutionary origins of this gene family were studied using a combination of bioinformatics and molecular biology approaches. *Map-1* genes were solely identified in one single member of the phylum Nematoda, i.e., the genus *Meloidogyne*, and not detected in any other nematode, thus indicating that the *map-1* gene family is indeed a TRG family. A phylogenetic analysis of the distribution of *map-1* genes in RKNs further showed that these genes were specifically present in species that reproduce by mitotic parthenogenesis, with the exception of *M. floridensis*, and could not be detected in RKNs reproducing by either meiotic parthenogenesis or amphimixis. These results highlighted the divergence between mitotic and meiotic RKN species as a critical transition in the evolutionary history of these parasites. Analysis of the sequence conservation and organization of repeated domains in *map-1* genes suggested that gene duplication(s) together with domain loss/duplication had contributed to the evolution of the *map-1* family and that some strong selection mechanism might be acting upon these genes to maintain their functional role(s) in the specificity of the plant–RKN interactions.

Long terminal repeat (LTR) retrotransposons may be important contributors to host gene evolution because they contain regulatory and coding signals (Ganko et al. 2003). In an effort to assess the possible contribution of LTR retrotransposons to *C. elegans* gene evolution, they searched upstream and downstream of LTR retrotransposon sequences for the presence of predicted genes. Sixty-three percent of LTR retrotransposon sequences (79/124) were located within 1 kb of a gene or within gene boundaries. Most gene–retrotransposon associations were located along the chromosome arms. The findings were consistent with the hypothesis that LTR retrotransposons have contributed to the structural and/or regulatory evolution of genes in *C. elegans.*

3.19 Horizontal Gene Transfer in the Nematoda

Horizontal gene transfer occurs frequently in prokaryotes, but seems to be rare in eukaryotes. For example, ~1 % of the gene repertoire in the nematode *Meloidogyne* probably originated by

horizontal transfer (Scholl et al. 2003), compared to 1–5 % of single-copy genes and at least 22 % of gene duplicates in Y-proteobacteria. *Meloidogyne hapla*, a plant-parasitic nematode, seems to have gained at least a dozen genes by horizontal gene transfer from bacteria that occupy similar niches in the soil and roots. Those genes gained are useful for the nematode's parasitic lifestyle, such as cellulases for digesting plant material and signaling molecules that induce morphological changes in the plant, facilitating invasion. A distantly related plant parasite, *Bursaphelenchus xylophilus*, seems to have independently acquired a cellulase gene from a fungus. Perhaps horizontal transfer can spur the transition to parasitism. Several groups of parasitic nematodes, including *Brugia malayi*, live in symbiosis with specific bacteria carried by the nematodes (Blaxter 2003). Some of these are extracellular symbionts, but others are intracellular, such as *Wolbachia* living in *B. malayi* and other filarial nematodes. The capture of the *Wolbachia* gene set seems to have been adaptive for filarial nematodes, since killing *Wolbachia* with antibiotics reduces the growth and fecundity of the nematodes.

3.20 Identifying Parasitism Genes

Parasitism of plants and animals has evolved independently at least nine times in the history of the nematodes (Dorris et al. 1999). Four of the nematodes whose genomes are being sequenced are parasites: *Haemonchus contortus*, *Meloidogyne hapla*, *Brugia malayi*, and *Trichinella spiralis*. The adoption of parasitism in nematodes probably required adaptation of genes present in their free-living ancestors (Blaxter 2003). For example, modification of nutrient-acquisition genes found in *C. elegans*, such as digestive enzymes or secreted hydrolases, are likely to have been important for the evolution of parasitism. The ability of parasitic nematodes to survive immunological attack, some living in an infected individual for years, has long been a puzzle. In viral, bacterial, and protozoan parasites, genes involved in host immune evasion or recognition are often under positive selection and hence show patterns of rapid amino acid substitution. Indeed, *B. malayi* GPX-1 shows signs of positive selection. By scanning for *Haemonchus contortus* genes that have diverged sharply in sequence from their *Pristionchus* and *Caenorhabditis* orthologs and that bear secretory signals (Harcus et al. 2004), it may be possible to identify *H. contortus* genes that have adapted for a parasitic lifestyle.

Some genes essential for parasitism in worms may be novel genes. One possible source is gene duplication, which allows one duplicate to keep the original role and the other duplicate to take on a parasitic role. For example, the *alt* gene family of filarial nematodes, which has been implicated in establishing infection, has a single *C. elegans* assembled de novo or has been gained by horizontal gene transfer: plant-parasitic nematodes seem to have acquired "parasitism genes" from bacteria in their environment.

When a comparison was made between 36 *C. elegans* and *Drosophila* protein orthologs to their yeast counterparts, many *C. elegans* genes were found to be evolved twice as fast as their *Drosophila* orthologs. Nematode rRNA genes also seem to have a substitution rate that is 2–3 times that of other animal phyla (Aguinaldo et al. 1997). For example, the rRNA gene divergence between *Caenorhabditis* species is comparable to that between vertebrate species. To accurately estimate the evolutionary rate in nematodes, ideally we would divide the number of mutations between two closely related species by their divergence date. Many mysteries remain in eukaryotic genome evolution. Information is available on data set of ten nematode genome sequences that will be ideal for investigating unresolved questions, such as what are the forces governing the evolution of chromosome number, size, and structure; how does sex chromosome evolution differ from that of autosomes; how do differences in life history traits and reproductive strategy affect genome evolution; and what are the major genomic changes that enable species to adapt to new ecological niches such as parasitism. Looking forward, it seems very possible that once again these tiny animals will be first in revealing some of nature's deepest secrets.

3.21 Phylogeny and Introns

A phylogeny of *Caenorhabditis r*eveals frequent loss of introns during nematode evolution. The most popular model to explain how introns are lost involves homologous recombination between the genomic copy of a gene and an intronless cDNA copy produced by reverse transcription. Because retrotransposons can reverse-transcribe the cell's own mRNA, the required cDNA templates are expected to be present in eukaryotic cells. Furthermore, a cDNA could recombine with its corresponding gene, resulting in intron loss. However, other researchers have suggested that the loss of introns most often occurs by a simple deletion, caused by imprecise recombination. The most common mechanisms proposed to explain intron gain are the insertion of mobile genetic elements that contain splicing signals into a gene, "reverse splicing," and recombination between homologous copies of a gene. Finally, recent work indicates that some introns might be created by the activation of new splice sites in a degenerate coding region.

It has been a topic of curiosity to know how changes in intron/exon structure occur and what role these changes play in evolution. Cho et al. (2004) studied gene structure in nematodes related to *Caenorhabditis elegans*. They cloned a set of five genes from six different *Caenorhabditis* species and used their amino acid sequences to construct the first detailed phylogeny of this genus. They observed that nematode introns are lost at a very high rate during evolution, almost 400-fold higher than in mammals. These losses do not occur randomly, but instead favor some introns and do not affect others. In contrast, intron gains are far less common than losses in these genes. On the basis of the sequences at each intron site, we suggest that several distinct mechanisms can cause introns to be lost. The small size of *C. elegans* introns should increase the rate at which each of these types of loss can occur and might account for the dramatic difference in loss rate between nematodes and mammals.

Kelchner (2002) reported that group II introns comprise the majority of noncoding DNA in many plant chloroplast genomes and include the commonly sequenced regions *trnK/matK*, the *rps16* intron, and the *rpl16* intron. As demand increases for nucleotide characters at lower taxonomic levels, chloroplast introns may come to provide the bulk of plastome sequence data for assessment of evolutionary relationships in infrageneric, intergeneric, and interfamilial studies. Group II introns have many attractive properties for the molecular systematist: They are confined to organellar genomes in eukaryotes and the majority are single copy; they share a well-defined and empirically tested secondary and tertiary structure; and many are easily amplified due to highly conserved sequence in flanking exons. However, structure-linked mutation patterns in group II intron sequences are more complex than generally supposed and have important implications for aligning nucleotides, assessing mutational biases in the data, and selecting appropriate models of character evolution for phylogenetic analysis. These unique features might allow these animals to develop some nematode-specific ways of constructing and altering genomes. To learn how intron/exon structure changes during evolution, genomic and cDNA sequences for *fog-3* and the CPEB genes *fog-1*, *cpb-1*, *cpb-2*, and *cpb-3* were compared from several species in the genus *Caenorhabditis*. These comparisons elucidate the recent history of each intron. Furthermore, because the four CPEB genes were formed by earlier duplication events, comparisons between them revealed information about ancient changes in intron structure.

The rate of intron loss is very high in nematodes. From an evolutionary perspective, changes in intron/exon structure might be an important force for generating differences in gene function. However, a recent study showed that such as it seems likely, then the rate at which introns are lost in worms exceeds that of mammals by more than 400-fold. It was observed that the deleted introns in mammals were probably much smaller than the average human size of 2,500 bp. In *C. elegans*, most introns were about 50 bp long, and small introns were found in each of the other nematode species examined. Thus, it seems possible that the frequency at which introns are lost

is inversely proportional to their size. Because nematodes also have some large introns, this hypothesis can be directly tested when additional genome sequences from *Caenorhabditis* are finished. The rate at which introns are gained might also be higher in worms. No insertions were found in any human, mouse, or rat genes, whereas they observed either two or three insertions in the nematode genes analyzed. However, in the mammalian study, 16 introns in coding regions of low amino acid sequence conservation were not considered and some of these excluded cases might have involved insertions. For comparison, the two recently inserted introns we detected in *fog-1* are both located in a poorly conserved region that would have been excluded from the mammalian study.

3.22 Intron Losses

The rate of intron loss is very high in nematodes (Cho et al. 2004). From an evolutionary perspective, changes in intron/exon structure might be an important force for generating differences in gene function. However, a recent study showed that such changes are rare in mammalian evolution. For example, of 10,020 introns considered in a comparison between humans and mice, only five were lost in the mouse lineage, and none were lost in humans. Gene structures change much more rapidly in nematodes. First, a direct comparison of the *C. elegans* and *C. briggsae* genomes showed that they have significant differences in intron/exon structure. In a study, of 60,275 introns that were examined, 4,379 were unique to *C. elegans* and 2,200 were unique to *C. briggsae.* Because no outgroups were considered, it was unclear if these differences were caused by losses or gains. Several studies have documented dramatic changes in intron/exon structure within large *C. elegans* gene families. These data showed that intron losses were more common than gains, but could not determine the rate of loss, as the dates of each gene duplication were unknown. Intron losses occur by the following manner.

Recombination with cDNA: The most common hypothesis for how introns are lost is by recombination with reversed-transcribed copies of a message, which should lack all introns (Fig. 3.6). In its simplest form, this model implies that adjacent introns have a high probability of being lost together in a single

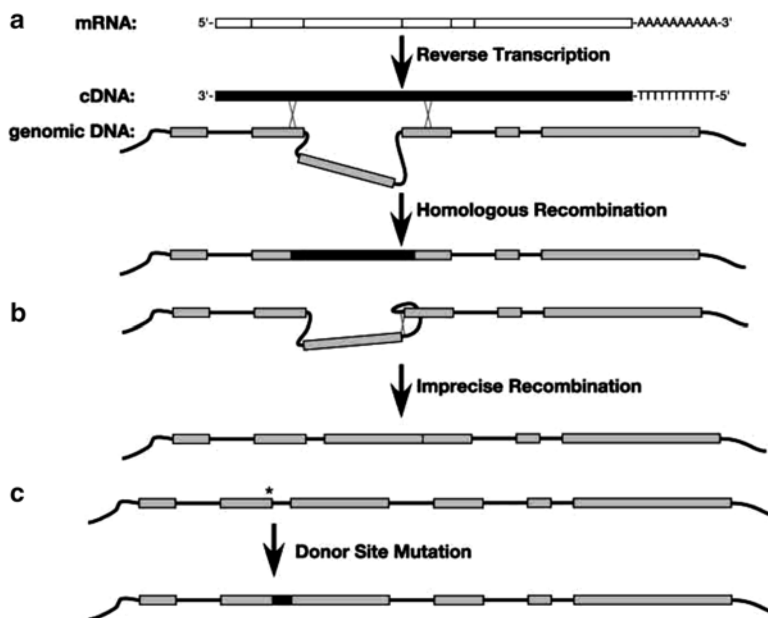

Fig. 3.6 Intron losses

event. Some data strongly support this model. For example, five adjacent introns seem to have been lost simultaneously during the evolution of the catalase 3 gene in *Zea mays*, and all of the introns in the *Oikopleura longicauda* EP-1 gene might have been lost in a single event (Wada et al. 2002). However, adjacent losses like these are rare in our data and in data from plants (Frugoli et al. 1998), insects, and deuterostomes. One potential explanation is that the cDNA templates that recombine with genomic DNA are usually small fragments rather than complete genes. For each species, the size of these fragments would determine which introns could be lost together. If so, in worms, these cDNA fragments are unlikely to be formed by partial reverse transcription starting from the 3_ end, as has been hypothesized for unicellular eukaryotes, as there is no bias toward loss of introns at the 3_ ends of genes.

Deletions: Introns could also be lost by spontaneous genomic deletions. In theory, these deletions could either be precise, which would yield a product indistinguishable from an intron lost by recombination, or imprecise. Such events are known to occur, as the *jingwei* gene of *Drosophila teissieri* has two alleles, one of which is an imprecise deletion that did not remove 12 nucleotides of the original intron. Similarly, intron #1 of the *C. elegans cpb-1* gene might have been formed by imprecise deletion, as it appears to have been lost along with some adjacent coding sequence. Because the probability of a 2,500-bp intron being exactly deleted is much lower than it is for a 50-bp intron, this mechanism should also favor the loss of short introns.

3.23 Changes in Splice Donor Sites

In regions that are tolerant of changes in amino acid sequence, one might also expect some introns to be lost by the mutation of a splice donor site. If this was to happen and no cryptic donor sites were activated, the associated intron would become part of the coding region. This mechanism could explain how intron #3 was lost from the *fog-1* gene of *C.* sp. CB5161. Because longer introns are more likely to contain in-frame stop codons, this mechanism should only work for very short introns. It was suspected that all of these mechanisms contribute to intron loss during evolution but that spontaneous genomic deletions are far more important than previously suspected. Once additional nematode genome sequences become available, a global comparison of the loss rate for introns was planned in germ line and somatic genes to test this hypothesis. Because of the high loss rate for nematode introns, such a comparison could also test the hypothesis that all mechanisms for intron loss favor the elimination of short introns over longer ones.

Martijn Holterman et al. (2006) observed that inference of evolutionary relationships between nematodes was severely hampered by their conserved morphology, the high frequency of homoplasy, and the scarcity of phylum-wide molecular data. To study the origin of nematode radiation and to unravel the phylogenetic relationships between distantly related species, 339 nearly full-length small-subunit rDNA sequences were analyzed from a diverse range of nematodes. Bayesian inference revealed a backbone comprising 12 consecutive dichotomies that subdivided the phylum Nematoda into 12 clades. The most basal clade is dominated by the subclass Enoplia, and members of the order Triplonchida occupy positions most close to the common ancestor of the nematodes. Crown clades 8–12, a group formerly indicated as “Secernentea” that includes Caenorhabditis elegans and virtually all major plant and animal parasites, show significantly higher nucleotide substitution rates than the more basal clades 1–7. Accelerated substitution rates are associated with parasitic lifestyles (clades 8 and 12) or short generation times (clades 9–11). The relatively high substitution rates in the distal clades resulted in numerous autapomorphies that allow in most cases DNA barcode-based species identification. *Teratocephalus*, a genus comprising terrestrial bacterivores, was shown to be most close to the starting point of Secernentean radiation. Notably, fungal-feeding nematodes were exclusively

found basal to or as sister taxon next to the three groups of phytonematodes, viz., Trichodoridae, Longidoridae, and Tylenchomorpha. The exclusive common presence of fungivorous nematodes and phytonematodes supports a long-standing hypothesis that states that plant-parasitic nematodes arose from fungivorous ancestors.

Meloidogyne species are known to reproduce either by cross-fertilization (amphimixis), facultative meiotic parthenogenesis, or obligatory mitotic parthenogenesis (Castagnone-Sereno et al. 1993). Among them, *M. incognita, M. arenaria*, and *M. javanica* are obligatory mitotic parthenogenetic species, while *M. hapla* can reproduce by both cross-fertilization and meiotic parthenogenesis. Phylogenetic relationships in this genus have been investigated by hybridization of *Bam*HI-digested genomic DNAs of 18 geographical isolates belonging to six species with three homologous repeated DNA probes cloned at random from a genomic library of one population of *M. incognita*. Due to the repetitive nature of the probes, the autoradiograms exhibited extensive restriction fragment length polymorphisms (RFLPs) both between and within nematode species. Genetic distance values estimated from hybridization patterns were analyzed by two phylogenetic tree-building distance methods, respectively, based on constant (UPGMA) and varying (FITCH) rates of nucleotide substitution, and the resulting dendrograms showed a very similar clustering of species and populations. Comparison of these results with the other sources of phylogenetic data available for this genus, i.e., cytogenetic, isoenzymatic, and mitochondrial DNA (mtDNA) data, revealed consistency with all but the mtDNA phylogeny. Due to the maternal inheritance of mtDNA, and the parthenogenetic reproductive mode of these organisms, which excludes any possibility of horizontal transfer, it was concluded that nuclear DNA phylogeny should represent a more likely evolutionary history of this particular genus and that interspecific hybridizations between sexual ancestors may account for the results with mtDNA. Thus, the early split-off of the mitotically parthenogenetic species cluster and *M. hapla* confirms the amphimictic ancestral mode of reproduction of root-knot nematodes.

Kaplan et al. (2000) compared the nucleic acid sequences of rDNA ITS1 and the rDNA D2/D3 expansion segment for 57 burrowing nematode isolates collected from Australia, Cameroon, Central America, Cuba, Dominican Republic, Florida, Guadeloupe, Hawaii, Nigeria, Honduras, Indonesia, Ivory Coast, Puerto Rico, South Africa, and Uganda. Of the 57 isolates, 55 were morphologically similar to *Radopholus similis* and seven were citrus parasitic. The nucleic acid sequences for PCR-amplified ITS1 and for the D2/D3 expansion segment of the 28S rDNA gene were each identical for all putative *R. similis.* Sequence divergence for both the ITS1 and the D2/D3 was concordant with morphological differences that distinguish *R. similis* from other burrowing nematode species. This result substantiated previous observations that the *R. similis* genome is highly conserved across geographical regions. Autapomorphies that would delimit phylogenetic lineages of non-citrus-parasitic *R. similis* from those that parasitize citrus were not observed. The data supported the concept that *R. similis* is comprised of two pathotypes, one that parasitizes citrus and one that does not.

Internal transcribed spacer (ITS) sequences of rDNA from 53 populations and species of gall-forming nematodes of the subfamily Anguininae, along with five populations of the *D. dipsaci* species complex, were used for phylogenetic analyses (Subbotin et al. 2001). The molecular analyses support a concept of narrow specialization for seed-gall nematodes and reveal distinction of at least nine undescribed species of *Anguina* inducing seed galls, previously identified as *A. agrostis*, and two species within the *D. dipsaci* species complex. Both the maximum parsimony and maximum likelihood analyses of the ITS data strongly support monophyly of the genus *Anguina.* Also, non-monophyly of *Subanguina* in the broad sense of Brzeski (1981) and of *Mesoanguina* and *Heteroanguina* according to the classification by Chizhov and Subbotin (1985, 1990) was indicated. Morphological and biological characters are congruent with the anguinid groups supported by the ITS phylogeny. The test of topologies conducted by maximum likelihood analyses showed that the monophyletic origin of

anguinids parasitizing grasses and sedges could not be rejected. The main anguinid groups are generally associated with plant hosts belonging to the same or related systematic groups.

3.24 Evolution of Parasitism in Nematodes

Despite extraordinary diversity of free-living species, a comparatively small fraction of nematodes are parasites of plants. These parasites represent at least three disparate clades in the nematode tree of life, as inferred from rRNA sequences (Baldwin et al. 2004). Plant parasites share functional similarities regarding feeding, but many similarities in feeding structures result from convergent evolution and have fundamentally different developmental origins. Although Tylenchida rRNA phylogenies are not fully resolved, they strongly support convergent evolution of sedentary endoparasitism and plant nurse cells in cyst and root-knot nematodes. This result has critical implications for using model systems and genomics to identify and characterize parasitism genes for representatives of this clade. Phylogenetic studies reveal that plant parasites have rich and complex evolutionary histories that involve multiple transitions to plant parasitism and the possible use of genes obtained by horizontal transfer from prokaryotes. Developing a fuller understanding of plant parasitism will require integrating more comprehensive and resolved phylogenies with appropriate choices of model organisms and comparative evolutionary methods.

3.24.1 Mode and Tempo of the Evolution of Parasitism

Branch length data from molecular phylogenies can be related to evolutionary time if a model of molecular evolution is applied that assumes clocklike accumulation of genetic change (Dorris et al. 1999). This assumption of a molecular clock allows inferred branch lengths to be read as time intervals, i.e., a time axis can be placed on the tree. Fossils are required to calibrate and validate the clock, and their absence in nematodes invalidates clock assumptions for the SSU nematode phylogeny. In addition, extreme rate differences in inferred accumulation of changes are seen between 25 taxa. These rate differences are even more problematic in that they can cause significant artifacts in the building of trees. Of note here is that the distances between genera within the Rhabditina (in clade 5) are similar to those seen between tetrapod classes; the Nematoda appears to be old and diverse (Fitch et al. 1995). The genetic divergence between taxa in the Strongylida is remarkably low. This pattern suggests either a relative slowdown in molecular evolutionary rates, correlated with the adoption of parasitic mode of life, or an increase in the relative rate of molecular evolution or both. One possibility may be that the rate of molecular evolution is correlated with generation time.

The diversity of parasitic lifestyles displayed by nematodes, and the diversity of hosts used, reflects both a propensity toward parasitism in the phylum and an adaptability to new and challenging environments (Blaxter 2003). Parasitism of plants and animals has evolved many times independently within the Nematoda. Analysis of these origins of parasitism using a molecular phylogeny highlights the diversity underlying the parasitic mode of life. Many vertebrate parasites have arthropod-associated sister taxa, and most invade their hosts as third-stage larvae: These features co-occur across the tree and thus suggest that this may have been a shared route to parasitism. Analysis of nematode genes and genomes has been greatly facilitated by the Caenorhabditis elegans project. However, the availability of the whole-genome sequence from this free-living rhabditid does not simply permit definition of “parasitism” genes; each nematode genome is a mosaic of conserved features and evolutionary novelties. The rapid progress of parasitic nematode genome projects focusing on species from across the diversity of the phylum has defined sets of genes that have patterns of evolution that suggest their involvement with various facets of parasitism, in particular the problems of acquisition of nutrients in new hosts and the evasion of

host immune defenses. With the advent of functional genomics techniques in parasites and in particular the possibility of gene knockout using RNA interference, the roles of many putative parasitism genes can now be tested.

3.25 Nematode Mating Systems and Evolution

In animals, sexual traits evolve rapidly. Ronald E. Ellis and Soochin Cho (2003) studied nematode mating systems. *C. elegans* (Ce) and *C. briggsae* (Cb) have male and hermaphrodite sexes, whereas *C. remanei* (Cr) has male and female sexes. They were interested to know why XX animals become self-fertile hermaphrodites in some species but females in others and how these different systems arose. The essential difference between hermaphrodites and females is that the former produce sperm and oocytes, but the latter only make oocytes. In Ce, they demonstrated that FOG-3 and the CPEB protein FOG-1 were required for germ cells to initiate spermatogenesis. To study the control of germ cell fate, homologues of these genes were cloned from Cb and Cr, which revealed that each species had four CPEB genes, just like Ce. In all three species, dsRNAi against the fog-1 homologue caused germ cells to become oocytes rather than sperm. The requirement for cpb-1 in early spermatogenesis has been conserved as well. Thus, the divergence of these proteins predated the origin of this genus. All three species had one fog-3 gene, which is required for germ cells to become sperm rather than oocytes. Since the levels of fog-3 transcripts were correlated with spermatogenesis, the control of fog-3 expression could be responsible for determining if XX animals become females or hermaphrodites. Experiments with chimeric transgenes showed that the fog-3 promoters from all three species could drive expression of fog-3 in Ce XX larvae.

Furthermore, these promoters each contained multiple binding sites for the sex determination protein TRA-1A. It was proposed that fog-3 controls germ cell fate in all caenorhabditids and that the activity of TRA-1A was modulated in hermaphrodite species to allow fog-3 expression in XX larvae. To test this hypothesis, they cloned tra-1 from Cr. Surprisingly, in Cr, tra-1(RNAi) males only produced oocytes. Similar results were found in Cb. These results suggested that TRA-1 plays an important role promoting spermatogenesis in these species. In Ce, the activity of TRA-1A is modulated by upstream factors like FOG-2, to allow hermaphrodite spermatogenesis. They screened for Fog mutants in Cb and identified a mutation, v35, with a similar phenotype; XX animals are female and XO animals are male. They used sequence analysis of fog-1, fog-3, cpb-1, cpb-2, and cpb-3 to establish a phylogeny for all caenorhabditids. It was observed that Ce and Cb were not sister species and that mating systems must have switched multiple times during the evolution of this genus. They also observed rapid evolution of intron number in these genes.

Self-fertilizing species often harbor less genetic variation than cross-fertilizing species, and at least four different models have been proposed to explain this trend (Graustein et al. 2002). To investigate further the relationship between mating system and genetic variation, levels of DNA sequence polymorphism were compared among three closely related species in the genus *Caenorhabditis*: two self-fertilizing species, *C. elegans* and *C. briggsae*, and one cross-fertilizing species, *C. remanei.* As expected, estimates of silent site nucleotide diversity were lower in the two self-fertilizing species. For the mitochondrial genome, diversity in the selfing species averaged 42 % of diversity in *C. remanei.* Interestingly, the reduction in genetic variation was much greater for the nuclear than for the mitochondrial genome. For two nuclear genes, diversity in the selfing species averaged 6 and 13 % of diversity in *C. remanei*. They argued that either population bottlenecks or the repeated action of natural selection, coupled with high levels of selfing, is likely to explain the observed reductions in species-wide genetic diversity.

The mechanisms by which new modes of reproduction evolve remain important unsolved puzzles in evolutionary biology (Chaudhuri et al. 2011). Nematode worms are ideal for studying the evolu-

tion of mating systems because the phylum includes both a large range of reproductive modes and large numbers of evolutionarily independent switches. *Rhabditis* sp. SB347 is a nematode with sexual polymorphism, which produces males, females, and hermaphrodites. To understand how the transition between mating systems occurs, the mechanisms that regulate female versus hermaphrodite fate in *Rhabditis* sp. were characterized. Hermaphrodites develop through an obligatory nonfeeding juvenile stage, the dauer larva. It was shown that by suppressing dauer formation, *Rhabditis* sp. SB347 develops into females. Conversely, larvae that under optimal growth conditions develop into females can be respecified toward hermaphroditic development into females if submitted to dauer-inducing conditions. These findings are of significance to understanding the complex mating system evolution in phytonematodes.

To analyze changes in gene structure during nematode evolution, the first detailed phylogeny of the *C. elegans* group was prepared (Blaxter et al. 1998). This phylogeny also showed that mating systems have changed multiple times during the evolution of this small group within the genus *Caenorhabditis*. This result dramatically extends previous analyses of the entire phylum Nematoda which showed that mating systems had changed many times during the long evolutionary history of the nematodes.

The mechanisms by which new modes of reproduction evolve remain important unsolved puzzles in evolutionary biology (Chauduri et al. 2011). Nematode worms are ideal for studying the evolution of mating systems because the phylum includes both a large range of reproductive modes and large numbers of evolutionarily independent switches. *Rhabditis* sp. SB347, a nematode with sexual polymorphism, produces males, females, and hermaphrodites. To understand how the transition between mating systems occurs, they characterized the mechanisms that regulate female versus hermaphrodite fate in *Rhabditis* sp. SB347. Hermaphrodites develop through an obligatory nonfeeding juvenile stage, the dauer larva. They observed that by suppressing dauer formation, *Rhabditis* sp. SB347 develops into females. Conversely, juveniles that under optimal growth conditions develop into females can be respecified toward hermaphroditic development if submitted to dauer-inducing conditions. These results are of significance to a better understanding of the evolution of complex mating systems present in parasitic nematodes.

The fact that most species in this genus use male/female mating systems suggests that the ancestor of the elegans group was male/female. Thus, if our phylogeny is correct, the simplest model is that *C. elegans* and *C. briggsae* each evolved hermaphroditism separately. However, it remains possible that the common ancestor of *C. briggsae*, *C. remanei*, *C.* sp. *CB5161*, and *C. elegans* acquired a male/hermaphrodite mating system, and this system then reverted to a male/female one in *C. remanei* and *C.* sp. *CB5161*. Although the second scenario involves more steps than the first one, it might be equally probable, because we do not know the relative likelihood of switching from a male/female mating system to a male/hermaphrodite one, or vice versa. In the past few years, a major effort has been launched to determine the molecular changes that have influenced the control of sex determination during nematode evolution.

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4 Major Phytonematodes Associated with Horticultural Crops and Their Diagnostic Keys

Since phytonematodes are microscopic, morpho-anatomical characters have to be precisely observed and interpreted. Detailed studies on morpho-anatomical characters including their variability in several populations from many hosts and geographical regions provide the basis for creating new species. Major criteria considered to diagnose a particular species of a nematode include morphometric data, sex and sexual dimorphism, body size and shape, cuticle, cephalic region, stylet and esophagus, intestine, prerectum, rectum and anus, male and female reproductive system, tail, and juvenile characters (Siddiqi 2001). Biological characters, especially host preference, can be used in the identification of some plant-parasitic nematodes. However, the effects of physical, chemical, and biological factors on host–parasite relationships are often great. The presence or absence of males is also a differentiating character, but it is known that at least in some species, males may arise as a response to environmental stress.

Biochemical, molecular, and cytogenetic techniques are being employed to discover the degree of genetic similarity in a taxonomic group. Such data must be obtained for several species to compare with both ingroup and outgroup members. A comparative study of such data provides valuable information about characters/features that are the result of common genetic material, as well as those that are unique for a particular member of the group. The uniqueness determines the identity of the taxon. Genetically dependent molecular data can be used concordantly with the morpho-anatomical characters to determine natural groupings and evolutionary trends. Phytonematodes found associated with various horticultural crops can be identified with the help of characteristic and distinguishing morphological features of juvenile, adult male, and female stages.

Major morphological features of juvenile, adult male, and female stages of important phytonematodes are furnished below (Thorne 1961; Dropkin 1980; Kiryanova and Krall 1980).

4.1 Potato Rot Nematode (*Ditylenchus destructor*)

It is a major pest on potato and also on other host plants including garlic, onion, carrot, tomato, beet, watermelon, pumpkin, cucumber, brinjal, clover, peanuts, and pepper. This nematode is also known as iris nematode, potato tuber eelworm, and potato tuber nematode.

4.1.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Superfamily:	Tylenchoidea
Family:	Anguinidae
Subfamily:	Anguininae
Genus:	*Ditylenchus*
Species:	*D. destructor*

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4.1.1.1 Female Nematode

- The cuticle near the head is marked by transverse striae about 1 μ apart, while on the remainder of the body the striae are obscure unless the specimens are shrunken by cold fixative.
- Lateral fields with six incisures, which are reduced to two on the neck and tail.
- Deirids usually visible near base of neck.
- Hemizonid slightly anterior to excretory pore.
- Phasmids not observed.
- Cephalic papillae and amphids visible only from a face view, arranged in a manner similar to those figured for *Ditylenchus dipsaci*.
- Labial framework well sclerotized.
- Spear typical of the genus, with rounded knobs.
- Basal bulb of esophagus with three large and two small gland nuclei, generally extended in a lobe reaching back over the dorsal side of the intestine.
- This lobe may be either shorter or longer.
- Anterior end of intestine extending into base of esophagus, where it connects with the lumen by an obscure valvular apparatus.
- Intestine densely granular, ending in a distinct rectum and anus.
- Anterior ovary outstretched to near the base of esophagus, the developing oocytes often arranged in two lines, changing to tandem near the middle of the ovary.
- Eggs average slightly longer than body diameter and are about one-half as wide as long.
- Posterior uterine branch rudimentary, not observed to function as a spermatheca.
- Spermatozoa usually well up in the uterus.
- Lips of vulva thick and elevated.
- Vulva–anus distance 1 3/4 to 2 1/2 times the tail length.
- 0.8–1.4 mm; $a = 33–35$; $b = 8–10$; $c = 15–20$; $V = 78–83$.

4.1.1.2 Male Nematode

- Testis outstretched to near the base of esophagus with spermatogonia arranged mostly in a single line until near the middle of the body, where they become primary spermatocytes from which the spermatozoa are produced.
- Spicules with a distinctive sclerotized pattern, with two longitudinal rays, one of which is noticeably longer than the other.
- Bursa extending from a point about opposite the proximal ends of the spicula to about two-thirds the tail length.
- Lateral incisures usually reduced to four near the tail, forming a pattern similar to that figured for *D. dipsaci*.
- 0.8–1.3 mm; $a = 34–40$; $b = 7–8$; $c = 12–16$; $T = 73–80$.

4.2 Stem and Bulb Nematode (*Ditylenchus dipsaci*)

This nematode is a major pest on onion and garlic, which are its primary hosts. It is commonly known as the stem nematode, the stem and bulb eelworm, or onion bloat. Other hosts include carrot, strawberry, faba beans, alfalfa, oats, and ornamental plants including hyacinth and tulip.

4.2.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Superfamily:	Tylenchoidea
Family:	Anguinidae
Subfamily:	Anguininae
Genus:	*Ditylenchus*
Species:	*D. dipsaci*

4.2.1.1 Female Nematode

- Body marked by transverse striae, about 1 μ apart, which are easily visible under the oil immersion at any point on the body.
- Lateral field marked by four incisures.
- Body straight or almost so when relaxed.
- Head unstriated, continuous with adjacent body part. Stylet cone about half of stylet length, knobs rounded.
- Median esophageal bulb muscular, with thickenings of lumen walls about 4–5 μm long.

- Basal bulb offset or overlapping intestine for a few micrometers.
- Excretory pore opposite posterior part of isthmus or glandular bulb.
- Postvulval part of uterine sac about half of vulva–anus distance long or slightly more.
- Deirids usually visible near base of neck.
- Hemizonid adjacent to excretory pore, about six annules wide.
- Phasmids rarely visible and if visible, then only from a dorsal or ventral view on favorable specimens.
- Amphid apertures on apices of lateral lips, where they appear as minute refractive dots which can be seen only from a face view.
- Spear with strongly developed knobs from which protrudor muscles lead to the well-sclerotized cephalic framework.
- Basal esophageal bulb with the usual three prominent and two inconspicuous, gland nuclei.
- Intestine connected to esophageal lumen by a very small valvular apparatus.
- Ovary outstretched, sometimes reaching to median esophageal bulb, but more often near the basal bulb, rarely with one or two flexures.
- Oocytes lie largely in tandem and develop into eggs which are two to three times as long as the body diameter.
- Rudimentary posterior uterine branch present, extending about halfway back to the anus.
- Vulva–anus distance equal to 1 3/4 to 2 1/4 times the tail length.
- Terminus always acute.
- 1.0–1.3 mm; $a=36–40$; $b=6.5–7.1$; $c=14–18$; $V=80$.

4.2.1.2 Male Nematode

- Testis outstretched, with spermatocytes arranged in single file except for a short region of multiplication.
- From a perfectly lateral view, the spicula exhibits a sclerotized pattern that apparently is characteristic of the species, but the proper angle of observation is so difficult to obtain that the pattern is rarely of taxonomic value.
- Bursa rising opposite proximal ends of spicula and extending about three-fourths the length of the tail.
- Lateral incisures end in a typical pattern.
- Male cloacal alae envelop about three-quarters of tail length.
- Spicules 23–28 μm long. Tail of both sexes conical, always pointed.
- 1.0–1.3 mm; $a=37–41$; $b=6.5–7.3$; $c=11–15$; $T=65–72$.

4.3 Awl Nematode (*Dolichodorus* spp.)

It is a common pest on a wide range of hosts like bean, Bermuda grass, cabbage, carnation, celery, centipede grass, corn, anubias, balsam, cotton, cranberry, hydrilla, impatiens, St. Augustine grass, sugarcane, tomato, palms, pepper, potato, and water chestnut (Paracer 1968). Usually, awl nematodes are found in moist to wet soil, in low areas of fields and near irrigation ditches, and in other bodies of freshwater.

4.3.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Suborder:	Tylenchina
Family:	Dolichodoridae
Subfamily:	Dolichodorinae
Genus:	*Dolichodorus*

4.3.1.1 Female Nematode

- Very large nematodes
- Head sharply offset from the rest of the body
- Vulva not raised; vaginal sclerotization appearing symmetrical in lateral view
- Female tail convex–conoid anteriorly, spicate posteriorly; terminal portion not annulated; phasmids a little behind anal level
- Post-rectal intestinal sac present
- Juvenile tail similar to that of female
- Labial region rounded, striated, offset, roughly quadrangular to prominently four lobed in en face view

- Labial disc more often prominent; subdorsal and subventral lip sectors distinct; lateral lip sectors reduced or absent
- Amphid aperture small slit laterally directed
- Stylet long (50–160 μm), strong; lacks a nerve ring
- Lateral field with three lines, areolated
- Tail hemispherical spiked, rarely conoid
- Phasmids postanal
- 2.6–3.6 (3.2) mm; a=48–73.4 (63.5); b=7.9–11.4 (9.4); c=28.7–64.6 (39.4); V=48.8–57.1 (53.6)%; stylet 132–162 μ (*D. silvestris*)

4.3.1.2 Male Nematode

- Male tail short, conical, with a trilobed bursa whose lateral lobes are well developed, striated, and posteriorly tapering
- Spicules, most generally with prominent flanges; robust, with distal half appearing clawlike due to large flanges
- Gubernaculum also robust, protrusible
- 2.3–3.1 (2.7) mm; a=50–74.2 (63.9); b=7.4–10.1 (8.4); c=54.4–87 (69.6); stylet 137–159 μ; spicules 42–75 (53) μ; gubernaculum 17–25 (20) μ (*D. silvestris*)

4.4 Golden Cyst Nematode of Potato (*Globodera rostochiensis*)

It is a major pest of crop plants in the family Solanaceae and economically important on potato and tomato besides other root crops. Other common names include the golden nematode, golden eelworm, or yellow potato cyst nematode.

4.4.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Super family:	Tylenchoidea
Family:	Heteroderidae
Subfamily:	Heteroderinae
Genus:	*Globodera*
Species:	*G. rostochiensis*

4.4.1.1 Female Nematode

- Length 0.47 mm; width 0.32 mm; L/W ratio 1.4.
- Stylet 23.8 μ; outlet of dorsal esophageal gland 6.4 μ.
- Excretory pore at base of neck and 130 μ from anterior end.
- Vulva slit 10 μ in length.
- Anus 41 μ from nearest edge of the hyaline vulval membrane, the latter measuring 13 μ in length (on the longer axis) and 7 μ in width.
- Body pearly white, ovate to subspherical in shape, with elongate, protruding neck, rounded posteriorly.
- As maturity continues toward the cyst stage, body undergoes color changes through yellow to light golden.
- Cuticle thick, outer layer rugose, and punctations near or just beneath the surface.
- Head slightly set off, bearing two annules.
- Cephalic framework weakly developed.
- Stylet fairly strong, with slight curvature, and well-developed basal knobs, sloping posteriorly.
- Median bulb large, nearly spherical, with well-developed valve.
- Esophageal glands often obscured but appearing clustered (sic) near base of neck.
- Excretory pore prominent, located 131 μ (105–175) from anterior end and always at or near base of neck.
- Vulva ellipsoid in shape, quite small, and measuring 12 μ (7–14) in length and 7 μ (5–11) in width
- Vulva slit 9 μ (6–11) in length.
- Underneath the vulva and generally in a cluster are vulval bodies, being highly variable in size and shape.
- Anus is much smaller than the vulva and is located 47 μ (39–80) from the nearest edge of vulva and generally opposite the long axis of the latter.
- Length (including neck) 0.52 mm (0.42–0.64); width 0.34 mm (0.27–0.43); L/W ratio 1.5.
- (1.2–2.0); stylet 23 μ (22–24); outlet of dorsal esophageal gland 6.2 μ (5.8–7.0).

4.4.1.2 Male Nematode

- Body slender, vermiform, tapering slightly at both extremities

- Cuticle with prominent annulation; subcuticular annulation less distinct and occurring twice as often as on cuticle
- Lateral field measuring 7.0 μ (6.7–8.4) in width at midbody, with four equally spaced lines except at its beginning in anterior portion
- About midway, body measures 39 μ (31–46) in width
- Head slightly set off
- Hemispherical with six annules
- Cephalic framework heavily sclerotized
- Stylet very strong, with prominent knobs
- Stylet guide seen anteriorly as the usual lyre-shaped structure with a ring at its base encircling the stylet; attached to the base of this lyre-shaped guide is a membranous, sleeve-like extension of the guide reaching about half the length of the basal stylet shaft, ending in another ring encircling the stylet at that point
- Anterior and posterior cephalids present
- Median bulb ellipsoidal with its center located 99 μ from anterior end
- Excretory pore about two annules posterior to commonly distinct hemizonid
- One testis
- Spicules slightly arcuate, with tips rounded, unnotched
- Tail short, variable in both length and shape
- Length 1.08 mm (0.89–1.27); $a=27$ (22–36); $b=5.9$ (4.9–7.3); $c=267$ (161–664); stylet 26 μ (25–27)
- Outlet of dorsal esophageal gland 6.4 μ (5.3–7.0)
- Spicules 35 μ (32–39)
- Gubernaculum 12 μ (10–14); tail 4.4 μ (1.7–6.7)

4.4.1.3 Second-Stage Juveniles

- Length 0.43 mm (0.37–0.47); $a=19$ (16–23); $b=2.3$ (2.2–2.5); $c=8$ (7–9); stylet 22 μ
- Outlet of dorsal esophageal gland 5.5 μ (5.0–6.7)
- Tail 51 μ (44–57); hyaline tail terminal 24 μ (18–30)
- Caudal ratio A = 3.4 (2.8–4.4); caudal ratio B = 10.8 (5.5–17.0)
- Body tapering at both extremities but much more so posteriorly
- Subcuticular annulation twice as frequent as on cuticle
- Lateral field with four lines for most of body length, the outer two crenate but without aerolation
- Body measures 23 μ (19–26) at widest part
- Head slightly set off, bearing five annules and considerably wider at its base than in height, presenting a rounded though rather anteriorly flattened appearance
- Cephalic framework heavily sclerotized
- Stylet well developed, with prominent knobs appearing in lateral view
- Stylet guide as described above for males
- Anterior and posterior cephalids present, located about as shown
- Valvate median bulb prominent, ellipsoidal with its center located 68 μ (64–76) from the anterior end
- Isthmus and esophageal glands typical for the genus
- Excretory pore posterior and almost adjacent to hemizonid
- Genital primordium located slightly posterior to midbody and commonly consists of four cells
- Tail tapering to small, rounded terminus
- Phasmids generally difficult to see, located about halfway on tail

4.4.1.4 Cysts

- Length (including the neck) 0.68 mm (0.45–0.99)
- Width 0.54 mm (0.25–0.81)
- L/W ratio = 1.27 (1.0–1.8)
- Diameter or longest axis of fenestra (A) 15 μ (8–20)
- Distance from anus to nearest edge of fenestra (B) = 68 μ (29–116); B/A ratio (Granek's ratio) = 4.5 (2.0–7.0)
- Cysts brown in color, ovate to spherical in shape, with protruding neck
- Circumfenestrate, abullate, and without the distinct "vulval bodies" commonly seen in white females
- Fenestra much larger than the small but distinct, V-shaped anus
- Cyst wall pattern basically as in female but often more prominent, and especially near

midbody, tends to form wavy lines going latitudinally around body
- Punctation generally present but variable in intensity and arrangement

4.4.1.5 Eggs
- Length 105 μ (5–115)
- Width 45 μ (42–48)
- L/W ratio = 2.3 (2.0–2.6)
- Egg shell hyaline, without visible markings

4.5 Pale Cyst Nematode of Potato (*Globodera pallida*)

This species differs from the golden nematode (*Globodera rostochiensis*) in that females lack the golden phase. They are pale/white. Other hosts include many *Solanum* species, oca (*Oxalis tuberosa*), Jamestown weed (*Datura stramonium*), tomato (*Lycopersicon* spp.), and *Salpiglossis* spp.

4.5.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Super family:	Tylenchoidea
Family:	Heteroderidae
Subfamily:	Heteroderinae
Genus:	*Globodera*
Species:	*G. pallida*

- Sedentary females.
- Smoothly rounded with small projecting neck, no terminal cone present, diameter + 450 μm, ranging in color from white to yellow.
- Cysts are similar in shape but have a tanned brown skin.
- Cuticle surface with zigzag pattern of ridges; a distinct D-layer is present.
- The perineal area consists of a single circumfenestration around the vulval slit, perineal tubercles on crescents near vulva.
- Anus subterminal without fenestra, vulva in a vulval basin, and underbridge and bullae rarely present.
- Eggs retained in cyst, no egg mass present.
- The non-sedentary second-stage juveniles are vermiform, annulated, and tapering at both ends.
- Body length ranging from 445 to 510 μm, stylet length 19–25 μm, tail length 37–55 μm, and a hyaline tail part of 21–31 μm.
- Use of a combination of cyst and second-stage juvenile characteristics is recommended for reliable identification.
- These stages are normally present in most soil samples infested with potato cyst nematodes.

4.5.1.1 Female Nematode
- Body subspherical with a projecting neck bearing the head and containing the esophagus and part of the esophageal glands.
- Color white, some populations passing through a 4–6-week cream stage, turning glossy brown when dead.
- Head with amalgamated lips and one or two prominent annules, deep irregular annulations on the neck, changing to reticulate pattern of ridges over most of the body surface.
- Lateral incisures absent.
- Head skeleton weakly developed, hexaradiate.
- Anterior part of stylet about 50 % of total stylet length and frequently separated from posterior part in fixed specimens.
- Stylet knobs backward sloping.
- Stomal lining forming a tube-shaped “stylet guide” extending from the head skeleton to about 75 % of stylet length.
- Very large median esophageal bulb, almost circular with large crescentic valve plates.
- Lobe of esophageal glands broad, frequently displaced forward; 3 nuclei.
- Prominent excretory pore situated at base of neck.
- Internal structures in neck region often obscured by hyaline secretions on cuticle surface
- Ovaries paired, occupying most of body cavity.

- Vulva a transverse slit at the opposite pole of body to neck, set in a slight circular depression, the vulval basin.
- Vulval orifice set between two finely papillated crescentic areas occupying most of the vulval basin.
- Cuticle surface between anus and vulval basin thrown into about 12 parallel ridges with a few cross connections.
- Subsurface punctations, irregularly arranged, are visible over much of the body surface and may be confused with surface papillae on the vulval crescents.
- Stylet length = 27.4 + 1.1 μ.
- Head width at base = 5.2 + 0.5 μ.
- Stylet base to dorsal gland duct entry = 5.4 + 1.1 μ.
- Head tip to median bulb valve = 67.2 + 18.7 μ.
- Median bulb valve to level of excretory pore = 71.2 + 21.9 μ.
- Head tip to level of excretory pore = 139.7 + 15.5 μ.
- Mean diameter of median bulb = 32.5 + 4.3 μ.
- Mean diameter of vulval basin = 24.8 + 3.7 μ.
- Length of vulval slit = 11.5 + 1.3 μ.
- Anus to vulval basin = 44.6 + 10.9 μ.
- Number of cuticular ridges on anal–vulval axis = 12.5 + 3.1.

4.5.1.2 Male Nematode

- Vermiform body with short tail ending in bluntly rounded terminus of variable shape
- Heat-relaxed specimens C or S shaped, posterior part twisted 90–180° about longitudinal axis
- Cuticle with regular annulations and four incisures in lateral field, terminating on the tail
- Annules sometimes crossing the outer incisures but not the inner pair
- Offset rounded head with large oral disc, six irregular lips, six or seven annules, and heavily sclerotized hexaradiate skeleton
- Anterior part of cephalids at 2nd to 4th annules behind the head, posterior pair at 6th to 9th annules
- Stylet well developed with backward-sloping basal knobs and anterior part about 45 % of total stylet length
- Lining of stoma lyre shaped anteriorly and forming a simple tube-shaped "stylet guide" posteriorly to about 70 % of stylet length
- Ellipsoid median esophageal bulb with strong crescentic valve plates linked to a narrow, ventrally situated esophageal gland lobe by a narrow isthmus encircled by a broad nerve ring
- Three esophageal gland nuclei, the dorsal one most prominent; lobe terminating near the excretory pore about 15 % of body length from the head
- Hemizonid two annules long situated 2–3 annules behind the excretory pore
- One testis, commencing with single cap cell 40–65 % of body length from the head and terminating in a narrow vas deferens with glandular walls
- Cloaca with small raised circular lip containing two stout arcuate spicules terminating distally in single pointed tips
- Small dorsal gubernaculum without ornamentation 2 μ thick laterally, slightly wider in dorsoventral aspect
- Phasmids and caudalids not observed
- Body length = 1,198 + 104 μ
- Body width at excretory pore = 28.4 + 1.3 μ
- Head width at base = 12.3 + 0.5 μ
- Head length = 6.8 + 0.3 μ
- Stylet length = 27.5 + 1.0 μ
- Stylet base to dorsal gland duct entry = 3.4 + 1.0 μ
- Head tip to median bulb valve = 96.0 + 7.1 μ
- Median bulb valve to excretory pore = 81.0 + 10.9 μ
- Head tip to excretory pore = 176.4 + 14.5 μ
- Tail length = 5.2 + 1.4 μ
- Tail width at anus = 13.5 + 2.1 μ
- Spicule length along axis = 36.3 + 4.1 μ
- Gubernaculum length = 11.3 + 1.6 μ

4.5.1.3 Cysts

- New cysts glossy brown in color, subspherical with protruding neck
- Head frequently lost leaving the terminus of the neck open
- Vulval region intact or fenestrated with single circumfenestrate opening occupying all or part of the vulval basin

- Vulval bridge, underbridge, and other remains of internal genitalia absent
- Abullate, but small darkened or thickened “vulval bodies” sometimes present in the vulval region
- Anus visible in most specimens, often at apex of a V-shaped mark
- Cuticular pattern as in female, but more accentuated
- Subcrystalline layer absent
- Width = 534 + 66 μ
- Length excluding neck = 579 + 70 μ
- Neck length 118 + 20 μ
- Mean fenestral diameter = 24.5 + 5.0 μ
- Anus to fenestra distance = 49.9 + 13.4 μ
- Granek’s ratio = 2.1 + 0.9

4.5.1.4 Second-Stage Juveniles

- Vermiform, folded four times within the egg.
- Tail tapering uniformly with a finely rounded point, body cavity extending about halfway along the tail length, remainder forming a hyaline tail region.
- Regular cuticular annulations.
- Lateral field with four incisures beginning and terminating with three and occasionally completely areolated.
- Cuticle thickened for first 7–8 body annules.
- Rounded head slightly offset with 4–6 annules.
- Oral disc surrounded by two lateral lips bearing amphid apertures and dorsal and ventral pairs of sublateral lips which are often fused.
- Contour of lips and oral disc subrectangular.
- Heavily sclerotized hexaradiate head skeleton; the dorsal and ventral radii bifurcate at tips in 60 % of specimens.
- Anterior cephalids at 2nd to 3rd body annule from the head, posterior at 6th to 8th annule. Stylet well developed, basal knobs with distinct forward projection on anterior face in lateral view.
- Anterior part of stylet approximately 50 % of total stylet length.
- Stomal lining as in male.
- Esophagus and nerve ring as in male.
- Gland lobe extended posteriorly for approximately 35 % of body length.
- Excretory pore approximately 20 % of body length behind head.
- Distinct hemizonid two annule-widths long, one annule before excretory pore.
- Hemizonian 5–6 annules behind excretory pore.
- Four-celled gonadal primordium at approximately 60 % of body length behind the head. Phasmids and caudalids not observed.
- L = 486 + 23 μ.
- Body width at excretory pore = 19.3 + 0.6 μ.
- Head width at base = 10.6 + 0.5 μ.
- Head length = 5.5 + 0.1 μ.
- Stylet length = 23.8 + 1.0 μ stylet base to dorsal gland duct entry = 2.7 + 0.9 μ.
- Head tip to median bulb valve = 68.7 + 2.7 μ.
- Median bulb valve to excretory pore = 39.9 + 3.3 μ.
- Head tip to excretory pore = 108.6 + 4.1 μ.
- Tail length = 51.1 + 2.8 μ.
- Tail width at anus = 12.1 + 0.4 μ.
- Length of hyaline tail = 26.6 + 4.1 μ.

4.5.1.5 Holotype Juveniles

- L = 510 μ
- Body width at excretory pore = 19 μ
- Tail length = 53 μ
- Hyaline tail length = 31 μ
- Tail width at anus = 13 μ
- Head annules = 4
- Head height = 5 μ
- Head width = 9 μ
- Stylet length = 25 μ
- Stylet base to dorsal gland duct entry = 2 μ
- Head tip to median bulb valve = 74 μ
- Median bulb valve to excretory pore = 32 μ
- Head tip to excretory pore = 106 μ

Globodera pallida resembles *G. rostochiensis*, but they differ in the following physical aspects:

1. Larvae of *G. pallida* usually larger, stylet longer (21–26 (23.6) μ compared with 21–23 (21.8) μ), body longer (440–525 (484) μ compared with 425–505 (468) μ), and tail longer (46–62 (51.9) μ compared with 42–50 (43.9) μ).
2. Profile of larval stylet knob of *G. pallida* with anterior face pointed but rounded in *G. rostochiensis*.
3. The rectangular contours of oral disc and lips also distinguish *G.* pallida larvae from those of *G. rostochiensis* which have ovate contours.
4. Males of *G. pallida* have an average shortened distance between stylet knobs and junction of

the dorsal gland duct than those of *G. rostochiensis,* 2–7 (3.5) µ and 4–8 (5.3) µ, respectively.

5. Females of *G. pallida* are distinguished from those of *G. rostochiensis* by their longer stylets (23–29 (26.7) µ compared with 21–25 (22.9) µ), by the shorter anal–vulval distance (22–67 (43.9) µ compared with 37–77 (60.0) µ), and by the smaller number of cuticular ridges between the anus and vulva (8–20(12.2) µ compared with 16–31 (21.6) µ).
6. The cream or white internal color of females of *G. pallida* also distinguishes them from *G. rostochiensis* in which the color is golden.
7. Cysts are distinguished by the same cuticular characters as the female; since differences in larval morphometrics between the two species are small, preparation methods which cause dimensional changes are undesirable.

4.6 Spiral Nematode (*Helicotylenchus* spp.)

This is a widely distributed phytonematode on a wide range of crop plants (Zuckerman and Strich-Hariri 2002). Among its several species, *H. multicinctus* is the major pest on banana and plantains.

4.6.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Super family:	Tylenchoidea
Family:	Hoplolaimidae
Subfamily:	Hoplolaiminae
Genus:	*Helicotylenchus*
Species:	*H. multicinctus*

4.6.1.1 Female Nematode

- Body vermiform, spiral to straight.
- Labial region continuous to slightly offset, rounded, or anteriorly flattened, generally annulated but never longitudinally striated.
- Anterior lip annulus generally not divided into sectors, with elongate amphid apertures rarely faint or marked lip sectors are present.
- Lateral field with four lines.
- Phasmids small, near anus.
- Cephalids and caudalid present.
- Tail 1 to 2 1/2 body diameters long, typically more curved dorsally, with or without a terminal ventral process, sometimes rounded.
- Stylet and labial framework average sized.
- DGO from 6 to 16 µm from the stylet base.
- Median bulb rounded with average-sized valve.
- Glands overlap the intestine dorsally and ventrally, all three glands of about the same length. Two genital branches, the posterior one sometimes degenerated or reduced.
- Epiptygma present, inconspicuous.
- 0.47–0.53 mm; $a = 24–30$; $b = 4.7–5.4$; $c = 35–46$; $V = 65–69$ %; stylet 22–24 µ (*H. multicinctus*)

4.6.1.2 Male Nematode

- Slight secondary sexual dimorphism seen in smaller anterior end
- Caudal alae enveloping tail end
- 0.47 mm; $a = 29–30$; $b = 5.2$; $c = 28–31$; stylet 22 µ; spicules 17–18 µ (*H. multicinctus*)

4.7 Sheath Nematode (*Hemicycliophora thienemanni*)

Its major hosts include pepper, squash, citrus, tomato, beans, celery, Tokay grape, cheesebush (*Hymenoclea salsola*), and coyote melon (*Cucurbita palmata*) on virgin desert soil.

4.7.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Suborder:	Tylenchina
Super family:	Criconematoidea
Family:	Hemicycliophoridae
Genus:	*Hemicycliophora*
Species:	*H. thienemanni*

4.7.1.1 Female Nematode

- Body well formed; curls into a C shape when the nematode dies.
- Ovary straight and anterior.
- Number of cuticular annulations 273–307.
- Stylet extends to the 29th or 30th cuticular annulation.
- Lateral field in the form of a single longitudinal line.
- Vulval lips protrude slightly above the body contours; vulva occurs on the 67th to the 73rd annulation from the posterior end of the body (55 %).
- 0.72–1.19 mm; $a=25$–34; $b=5.2$–6.7; $c=7.8$–10.6; $V=78$–84 %; spicules 24–27 μ.

4.7.1.2 Male Nematode

- Rarely found.
- Body straight and remains so after death.
- Tail very long, 130–184 μ, i.e., equal to 1–12 anal diameters of the body.
- Cuticle finely annulate.
- Lateral fields have four incisures.
- Esophagus not developed.
- Spicule arcuate.
- Bursa narrow.
- Spicules curved at a 90° angle.
- 0.79–0.93 mm; $a=40$–51; $b=6.1$–7.3; $c=5.0$–6.3; spicules 24–27 μ.

4.8 Sugar Beet Cyst Nematode (*Heterodera schachtii*)

It is also known as beet cyst eelworm. It is economically very important on sugar beet. Other important hosts are cabbage, broccoli, celery, turnip, rapeseed, and radish.

4.8.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Suborder:	Tylenchina
Super family:	Tylenchoidea
Family:	Heteroderidae
Genus:	*Heterodera*
Species:	*H. schachtii*

4.8.1.1 Female Nematode

- Typically they are lemon shaped and usually vary from 0.5 to 0.8 mm in length, but some specimens may be smaller or larger.
- Young females are yellowish brown in color rapidly darkening to deeper shades of yellow or yellowish brown.
- Cuticular markings of adult females and cysts are rugose, chaotic, short, zigzag elements without order of arrangement.
- Head region annulated, the terminal annule almost round, surmounted by a squarish labial plate.
- Spear straight or slightly curved, averaging 26 μ in length and bearing ovoid basal knobs.
- Median esophageal bulb spheroid.
- Basal portion of esophagus extended in lobe-like glands reaching back over the intestine.
- In early stages of development, the slender ovaries are coiled in the body cavity.
- As egg production proceeds, they completely fill the body.
- The cervix is supported by a single band of muscles attached to the lateral regions.
- Vagina sheaflike from a dorsal view, ending in a deeply cleft vulva.
- Fenestrae average 45 μ long.
- The white or pale yellow females of the sugar beet nematode are easily observed with the unaided eye as they lie attached to roots.
- Many individuals diverge from the typical form.
- Usually the subcrystalline layer is a prominent feature, appearing as a white incrustation completely covering that part of the body protruding from the root.
- A mucoid mass extruded from the vulval region surrounds the posterior end and is covered with adhering soil particles.
- The subcrystalline layer and mucoid mass give the female protection from mononchs and other predators.
- 0.4–1.1 (0.8) × 0.2–0.8 (0.5) mm; stylet 18–20 μ.

4.8.1.2 Male Nematode

- Length usually ranges between 1.3 and 1.6 mm.
- Annules of the lip region, three or four, including the labial disc.
- Spear 25–28 μ long, with strong basal knobs.
- Tip of the spicule bears two denticles and hence appears bifurcate.
- Gubernaculum less than half the size of the spicule.
- The male begins to develop in much the same manner as the female.
- It first becomes an elongated, cylindroid body slightly shorter than the length of the original larva from which it was formed.
- The testis appears as a series of cells roughly arranged in rows, with the terminus reflexed.
- From the indefinite mass of cells and granules surrounding the testis, the male body gradually takes form, finally appearing as an obese wormlike organism flexed once within the cuticle.
- From this third stage, the male elongates until it is flexed three or four times within the greatly expanded larval cuticle.
- Possesses a well-developed spear with strong basal knobs and all other organs typical of nematodes of this sort.
- Development takes place in the root cortex, and after the final molt, the male emerges into the soil
- Lobes of esophageal glands extending back ventrally along the intestine.
- Testis single, outstretched; spicula bidentate, resting on a slightly arcuate, trough-like gubernaculum.
- 1.3–1.6 (1.4) mm; $a=37$; $b=5–7$; $c=99$; stylet 28–30 μ; spicules 33–36 μ; gubernaculum 10–15 μ.

4.8.1.3 Juveniles

- Range from 450 to 500 μ in length, with a general average of about 460 μ.
- Under- and oversized larvae are not unusual, depending no doubt on the suitability of the host and on natural variation.
- Spear averaging about 25 μ length, which usually is about the length of the hyaline portion of the tail.

4.8.1.4 Eggs

- Within the body, may vary from less than 10 to over 600, with an average of 286.
- Most females eject some eggs into the mucoid mass before encystment occurs.
- Number varies from 1 to more than 200.
- They play an important part in the life history because they hatch immediately, enter roots, and produce another generation.
- Eggs within mature females and newly formed cysts hatch immediately under favorable environmental conditions and complete the life cycle in about 30 days.
- Rapid multiplication through several generations in a season makes it possible for a relatively small population remaining after a rotation to increase to enormous numbers before the end of the growing season.

4.9 Lance Nematode (*Hoplolaimus gaelatus*)

Major hosts of this nematode include cotton, pine, oak, wheat, corn, beans, bananas, peas, cabbage, sweet potatoes, peanuts, chrysanthemums, sycamore, apple, clover, alfalfa, and lawn grasses.

4.9.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Super family:	Tylenchoidea
Family:	Hoplolaimidae
Genus:	*Hoplolaimus*
Species:	*H. gaelatus*

4.9.1.1 Female Nematode

- Body straight, large (1–2 mm long).
- Lip region offset from body, wide, anteriorly flattened, with clearly marked annuli, and with longitudinal striae.
- Lateral field with four lines or less, generally areolated at level of phasmids and anteriorly,

sometimes with striae irregularly scattered over the entire field, rarely not areolated.
- Labial framework and stylet massive.
- Stylet knobs anchor or tulip shaped.
- DGO 3–10 μm from the stylet base.
- Esophageal glands overlap the intestine dorsally and laterally.
- Gland nuclei may be duplicated to a total of six nuclei.
- Intestine symmetrically arranged, equally developed.
- Tail short, rounded, phasmids enlarged to scutella erratically situated on body, anteriorly to the anus level and sometimes anterior to the vulva level, not opposite each other.
- Female tail shorter than anal body diameter.
- $L = 1.1–1.5$ mm; $a = 22–26$; $b = 7.4–8.6$; $c = 48–54$; $V = 55$.
- 0.9–1.3 mm; $a = 27–30$; $b = 6.5–7.0$; $c = 28–32$; $T = 42–50$.

4.9.1.2 Male Nematode

- Caudal alae enveloping tail, regular.
- Secondary sexual dimorphism visible in labial region and esophageal structures smaller in males.
- Lip region without tiling, or with only the basal annule divided.
- Cephalic framework massive, often yellow.
- Spear knobs with anterior projections.
- Esophageal glands overlapping the intestine dorsally and laterally, with 3–6 nuclei.
- One phasmid (scutellum) in the anterior portion of body, the other posterior.
- Spicule slightly arcuate.
- Gubernaculum with titillae.
- Bursa broad, striated, enveloping tail.
- 1.05–1.56 mm; $a = 25–32$; $b = 8.3–10.3$; $c = 28–40$; spicules 40–52 μ.
- Gubernaculum 20–28 μ; stylet 40–48 μ.

4.10 Needle Nematode (*Longidorus* spp.)

This is a widespread phytonematode with a broad range of host cops like grapevine, corn, citrus, pine, coffee, garlic, potato, lettuce, tomato, wild rose, eggplant, banana, sweet orange, pomelo, coconut, cucumber, and strawberry. This genus is involved in transmitting viral pathogens.

4.10.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Dorylaimida
Super family:	Dorylaimoidea
Family:	Longidoridae
Genus:	*Longidorus*
Species:	*L. elongatus*

4.10.1.1 Female Nematode

- Female body often in loose open C when relaxed by gentle heat, but sometimes irregular
- Body slender, of uniform width 50 μm (49–54) except for tapering extremities and, frequently, a slight protrusion at vulva
- Lips flattened, set off by a knob-like expansion
- Wide bilobed amphidial pouches extending nearly to or reaching the guiding ring
- Amphidial apertures obscure, just behind lateral lips
- Amphids abnormally large, almost encircling the head
- Cuticle and subcuticle 2 μm thick for most of body length, becoming thicker at extremities, markedly so at the tail
- Hemizonid 159 μm (150–173) from the anterior end and seen best on fresh nematodes or those relaxed and fixed in 5 % formalin
- Esophagus 292 μm (195–405), with anterior part often convoluted
- Cardia bluntly conoid
- Intestine about six cells in circumference
- Prerectum length variable, but 3.4 (0.6–4.8) times the body width
- Rectum shorter than anal body diameter
- Tail conoid, but sometimes with short broad peg in adults
- Ovaries didelphic, reflexed
- Vagina reaching about halfway across the body

- 5.2–7.3 (6.1) mm; *a* = 120–210 (158); *b* = 12.7–18.9 (15.2); *c* = 82–131 (97); *V* = 44–55 (49 %) (*L. attenuatus*)
- Odontostyle = 85 µm; spear extension = 35 µm; tail = 37 µm
- *V* = 46.4; guiding ring from anterior of nematode = 24 µm

4.10.1.2 Male Nematode (Not Common)

- Spicula blunt, arcuate with small, furcated lateral guiding pieces.
- Protractor muscle an unusually broad band.
- Supplements consisting of the adanal pair and 10–16 ventromedian ones, the series beginning within range of the spicula and being rather uniformly spaced.
- Oblique copulatory muscles are a prominent feature.
- 5.5 mm; *a* = 112; *b* = 12; *c* = 105.

4.11 Southern Root-Knot Nematode (*Meloidogyne incognita*)

This is one of the most predominant, economically important phytonematodes occurring on a wide range of crop plants. Vegetables are the most preferred hosts for this nematode, apart from fruits, ornamentals, plantation crops, polyhouse-grown crops, and medicinal and aromatic crops. Most cultivated crop plants are susceptible to this nematode.

4.11.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Super family:	Tylenchoidea
Family:	Heteroderidae
Subfamily:	Meloidogyninae
Genus:	*Meloidogyne*
Species:	*M. incognita*

4.11.1.1 Female Nematode

- Body melon/flask shaped, often with a very long cervix which may be bent sideways in fixed specimens.
- Stylet hard with large round basal knobs; width of base 4–5 µ and height 1.8–2 µ.
- Opening of the dorsal esophageal gland situated at a distance of 2–4 (3) µ from the base of the stylet.
- Excretory pore situated at the level of the opening of the dorsal esophageal gland.
- Anal valve plate rounded–oval shaped.
- Anal arc high with compact sinuate and zigzag lines.
- Right and left sides of the anal arc often asymmetric.
- Rudimentary tail marked with whorled, unbroken lines.
- Two short straight folds usually protrude from the posterior lip of the vulva toward the anal pore, lying vertical to the vulval slit.
- The lateral folds may be very poorly expressed; they are delineated by double lines on the dorsal and ventral sides of the anal–vulval Plate.
- 0.51–0.69 mm × 0.30–0.43 mm; stylet 15–16 µ.
- Perineal patterns of females typically have a high, squarish dorsal arch.
- The male head has a characteristic large, rounded labial disc, which appears concave in lateral view.

4.11.1.2 Male Nematode

- The head has one labial annulation and three more or less distinct postlabial annulations.
- Height of lateral gene 6 µ.
- Stylet usually 25–26 µ in length; width at base 5.5–6.5 µ and height 3.0–3.5 µ; knobs are rounded and sometimes appear anteriorly bifurcate.
- Opening of the dorsal esophageal gland located at a distance of 1.7–3.5 (2.5) µ from the base of the stylet.
- Males produced from eggs of a single female have either one or two testes.
- 1.2–2.0 mm; *a* = 39–48; *b* = 8–17; stylet 23–26 µ; spicules 34–36 µ.

4.11.1.3 The Second-Stage Juveniles

- The head has four annulations as in the male.
- Stylet 10 μ long with very rounded basal knobs; width of base about 2 μ and height 1.3–1.5 μ.
- Opening of the dorsal esophageal gland located at a distance of 2.0–2.5 μ from the base of the stylet.
- Tail simple.
- Length 360–393 μ; $a = 29–33$; $b = 5.6–6.4$; $c = 8.0–9.4$

4.11.1.4 Eggs

Relatively small, 80–98 μ × 30–38 μ

4.11.1.5 Perineal Pattern

Perineal pattern somewhat oval with a high, irregular arch composed of closely spaced wavy lines (Fig. 4.1). Interior portion of the arch above anus marked by numerous zigzag and broken striae which sometimes form a whorl. There is little evidence of lateral lines, but along these areas, both the dorsal and ventral striae have a tendency to become forked. Often there are short transverse striae extending from the inner striae toward the vulva.

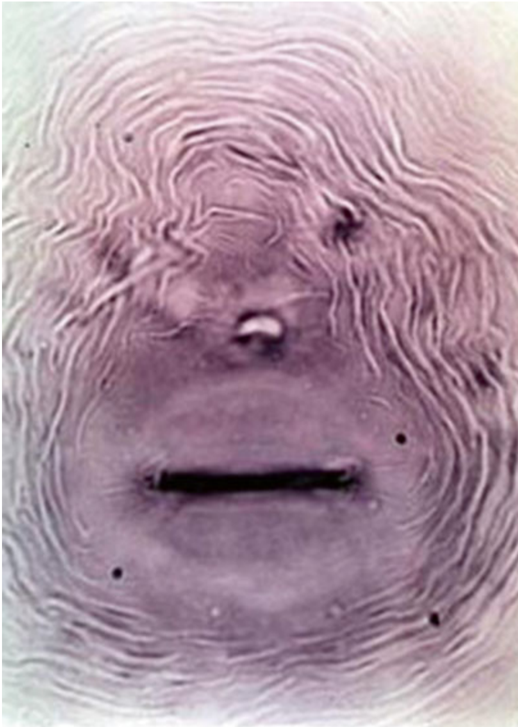

Fig. 4.1 Perineal pattern of *M. incognita*

4.12 Javanese Root-Knot Nematode (*Meloidogyne javanica*)

Next to *M. incognita,* it is one of the potential pests of several host plants, and this nematode is most prevalent in hot, dry regions causing significant damage and yield loss.

4.12.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Super family:	Tylenchoidea
Family:	Heteroderidae
Subfamily:	Meloidogyninae
Genus:	*Meloidogyne*
Species:	*M. javanica*

4.12.1.1 Female Nematode

- Body flask shaped, usually with a long cervix
- Stylet base 4–5 μ wide and 2 μ high
- Stylet knobs rounded
- Opening of the dorsal esophageal gland orifice located at a distance of 3–4 μ behind the base of the stylet
- In typical cases, the anal–vulval plate is round with simple circular lines interrupted in the region of the lateral fields.
- Lateral field in the form of a distinct band which is not intersected by the dorsal and ventral lines of the anal–vulval plate.
- Characteristically, the lateral field is very clearly visible beyond the anal–vulval plate and extends far forward along both sides of the body of the nematode.
- Anal arc low.

- Rudimentary tail clearly expressed.
- Phasmids distinct and arranged on both sides of the tail up to a distance of 19–26 µ from it.
- 0.54–0.85 mm × 0.30–0.55 mm; stylet 16–17 µ

4.12.1.2 Male Nematode

- The male head is diagnostic in its high, rounded headcap formed by the labial disc and medial lips with four cuticular annulations.
- Labial annulation wide and rather flat; three postlabial annulations equal in width.
- Lateral gene 4 µ high and 2 µ wide.
- Stylet base 5 µ wide and 3.0–3.5 µ high.
- Basal knobs rounded.
- Opening of the dorsal esophageal gland orifice located at a distance of 3 µ behind the base of the stylet.
- Phasmids asymmetric and anterior to but on the same level as the anus.
- Hermaphrodite specimens may be sometimes observed in the males along with the normal development of the male reproductive organs; a vulva or its rudiment is observed at some distance above the cloacal opening.
- 0.94–1.44 mm; *a* = 26–42; *b* = 7–13; stylet 20–21 µ; spicules 30–31 µ.

4.12.1.3 The Second-Stage Juveniles

- Length 340–400 µ; *a* = 24–26; *b* = 8; *c* = 5.8–6.6; stylet 10 µ
- Opening of the dorsal esophageal gland orifice located at a distance of 4 µ behind the stylet base

4.12.1.4 Eggs

Size variable: 76–100 µ × 31–40 µ; 125 µ × 45 µ; 84–101 µ × 32–45 µ

4.12.1.5 Perineal Pattern

The perineal pattern is often very diagnostic for the species because many specimens have distinct lateral lines that clearly delineate the dorsal and ventral regions of the pattern (Fig. 4.2). Patterns that resemble those of *M. arenaria* and *M. incognita* can be easily identified as *M. javanica* by the presence of distinct lateral lines. The overall shape of the perineal pattern is round or oval to slightly squarish.

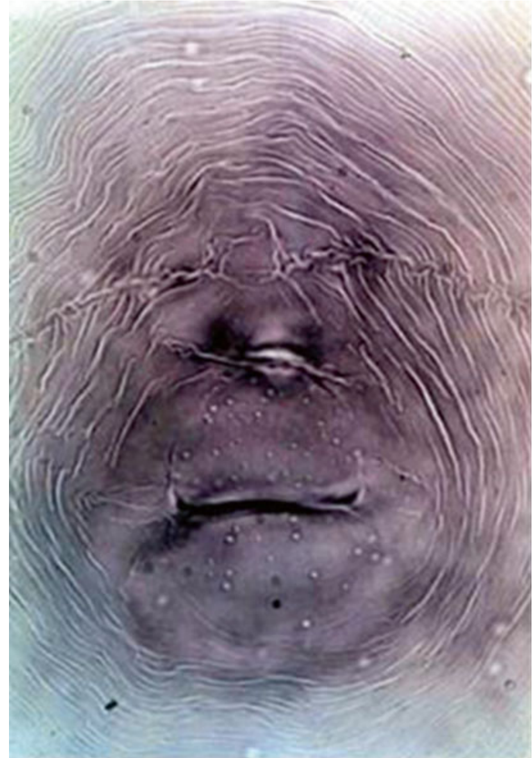

Fig. 4.2 Perineal pattern of *M. javanica*

4.13 Peanut Root-Knot Nematode (*Meloidogyne arenaria*)

The peanut root-knot nematode is the most serious nematode pest of the crop and questionably the most serious soilborne disease problem. *M. arenaria* is considered one of the “major” *Meloidogyne* species due to its worldwide economic importance. It also attacks crops like vegetables, fruits, and ornamentals.

4.13.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Super family:	Tylenchoidea
Family:	Heteroderidae

(continued)

Subfamily:	Meloidogyninae
Genus:	*Meloidogyne*
Species:	*M. arenaria*

4.13.1.1 Female Nematode

- Round body or oval body with a cervix of moderate length.
- Stylet hard with rounded basal knobs; base about 4–5 μ wide and 2 μ high.
- Opening of the dorsal esophageal gland orifice located at a distance of 4–6 μ from the base of the stylet.
- Anal–vulval plate more or less round.
- Anal arc low.
- Well-developed lateral fields; breaks and irregularities in the circular lines may be seen often.
- The dorsal and ventral lines in the region of the lateral fields may merge into an angle and sometimes form "wings" as in the northern root-knot nematode.
- Numerous additional lines are usually scattered near the lateral fields.
- Punctation is never found in the caudal region.
- 0.51–1.00 mm × 0.4–0.6 mm; stylet 14–16 μ.

4.13.1.2 Male Nematode

- Cephalic cap (labial ring) is very wide and when viewed laterally possesses a rectangular shape.
- Out of four rings, the first one is the widest.
- Pointed stylet; conical part notably shorter than the posterior cylindrical part.
- Basal knobs are round and fused with the stylet base about 4–5 μ and a height of 3 μ.
- Opening of the dorsal esophageal gland orifice located at a distance of 4–7 μ behind the stylet base.
- Phasmids preanal or lateroanal.
- Testes two and either straight or curved.
- 1.27–2.00 mm; $a = 44$–65; $b = 11$–16; stylet 20–24 μ; spicules 31–34 μ.

4.13.1.3 The Second-Stage Juveniles

- Length 450–490 μ; $a = 26$–32; $b = 7.2$–7.8; $c = 6.0$–7.5; stylet 10 μ; basal knobs 2 μ wide and 1 μ high; junctions of knobs with the stylet rod indistinct
- Opening of the dorsal esophageal gland orifice located at a distance of 3 μ from the stylet base

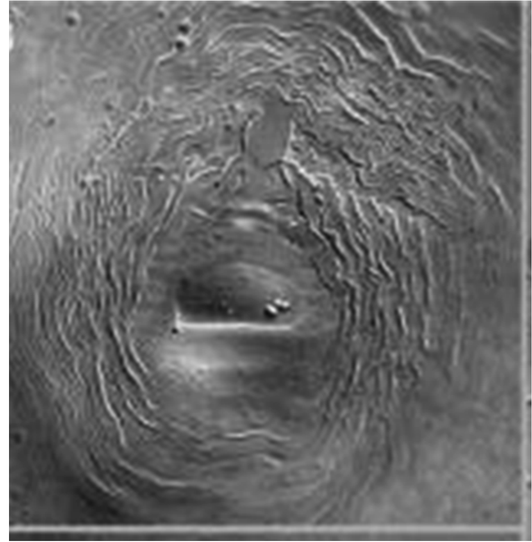

Fig. 4.3 Perineal pattern in *M. arenaria*

4.13.1.4 Eggs

Size 77–105 μ × 33–44 μ

4.13.1.5 Perineal Pattern

A highly variable perineal pattern often is characterized by a dorsal arch that is low and rounded, with striae that range from smooth to wavy; the female stylet (13–17 μm) is robust and characteristically wide near the knobs (Fig. 4.3).

4.14 Northern Root-Knot Nematode (*Meloidogyne hapla*)

Meloidogyne hapla has been referred to as the northern root-knot nematode because it commonly occurs in cooler environments. It is also found in the tropics and subtropics at higher elevations. It has a wide host range including vegetables, ornamentals, clover, and alfalfa.

4.14.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Super family:	Tylenchoidea
Family:	Heteroderidae
Subfamily:	Meloidogyninae
Genus:	*Meloidogyne*
Species:	*M. hapla*

4.14.1.1 Female Nematode

- At the body terminus just above the anus, distinct punctations are found.
- Lateral lines may be marked by only slight irregularities in the striae, or the striae of the dorsal and ventral sectors may meet at a slight angle along the lines.
- Low and rounded arch.
- Ventral striae frequently extended laterally to form wings on one or both sides; breaking of the symmetry of the perineal pattern.
- Striae of both sectors smooth or slightly wavy.

4.14.1.2 Male Nematode

One or two testes

4.14.1.3 Second-Stage Juveniles

The juvenile length ranges from 350 to 470 μm, and the stylet is fine with small, rounded knobs.

4.14.1.4 Eggs

Size 70–92 μ × 32–44 μ; length to width ratio is 2:1.

4.14.1.5 Perineal Pattern

Perineal patterns of females are often diagnostic of the species by the generally rounded pattern with distinct punctations near the tail (Fig. 4.4).

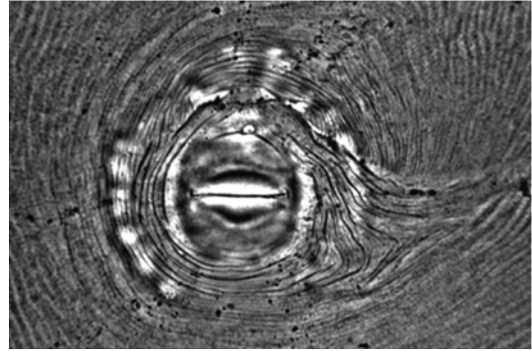

Fig. 4.4 Perineal pattern in *M. hapla*

4.15 False Root-Knot Nematode (*Nacobbus* spp.)

The false root-knot nematode has also been referred to as the Nebraska root-galling nematode and Cobb's root-galling nematode. Nematodes in this genus induce galls on roots of hosts, as do species of *Meloidogyne*; thus, they are familiarly known as false root-knot nematodes. Carrot, peas, lettuce, tomato, and members of Cruciferae and Cucurbitaceae are few major hosts. *Nacobbus aberrans* is one of the major species.

4.15.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Super family:	Tylenchoidea
Family:	Pratylenchidae
Subfamily:	Nacobbinae
Genus:	*Nacobbus*
Species:	*N. aberrans*

4.15.1.1 Young Female Nematode

- Vermiform, elongate–slender, about 1 mm long.
- Cuticle distinctly annulated.
- Lateral fields each with four incisures, irregularly areolated.
- Phasmid pore-like, anterior to middle of tail.
- Cephalic region broadly rounded, continuous, with three to four annules.
- Median bulb rounded, with large refractive thickenings.
- Stylet strong, about 21–25 μm long, knobs rounded.

- Esophageal glands elongate, extending for more than two body widths.
- Subventral glands asymmetrical, extending past the dorsal gland.
- Nuclei of three glands lie in tandem behind the esophago-intestinal junction.
- Vulva, a transverse slit, located within two anal body widths of the anus.
- Ovary immature.
- Tail tapering to a broadly rounded terminus, one to two anal body widths long.
- Migratory in soil and roots.

4.15.1.2 Mature Female Nematode

- Body saccate tapering anteriorly from the median bulb and posteriorly from the uterine region, often appearing spindle shaped.
- Early stage of mature female usually batatiform, with subterminal anus and vulva, very long tubular uterus, and a long, serpentine ovary reaching the esophagus.
- Swollen body may contain several dozen eggs.
- Sedentary endoparasites inciting root galls.
- 0.70–1.4 mm; stylet 15–18 μ (*N. batatiformis*).

4.15.1.3 Male Nematode

- Vermiform, with well-developed cephalic sclerotization
- Stylet about 23–27 μm long and esophagus structurally similar to that of immature female
- Esophageal glands elongated, mostly dorsal to intestine
- Testis single, outstretched
- Spicules cephalated, ventrally arcuate, 20–35 μm long
- Gubernaculum simple, linear to trough shaped, fixed
- Cloacal lips not modified
- Male tail completely enveloped by a bursa
- 0.8–1.2 mm; $a = 32$; $c = 35–45$ μ; stylet 20–25 μ (*N. batatiformis*)

4.15.1.4 Eggs

Length 49–83 μ; laid before cleavage begins (*N. batatiformis*)

4.16 Stubby-Root Nematode (*Trichodorus* spp.)

This phytonematode has a wide host range depending upon the species, viz., corn, clover, potato, other vegetables, etc. Host range of *T. obtusus* includes Bermuda grass, St. Augustine grass, tomato, big bluestem (*Andropogon gerardii*), side oats grama (*Bouteloua curtipendula*), Kentucky bluegrass (*Poa pratensis*), rhododendron (*Rhododendron* sp.), Sabal palm (*Sabal palmetto*), little-leaf linden (*Tilia cordata*), and sweetbay magnolia. This genus is involved in transmitting viral pathogens.

4.16.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Adenophorea
Order:	Triplonchida
Super family:	Diphtherophoidea
Family:	Trichodoridae
Subfamily:	Trichodorinae
Genus:	*Trichodorus*
Species:	*T. christiei*

4.16.1.1 Female Nematode

- Thick, almost cylindrical nematode whose body negligibly tapers toward both ends.
- Tail obtusely rounded.
- Cuticle smooth; frequently swells in fixed specimens.
- The excretory pore opens at the level of the base of the esophagus, which is a distinguishing character of *T. christiei*.
- The posterior part of the esophagus ventrally protrudes a little beyond the commencement of the mid-intestine.
- Lateral and caudal hypodermal pores absent.
- Posterior ventrosublateral esophageal glands overlap the intestine, or the intestine anteriorly partly overlaps the esophageal bulb, or both types of overlaps occur.

- Dorsal esophageal gland nucleus usually near the beginning of esophageal enlargement.
- Female reproductive system amphidelphic.
- Spermatheca present or absent.
- Vaginal sclerotization weakly developed.
- 0.46–0.71 mm; *a* = 15–20; *b* = 4.6–6.0; *c* = 110–140; *V* = 50–56 %; stylet 33–47 μ (*T. christiei*).

4.16.1.2 Male Nematode

- Possess a fully developed bursa which terminates slightly ahead of the tip of the tail.
- Lie straight with fixation.
- Caudal alae present.
- Copulatory muscles not extending anterior to the caudal alae.
- Spicules straight to arcuate, regularly tapering, transversely striated.
- Suspensor muscles not prominent.
- Two ventromedian supplements within caudal alae region, well separated from anterior third supplement, which is often reduced.
- As an exception, only one supplement may also be present.
- One or two pairs of large post-cloacal ventrosubmedian papillae present.
- Tail obtusely rounded.
- Lateral cervical pores, if present, at or close to level of excretory pore but not near base of onchiostyle.
- Ventromedian cervical pores rarely more than one, never located in the region of the onchiostyle.
- 0.60–0.67 mm; *a* = 16–20; *b* = 5.0–6.5; *c* = 30–37; spicules 60–65 (63) μ; gubernaculum 18–20 μ; stylet 35–39 μ (*T. christiei*).

4.17 Pin Nematode (*Paratylenchus* spp.)

These nematodes are among the smallest plant parasites, common in native and cultivated soils, especially around roots of woody plants and shrubs. Grape, pine, apricot, almond, bean, spinach, tomato, radish, okra, carnation, and coffee are some of major hosts.

4.17.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Super family:	Criconematoidea
Family:	Tylenchulidae
Subfamily:	Paratylenchinae
Genus:	*Paratylenchus*
Species:	*P. projectus*

4.17.1.1 Female Nematode

- Small nematodes, under 0.5 mm long.
- Female may swell slightly and become sessile parasite.
- Neck tapering rapidly to a pointed lip region which is marked by several fine annules.
- Spear strong, variable in length but may be relatively very long, fore part longer than the shaft; basal knobs rounded.
- Procorpus and median bulb amalgamated, containing large crescentic valve plates. Isthmus narrow but relatively long, leading to a rounded or spatulate terminal bulb.
- Body distinctly annulated; lateral field usually with four incisures.
- Vulva posterior, protected on either side by a membrane of cuticle leading into a vagina which stretches halfway or more into the body.
- Uterus composed of a number of large cells, sometimes with an open chamber adjoining the vagina.
- A spermatheca, packed with sperms, present apparently as a diverticulum of the upper end of the uterus.
- Ovary outstretched, prodelphic.
- No postvulval sac.
- Anus often obscure, tail conical to a variously shaped point.
- Body becomes C shaped when killed by heat.
- 0.26–0.5 mm; *a* = 11–46; *b* = 11–26; *c* = 11–21; *V* = 44–64 (*P. goodeyi*)

4.17.1.2 Male Nematode

- Male more slender and spear and esophagus usually faint and ill-defined or non-existent.

- Spicules long, slender, and pointed.
- Gubernaculum short.
- At the anus, a short sheath usually protrudes, which may have a process at its posterior edge.
- Tail with slight or without bursa.
- Male tail conoid to a variously shaped point.
- 0.36 mm; *a* = 33; *b* = 3.9; *c* = 10.5 (*P. goodeyi*).

4.18 Lesion Nematode (*Pratylenchus coffeae*)

It is the major, economically important pest on coffee worldwide. It has a wider host range. Apart from coffee, banana and citrus are the major hosts for this nematode.

4.18.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Adenophorea
Order:	Tylenchida
Super family:	Tylenchoidea
Family:	Pratylenchidae
Subfamily:	Pratylenchinae
Genus:	*Pratylenchus*
Species:	*P. coffeae*

4.18.1.1 Female Nematode

- Body rather slender in young females, fat in old ones.
- Cuticular annulation fairly conspicuous.
- Lateral field distinct, normally with 4–5, occasionally with 6 incisures.
- Lateral field marked by four incisures.
- Lip region slightly set off from the body, rounded, bears two annules.
- Outer margins of heavily sclerotized labial framework extend into the body about one body annule.
- The spear-guiding apparatus extends posteriorly from basal plate about three annules.
- Spear 18 μm long, with well-developed, broadly rounded basal knobs.
- Dorsal esophageal gland orifice about 2 μm behind spear base.
- In young specimens, the ovary extends over one-quarter of body length and in old ones over more than one-half.
- Spermatheca broadly oval to nearly round.
- Posterior uterine branch variable in length, sometimes reaching 50 μm.
- In about 20 % of the females examined (especially older ones), it carries a distinct rudimentary ovary.
- Tail tapering slightly, its length in young specimens 2–2.5×, in old ones 1.5–2× anal body diameter.
- Tip broadly rounded, truncate, or indented; in some specimens appearing weakly and irregularly annulated.
- Intrauterine eggs often contain embryos.
- Hemizonid just anterior to the excretory pore, about two body annules long.
- The ovary does not extend to the esophageal gland, consisting of a single row of oocytes, except for the double row near the anterior end.
- Spermatheca oval.
- Cellular oviduct long, uterus short.
- Posterior uterine branch about one and a half times the width of the body at the vulva.
- Phasmid slightly posterior to middle of tail.
- Four lateral lines extend past the phasmid.
- Tail tapering, terminus rounded.
- No striations around the terminus.
- *L* = 529 μm; *a* = 23.7; *b* = 6.8; *c* = 19.0; *V* = 80.1.
- *L* = 480 μm; *a* = 27.4; *b* = 6.5; *c* = 19.1; *T* = 48.

4.18.1.2 Male Nematode

- Similar to female.
- Phasmids slightly posterior to middle of the tail, extend slightly into the bursa.
- Spicula very slender, shaft ventrally concave.
- The gonad extends over about one-half the body length.
- Testis shorter than the vas deferens.
- Bursal edge faintly crenate.
- Single outstretched testis does not extend to esophageal glands, spermatocytes in single or multiple rows.
- *L* = 0.45–0.70 mm; *a* = 25–35; *b* = 5–7; *c* = 17–22; *V* = 76–83; spear = 15–18 μm.

4.19 Burrowing Nematode (*Radopholus similis*)

It is an economically important nematode pest on several horticultural crops causing significant damage and yield loss. Its host range consists of banana, citrus, coconut, areca nut, black pepper, sugarcane, ginger, tea, avocado, ornamentals, etc. It causes "toppling-over" disease of banana, "yellows" disease of pepper, and "spreading decline" of citrus.

4.19.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Family:	Pratylenchidae
Genus:	*Radopholus*
Species:	*R. similis*

4.19.1.1 Female Nematode

- Head composed of three to four annules.
- Lip region rounded, marked by three striae, set off by a slight narrowing of the head contour.
- Cuticle distinctly striated.
- Stylet strong, with well-developed knobs (=17–20 μm).
- Lateral field with four longitudinal lines, the lateral ones minutely crenate.
- Median bulb of esophagus subspherical.
- Isthmus about as long as the body width.
- Junction of esophageal lumen and intestine very obscure.
- Basal lobe of esophagus two or three times as long as the body width, extending back over the anterior end of the intestine.
- This lobe usually is in a dorsal position and contains the three gland nuclei.
- Anterior ovary frequently extending forward to median bulb of the esophagus.
- Posterior ovary sometimes reaching into the tail and occasionally reflexed forward one to three body widths.
- Oocytes in single file except for a short region of reproduction.
- Eggs about twice as long as body diameter.
- Deirids not observed.
- Spermatheca round, with small, rod-shaped sperm.
- Hyaline part of tail 9–17 μm long; terminus striated.
- Phasmids in anterior third of tail.
- Tail conoid to the blunt, rounded terminus.
- $L = 0.52–0.88$ mm; $a = 22–30$; $b = 4.7–7.4$; $b' = 3.5–5.2$; $c = 8–13$; $c' = 2.9–4.0$; $V = 55–61$.

4.19.1.2 Male Nematode

- Head four-lobed, lateral sectors strongly reduced.
- Lip region subspheroid, with or without fine striae, set off by constriction.
- Cuticle distinctly annulated.
- Lateral fields marked by four incisures, ending on the tail.
- Phasmids near the base of the bursa, about one body width posterior to the latitude of the anus.
- Spear slender with tiny basal knobs.
- Reduced esophagus, the median bulb valveless.
- Testes outstretched, one-fourth to one-third the body length.
- Gubernaculum with small titillae.
- Bursa extends over about two-thirds of the tail.
- Spicules slightly arcuate, cephalated (=18–22 μm).
- Gubernaculum thin, trough-like, slightly less half as long as spicula (=8–12 μm).
- $L = 0.54–0.67$ mm; $a = 31–44$; $b = 6.1–6.6$; $b' = 4.1–4.9$; $c = 8–10$; $c' = 5.1–6.7$; stylet = 12–17 μm.

4.20 Reniform Nematode (*Rotylenchulus reniformis*)

This nematode has a worldwide distribution on several crop plants and causes significant yield loss. Cotton, castor, cowpea, soybean, pineapple, tea, banana, crucifers, cucurbits, citrus, lettuce, guava, mango, okra, and various vegetables are also hosts.

4.20.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Super family:	Tylenchoidea
Family:	Hoplolaimidae
Genus:	*Rotylenchulus*
Species:	*R. reniformis*

4.20.1.1 Female Nematode

- Juveniles, males, and young females vermiform, arcuate to spiral upon relaxation.
- In the adult female, posterior part of the body becomes swollen and reniform and kidney shaped, with an irregular, less swollen neck, a postmedian vulva, and a short pointed tail.
- Tip of the body in the form of a tapered protrusion.
- Cuticle annulated.
- Lateral fields each with four incisures, non-areolate, obliterated in a mature female.
- Median bulb strongly developed; excretory pore directly posterior to it.
- Posterior lobe of esophageal glands extends far beyond the commencement of the middle intestine.
- Cephalic region high, continuous.
- Stylet in juveniles and female two to three times cephalic region width long.
- Orifice of the dorsal esophageal gland usually about one stylet length behind the stylet base.
- Subventral glands in the normal position, the dorsal gland shifted laterally to subventrally, former much longer than the latter.
- Immature female, vermiform, migratory.
- Ovaries paired, with double flexures; total length of the ovary exceeds the nematode's total body length.
- Tail elongate–conoid, with prominent hyaline terminal portion.
- *Young female*: 0.34–0.42 mm; $a = 22–27$; $b = 3.6–4.3$; $c = 14–17$; $V = 68–73$ %; stylet 16–18 μ
- *Adult, swollen female:* 0.38–0.52 mm (length of reniform part); 0.10–0.14 mm (width at vulva); $V = 68–73$ %

4.20.1.2 Male Nematode

- Stylet and esophagus regressed
- Vermiform with a narrow bursa that does not extend to the tip of the tail
- Tail similar to that of young female; bursa subterminal, low, not quite projecting beyond tail contour in lateral view
- Phasmids located on tail in its anterior region
- Spicules slender, lacking distal flanges
- Gubernaculum fixed, devoid of titillae and telamon
- Cloacal lips pointed, not forming a tube
- Hypoptygma absent
- Juvenile tail more rounded terminally and with shorter hyaline terminal portion than that of a female
- 0.38–0.43 mm; $a = 24–29$; $b = 2.8–4.8$; $c = 12–17$; stylet 12–16 μ

4.21 Yam Nematode (*Scutellonema* spp.)

It is an economically important nematode pest on several host crops. The major species is *Scutellonema brachyurum* (British spiral nematode/Carolina spiral nematode). Its major host is yam (*Dioscorea*) apart from banana, coconut, cotton, corn, etc.

4.21.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Super family:	Tylenchoidea
Family:	Hoplolaimidae
Subfamily:	Hoplolaiminae
Genus:	*Hoplolaimus*
Species:	*H. brachyurum*

4.21.1.1 Female Nematode

- Body spiral to C shaped or almost straight
- Presence of three to five cuticular annulations on the head, with six longitudinal bands on the lowest one

- Labial region narrow truncate to offset rounded, annulated, with or without longitudinal striae
- First labial annulus divided into six sectors, lateral sectors smaller than the others
- Amphid apertures oval between labial disc and lateral sectors
- Lateral field with four lines usually areolated near phasmids and anteriorly, sometimes transverse striae scattered over average sized for the family
- Knobs rounded to indented
- DGO 4–8 μm from stylet base
- Esophageal gland overlap dorsal and lateral
- Two genital branches outstretched, equally developed
- Epiptygma present
- Tail short and rounded
- Phasmids enlarged (scutella) situated opposite each other, near the anus level
- 0.65–0.84 mm; *a* = 24–32; *c* = 67–99; *V* = 57–61 %; stylet 26–29 μ (*S. brachyurum*)

4.21.1.2 Male Nematode

- Caudal alae enveloping tail tip, regular or rarely deeply lobed
- No secondary sexual dimorphism
- 0.63–0.85 mm; *a* = 25–33; *b* = 6.1–7.5; *c* = 45–85; stylet 24–27 μ (*S. brachyurum*)

4.22 Stunt Nematode (*Tylenchorhynchus* spp.)

This is a major nematode on a wide range of crop plants with worldwide distribution. Major hosts for several species of this genus include grape, elms, grapes, tea, sweet potato, rose, and lettuce.

4.22.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Super family;:	Tylenchoidea
Family:	Belonolaimidae
Subfamily:	Teotylenchinae
Genus:	*Tylenchorhynchus*
Species:	*T. claytoni*

4.22.1.1 Female Nematode

- The head has four cuticular annulations and a slight sclerotization and is well offset from the rest of the body.
- Lip region set off by constriction or continuous with contour.
- Lateral fields marked by 4, 5, or 6 incisures; 20 longitudinal lines occur additionally in the midbody region, which decreases in number toward the head and the tail.
- Tail tip has 8–16 cuticular annulations.
- Phasmids conspicuous, located well behind anal region.
- Spear usually strong with heavy basal knobs.
- Basal bulb of the esophagus connected to the intestine by the cardia.
- Vulva near the middle of body.
- Ovaries two, outstretched.
- Female tail conoid, blunt, usually two or more times anal body diameter.
- No sexual dimorphism.
- Esophageal glands contained tin basal bulb, sometimes a lobe of the basal bulb slightly overlapping the intestine.
- Lateral fields marked by four, five, or six incisures.
- Stylet well developed, with conspicuous basal knobs.
- Deirids usually inconspicuous.
- Phasmids conspicuous, located near the middle of the tail.
- Vulva near the middle of the body.
- Ovaries two, outstretched.
- Female tail cylindrical, conoid, with terminus usually bluntly rounded, not acute.
- 0.64–0.73 mm; *a* = 24; *b* = 5.6; *c* = 18–19; *V* = 55–57 %

4.22.1.2 Male Nematode

- Tail slightly arcuate, enveloped by the bursa.
- Phasmids about middle of the tail.
- Spicula and gubernaculum tylenchoid.
- The bursa commences anterior to the cloaca at a distance equal to twice the body diameter and envelopes the tail.
- Cephalic framework lightly to heavily sclerotized.
- 0.57 mm; *a* = 26; *b* = 5.8; *c* = 14.6.

4.23 Dagger Nematode (*Xiphinema americanum*)

This nematode is a major pest on several hosts, which is also involved in virus transmission. Economically important hosts include grapevine, perennial orchards, strawberry, forest tress, nectarine, oak, rose, grapevine, raspberry, carrot, cherry, peach, and soybean.

4.23.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Adenophorea
Order:	Dorylaimida
Super family:	Dorylaimoidea
Family:	Longidoridae
Subfamily:	Xiphineminae
Genus:	*Xiphinema*
Species:	*X. americanum*

4.23.1.1 Female Nematode

- Body spiral when relaxed, cylindroid except at extremities.
- Lateral field 1/4 to1/3 body width, composed of a series of chain-like cells, each with a distinct pore.
- Lip region set off by slight depression, the papillae slightly elevated.
- Stylet 80–90 μ long with 44–50 μ long extensions which are modified into elongate, flange-like expansions.
- Guiding ring fragile, double.
- Cuticle about the vestibule must be very strong for it functions as a spear guide without being sclerotized.
- Esophagus basal portion 2 1/2 to 3 times as long as the neck width.
- Cardia simple, conoid.
- Intestinal cells packed with coarse, hyaline granules.
- Prerectum length 4–6 times the body width.
- Rectum length near anal body diameter.
- The tail varying from bluntly conoid to dorsally convex–conoid, sometimes almost subacute.
- 1.4–1.9 (1.6) mm; $a = 33.6–44.6$ (42.3); $b = 4.7–7.2$ (6.3); $c = 36.5–52.8$ (44.7); $V = 46–54$ (51)%; stylet 117 μ (true stylet, 72 μ, and its elongation, 45–47 μ).

4.23.1.2 Male Nematode

- Many have normally developed testes producing spermatozoa, while in others the testes are rudimentary.
- Supplements 5–8, mammiform.
- Spicula slightly arcuate.
- Muscle bands 14–22.
- The tail slightly arcuate, usually somewhat shorter than that of the female.
- 1.5–1.7 (1.6) mm; $a = 39.7–51.6$ (47.2); $b = 6.1–6.3$ (6.2); $c = 37.8–50.1$ (43.6); stylet 111 μ (together with its elongation); spicules 27–33 μ.

4.24 Strawberry Foliar Nematode (*Aphelenchoides fragariae*)

Other common names of this nematode are begonia leaf nematode, fern nematode, spring crimp nematode, strawberry bud nematode, strawberry nematode, and summer dwarf nematode. It is an economically important pest on strawberry. It has a wide host range including begonia, ferns, lily, spearmint, ornamentals, narcissus, sage, and primrose.

4.24.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Super family:	Aphelenchoidea
Family:	Aphelenchoididae
Subfamily:	Aphelenchoidinae
Genus:	*Aphelenchoides*
Species:	*A. fragariae*

4.24.1.1 Female Nematode

- Body slender, cylindrical, tapering near extremities

- Lateral field, a narrow band occupying about one seventh of body width, marked by two lines
- Lip region almost continuous with head contour, not marked by striae
- Labial framework delicate, obscure, except for hexaradiate oral armature
- Stylet slender, 10 μ long with small but distinct knobs
- Nerve ring about one body width behind median bulb, encircling both intestine and esophageal gland ducts
- Excretory pore opposite the nerve ring
- Esophageal glands extending about five body widths posterior to median bulb
- Anterior portion of the intestine, a slender tube crowded ventrally by esophageal gland lobes
- Ovary outstretched, the oocytes arranged in single file
- Posterior uterine branch, an elongated pouch serving as a reservoir for spermatozoa
- Tail tapering uniformly to an abruptly conoid, acute terminus
- 0.45–0.8 mm; $a=45–60$; $b=9–11$; $c=12–22$; $V=64–71$ %

4.24.1.2 Male Nematode

- The tail slightly curved when relaxed, bearing three pairs of ventrosubmedian papillae
- The first pair slightly postanal, second pair near the middle of the tail, and third pair near the terminus
- 0.48–0.65 mm; $a=46–63$; $b=9–11$; $c=16–19$; $T=44–61$

4.25 Chrysanthemum Foliar Nematode (*Aphelenchoides ritzemabosi*)

This is a major pest on chrysanthemum. It is also known as black currant nematode, chrysanthemum leaf nematode, chrysanthemum nematode, and chrysanthemum foliar eelworm. It has a wide host range, among which important crops affected are chrysanthemums and strawberries. Its several species attack garlic, celery, coleus, ornamentals, bean, etc.

4.25.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Super family:	Aphelenchoidea
Family:	Aphelenchoididae
Subfamily:	Aphelenchoidinae
Genus:	*Aphelenchoides*
Species:	*A. ritzemabosi*

4.25.1.1 Female Nematode

- Lateral fields have four incisures and occupy one-fourth of the body of width.
- Lip region expanded.
- Spear 12 μ long with distinct knobs.
- The head offset by a constriction from the rest of the body.
- Nerve ring located beyond the median esophageal bulb at a distance equal to 1.5 times the body width.
- Hemizonid six or eight annules posterior to excretory pore.
- Esophageal glands four times as long as the body width.
- Excretory pore located at a distance of one body width beyond the nerve ring.
- Anterior end of the intestine, a slender tube joining the esophagus immediately behind bulb.
- Cells arranged in two or more rows in the ovary.
- Posterior uterus present; strongly elongated and usually contains spermatozoa.
- The tail tapers gradually; four mucrones occur at the tip.
- 0.77–1.2 mm; $a=40–50$; $b=10–13$; $c=18–24$; $V=66–75$ %; stylet 12 μ.

4.25.1.2 Male Nematode

- Tail curvature about 180° when relaxed by gentle heat
- Three pairs of ventrosubmedian papillae arranged in the usual manner

- Spicula simple, arcuate, the ventral shaft only about one-third as long as the dorsal
- 0.71–0.93 mm; *a* = 31–50; *b* = 10–14; *c* = 16–30

4.26 Mushroom Nematode (*Aphelenchoides composticola*)

This is a major pest on mushroom, which is also a fungal feeder. It induces considerable damage to mushrooms resulting in significant yield loss.

4.26.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Super family:	Aphelenchoidea
Family:	Aphelenchoididae
Subfamily:	Aphelenchoidinae
Genus:	*Aphelenchoides*
Species:	*A. composticola*

4.26.1.1 Female Nematode

- The head offset from the rest of the body; cuticular annulations (width 0.9–1.0 μ) absent on the head.
- Stylet has delicate basal knobs.
- Esophageal glands form a lobe which dorsally overlaps the commencement of the intestine for a distance equal to three times the body width.
- Lateral fields occupy 0.2 of the total body width and have three incisures.
- The number of incisures decreases to two and then one on the tail and anterior to the median esophageal bulb.
- The tail has a mucro which is usually located on the ventral side.
- Ovary straight; oocytes arranged in one row.
- Posterior uterus extends to one-half to two-thirds the distance between the vulva and the anus.
- 0.45–0.61 (0.52) mm; *a* = 30–42 (34); *b* = 8–10 (9); *c* = 11–17 (14); *V* = 67–72 (70) %; stylet 11 μ.

4.26.1.2 Male Nematode

- Presence of the typical aphelenchoidal spicules
- Gubernaculum absent
- Three pairs of caudal papillae usually present
- 0.41–0.58 (0.49) mm; *a* = 28–41 (34); *b* = 7–9 (8.5); *c* = 11–20 (15); spicules (dorsally) 21 μ; stylet 11 μ

4.27 Mushroom Spawn Nematode (*Ditylenchus myceliophagus*)

This is a major pest in commercial mushroom production. It feeds on a wide range of fungal hyphae, swarms, and aggregates on mushroom beds. It becomes cryptobiotic if drying is slow and remains viable for 3 1/2 years. It has a sticky surface that sticks nematode to insect or human vectors. It normally withdraws contents of fungal cells, and mycelium disappears. Mushroom beds become soggy and foul smelling.

4.27.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Super family;:	Tylenchoidea
Family:	Anguinidae
Subfamily:	Anguininae
Genus:	*Ditylenchus*
Species:	*D. myceliophagus*

4.27.1.1 Female Nematode

- Cuticular annulations fine and observable only under immersion magnification; width of individual annulations 1.4 μ.
- Lateral fields have six poorly defined incisures with, sometimes, additional lines (11 incisures).
- The head is flat and continuous with the body.
- Opening of the dorsal esophageal gland located very close to the base of the stylet.
- Posterior glandular bulb of the esophagus is wide and spade shaped; on one side, it overlaps the commencement of the intestine.

- Ovary consists of a single row of cells except for the extreme anterior part (two rows).
- Spermatheca is oval.
- Posterior uterus extends to midway between the vulva and the anus.
- Tail tapers gradually; tip of the tail is digitiform and rounded.
- 0.60–1.38 (0.89) mm; a=22.6–44.4 (30.1); b=5.4–11.7 (7.3); c=10.5–20.5 (14.4); V=78–90 (82.5) %; stylet 7–9 μ.

4.27.1.2 Male Nematode

- General body structure similar to that of the female
- Proximal part of the spicules Notable
- 0.58–0.88 (0.71) mm; a=21.4–44.4 (33.3); b=4.5–8.7 (5.9); c=9.5–15.3 (12.3); stylet 7–9 μ

4.28 Bent Grass Nematode/Purple Nematode (*Anguina agrostis*)

Anguina agrostis was one of the first phytonematodes to be taxonomically described by J. G. Steinbuch in 1979. Galls caused by *A. agrostis* have glumes that are 4–5 times longer than normal and can cause yield losses of up to 40–70 %. In addition to crop loss, *A. agrostis* associates with pathogenic bacteria *Rathayibacter rathayi* (*Corynebacterium rathayi*) to cause annual ryegrass toxicity. *A. agrostis* infects bent grasses within the genus *Agrostis* as well as annual and perennial ryegrass (*Lolium* spp.). The nematode can also infect 14 other genera of grasses.

4.28.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Superfamily:	Tylenchoidea
Family:	Anguinidae
Subfamily:	Anguininae
Genus:	*Anguina*
Species:	*A. agrostis*

4.28.1.1 Female Nematode

- Body spirally coiled or crescentic (C shaped).
- Cuticle with fine annulation.
- Lip region set off, 3–4 μm high.
- Procorpus cylindrical, somewhat enlarged in the middle part with a constriction before joining the median bulb; the latter ovoid.
- Isthmus short and narrow.
- Basal bulb well developed, trapezoid, although not overlapping the intestine.
- In more slender specimens from *Agrostis* (bent grass), the maximum width of the body is reached only at the level of the spermatheca.
- Ovary reflexed two or three times.
- Oocytes in the zone of multiplication in two or three rows.
- Spermatheca elongate, sometimes with up to 10–12 synchronous oocytes, separated from the preuteral gland by a short constriction.
- Preuteral gland long, with up to 20 synchronous eggs, separated from the uterus by a constriction (oviduct).
- Postvulval uterine branch reaching half the distance from the vulva to the anus, filled with sperms.
- The tail short, conoid, with acute terminus.
- L=1.39–2.60 mm; a=13.8–25.4; b=12.6–28.7; c=25.2–43.0; V=87–92; stylet=10–12 μm.

4.28.1.2 Male Nematode

- The body after heat relaxation is almost outstretched.
- Testis is usually with one or two flexures.
- Bursa is subterminal and does not reach the tail tip.
- L=1.05–1.45 mm; a=23.8–30.0; b=6.5–8.9; c=21.5–28.4; spicules=25–32 μm.
- Gubernaculum=10–13 μm; stylet=10–12 μm.

(a) *Eggs*
67–92 (79) x 33–38 (35) um

(b) *Second-Stage Infective Juveniles*
L=0.55–0.82 mm; a=47.2–65.0; b=3.2–4.5; c=11.7–20.0; stylet=10 μm

(c) *Third-Stage Juveniles*
L=0.80–1.05 mm; a=27.1–30.4; b=4.7–6.5; c=14.6–16.7; stylet=10–12 μm

(d) *Fourth-Stage Juveniles*
L=0.97–1.70 mm; a=22.4–26.6; b=6.2–11.5; c=16.4–31.0; stylet=10–12 μm

4.29 Sting Nematode (*Belonolaimus longicaudatus*)

This is a common parasite of several crop plants and grasses. It is the most destructive nematode pest of turfgrass, and it also attacks a wide range of fruits, vegetables, and fiber crops including citrus, ornamentals, cotton, and forage. This is a migratory ectoparasite of roots. The sting nematode is mostly present in very sandy soils.

The first sting nematode described was *Belonolaimus gracilis*, which was collected from the rhizosphere of a pine tree (*Pinus* sp.) in Marion County, FL. Later this nematode was reported to damage various horticultural crops in the Atlantic coast states as far north as Virginia. Most of the crop damage that was originally attributed to *B. gracilis* is now recognized as having been caused by *B. longicaudatus*. Currently there are nine described species of *Belonolaimus. Belonolaimus longicaudatus, B. gracilis, B. euthychilus, B. maritimus,* and *B. nortoni* are found in the USA, whereas *B. anama, B. jara*, *B. lineatus*, and *B. lolii* are found elsewhere. Other populations of *Belonolaimus* that differ from described species by host range, morphology, and molecular characterization are reported and may represent new species.

4.29.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Chromadoria
Order:	Rhabditida
Superfamily:	Tylenchoidea
Family:	Dolichodoridae
Subfamily:	Belonolaiminae
Genus:	*Belonolaimus*
Species:	*B. longicaudatus*

4.29.1.1 Female Nematode

- Lateral field marked by a single deep incisure extending from the base of lip region to near the terminus.
- Lip region set off by constriction, with deep lateral grooves aligned with those in the lateral field.
- In these grooves, near the margin of the head, the amphid apertures are located.
- Dorsal and ventral indentations less conspicuous than lateral.
- From a face view, the lip region is of the typical quadrangular form, with four rounded sublateral lips, on each of which is a single conspicuous papilla.
- Labial disc low, rounded. Spear 107–115 μ long, with well-developed, rounded knobs. Median esophageal bulb more than half as wide as the neck.
- Isthmus shorter than neck width, with nerve ring adjacent to bulb.
- Esophageal glands lobe-like, extending over anterior end of the intestine.
- Excretory pore about one neck width behind median bulb.
- Hemizonid slightly anterior to excretory pore.
- Intestine extending into the tail, with the rectum attached to ventral side.
- The tail four to five times as long as the anal body diameter.
- Phasmids about one-third distance from cloaca to terminus, located at base of the bursa
- *Belonolaimus longicaudatus* is distinguished from *B. gracilis* by the slenderer body, shorter spear, and longer tail.
- 2.0–2.6 mm; $a = 56–75$; $b = 7.3–9.9$; $c = 14.5–19$; $V = 46–54$.
- Vulva transverse slit.
- Ovaries outstretched, each uterus containing a spermatheca.

4.29.1.2 Male Nematode

- 1.6–2.1 mm; $a = 54–76$; $b = 6.3–8.1$; $c = 12.9–16.9$
- Male with caudal bursa extending from slightly anterior to the spicula to the terminus

4.30 Coconut Red Ring Nematode or Palm Nematode (*Rhadinaphelenchus/ Bursaphelenchus cocophilus*)

This nematode causes serious damage to coconut and oil palms, which become stunted and eventually killed by the nematode infection. Red ring disease is one of the most important

wilt diseases of coconut palm and African oil palms in the neotropics causing up to 10–15 % annual losses (Baujard 1989). Hosts of *B. cocophilus* are confined to the family Palmae where the nematode is known to infect over 17 species. Most palm species appear to be susceptible to inoculation by red ring nematode, but disease severity and symptoms are variable. The most economically important species with red ring disease susceptibility are coconut palm (*Cocos nucifera* L.), the African oil palm (*Elaeis guineensis* Jacquin), and the date palm (*Phoenix dactylifera* L.). Under greenhouse conditions, West Indian royal palm (*Roystonea oleracea*), grugru palm (*Acrocomia aculeata*), moriche palms (*Mauritia flexuosa*), and cocorite palm (*Maximiliana maripa*) are also infected artificially.

Highest incidence of red ring disease caused by *B. cocophilus* occurs in low, poorly drained areas. *B. cocophilus* are susceptible to desiccation, and drought conditions keep the disease in check. *B. cocophilus* survive best in wet, swampy areas and in clay rather than sandy soil. *Bursaphelenchus cocophilus* is associated with the palm weevil (*Rhynchophorus palmarum*) which transmits it to the coconut palm (*Cocos nucifera*) and the African oil palm (*Elaeis guineensis*). The red ring nematode is co-distributed with the palm weevil in the lower Antilles and Mexico southward into South America. Other beetles, *Dynamis borassi* and *Metamasius hemipterus* [L.], are also reported to vector the red ring nematode.

Adult female weevils which are internally infested with *B. cocophilus* disperse to a healthy coconut palm and deposit the juvenile stage of the nematode during oviposition. Nematodes enter the wounds, feed, and reproduce in the palm tissues, causing the death of the infected trees. The weevil larvae are parasitized by juveniles of *B. cocophilus* which persist in the insect through metamorphosis and appear to aggregate around the genital capsule of the adult weevil. The adult weevils emerge from their cocoons in the rotted palm and disperse to apparently healthy or stressed and dying palms, completing the life cycle.

4.30.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Aphelenchida
Superfamily:	Aphelenchoidoidea
Family:	Parasitaphelenchidae
Subfamily:	Bursaphelenchinae
Genus:	*Bursaphelenchus/Rhadinaphelenchus*
Species:	*B. cocophilus*

4.30.1.1 Female Nematode

- Exceptionally well-formed worm, smooth head and a little narrower than the rest of the body.
- Cuticle finely annulate; width of annulations 0.5 μ.
- Lateral fields have four incisures.
- Lip region high, offset with six lips.
- Stylet with small basal swellings; very slender but posteriorly dilated.
- Esophageal glands slender, 3–4 body widths long, overlapping dorsally.
- Excretory pore opposite junction of the esophagus and intestine, sometimes at the level of the nerve ring.
- Hemizonid conspicuous, about two-thirds the body width behind the median bulb.
- Ovary unpaired and anterior, outstretched, oocytes usually in single file.
- Post-uterine sac long, extending three-fourths distance to the anus.
- Posterior rudimentary uterus very long.
- Vulva anteriorly covered with a cuticular fold.
- The tail subcylindrical, usually with broadly rounded terminus.
- 0.97–1.18 mm, $a = 78–96$; $b = 8.4–8.7$; $c = 9.5–13.2$; $V = 64–68$ %; stylet 12–13 μ.

4.30.1.2 Male Nematode

- Spicules large, uniquely arcuate, paired, with sharply pointed prominent rostrum; distal ends of spicules with typical disclike expansions
- Shape of the spicules: the distinct vulval flap and the lack of a digitate tail tip which is present in *B. mucronatus*
- Tail arcuate, terminus pointed, appearing talon-like on lateral view, surrounded by short, oval caudal alae

- Seven caudal papillae, one adanal pair just preanal, single papilla just preanal centered; two postanal pairs just before caudal alae origin
- 0.84–1.16 mm; $a = 100–179$; $b = 6.5–7.8$; $c = 24–35$; spicules 12 μ (dorsal side) and 8 μ (ventral side); stylet 12–13 μ

4.30.1.3 Juveniles

- Third-stage dauer juveniles from coconut palm usually range from 700 to 920 μm.
- Possess a pointed tail with or without a mucro.
- The metacorpus is usually not well developed in juveniles from the palm or the weevil vector
- Have high, dome-shaped heads that are not offset from the body.
- The tails of the second- and third-stage juveniles are conoid with or without sharply mucronate tips.
- Those of fourth-stage juveniles have dimorphic tips: in female juveniles, they are rounded as in the adult female, and in male juveniles, they are "sharply drawn out."
- The third larval stage is 0.84 mm (0.03 in.) and characterized by a tapered terminal end of the body.

4.31 Ring Nematode (*Criconema* spp.)

Criconemella spp. are widespread and often occur at high population densities. Host plants include grapevine, peaches, apple, walnut, peanuts, ornamentals, soybeans, corn, and other turfgrasses. One species of ring nematode, *C. xenoplax*, has been associated with peach tree.

4.31.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Suborder:	Tylenchina
Superfamily:	Criconematoidea
Family:	Criconematidae
Genus:	*Criconemoides*
Species:	*C. xenoplax*

4.31.1.1 Female Nematode

- Body small to rather large (0.24–0.74 mm).
- Annuli 24–134; smooth or variously ornamented:
  1. Finely crenate
  2. Scalelike projections, if present, only on the posterior part of body
  3. Irregular platelike coverings on cuticle over entire body (paradoxiger, shepherdae) or on part of annuli (lamellatum)
  4. Ruffled, ribbonlike ornamentation encircling annulus on the anterior surface (giardi) or both anterior/posterior surfaces (psephinum)
  5. Cuticular fringe extending from the posterior margin of annuli (brevicaudatum, giardi)Annuli of labial region smooth; usually with one annulus wider and clearly set off from the next succeeding body annulus; occasionally separation is not distinct and labial region appears to bear two annuli.
- Labial region usually with six pseudolips rounded and projecting forward from first annulus.
- Stylet 40–132 μm.
- Vulva on 4th–21st annulus from the terminus, slit-like or completely closed by overhanging anterior lip.
- Tail conoid pointed to bluntly rounded.
- 0.2–0.76 mm long.
- Annules 37–134, round or retrorse, with smooth or crenate margins.
- Cephalic region with two annules; lip region elevated, with six pseudolips; submedian lobes poorly developed or usually absent.
- Stylet 45–132 μm long.
- Vulva closed 3–21 annules from terminus. VL/VB 0.5–3.
- Tail conically pointed or rounded.

4.31.1.2 Male Nematode

- Three or four incisures in lateral fields and a low bursa, which may be absent
- Fourth-stage juveniles with 8–18 rows of scales each tipped with a pointed spine
- Two to four lateral lines; bursa small, strongly reduced or lacking

4.31.1.3 Juveniles

Cuticle with scalelike cuticular appendages over the entire body, usually with refractive elements or spinelike extensions at distal ends, arranged in eight to twenty-four longitudinal rows

4.32 Ring Nematode (*Criconemoides* spp.)

This is a major pest on several crops. Host range includes all *Prunus* species, viz., peach, almond, apricot, plum, and cherry apart from carnation, pine, grapevine, and lettuce. Landscape and ornamental plantings are also infested by this nematode. This slow-moving nematode can build to its highest population levels in highly porous soils, including coarse sands and well-aggregated silt or clay soils.

4.32.1 Scientific Classification

Kingdom:	Animalia
Phylum:	Nematoda
Class:	Secernentea
Order:	Tylenchida
Superfamily:	Criconematoidea
Family:	Criconematidae
Subfamily:	Criconematinae
Genus:	*Criconemoides*
Species:	*C. xenoplax*

4.32.1.1 Female Nematode

- Small to moderately large (about 0.3–1 mm), sausage- or ringlike when relaxed.
- Annules crenate, rough or smooth, with round to pointed edges, 48–200 in number. Cephalic annules two to three, smaller than and not differentiated or separated by a collar from body annules.
- Submedian pseudolips may be modified to appear as lobes, connected laterally (dorsal and ventral pseudolip lobes partially fused); lateral pseudolips present but reduced.
- True submedian lobes as outgrowths on submedian lobes absent.
- Stylet moderately long (about 38–80 μm), usually rigid.
- Ovary outstretched, the oocytes arranged in tandem.
- Vulva lips closed, conical (anterior lip not ornamented, not overhanging), protruding to or slightly beyond body contour; 5–10 annules anterior to the terminus.
- Vagina straight.
- Tail short, conoid, convex–conoid, or hemispheroidal.
- 0.4–0.6 mm; $a = 8.1–13.6$; $b = 3.1–4.8$; $c = 23–56$; $V = 90–95$ (*C. xenoplax*).

4.32.1.2 Male Nematode

- Cephalic region rounded or conoid, lateral field with three to four incisures
- Spicula straight to slightly arcuate
- Bursa distinct, subterminal, narrow, enveloping most of tail
- 0.5–0.6 mm; $a = 23–28$; $c = 12–15$; $T = 28–35$ (*C. xenoplax*)

4.32.1.3 Juveniles

- Annules crenate, sometime smooth, never with scales or spines.
- Male fourth-stage juveniles usually lacking stylet.
- Members of Criconematidae possess vermiform stages.
- Small to large animals (up to 1.9 mm).
- Most closely related to the Tylenchulidae by the esophagus with well-developed median bulbar area with massive valvular apparatus, short isthmus, and small posterior glandular region symmetrically arranged, not overlapping the intestine.
- Strong sexual dimorphism, males degenerate mostly without stylet.
- The families are distinguished by strong annulation and thick cuticle Criconematidae (fine annulations, mostly thin cuticle in Tylenchulidae); swollen females in many Tylenchulidae not found in Criconematidae.

4.33 A Summarized Diagnostic Key to Major Genera of Phytonematodes

1. Female spherical, pyriform, reniform, saccate, or lemon shaped	28
1. Female vermiform	2
2. Esophagus without middle bulbus but with basal swelling	24
2. Esophagus with distinct middle bulbus	3
3. Middle bulbus somewhat angular and large, occupies almost entire body width; duct of dorsal esophageal gland discharges into middle bulbus	22
3. Middle bulbus round or oval; duct of dorsal esophageal gland discharges into esophageal lumen immediately posterior to stylet	4
4. Procorpus and metacarpus swollen and fuse into single middle bulbus, which is anteriorly extended. Stylet long	18
4. Middle bulbus round or slightly oval and not anteriorly extended. Stylet either highly or poorly developed but not very long	5
5. Basal part of esophagus forms the bulbus, which is distinctly differentiated from the intestine	13
5. Basal part of esophagus is lobular and covers the initial portion of intestine	6
6. Stylet longer than twice the width of the head in the labial region. Ovaries two	10
6. Stylet considerably exceeds twice the width of the head in the labial region. Ovaries one or two	7
7. Ovary one; vulva situated in posterior third of the body	*Pratylenchus*
7. Ovaries two; vulva situated somewhat below the middle of the body	8
8. Site of discharge of esophageal glands not notably posterior	*Pratylenchoides*
8. Esophageal glands cover the intestine and site of discharge notably posterior	9
9. Esophageal glands cover the intestine dorsally. Head separate. Tip of the tail rounded. Length of the body less than 0.8 mm	*Radopholus*
9. Esophageal glands cover the intestine ventrally. Head not separate. The tail has a terminal spine (mucro). Length of body more than 1.0 mm	*Hirschmanniella*
10. Body of dead/fixed nematodes spiral. Phasmids small and pore-like	12
10. Body of dead/fixed nematodes straight. Phasmids large and scalelike (scutella)	11
11. Both phasmids located posterior to the vulva. Stylet knobs rounded	*Scutellonema*
11. One phasmid situated anterior to the vulva and the other posterior to it. Stylet knobs terminate in anteriorly directed tips	*Hoplolaimus*
12. Esophageal glands extend far backward; primarily on ventral side; duct of dorsal esophageal gland discharges into the esophageal lumen at a distance of one-third or more of the length of stylet from its base	*Helicotylenchus*
12. Esophageal glands dorsally extend far backward; duct of dorsal esophageal gland discharges into the esophageal lumen at a distance of one-fourth of the length of stylet from its base or somewhat below its base	*Rotylenchus*
13. Ovary one; vulva located at posterior half of the body	16
13. Ovaries two; vulva located approximately in the middle of the body	14
14. Distance from the cephalic end to the middle bulbus greater than the distance from the middle bulbus to the intestine. Tip of tail frequently clavate	*Psilenchus*
14. Distance from the cephalic end to the middle bulbus less than the distance from the middle bulbus to the intestine	15

(continued)

15. Tail of female obliquely rounded	*Tylenchorhynchus*
15. Tail of female tapers	*Tetylenchus*
16. Female well formed and mobile. Oocytes arranged in one or two rows. Bursa of male leptodermal	17
16. Female vermiform but highly swollen; mobile or sedentary. Multiple arrangement of oocytes (more than two rows). Bursa of male pelodernal. Parasites dwell in stem galls, leaf galls, or galls formed on inflorescences	*Anguina* (*Paranguina*)
17. Tail very long and thin with terminal point; length six or more than six times the diameter of the body at the level of the anus; lips annulate	18
17. Tail less than six times the diameter of the body at the level of the anus; elongated and conical. Lips not annulate or indistinctly so. Bursa longer, one-third to three-fourths of the tail	*Ditylenchus*
18. Cuticle finely annulate, isthmus narrow	*Paratylenchus*
18. Cuticle coarsely annulate, isthmus wide	19
19. Mature female with simple cuticle	21
19. Mature female with double cuticle (cuticle of last molt remains)	20
20. More than 200 annulations present on cuticle. Basal knobs of stylet directed backward	*Hemicycliophora*
20. Less than 200 annulations present on cuticle. Basal knobs of stylet directed forward	*Hemicriconemoides*
21. Cuticular annulations armed with backward directed spinescent processes	*Criconema*
21. Cuticular annulations smooth, unarmed	*Criconemoides*
22. Esophageal glands situated in esophageal tissue and distinctly separate from the intestine	*Paraphelenchus*
22. Esophageal glands cover the initial portion of the intestine	23
23. Stylet without basal knobs. Tail of female obliquely rounded. Male bursa supported on rays	*Aphelenchus*
23. Stylet has small basal knobs. Tail of female tapers. Males without bursa and tail more or less ventrally curved	*Aphelenchoides*
24. Spear short, anteriorly obliquely cut in the form of goose quill, devoid of basal knobs	*Dorylaimus*
24. Spear very long, thin, and pointed	25
25. Spear curved; posterior half split and basal part thickened	*Trichodorus*
25. Spear straight and highly elongated	26
26. Basal part of spear elongation broadens in the form of wing; guide ring of spear short and located in front of elongation	*Xiphinema*
26. Elongated section of spear without basal widening; guide ring of spear located at a distance of twice body width in labial region from cephalic end	27
27. Amphid openings very small, slit-like, and indistinct. Amphids large and pocket shaped	*Longidorus*
27. Amphid openings large and distinct. Amphids funnel or stirrup shaped	*Paralongidorus*
28. Female spherical, pyriform, or lemon shaped; transforms into brownish-colored cyst which lodges on roots	*Heterodera*
29. Female predominantly endoparasitic causing formation of root galls	31
29. Only the anterior part of the body of female buried in the cortex of root. Sedentary ectoparasites which do not induce gall formation	30
30. Sexually mature female reniform, ovaries two. Excretory pore located posterior to the middle bulbus at a distance of 1.5 length of the bulbus	*Rotylenchulus*

(continued)

30. Posterior half of sexually mature female swollen and saccate. Ovary one. Excretory pore located at the middle of the body or posterior to the middle of the body	*Tylenchulus*
31. Female highly swollen except for head. Ovaries two	*Meloidogyne*
31. Only the anterior part of female swollen and saccate. Ovary one	*Nacobbus*

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5 Symptoms Caused by Phytonematodes and Disease Diagnosis

Phytonematodes are nearly microscopic, worm-shaped animals virtually invisible to the naked eye when in the soil. They are rightly called as "hidden enemies" of crops. They can cause significant plant damage ranging from negligible injury to the total destruction of plant material. The severity of plant injury resulting from nematode activity depends on several factors such as the combination of plant and nematode species and prevailing environmental factors including rainfall, soil types, land contour, and cultural practices. Although a few nematode species feed on aboveground plant parts, such as leaves, stems, flowers, and seeds, the majority of these parasites feed on underground parts of plants, including roots, bulbs, and tubers. Because of this belowground, "hidden" feeding activity, nematode damage to plants cannot always be diagnosed readily.

5.1 Damage

Many plant-parasitic nematodes have a wide host range, while a few are host specific. Nematodes also feed and reproduce on many different weeds. The survival of some nematodes for at least 1 year in moist soil without a host plant is common (Ravichandra 2008). Many kinds of plant-feeding nematodes can survive freely in the soil through winter – even if the soil is frozen. A few plant-parasitic nematode species can survive in the soil in a dormant state within cysts for many years. Damage to plants from these root-feeding nematodes is progressive and often results in poor growth, low vigor, yellowing or bronzing of the foliage, loss of leaves, stem dieback, failure to respond to fertilizer because of root damage, and, eventually, death.

Extensive root damage has often occurred before aboveground symptoms become obvious on established woody plants. Symptoms include root galls, stunting, root decay, and darkened roots. Aboveground symptoms are similar to those resulting from many kinds of root injury. Foliage loses its luster and wilts more readily than it should. Prolonged root stressed caused by nematodes may result in yellowing and eventual loss of foliage. New flushes of growth are weak, with fewer and smaller leaves than healthy plants. The damage is usually distributed irregularly, since nematodes are rarely distributed evenly in the soil. Root symptoms vary widely. Root-knot and some foliar nematodes cause tissues on which they feed to grow strangely. Some nematodes stop the growth of the roots, while others kill the cells on which they feed, resulting in sporadic dead tissue. Fungi and bacteria often infect nematode-damaged roots more readily and more severely than roots that have not been injured. The death of nematode-damaged plants often occurs during or following drought or cold injury.

Phytonematodes, when feeding or moving through the tissues of the host, cause a certain amount of mechanical injury. For some of the internal parasites, like the lesion nematodes and the lance nematodes, this mechanical injury appears to be quite substantial, while for some of

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the external feeders, like stubby-root nematode, it appears very slight. A considerable part of the injury caused by nematode is due to the plant tissue reaction to the secretion injected into them while the parasites are feeding. This secretion affects plant tissues in various ways. When a stubby-root nematode feeds at a root tip, the only obvious effect in many cases is the suppression of cell division in the apical meristem – the root stops growing. The feeding of some nematodes kills the surrounding tissues and causes a necrotic lesion, usually quite small but sometimes of appreciable size. The reaction of the surrounding tissues to the secretions ejected by such sedentary parasites as the root-knot nematodes is varied and complicated. Cell walls near the head of the parasite may be destroyed, and several adjacent cells may coalesce to form a so-called giant cell. Farther away from the parasite, the cells may undergo hypertrophy and proliferation that results in a swelling or gall.

The symptoms of nematode injury vary with the kind of nematode, the kind and age of the plant, and the plant part where the injury occurs. Typical symptoms of nematode injury can involve both aboveground and belowground plant parts. Foliar symptoms of nematode infestation of roots generally involve stunting and general unthriftiness, premature wilting, and slow recovery to improved soil moisture conditions, leaf chlorosis (yellowing), and other symptoms characteristic of nutrient deficiency. An increased rate of ethylene production, thought to be largely responsible for symptom expression in tomato, has been shown to be closely associated with root-knot nematode infection and gall formation. Plants exhibiting stunted or decline symptoms usually occur in patches of nonuniform growth rather than as an overall decline of plants within an entire field.

The time in which symptoms of plant injury occur is related to nematode population density, crop susceptibility, and prevailing environmental conditions. For example, under heavy nematode infestation, crop seedlings or transplants may fail to develop, maintaining a stunted condition, or die, causing poor or patchy stand development. Under less severe infestation levels, symptom expression may be delayed until later in the crop season after a number of nematode reproductive cycles have been completed on the crop. In this case aboveground symptoms will not always be readily apparent early within crop development, but with time and reduction in root system size and function, symptoms become more pronounced and diagnostic.

Root symptoms induced by sting or root-knot nematodes can oftentimes be as specific as aboveground symptoms. Sting nematode can be very injurious, causing infected plants to form a tight mat of short roots, oftentimes assuming a swollen appearance. New root initials generally are killed by heavy infestations of the sting nematode, a symptom reminiscent of fertilizer salt burn. Root symptoms induced by root-knot cause swollen areas (galls) on the roots of infected plants. Gall size may range from a few spherical swellings to extensive areas of elongated, convoluted, tumorous swellings which result from exposure to multiple and repeated infections. Symptoms of root galling can in most cases provide positive diagnostic confirmation of nematode presence, infection severity, and potential for crop damage.

For most crop and nematode combinations, the damage caused by nematodes has not been accurately determined. Most vegetable crops produced are susceptible to nematode injury, particularly by root and sting nematodes. Plant symptoms and yield reductions are often directly related to preplant infestation levels in soil and to other environmental stresses imposed upon the plant during crop growth. As infestation levels increase, so does the amount of damage and yield loss. In general, the mere presence of root-knot or sting nematodes suggests a potentially serious problem, particularly on sandy ground during the fall when soil temperatures favor high levels of nematode activity. At very high levels, typical of those which might occur under doubling cropping, plants may be killed. Older transplants, unlike direct seed, may tolerate higher initial population levels without incurring as significant a yield loss.

It can be very difficult to decide if nematodes are causing or are likely to cause significant crop injury. If a particular nematode pest was previously found in a site, it probably is still present. Plan to continue to take steps to manage it. In a location for which a complete history is lacking, the identities and population densities of nematode pests, hence the severity of damage which might be expected, can usually be determined by laboratory assay of soil and/or plant samples.

Another common diagnostic problem is determining the role of nematodes when established plants are making unsatisfactory growth. This task is often difficult because few nematodes cause distinctive diagnostic symptoms. A sound diagnosis should be based on as many as possible of symptoms above- and belowground, field history, diagnostic nematicide tests, and laboratory assay of soil and/or plant samples. Plant nematodes, when feeding or moving through the tissues of the host, cause a certain amount of mechanical injury. For some of the internal parasites, like the lesion nematodes and the lance nematodes, this mechanical injury appears to be quite substantial, while for some of the external feeders, like the stubby-root nematode, it appears very slight. A considerable part of the injury caused by nematodes is due to the plant tissue reaction to the secretion injected into them while the parasites are feeding. This secretion affects plant tissues in various ways. When a stubby-root nematode feeds at a root tip, the only obvious effect in many cases is the suppression of cell division in the apical meristem – the root stops growing. The feeding of some nematodes kills the surrounding tissues and causes a necrotic lesion, usually quite small but sometimes of appreciable size. The reaction of the surrounding tissues to the secretions ejected by such sedentary parasites as the root-knot nematodes is varied and complicated. Cell walls near the head of the parasite may be destroyed and several adjacent cells may coalesce to form a so-called giant cell. Farther away from the parasite, the cells may undergo hypertrophy and proliferation that results in a selling or gall.

The symptoms of nematode injury vary with the kind of nematode, the kind of plant, the age of the plant, and the place on the plant where the injury occurs. While the following classification of symptoms is, in some respects, an oversimplification and may not include all the numerous kinds of injury that occur, it at least organizes the discussion and explains the terms that have been used.

5.2 General Aboveground Symptoms

Where the effect is indirect and caused by nematode injury that occurs belowground, aboveground symptoms are essentially the same as those caused by any condition that deprives a plant of an adequate and properly functioning root system. Affected plants that lack vigor have reduced ability to withstand drought, lack of fertilizer, and other adverse conditions. With some plants, especially trees, the foliage may be small and off-color, and there may be leaf drop and dieback. In regions where the soil is deficient in some essential element, affected plants have an increased tendency to develop symptoms of this deficiency. These are rarely, if ever, sufficient evidence to diagnose a root nematode problem. However, they are important because possible nematode problems are almost always first noticed because of abnormal top growth. Certain kinds of symptoms are typical of nematode injury to roots and should always make one consider nematodes as a possible cause for the inferior performance. They can also be used to help locate the most severely affected areas in the planting after the problem is diagnosed.

Since most phytonematodes affect root functions, characteristic symptoms associated with them are the result of inadequate water supply or mineral nutrition to the tops, chlorosis (yellowing) or other abnormal coloration of foliage, stunted top growth in patches (Fig. 5.1), failure to respond normally to fertilizers, small or sparse foliage, a tendency to wilt more readily than healthy plants, and slower recovery from wilting.

Fig. 5.1 Plants showing stunted growth with yellowing and wilting

Woody plants in advanced stages of decline incited by nematodes will have little or no new foliage when healthy plants have substantial flushes and eventually exhibit dieback of progressively larger branches. "Melting out" or gradual decline is typical of nematode-injured turf and pasture. Plantings which are stunted by nematodes often have worse weed problems than areas without nematode injury, because the crop is less able to compete with weeds than it should be.

The distribution of nematodes within any site is very irregular. Therefore, the shape, size, and distribution of areas showing the most severe effects of nematodes will be highly irregular within the field. Nematodes move very few feet per year on their own. In the undisturbed soil of groves, turf, and pastures, visible symptoms of nematode injury normally appear as round, oval, or irregular areas which gradually increase in size year by year. In cultivated land, nematode-injured spots are often elongated in the direction of cultivation, because nematodes are moved by machinery. Erosion, land leveling, and any other force which moves masses of soil or plant parts can also spread a nematode infestation much more rapidly than it will go by itself. Nematode damage is often seen first and most pronounced in areas under special stresses, such as heavy traffic, excessive drainage because of slope or soil and dry areas outside regular irrigation patterns.

5.3 Abnormalities Resulting from Injury to Buds, Growing Points, and Flower Primordia

5.3.1 Dead or Devitalized Buds

A nematode infection may kill a bud or growing point and result in a "blind" plant. This happens occasionally with strawberry plants infected with one of the strawberry bud nematodes. The terminal growing point of a seedling may become infected before it emerges from the soil, and the infection may be carried up and eventually result in a "blind" plant. Even such weak parasites as *Aphelenchoides parietinus* have been observed to "blind" cotton seedlings in this manner. Flower buds may be killed and eventually drop off, as happens when certain orchids become infected with *Aphelenchoides besseyi.* Stem and bulb nematode (*Ditylenchus dipsaci*) infects growing buds and causes "necrotic crown" and "white flagging" symptoms (Fig. 5.2).

Fig. 5.2 Necrotic crown with infected buds and white flagging

5.3.2 Crinkled and Distorted Stems and Foliage/"Crimp"

When an infected growing point is not killed but continues to function, the stems, foliage, and other structures that develop from it are likely to be malformed, viz., crinkled, twisted, and distorted (Fig. 5.3). Stem and bulb nematode or bud nematodes of the genus *Aphelenchoides* are examples of species that may cause this type of injury.

5.3.3 Seed Galls

Seed galls are caused by the species of *Anguina* on grasses and other crops. When the flower heads begin to form, almost fully grown nematode larvae enter the flower primordia where they quickly mature and reproduce. Instead of developing into a normal seed, an infected flower primordium develops into a gall containing a large number of larval nematodes capable of surviving long periods of desiccation (Fig. 5.4). When compared to normal wheat seeds, galls are smaller in size and are lighter, and their color ranges from light brown to black (normal wheat seeds are tan in color). Large numbers of motile juveniles are present within the galls and become active after the galls have been moistened. Galls are weakly attached and fall to the ground during harvest to continue infestation. Galls harvested with seed may be inadvertently sown with seed to infest new fields, for example, *Anguina agrostis* on orchard grasses/lawn/meadow.

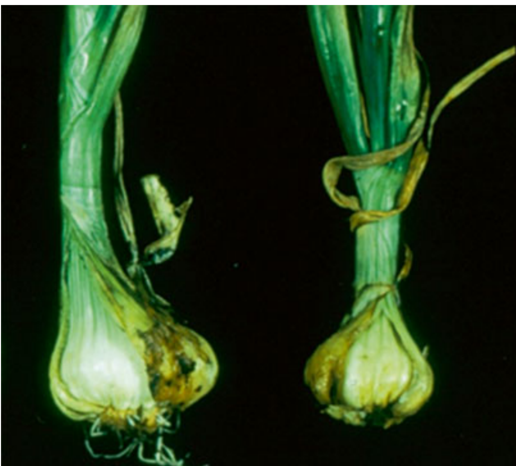

Fig. 5.3 Distorted stems and foliage

Fig. 5.4 Galls in orchard grass

5.4 Abnormalities Resulting from Internal Injury to Stems and Foliage

5.4.1 Necrosis and Discoloration

Some nematodes live and feed within the tissues of stems and leaves and cause varying degrees of necrosis and discoloration. This injury may result in twisting and distortion, although usually this is not very pronounced if the growing point is not involved. The "red ring" disease of coconut palms, caused by *Bursaphelenchus cocophilus*, is an example of this type of injury occurring in the trunk of a tree, in which discoloration of the inner trunk tissues assumes a ring pattern (Fig. 5.5). The red-colored band is approximately 3–5 cm wide, variable with the size of the tree. The surface of the cut in a healthy tree appears a solid, creamy white. The most common color of the band is bright red, although the shade can vary from light pink or cream to dark brown in infected African oil palms. *Ditylenchus dipsaci*, the stem nematode, causes injury of this kind in the stems and foliage of various kinds of plants. The narcis-

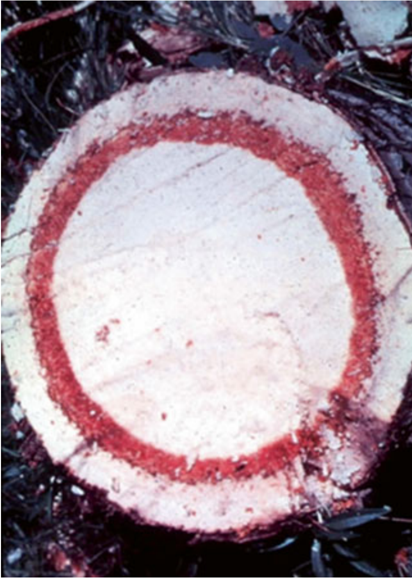

Fig. 5.5 “Red ring” symptom in coconut

sus race of *D. dipsaci* may cause small, yellowish, slightly raised, pimple-like spots on the leaves of narcissus known as “spikkels.” Branches from a peony plant may show abortive and distorted foliage resulting from an infection of *Aphelenchoides fragariae* in the buds.

5.4.2 Leaf Spots and Lesions

These are caused by nematodes that penetrate the foliage of broad-leafed plants, often through the stomata, to feed on and destroy the leaf parenchyma. The chrysanthemum foliar nematode, *Aphelenchoides ritzemabosi*, and the fern and begonia race of *Aphelenchoides fragariae* are examples of species that cause leaf spots (Fig. 5.6).

5.4.3 Leaf Galls

While most species of the genus *Anguina* produce seed galls, a few species, like *Anguina balsamophila* and *A. millefolii*, produce galls on the leaves of their host plants. These are comparable to seed galls, and they serve as structures in which the parasites mature and reproduce.

Fig. 5.6 Spots and discoloration on foliage

Fig. 5.7 Discoloration of foliage

5.4.4 Twisting of Leaves and Stem

These symptoms are common features of stem and bulb nematode (*Ditylenchus dipsaci*) on onion or rice infested by *D. angustus*, where emerging foliage shows mosaic or chlorotic discoloration or brown to dark spots on leaves with margins becoming corrugated (Fig. 5.7). Foliage and stem become abnormal exhibiting twisting and other distortions.

Fig. 5.8 Distortion of foliage

5.4.5 Crinkled and Distorted Stem and Foliage

The infection of growing point of wheat and other grasses by *Anguina tritici* leads to the distortion of stems and leaves (Fig. 5.8).

5.5 Symptoms Due to Belowground Feeders

Phytonematodes which feed on the belowground plant parts like roots, rhizomes, corms, suckers, and bulbs destruct these parts. As a result, there will be improper supply of water and nutrients to the aboveground parts of a plant. Hence aboveground parts exhibit certain symptoms.

5.5.1 Symptoms on Aboveground Plant Parts

5.5.1.1 Stunting

It mainly indicates reduced growth of plants, which makes them prone to adverse conditions. Plants appear sickened; dwarfing in patches (Fig. 5.9) is the characteristic symptom in most fields, for example, vegetables due to *M. incognita,* potato due to *G. rostochiensis,* and wheat due to *H. avenae*.

Fig. 5.9 Stunted growth of plants

5.5.1.2 Yellowing/Discoloration

This is the most common symptom of nematode injury. Either partial or complete discoloration may be observed depending upon several factors including the host plant, soil conditions, and nematode species involved (Fig. 5.10), for example, coffee infected with *Pratylenchus coffeae*, orange and lemon leaves infected with *T. semipenetrans*, and potato foliage infected with *G. rostochiensis*.

Fig. 5.10 Discoloration of plants

5.5.1.3 Wilting

Drooping of leaves or sometimes entire seedling/plant is an important symptom. Extensive damage to the root system leads to nonfunctioning of roots, leading to wilt symptoms even in the presence of moisture in soil (Fig. 5.11), e.g., root-knot infected vegetable crops.

5.5.1.4 Decline and Dieback

These symptoms are commonly found in crops like banana infected with *R. similis*, citrus infected with *R. citrophilus* (spreading

Fig. 5.11 Wilting of plants

Fig. 5.12 Decline in citrus

decline) and *T. semipenetrans* (slow decline) (Fig. 5.12), and grape infected with *M. incognita.*

5.6 Symptoms on Belowground Plant Parts

These may be more useful than aboveground symptoms for diagnosing phytonematode problems. Galls, abbreviated roots, necrotic lesions in the root cortex, and root rotting may all help in diagnosing/confirming nematode diseases.

5.6.1 Root Galls/Root Knots

These are the characteristic symptoms of the injury caused by the root-knot nematodes of the genus *Meloidogyne*. They refer to the swellings

Fig. 5.13 Knots on the roots of gherkin

Fig. 5.14 Malformation in carrot

of the root portion infested by nematode, which may range from small to large in size (Fig. 5.13). Root knots have become associated in the minds of many people with nematode injury, in general. This shows the importance of root knots and the wide spread occurrence of this nematode. It has a very wide host range. On some crops like carrot, the severe infestation may also lead to the malformation of the structure (Fig. 5.14).

Actually only a very few of the plant parasites cause conspicuous galls on roots and other underground structures. Nematodes of the genus *Nacobbus* cause galls on the roots of some kinds of plants that are very similar to, and have been mistaken for, root-knot galls, e.g., *N. batatiformis* on sugar beet and tomato, *Hemicycliophora arenaria* causing minute galls on lemon roots,

Xiphinema diversicaudatum on rose roots, and *Ditylenchus radicicola* causing small galls on the roots of oats, barley, rye, wheat, and some of the grasses. Many nematodes, including some of the external feeders, may cause moderate swelling of the roots which, if localized, results in small gall-like enlargements.

5.6.2 *Rhizobium* Nodules/ Nematode Galls

Root-knot nematodes are easy to recognize by the symptoms they cause on roots. Swollen "knots" or "galls" usually form where root-knot nematodes feed and develop. Taking a gall from the root usually tears the root apart. Galls vary in size and shape and have firm tissues. Some swellings that occur on plant roots are beneficial. The most common are nitrogen-fixing *Rhizobium* nodules found on roots of plants in the bean family. They normally are more uniform in shape and size than nematode galls, are very loosely attached to the main root, and are hollow with a milky liquid in the center.

5.6.3 Other Root Swellings

The most distinctive nematode symptoms on roots are galls caused by *Meloidogyne* spp. They are small, individual, bead-like, or fusiform swellings in some hosts. In other plants, galls may be massive lumps of fleshy tissue more than 1 in. in diameter, containing dozens of nematodes. Some hosts, including many grasses, may not form any visible root swelling even though the nematodes successfully establish giant cells, mature, and reproduce. In such cases, an absence of galls does not necessarily prove that there are no root-knot nematodes present or that the plant species in question is not a host for that root-knot nematode. Other root swellings must not be mistaken for root-knot galls. Some ectoparasitic nematodes may cause root tips to swell; by the time root-knot galling is visible, at least some of the galls are found distributed along the roots, away from the tip. Nitrogen-fixing *Rhizobium* bacteria cause swellings on the roots of most legumes (such as clovers, peas, and beans). These swellings, called nodules, are easily distinguished from root-knot galls by differences in how they are attached to the root and their contents. Nitrogen nodules are loosely attached to the root and can generally be very easily removed; root-knot galls originate from infection at the center of root, so they are an integral part of the root whose removal requires tearing the cortex apart.

In addition, fresh nodules should have a milky pink to brown liquid inside them, while root-knot galls have firmer tissues and contain female root-knot nematodes (creamy white beads less than 1/32 in. in diameter) inside the gall tissues, near the fibrous vascular tissues of the root. Abbreviated root systems may be caused by several kinds of nematodes. Many ectoparasites which feed on root tips, such as sting, awl, and stubby-root nematodes, cause root elongation to stop. These root tips sometimes swell to greater diameters than usual when they stop growing in length and often become much darker in color than uninjured root tips. Lateral roots often emerge from the root a short distance behind the injured main root tip; if a series of lateral roots are injured as they emerge, the end of the root acquires a bunchy or bushy arrangement of very short roots that is very characteristic of sting or awl nematode injury to grasses and some other plants.

5.6.4 Rotting of Fleshy Parts

Nematodes that enter fleshy planting parts may initiate injury resulting in extensive tissue destruction that can best be described as rot. While nematodes initiate the damage, the destruction may be continued by other organisms of decay that enter as secondary invaders. The potato rot nematode, *Ditylenchus destructor* (Fig. 5.15), and the yam nematode, *Scutellonema bradys*, are examples of species that cause this type of injury.

Fig. 5.15 Rotting of potato tuber

Fig. 5.16 Surface necrosis on roots

5.6.5 Surface Necrosis

Nematodes that feed on roots from the outside may kill the surface cells over large areas. Sometimes this results in superficial discoloration of the tissues. When present in large numbers, even a weak parasitic species like *Aphelenchoides parietinus* may kill enough epidermal cells to give a young succulent root a yellowish to brownish color (Fig. 5.16). On the other hand, injury of this kind caused by some nematodes may be very severe and extend so deep that roots may be completely decorticated. Dagger nematode (*Xiphinema* spp.), citrus nematode (*Tylenchulus semipenetrans*), and spiral nematodes (*Helicotylenchus* spp.) frequently are found associated with injury of this kind.

Fig. 5.17 Lesions on banana roots

5.6.6 Lesions

These are more or less distinctly circumscribed necrotic areas, usually small to moderate in size and normally reddish brown in color (Fig. 5.17), that frequently originate internally. Lesions are typical symptoms caused by nematodes that penetrate the roots and congregate in limited areas, e.g., the lesion nematode, *Pratylenchus coffeae* (on coffee); the burrowing nematode, *Radopholus similis* (on banana); and the spiral nematode, *Helicotylenchus multicinctus* (on banana and other plantation crops). Sometimes small lesions are caused by external feeders, such as the ring nematode, *Criconemoides* spp. As lesions enlarge, small roots may be girdled and the distal parts killed, causing extensive, root pruning.

5.6.7 Excessive Root Branching

Some phytonematodes may stimulate the development of branch rootlets near the region of invasion (Fig. 5.18). The northern root-knot nematode, some of the cyst nematodes, lesion nematodes, and the species of the genera *Nacobbus, Trichodorus, Heterodera, Meloidogyne, Pratylenchus*, etc., induce this type of symptom. The resulting symptoms may be much the same as stubby root, but the manner of origin may be somewhat different. When the distal part of a root is killed by girdling,

Fig. 5.18 Excessive root branching

the part that remains alive usually develops branches without any stimulation by the nematode. Despite their origin, such terms as "witch's broom," "hairy root," and "bearding" have been applied to the resulting abnormalities.

5.6.8 Injured/Reduced/Devitalized Root Tips

After a nematode has fed on it, a root tip often stops growing even though the feeding may not actually kill the tissues or cause the tip to turn brown. A root thus affected usually branches and the growth of these branches also may be stopped. There are different kinds of devitalized roots as follows.

5.6.8.1 Stubby Root

If the branch rootlets manage to attain a moderate length before their growth is stopped, the resulting root system may be composed of numerous short stubby branches often arranged in clusters (Fig. 5.19). The term "stubby" means "cigarette stub" because of the resemblance, e.g., stubby-root nematode, *T. christiei*.

Fig. 5.19 Stubby root

5.6.8.2 Coarse Root

If the growth of lateral roots is stopped just as most of them are breaking through the cortex or while they are very short, an open system made up mostly of the main roots largely devoid of small branch rootlets may result (Fig. 5.20). Such symptom is referred to coarse root, e.g., *Paratrichodorus* spp.

5.6.8.3 Curly Tip/Fish Hook

Injury at the side of a root close to the tip may retard growth and elongation on that side and result in curling (Fig. 5.21). Curly tip may be caused by dagger nematodes, root-knot nematodes, and others that feed near the tip.

5.6.9 Abbreviated Root Systems

Migratory endoparasites can also cause abbreviated roots, referring to the shortened root system without secondary/feeder roots or with reduced secondary/feeder roots (Fig. 5.22). When lesion, burrowing, root-knot, or lance nematodes injure the fleshy cortex of roots, fungi which ordinarily could not penetrate the intact root are often able to colonize the injured tissues and infect the entire root. Sometimes, rotted mature tissues at

Fig. 5.20 Coarse root

Fig. 5.21 Curly tips of roots

Fig. 5.22 Abbreviated root system

Fig. 5.23 Necrosis of banana roots

the tip of the root are a clue that endoparasitic nematodes and/or root-rot fungi, rather than ectoparasitic nematodes, may have shortened the roots.

5.6.9.1 Root Rot

Rot of large roots and storage organs (tubers/suckers/bulbs) is sometimes caused by severe infestation by nematodes alone like the potato rot nematode, yam nematode, and burrowing, and root-knot nematodes often cause extensive necrosis, which looks much like dry rot of fleshy tissues of large roots such as those of bananas (Fig. 5.23) and many tropical foliage plants.

Reference

Ravichandra, N. G. (2008). *Plant nematology* (695 pp). New Delhi: I.K. International Pvt. Ltd.

6 Nematode Population Threshold Levels

Although hundreds of different nematode species are associated with plants, not all are plant parasites. In several cases, phytonematode populations occur in numbers too small to cause serious plant injury. Limited information is available regarding potential economic losses associated with synergistic and antagonistic interactions between nematode species and the involvement of them in disease complexes. The evaluation of population threshold levels and the damage is of much significance. Nematodes from soil samples or infected plant parts must be extracted, identified, and counted in order to determine if one or more nematode species are causing poor plant growth. Hence, much emphasis has been given to the characterization of host sensitivity, host efficiency, and models in terms of population counts (Barker et al. 1985); with regard to host sensitivity, the development of tolerance limits or damage thresholds and the maximum number of nematodes that the plant may support without damage have received much attention. Major factors that influence host efficiency are reproduction factor of nematode, the nematode equilibrium density, and the maximum rate of nematode reproduction apart from the final population.

A population is a collection of individuals of a single species of organisms spatially or temporally isolated from other such groups (Ferris and Wilson 1986). In some cases, populations are geographically contiguous but exhibit gradual genetic variation across their range (clinal variation). In a qualitative sense, a population can be considered at various levels of spatial resolution and will exhibit different characteristics in terms of the means and variances of parameters descriptive of its morphology, behavior, biological attributes, and distribution. In comparing two or more populations within a species, such parameters can be used to quantify degrees of difference or similarity. For instance, if the thermal optimum for development of *M. incognita* is calculated from the mean for samples of the population taken from each state in which it occurs, the mean of all the populations will have a large associated variance. If the mean is determined for the population in a single state, it will probably differ from the national average and have a much smaller variance. Similarly, if the mean is determined for the population in a single field, it will vary from both state and national averages and have a still smaller variance.

6.1 Threshold Levels

Determination and use of economic thresholds are considered essential in nematode pest management programs. Economic thresholds refer to the population density of a pest at which the value of the damage caused is equal to the cost of management. The economic efficiency of control measures is maximized when the difference between the crop value and the cost of pest control is greatest. Since the cost of reducing the nematode population varies with the magnitude of the reduction attempted, an economic (optimizing) threshold can be determined graphically

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or mathematically if the nature of the relationships between degree of control and cost and nematode densities and crop value are known (Ferris 1978). Economic thresholds then vary according to the nematode control practices used, environmental influences on the nematode damage function, and expected crop yields and values. A prerequisite of the approach is reliability of nematode population assessment techniques.

An action threshold is the nematode population density at which control measures must be implemented to prevent economic loss due to nematodes. Thresholds may vary due to differences in sample collection methods, nematode assay methods, soil types, climate, etc. Some differences may result from simple differences of opinion. For each crop, there may be certain plant-parasitic nematode species present in a sample that are not known to cause damage even at high levels. If a species has not been shown to cause a loss of yield or quality, no threshold is given and it is assumed that no management is necessary even if that species is present in high levels. Once a damaging species is identified in a field, it will be present in all subsequent years, although population levels will fluctuate. Proper sampling of such fields is critical to ensure that the management system being used is keeping known nematode pests in that field below threshold levels.

Accurate and detailed records should be kept for each field to track where each nematode species occurs and where populations tend to be highest. In fields where a damaging nematode species is detected at subthreshold levels, it is possible that "hot spots" with above-threshold levels can occur. If such hot spots are identified, localized nematicide applications may be profitable where application to the whole field would not be. It is common for a sample to contain two or more nematode species each at subthreshold levels. In these situations, some common sense must be exercised. As an example, if two species are each present at 90 % of their threshold levels, then management tactics probably should be implemented. This does not imply that nematode damage is necessarily additive. One cannot simply add up the threshold percentages for all species present to see if it surpasses 100 %. That is, if four species are each present at 30 % of their respective threshold levels, do not assume that their combined effect warrants control measures. If nematodes are below but approaching threshold levels, check and correct other limiting factors such as pH, fertility, and hardpan before implementing nematode control measures. Healthy plants that are not under other significant stresses are better able to withstand nematode feeding pressure.

Conversely, plants that are under significant stress (such as low pH soil with a hardpan and inadequate moisture) may be damaged by nematode population levels below those needed to damage otherwise healthy plants. Nematode management is usually based on economic value where one considers both the potential gain from controlling nematodes and the potential loss from not controlling them. As the value of the crop increases, controlling nematodes is more likely to provide economic benefit and thresholds are likely to be lower; low-value crops may not be able to recoup the cost of control measures. The cost and the potential benefits of control will determine which control measures (rotations, nematicides, etc.) are preferable. If either the cost of management or the value of the crop changes significantly, management recommendations and threshold levels may need to be reevaluated.

The terms "tolerance limit," "damage threshold," and "economic threshold" are used by nematologists when characterizing relationships between nematode density and yield. The tolerance limit is the population density below which no yield loss occurs, while the economic threshold is the population density at which it is economically justifiable to implement management measures, i.e., when expected yield loss is equal to the cost of management. Damage threshold is defined in different ways but usually refers to the population density that will cause about 10 % reduction in yield. Economic threshold refers to the population density at which it is economically justifiable to implement control measures, i.e., point at which returns equal control costs. Populations of phytonematodes in soils keep changing constantly. Because plant-parasitic

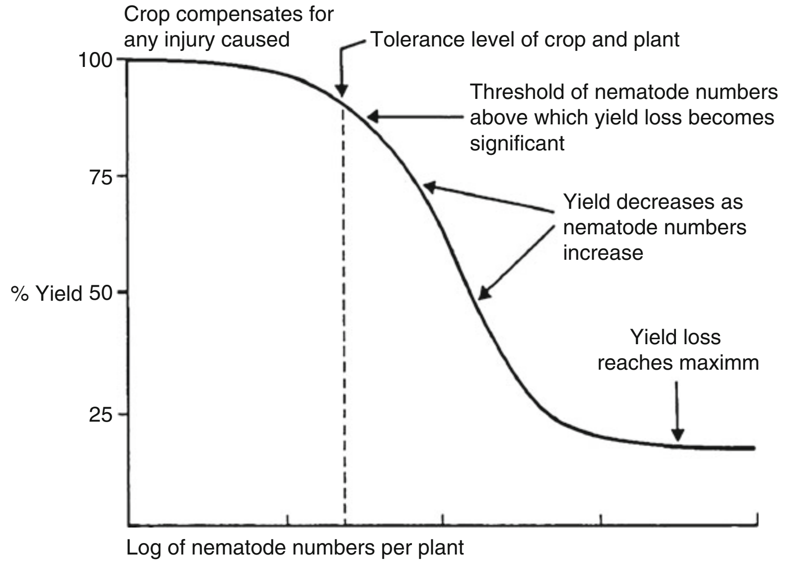

Fig. 6.1 Relationship between number of nematode and yield

nematodes are obligate parasites, they decrease in number when a host is not present or when conditions are not favorable for reproduction. Therefore, the correlation between nematode populations and plant yield can change during the season.

Multiple-point approaches to nematode population assessment for management purposes possess much potential, particularly with perennial crops. Knowing the number of phytonematodes present helps to determine the control strategies. If the population level is high enough to cause economic damage, i.e., at or above the "economic density threshold" for that species, then application of management strategies is recommended. Monitoring nematode populations in order to utilize damage thresholds involves many approaches (Barker and Noe 1987). The intensity of sampling and precision of extraction techniques may be increased as the value of a crop increases. Increased mechanization and computerization of assay techniques may lead to improved efficiency and precision. In this regard, digital sensing techniques for nematode counting including eggs and galls, image-enhanced, remote sensing for detecting large-scale nematode infestations, and measurement of environmental factors play a major role.

Some fundamental principles relating to economic thresholds include the following: The economic benefit and practical suitability of a control or management practice is related to the magnitude of the area under tile damage function (considering production overheads) less the area under the control cost function or the difference between the integrals of tile two functions. If this difference is negative, the population is below the economic threshold to that practice (Ferris 1978). The optimizing threshold is the population level at which the derivatives of the two functions are equal. For management practices resulting in anything less than pest population eradication, the control cost function shifts, relative to the damage function, with different field population densities. If the derivatives of the cost and damage functions intersect at a population level below the tolerance level, the optimizing threshold will be at the tolerance level; that is, profits will be maximized by controlling the population down to the tolerance level or the point below which nematode damage is not measurable.

An important factor is the population dynamics of the nematode species involved, particularly the relationship between nematode numbers of a particular species and plant yield (Fig. 6.1).

Three concepts are useful in interpreting this relationship. Firstly, the minimum density of nematodes which causes symptoms or yield loss is known as the tolerance limit or level. The nematode density at planting which will eventually reach the tolerance limit and cause yield loss is the threshold level. Finally, the nematode density which will cause yield loss equal to the cost of control is the economic (or treatment) threshold level. This determines the need for nematode control such as nematicide application, which usually occurs before or at planting, or the use of resistant cultivars. The economic threshold refers to the nematode density at the time of making decisions about nematode control options. In many crops, this occurs before planting. For other crops, particularly for perennial crops, decisions must also be made long after planting.

Economic threshold level depends upon several factors including the type of parasitism, life cycle, rate of reproduction and survival of the nematode, tolerance of the crop, length of the growing period of the host plant, and environmental conditions, because under good growing conditions, type of soil with adequate moisture and nutrition, plants can tolerate more nematodes than under stress conditions. Economic thresholds are often difficult to determine because environment and market values cannot be predicted with certainty.

Natural field infestations of phytonematodes offer the most realistic system for determining nematode damage thresholds and reproductive curves. It is a known fact that most epidemiological elements of nematode–plant relationships are dependent on density. Hence, it is essential to establish or recognize a wide range of population densities in field. Efforts have been made regarding exploitation of contagious spatial patterns of nematode populations in estimating damage thresholds and characterizing related disease epidemics (Noe and Barker 1985). It is essential to characterize soil and other environmental parameters to estimate damage thresholds for field experiments, as several soil parameters exhibit clustered spatial patterns that may overlap those of nematodes. In improving the reliability of nematode damage functions through multiple or multivariate regression analyses, data on soil characteristics are very much helpful. Wallace (1983) opines that damage thresholds of nematodes in the presence of interacting pathogens may be sometimes misleading as much of the damage associated with nematodes undoubtedly is due to the latter. Nematode population assays need higher precision for the development of economic thresholds than that currently available. Tolerance limits of several crops for highly aggressive nematodes may be near or below reliable detection levels with present assay techniques (Seinhorst 1981).

Tolerance limits have been estimated under limited experimental situations for several nematode species (Barker and Noe 1987). Cooperative, multidiscipline research with economic analysis is needed to develop predictive models of crop–yield–nematode and nematode–environment interactions, nematode efficiency, nematode population models, and optimal management. The tolerance limits and minimal yields depend upon various factors including soil type, moisture, crop variety, and management measures. At the grower's level, nematode bioassay threshold systems based on both plant symptoms and nematode signs will be of much significance.

The number of nematodes in a field is highly dependent on temperature and the current and previous crop species. Relatively hot years may result in several generations of nematodes, whereas cool years may only allow one generation of reproduction. Though the sugar beet nematode is somewhat generalist and can survive to some degree on many different hosts, it is greatly favored by planting sugar beets. Crop rotation may be an effective way to reduce the population of the sugar beet cyst nematode. Knowledge of previous crop rotation and temperature may be used to forecast the amount of disease in a given sugar beet field over a season (Schmidt et al. 1993).

The following Table 6.1 gives a general economic threshold level for important nematodes. However, these numbers may need adjustment when other variables are considered because density thresholds vary with crop, location, soil type, and cropping history. For example, density thresholds for economic damage to peach in clay loam to clay soils would be >39 ring nematodes,

Table 6.1 ETL for major phytonematodes

Nematode	Threshold/100 cc soil	Nematode	Threshold/100 cc soil
Root lesion nematode	500–1,000	Spiral nematode	300–500
Root lesion nematode in dry bean	>250/root	Alfalfa stem nematode	>1,000
Dagger nematode	50–100	Root-knot nematode	100
Lance	40–150	Stunt nematode	150–300
Ring nematode	250–600	Citrus nematode	10–100
Pin nematode	5,000		

>99 root-knot nematodes, and >49 dagger nematodes per 100 cc of soil; this will be different in other soil types and for other crops.

In contrast to fungal diseases, which are unacceptable at any level, low to moderate levels of phytonematodes should be expected in any stand of turf. Economic thresholds for nematodes are based on initial soil population levels that build up to damaging levels during the growing season (Pattison et al. 2002). Economic thresholds refer to the number or population of nematodes in a kilogram of soil that multiply over the growing season and cause economic damage to the crop. The economic threshold is often different for each crop and each nematode species. If the nematode soil analysis report indicates populations higher than the threshold, implement an integrated nematode management strategy.

The difficult part of managing a potential nematode problem is determining when the nematode population is actually a cause for concern. The point at which nematode populations become a problem is called the population, disease, or damage threshold. The threshold concept is used widely in plant pathology and is often the underlying mechanism of an integrated pest management strategy. Many factors contribute to nematode thresholds. While some of these factors are obvious, others are not. Additionally, interactions between these factors have the potential to dramatically influence a threshold. In short, thresholds are not as absolute as they may seem.

6.1.1 Pathogens

A nematode population's species is commonly recognized as the most important consideration in determining a nematode threshold. However, it is extremely difficult to identify the majority of individual nematodes to species in any soil sample. As a result, identifications are usually made to the genus level even though management recommendations are made using numbers published for an explicit species. While this would seem to be a major problem, it is generally considered an acceptable practice. The biological differences between species within most genera of turf pathogenic nematodes are considered to be slight. For instance, few morphological differences exist between most turf pathogenic species of the lance nematode, *Hoplolaimus*, and the assumption is usually made that pathological differences are also few.

Jatala et al. (1973) demonstrated that it could only parasitize annual bluegrass, while bentgrass in mixed stands will be unaffected. Discrete pathogenicity is often the rule in other agronomic crops but exceptions do also exist. Cultivar also plays a role in susceptibility to nematodes, as it does with most plant pathogens. Different levels of varietal resistance within turfgrasses of many different species have been observed (Townshend et al. 1973). Such relationships can potentially affect the reliability of a nematode threshold.

6.1.2 Soil Type

Soil type is another factor that may affect nematode thresholds. Nematodes are aquatic organisms. Even terrestrial nematodes live in films of water. The available water, the size of pores, and level of soluble inorganic molecules will all be affected by soil characteristics. Most turf-parasitic nematodes, for instance, are ectoparasitic; that is, they spend their lives in the soil and only their

stylets ever enter into the plant. Even migratory endoparasites (those that move around side of plants) will spend some time in the soil. Soil characteristics are critical to nematode success. Nematode thresholds are determined under precise experimental conditions, including specific soil parameters. If these parameters are changed, as is likely to occur when a threshold is taken into a field situation, nematode viability, reproduction, and pathogenicity may be affected. The same number of nematodes may be found in multiple soil types, but the nematodes may be more pathogenic in one soil type than another.

It is difficult to determine exactly which parameter is exerting the most influence on nematode populations because water-holding capacity, bulk density, organic matter, and many other factors are inherent characteristics of each soil. Even when thresholds are not directly affected by soil type, whether a nematode population can increase to the threshold level is clearly a function of soil type (Walker and Martin 2002).

6.1.3 Vigor

Plant health and vigor have a major impact on nematode thresholds. Nematodes are often considered stress-related pathogens. They usually do not cause significant damage but can cause visible disease symptoms when plants are under excessive stress. The same population of nematodes that can kill stressed turf may go unnoticed on a vigorously growing stand. Turf-parasitic nematodes feed on plant roots. When fewer roots are produced, nematode populations are concentrated on less root surface. This can further stress turf and exacerbate a decline problem. When turf is cut at a lower height, it produces shallower roots and nematode populations are quickly concentrated. As a consequence, nematode problems are most often seen on putting greens.

6.1.4 Antagonism

Microbial antagonists surely play a role in limiting nematode populations and may affect threshold values. Many species of fungi live off plant-parasitic nematodes and can reduce total nematode numbers. While this may not affect a nematode threshold, antagonists like the bacterium *Pasteuria penetrans* certainly do. *Pasteuria* attach to the cuticle of nematodes and slowly invade and reproduce inside the host. During this process, the nematode continues to survive but its pathogenicity, vigor, and fecundity will decline.

An uninfected nematode can produce more damage and more offspring than an infected nematode. If half a population of turf-parasitic nematodes is infected with *Pasteuria*, the pathogenicity of that population will be greatly diminished and the actual threshold value is likely to rise. Unfortunately, quantifying the degree to which *Pasteuria* affects any one population is difficult to measure and will change as the proportion of infected individuals changes.

6.1.5 Temperature

While climate and temperature are often overlooked, they may dramatically affect a threshold in the context of nematode vigor. Nematodes are invertebrates, and as such their life cycle is entirely dependent upon the environmental temperature. Nematode reproductive rates and metabolism are directly proportional and respond to fluctuations in temperature. Some nematode species have the ability to become dormant in colder climates and survive in frozen soils while others die. Some become quiescent at high soil temperatures while others thrive. To a certain point, nematodes that experience warmer temperatures will be more active and cause more damage. Thus, thresholds for the same nematode may vary from region to region.

In some, 150 nematodes per 3 cubic inches of soil is considered the threshold for *Hoplolaimus* (Couch 1995), while in several areas, *Hoplolaimus* does not start to produce damage symptoms until its population reaches about 400 nematodes per 3 cubic inches of soil. An unanswered question is whether this difference in threshold is attributable to altered nematode metabolism, the presence of different *Hoplolaimus* species in different climates, or both.

6.2 Population Dynamics

The term population dynamics is used to convey changes in the numbers, age class distribution, sex ratio, and behavior of a population through time and space, determined by inherent characteristics of the individuals and mediated by environmental conditions, food resources, and interacting biotic agents. Populations have characteristics that are the basis of their dynamics and that are definable and measurable. They exhibit age-specific rates of development, mortality, and reproduction. They migrate into or out of an area at a definable rate. They have a measurable sex and age composition which may or may not be stable and which is also determinant of the dynamics of change in the population. Intrinsic features of populations of Nematodes, like other organisms, have definable life history strategies. These strategies can be defined in terms of the number of life stages, the duration of the life stages, fecundity rates, life expectancy, sex ratios, and functionality of males. They can also be defined in terms of the feeding habits and mode of parasitism in the case of plant parasites. The strategies may be impacted by density-dependent and density-independent factors (Ferris and Wilson 1986). Thus, resource limitation may impact the survival of organisms, their reproductive potential, and their longevity. Temperature will affect the rates of metabolic processes and consequently duration of life stages and fecundity rates. Organisms have been categorized in terms of their life history strategy to aid in our understanding of the biology and in the development of concepts of the evolutionary and adaptive significance of these strategies.

Life history strategies may undergo tactical shifts in response to the impact of both density-dependent and density-independent conditions. Such alterations of the general strategies are examples of the plasticity and adaptability of the organism and are factors that promote its survival and success in its environment. At the population level, variability among individuals is important. The variability at the genetic level provides the basis for selection and adaptation to environmental change. It mediates and allows the evolution of the organism. Plasticity, in response to environmental shifts, can be measured in terms of tactical variation in life history strategies and genetic breadth to provide germplasm adapted to changed conditions.

Long-term discussions in ecology have explored the factors that govern the dynamics of populations. Nicholson and Bailey (1935) stressed the importance of density dependence in population regulation, that is, intraspecific competition for resources as a limitation in the size or growth of the population. In terms of an analytical model, their hypotheses centered around the carrying capacity (K) as a measure of environmental resources (food, space, pollution) and how close the current size of the population is to that carrying capacity. However, there are clearly instances at which the population of an organism is well below the apparent carrying capacity of the environment as determined by availability of resources. In these instances, competitive effects will have relatively small impact. In fact, in any other than microcosm situations, populations are frequently well below the carrying capacity of the environment due to biotic and abiotic factors (predation, parasitism, emigration, adverse conditions resulting in high mortality rates). Many extrinsic factors are considered density independent since, at least superficially, they do not appear affected by availability of resources. As an example, if the population is at a high density, there may be insufficient shelters or refuges for the organism to escape inclement conditions, or they may be more accessible to predation and parasitism.

The study of variability in populations and the impact of selection pressures on various components of the population of given genotype form the basis of population genetics. Population genotypic variability and distribution heterogeneity contribute to a complexity in biological systems that is difficult to capture in simple analytical models. Consequently, iterative, repeated calculation, approaches are used to solve the component equations of the increasingly complex models. The capabilities of high-speed computation have promoted the development of explanatory

simulation models, which may employ a large number of equations to describe the various complexities and minutiae of the system. Algorithms have been developed and adopted from other disciplines to allow incorporation and consideration of the variance of population parameter values into "distributed-delay" models (Manetsch 1976).

Although nematode thresholds are absolute values, thresholds also implicitly take into consideration the population's ability to reproduce and cause additional damage. Thus, sampling timing has an effect on how a threshold is used. Even though populations of specific nematodes are above a threshold at sampling, there may be no need to control the population, depending upon the climatic location and the time of the year. Indeed, the population may actually be declining. Population dynamics are critical to understanding whether a population is above a threshold. Nematode population levels begin at low levels in the spring and increase throughout the season. Populations of some nematodes crash in the summer and rebound in the fall. Others peak in the summer and decline in the fall. Unfortunately for the diagnostician, the dynamics of a population occur throughout time and space. A single absolute number provides only limited information. This highlights the point that appropriate use of nematode thresholds can only occur in context, incorporating a thorough case history.

Nematodes have various reproductive strategies. Some grow large and have long life cycles with low rates of population increase (*K* strategists), and others are relatively small and have short life cycles and potentially higher reproductive rates (*r* strategists). An endoparasitic habit with induction of giant cells or other rich and continuously available food sources reduces exposure to predation and other stresses and further increases reproductive potential. A reduction in the number of active juvenile stages further decreases development time, thereby reducing generation time and increasing the potential for multiple generations in a season. A wide host range completes the adaptation of pathogens such as some *Meloidogyne* spp., which can be regarded as the ultimate plant-parasitic nematode *r* strategists. Many *Longidorus* spp. are examples of *K* strategists. It is a characteristic of *K* strategists that they do best in stable environments where populations are usually close to the equilibrium density (the population density that can be sustained). In contrast, *r* strategists increase rapidly where the environment is favorable, often overshooting the equilibrium density. Severe damage to the host occurs and the population crashes. This can occur with repeated cropping of hosts as Pi increases, environmental influences on sex determination reduce multiplication, parasites of the nematode increase in number, and increasing damage to the host and competition for feeding sites progressively reduces multiplication.

Consequently, nematode multiplication rates are strongly density dependent. Again, the question of how density is defined arises. Usually it is expressed as the numbers of nematodes per gram or ml of soil, but the units that directly affect the nematode are those that are root related, e.g., number of root tips and/or length or weight. Hence, a cultivar with twice the root mass of another will, except at low densities where the multiplication rate is the maximum, support a higher multiplication rate. Similarly, tolerant cultivars that maintain a greater root mass as Pi increases than intolerant cultivars will have a greater equilibrium density and maintain a greater multiplication rate at high preplanting population densities. Overall multiplication rates are determined by the intrinsic maximum rate of multiplication, which is influenced by nematode species, the susceptibility (defined as all those qualities favoring the nematode) of the host, and the various environmental factors that influence both the nematode and the host. Nematode multiplication can be modeled in different ways. For migratory nematode species that multiply continuously, Seinhorst (1966) proposed the following formula derived from a logistic equation, $\mathrm{Pf} = a E \mathrm{Pi}/(a-1)\mathrm{Pi} + E$, where a is the maximum rate of increase and E is the equilibrium density at which $\mathrm{Pf} = \mathrm{Pi}$. For sedentary nematodes with one generation at a time, e.g., potato cyst nematodes, Seinhorst (1967) proposed an alternative model based on

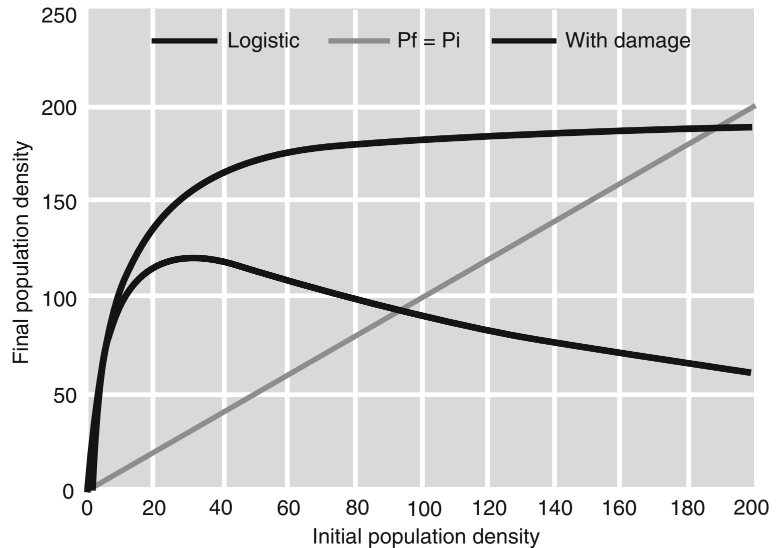

Fig. 6.2 The theoretical logistic relationship between initial population and final population density and the relationship when roots are damaged

the competition model, $Pf = a(a - q^{Pi})/-Inq$, where "a" is again the maximum rate of multiplication and "$1-q$" is the proportion of the available space which is exploited for food at a density of Pi = 1 (Nicholson 1933).

Jones and Perry (1978) also proposed a model for sedentary nematodes with a logistic basis derived from the observation that sex determination is density dependent. Their model includes parameters that reflect fecundity and the proportion of the population that does not hatch. All three models, in their most basic form, show maximum rates of multiplication at low initial densities. As Pi increases, the rate of multiplication is reduced as an upper asymptote is reached (Fig. 6.2). In reality, the shape of this curve is modified as Pi increases due to the increasing damage inflicted and the loss of roots. With the Jones and Perry model, this is exacerbated as space lost as a result of root damage increases the competition between invading nematodes, resulting in an even greater shift in the sex ratio toward male production than would otherwise be the case. Thus, the approach to the asymptote is slower and indeed the asymptote is reduced below the theoretical level. Further increases in Pi can inflict so much root damage that the population increase becomes negative and the population size is ultimately reduced.

All the equations mentioned require modifying by including a damage function such as that of Seinhorst, which also allows the differences in tolerance between cultivars to be taken into account. The damage functions used model proportional differences, and further modification may be required to account for absolute differences in plant size. Another plant characteristic that affects population increase is the host status of the plant. Differences can be modeled in terms of the maximum multiplication rate or the space required for successful multiplication (Seinhorst models) or in terms of fecundity or effects on the sex ratio (Jones and Perry model).

An important method of expressing and comparing the effects of different cultivars or cropping regimes is to consider the equilibrium density, i.e., the point at which Pf = Pi. This density is usually observed at a Pi which is larger than that which gives the largest Pf (Fig. 6.3). In practice, this equilibrium density is reached after a period of oscillation about the equilibrium density.

The size of the oscillations will be determined by the tolerance and resistance of the host. Tolerance and resistance will produce small oscillations, while susceptibility and intolerance can result in large oscillations. Indeed, these two factors can interact to the extent that a tolerant

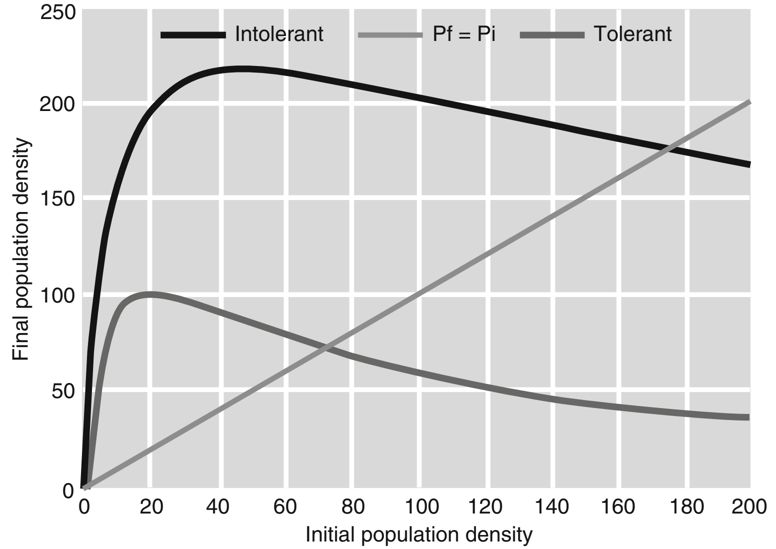

Fig. 6.3 The relationship between Pf and Pi when a tolerant and an intolerant host are cultivated

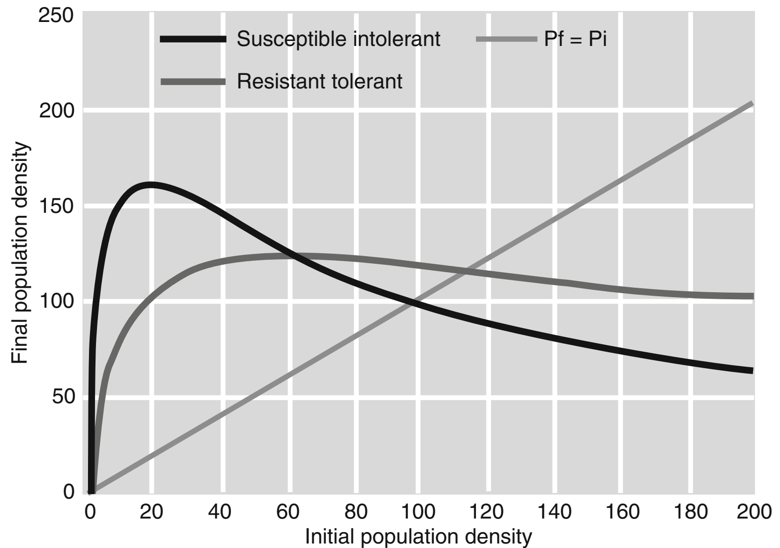

Fig. 6.4 The relationship between Pf and Pi contrasting the response when an intolerant susceptible host is grown to that which occurs when a tolerant but partially resistant host is cultivated

but partially resistant cultivar can produce a higher equilibrium density than an intolerant susceptible cultivar (Fig. 6.4).

Care needs to be taken in devising management strategies for the control of nematodes to balance the benefits of tolerance against the benefits of resistance, to ensure that while yields are maximized, nematode populations are not raised to levels that are damaging to other cultivars. Models can be used to examine and explore nematode management strategies, but need to take into account the effective population if this is less than the actual population, and the decline in the numbers of nematodes in the absence of a host crop.

6.2.1 Synergy

While interactions between nematode species can dramatically increase nematode damage, these interactions are rarely incorporated into

predicted nematode damage on turf. While stunt nematodes have a practical threshold of 800 nematodes per 3 cubic inches of soil and lance nematodes have a threshold of 400 nematodes per 3 cubic inches of soil in the Northeast, an algorithm does not exist that can predict whether a combination of 600 stunt nematodes and 300 lance nematodes per cubic inches of soil will cause observable damage. Nematode thresholds currently used for turf systems are extremely simple models. While this makes them easy to use, it ignores much of the inherent complexity of the soil microcosm. Diagnosticians, however, need to be cognizant of these potential interactions.

6.2.2 Sampling

Sampling location, specifically within a green, has a significant impact on nematode counts and thus the applicability of nematode thresholds. There are two general philosophies toward nematode sampling in turf. The first philosophy is to take two composite samples, one from affected areas and the other from unaffected areas, a process that we will call spot sampling. The other philosophy is to take a single composite sample from across an entire green, which we will call holistic sampling. While they are both valid, they serve two entirely different purposes and cannot be used interchangeably. Spot sampling can only be used when there is observable damage. The intent of spot sampling is to determine whether observable damage is caused by high populations of nematodes. Holistic sampling should not be used to make such a determination because a holistic sample actually dilutes the highest nematode population clusters with areas of low nematode density. While holistic sampling gives an estimation of nematode density across a green, it does not account for hot spots. The holistic approach is most useful in monitoring population density over multiple seasons or throughout a single season. Sampling methodologies can be complex, and these two methods can be modified in numerous ways.

6.2.3 Extraction

In order to count nematodes in soil, they must first be extracted from soil, which is an inherently error-prone process. Nematode extraction is largely performed by people, although some very expensive automatic and semiautomatic elutriators do exist. Technique and equipment vary from lab to lab, and this has a direct impact on the average extraction efficiency of each lab. Additionally, differences between each individual lab employee may have an effect on extraction efficiency. As a result, two labs that process exactly the same sample may generate different nematode counts. While these counts will often be close enough that they do not affect the final management recommendation, sometimes the management recommendation may vary based on a particular extraction, especially when counts hover close to threshold values.

6.2.4 Host

It is well established that phytonematodes often have very discrete host ranges. Even if a nematode species can cross over to another host, it is probable that its virulence on the new host will be different (and likely reduced) from its virulence on the original host. While pathogenicity is the ability to cause disease (a qualitative character), virulence is often regarded as severity (a quantitative character). Turf-parasitic nematodes are exceptional, however, because they often have wide host ranges, affecting creeping bentgrass, velvet bentgrass, annual bluegrass, Kentucky bluegrass, and others. As a result, it is often not always necessary to consider the turfgrass species when applying a nematode threshold. There are a few notable exceptions, however, that do have discrete host ranges including the root-gall nematode, *Subanguina radicicola*. Although this nematode is not frequently observed, it is common in the coastal areas of Rhode Island and New Jersey. Recently, it has been reported from as far north as New Brunswick, Canada (Mitkowski and Jackson 2003). This nematode

has a very discrete host range. However, many times threshold values are viewed as absolutes. Treatment is followed when levels are above the threshold for a particular nematode. The cost, availability, and environmental impact of nematicides should be seriously weighed before making such an application. But assembling the proper information to make an educated decision about control entails a significant amount of work with uncertain results.

Type of nematode, species of grass, soil composition, plant health, time of year, and a number of other factors will all influence management decisions. It is important to carefully monitor nematode populations annually. When numbers approach thresholds, treatment may be necessary. But populations can sometimes exceed threshold without disease symptoms ever being observed, given the variability discussed previously. Correlating nematode populations with observed damage is critical for making informed decisions about nematode management.

Patterns in the population dynamics of nematodes are determined by the intrinsic characteristics that regulate rates of births and deaths of individuals and modified by conditions of the environment in which the population functions. Intrinsic factors include the productive capacity of the gonad in relation to resource demands of somatic tissues, the rate and length of the reproductive period, and the life history strategy. Modifying factors include availability of food, sperm and other driving resources, and environmental conditions. Various models have been used to describe the dynamics of populations; some are primarily descriptive of observed trends and others more explanatory and mechanistic. All may be relevant in prescribed situations. Economic thresholds are management tools for minimizing economic losses due to nematodes. They are based on projections of expected crop performance in relation to population levels at a critical point in time or at multiple points in time. The economic threshold is that level to which the population of the target nematode species should be managed under prevailing economic and environmental conditions. In its most comprehensive sense, the economic threshold is based on the integral of expected returns from the current crop and from future crops, given the expected trajectory of the nematode population at this level of management. Phytonematodes feed and multiply on plant roots all through the growing season. Populations are usually highest in May–June and September–October.

6.3 Economic Threshold Based on Initial Nematode Population (Pi)

At its simplest, if the control cost is $100, it only makes sense (in the short term) to apply the control when the expected crop loss is >$100. Hence, the economic threshold is that population level of nematodes at which the cost of control is equal to the value of the crop loss. If the population is above that threshold, the value of the loss is greater than the cost of control so apply the control; if not, take the loss. The above reasoning assumes that the applied control will reduce the population to a non-damaging level. If that is not the case, the economic threshold is that population at which the cost of control is equal to the difference between the crop value with and without the control. So, if the cost of control is $100, the expected crop loss at this nematode population level without control is $120 and the expected crop loss with control (because the control is not 100 % effective) is $30, the population is below the economic threshold for that treatment, i.e., $100 of control cost is spent and only get $90 improvement in returns (Burt and Ferris 1996). At any initial population level, if one wants to plant a host crop, the population will increase. The amount of increase is determined by the reproductive capacity of the nematode population, the number of nematodes present, and the amount of damage they cause to the host. But, for any given population level, the amount of increase under a host crop should be predictable. In the absence of a host, the population of nematodes should decline. The rate of decline should be predictable for a given field, e.g., 60 % per year.

6.3.1 Population Change over Time

So for any starting population, say 1,000 nematodes, if one cultivates a host crop the population will increase, say to 10,000 nematodes (tenfold increase). If one follows that with one or more years of host crop, the population will decline. At some time in the future, determined by the rate of decline, it will be back to the starting population level. So in the example above, the 10,000 nematodes will be 60 % lower after 1 year on nonhost that is 4,000. After another year of nonhost, the population will be 1,600, and after a third year it will be 640. So, it took 3 years of nonhost to get back to around 1,000 nematodes that started with. If the start is with 100 nematodes, they may have increased 20-fold under the host crop, due to lower plant damage, to 2,000 nematodes. At 60 % loss per year under nonhosts, the progression of decline would be 800, 320, 128 and 52. Starting with 5,000 nematodes, may be only twofold increase on the host to 10,000 which would decline to 5,000 in only 1 year of nonhost.

6.3.2 Economics

For any starting population level, we should also be able to predict the amount of damage to the host crop. In the above examples, 5,000 nematodes may cause 90 % crop loss, 1,000 say 50 % loss, and 100 nematodes 10 % loss. So, if the potential value of the host crop is $1,000 and that of the nonhost is $500, the economics of these three scenarios may be examined as below.

Example 1*:* (starting with 1,000 nematodes) $500 from the host and 3×$500 for the nonhosts, that is, $2,000 in 4 years or $500/year

Example 2*:* (starting with 100 nematodes) $900 from the host and 4×$500 for the nonhosts, that is, $2,900 in 5 years or $580/year

Example 3*:* (starting with 5,000 nematodes) $90 from the host and 1×$500 for the nonhost, that is, $590 in 2 years or $295/year

If for every possible starting population level the calculation is done, the nonhost rotation length that maximizes the average annual returns is the optimum. The optimization calculation can be done more automatically with a spreadsheet algorithm.

Economic threshold level is an important tool in the integrated nematode management program. By establishing and knowing this, it is possible to reduce input cost by minimizing the number of nematicidal sprays and other inputs. It also creates the eco-friendly nematode management. However, economic threshold level has been largely ignored in nematology for obvious reasons like lack of information on the relationship between nematode densities and plant damage and damage functions, difficulties in assaying nematode densities in a field, work involved in arriving at the decision, and ready availability of low-cost nematicides.

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7 Crop Loss Assessment

Crop losses are influenced by several factors including the pathogenicity of the species of nematode involved, the nematode population density at planting, the susceptibility and tolerance of the host, and a range of environmental factors (Trudgill and Phillips 1997). Because of this, available models only estimate yield losses as proportions of the nematode-free yield. Estimating threshold levels further involves various economic calculations. Consequently, predicting yield losses and calculating economic thresholds for most nematode/crop problems is not yet possible. What is needed is more field-based information on the relationship between nematode population densities and crop performance, and various approaches to obtaining such data are described. Measuring the population density, especially of *Meloidogyne* species, is a major problem which needs addressing. Better loss estimates are needed for most crops, but good estimates are costly in time and money. Detailed surveys and accurate damage threshold relationships are essential for strengthening expert opinion for generating loss estimates, which is currently the most feasible way to approach this vast and complex topic. Costs of nematode management practices and quarantine and regulatory penalties also should be included more often in generating loss information in the future.

Reliable crop loss estimates are important for establishing research, extension, and budget priorities (Dunn 1984). Unfortunately, obtaining such estimates is difficult. Nematode population dynamics are also density dependent and are influenced by host growth, the reproductive potential of the species, and various environmental factors. Consequently, modeling nematode population dynamics is an equally impressive science. Good field data are required but the complicating effects of biological control agents, host susceptibility differences, and environmental factors and errors associated with measuring initial population densities may mean it is practically impossible to predict reliably the multiplication rates of most nematodes, especially those with several generations per season. Nematode population dynamics are also density dependent and are influenced by host growth, the reproductive potential of the species, and by various environmental factors. Consequently, modeling nematode population dynamics is an equally impressive science. Again, good field data are required but the complicating effects of biological control agents, host susceptibility differences, and environmental factors and errors associated with measuring initial population densities may mean it is practically impossible to predict reliably the multiplication rates of most nematodes, especially those with several generations per season.

7.1 Crop Losses Due to Nematodes

Annual estimated crop losses due to nematodes in India have been worked out to be about Rs. 242.1 billion. Plant pathogenic nematodes are responsible for an annual loss of over $100 billion worldwide (Sasser and Freckman 1987).

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Additional losses to grower revenues also occur from either the sampling costs to determine if the treatment is necessary or yield losses in crops, which are not treated due to the decisions of growers/the lack of a registered nematicide or where treatment does not fully compensate for nematode damage and finally the loss in revenue when land must be rotated out of a high-cash-value crop to a less profitable crop to reduce nematode populations and/or avoid nematode damage.

It will indeed be a wonder if any crop is free from plant-parasitic nematodes. Many a times in olden days, nematodes have caused people to migrate due to soil sickness. It has been estimated by the International *Meloidogyne* Project that nematodes cause annual losses of 78 billion US dollars in developed countries and more than 100 billion in the developing countries. Nematode problems are more severe and complicated in warmer than cooler areas, horticultural than field crops, monoculture than multiculture, and plantation crops than natural forests and vegetation. Horticultural crops are more efficient producers of biomass and harvestable produce than the agronomical crops. Nematodes pose a constraint to horticultural development and intensive cultivation. It has been estimated that annually an average 6 % loss in field crops, 12 % in fruit and nut crops, and 11 % in vegetable and 10 % in ornamental crops is due to nematode infections. Besides causing quantitative losses, nematodes are known to reduce vitamins and minerals in edible plant parts. Nematode damage is less obvious and many a times goes unnoticed. It causes gradual decline in yield (Seinhorst 1965). Nematodes cause complex diseases in association with other soilborne pathogens.

Additional losses to grower revenues also occur from either the sampling costs to determine if treatment is necessary or yield losses in crops, which are not treated due to the decisions of the growers/the lack of a registered nematicide or where treatment does not fully compensate for nematode damage and loss in revenue when land must be rotated out of a high-cash-value crop to a less profitable crop to reduce nematode populations and/or avoid nematode damage. Yield losses by nematodes are influenced by the pathogenicity of the species of nematode involved, by the nematode population density at planting, by the susceptibility and tolerance of the host, and by a range of environmental factors. Because of this, available models only estimate yield losses as proportions of the nematode-free yield. Estimating threshold levels further involves various economic calculations. Consequently, predicting yield losses and calculating economic thresholds for most nematode/crop problems is not yet possible. What is needed is more field-based information on the relationship between nematode population densities and crop performance and various approaches to obtaining such data are described. Measuring the population density, especially of *Meloidogyne* species, is a major problem, which needs addressing.

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7.2 Sampling for Nematodes

For estimation of nematode population, precise sampling is essential during which following risk of loss has to be assessed, which includes the determination of the following:

1. Presence or absence of nematodes. It may be assessment of long-term risk or virus vectors or root crops – direct damage or exotic pests.
2. Population abundance – relative/absolute (predict potential yield/damage or assess rate of population change (+ or −)).
3. Spatial patterns (pattern of potential loss or partial treatment/management).

Factors affecting the sampling for nematodes: Several factors which affect the nematode sampling include:

Factors affecting microdistribution: Life history strategies (feeding/parasitism, reproductive behavior, and motility) and food distribution (crop spacing and root morphology)

Factors affecting macrodistribution: Crop history, management, and field usage (crop sequence and spatial arrangement of previous crops); age of infestation (time to spread from a point source); edaphic conditions (soil texture patterns); and drainage patterns (soil moisture levels and soil aeration)

7.3 Damage by Nematodes

The nematode, the host, and the environment are the three interacting variables influencing the extent of yield loss in infested soils. An understanding of the mechanisms and principles involved in these interacting relationships is basic to being able to predict yield reductions from estimates of preplanting nematode population densities (Pi). The following Table 7.1 provides the loss caused by some phytonematodes on major crops (Sasser and Freckman 1987).

7.3.1 Components of Damage

Total energy consumption during the life cycle of a female root-knot nematode is 1 cal. The total biomass of a female root-knot nematode is 200 μg, including the egg mass. For say 100,000 nematodes in a root system, the total nematode biomass is 20 g! Allowing for 50 % production efficiency, total material extracted from the plant would be 40 g. So, the demand effect on the plant may be minimal unless plant is very stressed and resources are limited. An adult *Heterodera schachtii* consumes 11 nL/day of cell content. Hence, it would take 1,000,000 such females to remove 11 mL of cell content in a day.

Table 7.1 Crop losses due to phytonematodes

Life-sustaining crops	Annual loss (%)	Economically important crops	Annual loss (%)
Banana	19.7	Cacao	10.5
Cassava	8.4	Coffee	15.0
Coconut	17.1	Cowpea	15.1
Potato	12.2	Okra	20.4
Sugar beet	10.9	Tea	8.2
Sweet potato	10.2	Tomato	20.6

7.3.2 Mechanical Disturbance

7.3.2.1 Penetration of Cells

It is relative to length of stylet. Damage depends on types of cells affected, i.e., storage tissues, cortex, or functional vascular cells.

7.3.2.2 Migration Through Tissues

Migration may be intercellular and intracellular requiring dissolution of cell walls and middle lamellae. This suggests the involvement of cellulase and pectinase enzymes with spongy tissues and sloughing, e.g., damage caused by *Pratylenchus* and *Ditylenchus*. This may allow ingress of other organisms. Root-knot and cyst nematodes produce endoglucanase (cellulase) enzymes and pectate lyase which are presumably involved in the passage through plant tissues.

7.3.2.3 Leakage from Damaged Tissues

It is estimated that up to 20 % or more of photosynthate partitioned to roots may leak into rhizosphere soil without root damage. "Root exudation" nurturing organisms in rhizosphere-presumably to plant benefit- but speculate that selection has optimized the costs and benefits. Enhancing root leakage through nematode damage must reduce plant productivity.

7.3.3 Physiological Disturbance

7.3.3.1 Nematode Secretions

These are associated with the establishment and maintenance of feeding sites. Effects increase with sedentary endoparasitism. Secretions from the nematode digestive glands may polymerize into a feeding tube inside the cell. The feeding tube

remains associated with the stylet during ingestion. When the stylet is withdrawn, the opening in the cell wall is sealed with an electron-dense feeding plug.

Various disturbances include cell wall permeability and surface enlargement including transfer cells and cell wall thickening; increase in cytoplasm density and metabolic activity; hypertrophy (cell enlargement, 500–1,000× increase in volume (*Meloidogyne*), nuclear division without cytoplasmic division (karyokinesis without cytokinesis) – but different strategies are present in different genera, for example, cell wall dissolution in *Heterodera* – multinucleate cells and nuclei larger); hyperplasia (tissue enlargement, mitotic activity, galling, root tip galls, etc.); disruption of normal meristem activity (*Xiphinema, Trichodorus, Hemicycliophora* (different taxonomic groups)) and damage to meristems; and physiological control of carbohydrate partitioning (metabolic sink – McClure, $^{14}CO_2$ studies).

7.3.3.2 Physiological Effects

These include increased root respiration (more mass), increased growth respiration (repair), mobilization of defense mechanisms, and increased levels of plant hormones (indoleacetic acid, cytokinins) in galled tissue, but source is uncertain.

7.3.3.3 Whole-Plant Effects

They refer to the disturbance of the biochemical network. Photosynthesis is divided into two basic phases – a light phase when light energy is converted into chemical energy and a synthetic phase in which carbohydrates are formed in a series of reactions accelerated by light. Photosynthesis involves a chain of metabolic events cross-linked to other physiological processes, so disruption of one may have effects throughout the system. For example, photosynthesis is reduced in tomato by *M. javanica* by inhibiting production of cytokinins and gibberellins in roots and/or by increased stomatal resistance due to water stress. The response of potato to *Globodera rostochiensis* is due to stomatal closure through water stress; the result is reduced photosynthesis. However, generally the mechanisms by which root-infecting pathogens, including nematodes, affect physiological processes have been insufficiently studied.

7.3.3.4 Plant as an Integrator

This may comprise of metabolic pool concept, plant as an integrator, and concepts of demand and damage. Five effects of root-knot nematode infection in grape were characterized while exploring the impact in an energy partitioning and flow model:

1. Reduction (disruption) of water uptake: Seinhorst, however, asserted that there is little evidence of reduction of water uptake in response to nematodes. He measured daily water uptake on a water usage basis – rate of water loss from pots minus increase in dry wt minus evaporation from surface. The rate was a linear function of total dry wt. So, he argued that the rate of use per g tissue is constant. However, root damage could result in lower water uptake, and final dry weight could be a function of the rate of water uptake.
2. Reduction in rate of photosynthesis.
3. Reduced leaf expansion and total photosynthesis.
4. Alteration of partitioning of photosynthate: change in root/shoot ratio.
5. Increased leakage: Direct effect on other pathogens' energy supply (Garrett – inoculum potential as a function of the abundance of infective units and the energy resources available to them).

7.3.4 Molecular Events

These are plant responses to nematode infection (from various plant and nematode systems). It may include the induction of pathogenesis-related proteins (similar to those after fungal or viral infections), occurrence of nonlocal induction of proteinase inhibitor proteins, and local and systemic induction of a gene encoding catalase. Protection by catalase to the plant from oxidative stress is associated with nematode feeding and the expression of a gene encoding extensins, which are hydroxyproline-rich glycoproteins

that are components of plant cell walls. Changes in gene expression at feeding sites are probably localized in a few cells. Identifying the small amount of product is technically difficult. Use of reporter genes such as GUS which can be attached to promoter regions of other genes is among the useful new indicators of localized gene activity.

The genome of plant-feeding nematodes of the order Tylenchida includes genes that encode for endoglucanases. Endoglucanases are cellulases, a family of enzymes formerly thought to be restricted to prokaryotes. Other plant cell wall digesters such as termites and ruminants use symbiotic and commensal bacteria to dissolve cellulose. The presence of these and other genes suggests that horizontal or lateral gene transfer has occurred between bacteria and nematodes. The guidelines for obligate parasite nematodes include establishment of association (ecological phase), parasitic capabilities (biological phase), and host–parasite relations (etiological phase).

7.3.4.1 Predisposition

This comprises of the interaction with other organisms like other nematodes, fungi, bacteria, viruses, and abiotic/physical stresses. Actually, the term "interaction" is loosely used, which implies that the effect in combination is different than the sum of individual effects – not additivity. Three descriptions of the result of combinations of organisms are synergistic, suppressive, and no interaction.

7.3.5 Mechanisms of Interactions

(a) *Synergistic*: It includes the reduced tolerance (multiple stress); vectoring (Longidoridae and Trichodoridae); change in substrate, for example, in case of fungi; routes of ingress (fungi and bacteria); and leakage of energy source in fungi and bacteria.

(b) *Suppressive*: This comprises of induced resistance; reduction of stress (mycorrhizae); biological antagonists, but not an interaction of two pathogens; and reduced substrate availability.

Ranking of important nematode genera and relative weight

Genus	Relative damage importance
Meloidogyne	1.00
Pratylenchus	0.57
Heterodera	0.44
Ditylenchus	0.18
Globodera	0.19
Tylenchulus	0.17
Xiphinema	0.15
Radopholus	0.12
Rotylenchulus	0.10
Helicotylenchus	0.09

7.3.6 Damage Models

When modeling the damage caused to plants by root-feeding nematodes, certain basic principles apply: damage is proportional to the nematode population density, and the degree of damage is influenced by environmental factors.

The yield harvested is determined by the amount of light intercepted by the crop, by how efficiently the intercepted light is converted into dry matter, and finally by how that dry matter is partitioned into non-harvested and harvested yield. For some crops, significant variations in moisture content will also affect final yield. The above principles can be simply stated but are more complex in practice. Damage may be proportional to the nematode population density, but there are several qualifications of this statement. The relationship is usually curvilinear, increasing numbers of nematodes having proportionally diminishing effects. There is some evidence that at low densities the host plant can repair the damage and that growth may even be slightly stimulated. Seinhorst (1965) termed the population density (Pi) at which damage first became apparent as the tolerance limit (T).

Equally, at very high values of Pi, increasing numbers of nematodes may not further reduce dry matter productivity. Seinhorst termed this the minimum yield (m). There are various reasons why m may occur; there may be some growth before attack starts or after it finishes, and a significant biomass may be planted (e.g., potato

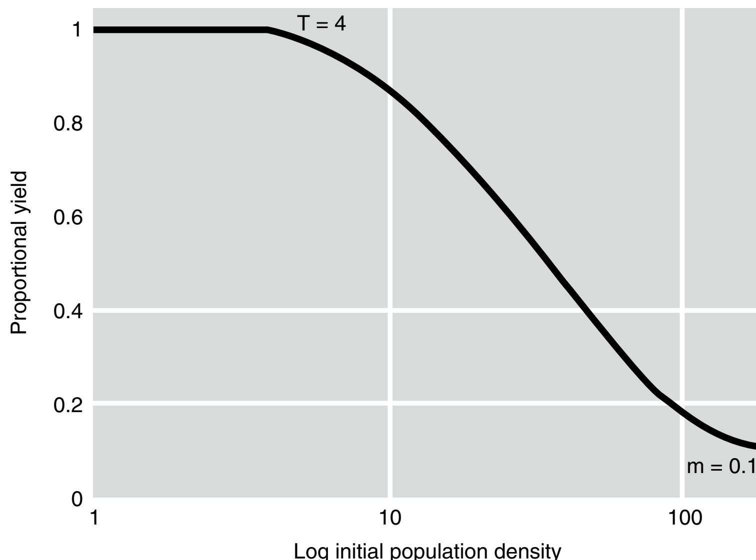

Fig. 7.1 The relationship between proportional yield loss and initial population density

tubers). However, m applies to total dry matter, and because of effects on partitioning, the harvest value of *m* may be greater or less than that for total dry matter.

The third parameter in the Seinhorst equation is *z*, a constant slightly less than one. The equation is

$$Y = m + (1 - m) z^{(\mathrm{pi} - T)} \quad \text{for}$$

$$\mathrm{Pi} > T$$

$$y = 1 \quad \text{where Pi} \leq T, \quad \text{where } y \text{ is the yield.}$$

An important qualification is that *y* is expressed as a proportion of the nematode-free yield. Hence, according to Seinhorst, the greater the yield potential, the greater the loss in tons per hectare for any value of Pi. The Seinhorst equation is usually plotted with Pi on a logarithmic scale, producing a sigmoidal curve (Fig. 7.1). In practice, *T* is usually small and the Pi value at which *m* is reached is so large that it is only the central part of the curve that is of practical use. Oostenbrink (1966) suggested that this approximated to a straight line. The equation for such a line is $y = y(\text{max}) - \text{slope constant} \times \log \text{Pi}$.

Even the simplified Oostenbrink relationship is not very helpful. Yield is still expressed in proportional rather than real (tons per hectare) terms. Also, there is no way of applying the relationship without considerable experimentation to determine the slope of the regression. The slope of the regression varies for several reasons. These include differences in pathogenicity (capacity to cause damage) between species, e.g., *Meloidogyne* spp. may be inherently more damaging than *Tylenchus*, but we have no measure of their relative pathogenicities. Different plant species and varieties within species differ in their tolerance (capacity to withstand nematode damage). Also, there are large environmental influences on the damage suffered and particularly how that damage is translated into effects on final yield.

An important consideration, often overlooked, is the basis of measuring Pi. Usually it is given as numbers per gram of soil. A more appropriate measure is per unit volume of soil as this allows for bulk density differences. Numbers per gram of root is probably the most appropriate, but is difficult to measure because it is always changing. This latter aspect becomes important when trying to relate results from experiments where root densities are very different, e.g., pot and field trials.

A further problem is encountered when considering damage by nematodes that have two or more generations in the lifetime of a crop. Usually

the Pi is measured at planting, but on a good host population of, for example, *Meloidogyne* spp., they can increase from below the value of T to a level in mid-season where they cause significant damage. Even so, it is a race between increasing Pi and increasing plant size that brings with it increasing tolerance (in Seinhorst terms, increasing m). In such situations, suitability as a host (susceptibility) and tolerance can have a marked effect on the degree of damage. To sum up, both the Seinhorst and Oostenbrink equations are, without the addition of a substantial amount of additional information, purely descriptive and cannot be used to predict actual yield losses.

7.3.7 Mechanisms of Damage and Environmental Effects on Damage

Damage is proportional to the intensity of attack, which is often proportionally greater in sandy soils where nematodes can move more freely than in heavier soils where movement is impeded. Adequate soil moisture is essential for free movement so attack is often limited as soils dry out later in the season. Temperature also influences the rate of nematode movement, but plant growth is usually equally affected.

Primary damage to the attacked roots can be attributed to mechanical damage associated with feeding or invasion, to withdrawal of nutrients, and/or to more subtle physiological effects. Generally, damage reduces the rate of root extension. This reduces the rate of uptake of nutrients and water and if any become limiting (and they usually do, even for crops without nematode damage), top growth rates are reduced. This reduces the rate of increase in light interception and carbohydrate synthesis and hence the capacity of the plant to generate more roots to overcome the limitations imposed by nematode damage. Such appears to be the main mechanism of damage by potato cyst nematodes (*Globodera* spp.) whose effect is further increased by reductions in root efficiency, revealed in a decrease in root/shoot ratio. Further damage is associated with withdrawal of nutrients by the developing females (resistant cultivars of potato are often less damaged than susceptible cultivars) and by secondary pathogens such as *Verticillium dahliae.* The central role of nutrient uptake is revealed, however, by the substantial ameliorating effect on damage of additional fertilizer.

With *Meloidogyne* spp., impaired water relations appear to contribute substantially to reduced rates of top growth. This is probably because the developing giant cell systems interfere with and disrupt the developing xylem. Clearly, with such damage, effects on growth and yield are likely to be greater where the plants are on the threshold of becoming moisture stressed. Other effects include reduced photosynthetic efficiency (Trudgill 1992).

7.3.7.1 Effects on Light Interception and Utilization

There is a good correlation in many crops between percent ground cover (i.e., the percentage of ground occupied by a plant or a crop, when viewed from above, that is covered by green leaves) and percent light interception. Most annual crops start as individual, separate plants, and a reduction in growth rate is directly reflected in ground cover and hence light interception. As they grow the leaves of neighboring plants merge to form a continuous canopy. Nematode damage that only delays the production of a continuous canopy, and hence 100 % light interception, will have a smaller effect on final yield than damage which prevents the crop achieving such full cover. Premature crop death will also proportionally reduce yield.

7.3.7.2 Environmental Effects

Several environmental interactions have already been mentioned. Soil type clearly has an effect because it influences nematode movement as well as being a nutrient and water supply to the host. It can also influence nematode survival during periods of stress and will certainly influence the species composition of nematode communities. The effect of fertilizer practice and of water availability has also been mentioned, but these in turn will interact with host genotype and husbandry factors such as spacing and time of planting. Recent studies of potato cyst nematodes

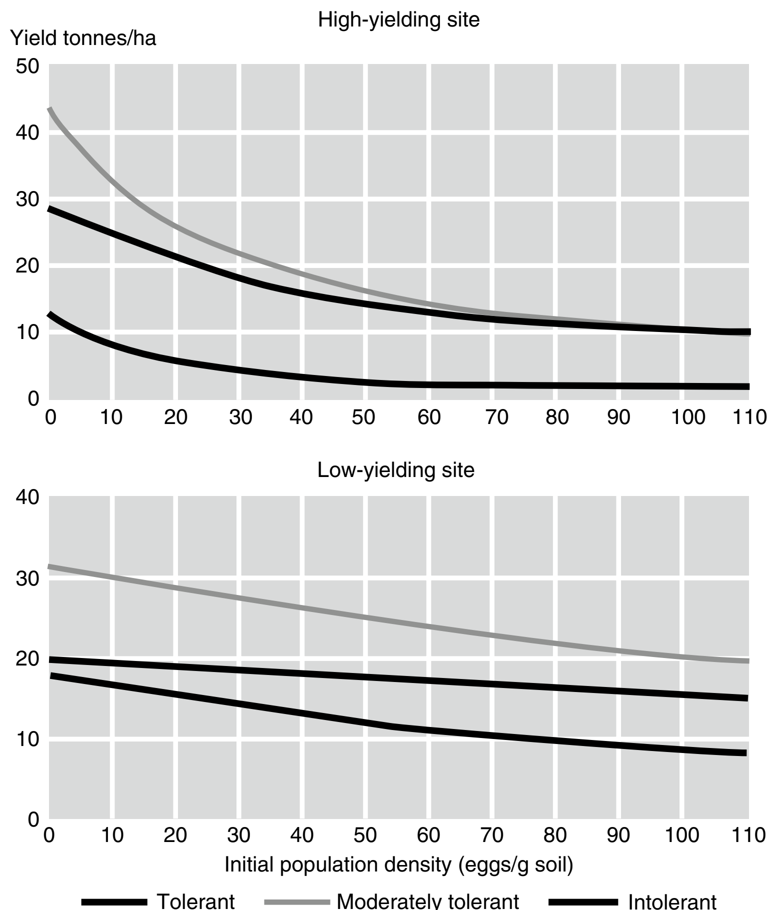

Fig. 7.2 The relationship between initial population of *Globodera pallida* and tuber yield for tolerant, moderately tolerant, and intolerant genotypes at two sites with contrasting yield potential

illustrate some of the interactions and are briefly summarized below.

The interaction between two potato cultivars of different tolerance and rates of compound fertilizer and the nematicide aldicarb was studied at a site with a sandy soil (Trudgill 1987). In this trial, the site was uniformly heavily infested with *Globodera pallida*, and the tolerant cv. Cara produced tops that were generally twice the size of intolerant cv. Pentland Dell. Consequently, Cara tended to produce many more leaves than were required to give 100 % ground cover. The yield of the Cara was increased equally by a half and a full rate of aldicarb, whereas that of the Pentland Dell was increased more by the full rate. Similarly, increasing rates of fertilizer proportionally increased the yield of Pentland Dell untreated with nematicide more than it did that of treated Pentland Dell or untreated Cara. This trial and several others showed that initially the *G. pallida* proportionally decreased the top growth of both cultivars to the same degree, supporting the basic proportional model proposed by Seinhorst.

A series of five trials on different soil types tested the same five potato genotypes in plots with a wide range of initial populations (Pi) of *G. pallida.* Excellent regressions between Pi and tuber yields were produced (Fig. 7.2) revealing differences in tolerance between genotypes and in overall rates of yield reduction at the different sites. Further analysis showed that variations from a basic model similar to a simplified Seinhorst curve (without T or m) could be partitioned into genotype and site effects. The former were common across sites and the latter across genotypes.

This information provides the basis for predicting the effects of *G. pallida* on the tuber yields of different cultivars classified on their

degree of tolerance and of sites classified by their soil type. However, the losses are still predicted as a proportion of the nematode-free yield. To have a prediction of the actual loss in tons per hectare requires an estimate of the yield potential of the cultivar and site, which requires yet further modeling. Only with this information can yield losses be accurately quantified in financial terms and the tolerance limit identified. The alternative is to extrapolate from the available trial data and make allowances on the basis of experience for the obvious possible environmental influences. Methods of estimating yield loss are therefore of central importance and are considered below.

7.3.8 Methods of Estimating Yield Losses

Pot studies can be used to determine some of the basic information on yield–loss relationships, but because of environmental differences and interactions, field studies are essential. There are two approaches, one is to use nematicides at relatively uniformly infested sites and the other is to work at sites with a range of population densities but which are uniform in other respects. A combination of both approaches is often a better compromise. The former gives practical information on the effectiveness and potential value of a particular treatment but tells little about the nature of the relationship. It also suffers from the criticism that nematicides have a range of side effects. The latter has the benefit of producing information on the relationship between Pi and yield, but it requires experimental errors to be minimized. Because Pi estimates have large errors, accuracy is improved by reducing plot size and by taking and processing multiple samples from each plot. However, plot size must be large enough to obtain a realistic yield and adequate guard plants are essential.

Another option is to establish many small plots in large but otherwise uniform fields. These can be at random, in a grid pattern or along known trends in Pi. The plots can be split and a nematicide applied to one half. For each plot the Pi and yield are determined. The results will produce a scatter of points, hopefully with yield decreasing as Pi increases. Much of the scatter is due to errors in estimating Pi and yield, and it can be minimized by taking the average of all the results within each error band. Such an approach needs a wide range of initial populations, a uniform field, a large number of plots (100 or more), and the plots to be part of an otherwise uniform crop.

7.3.8.1 Management Measures

Management measures aim to protect the treated crop from damage and to prevent nematode multiplication and so reduce the threat to the next susceptible crop in the rotation. The most cost-effective and successful is the growing of resistant varieties. However, while these will prevent nematode multiplication, they are often as vulnerable to damage as a susceptible variety. In yield–loss studies, resistant varieties can be a very useful tool for preparing plots with reduced populations without the side effects associated with other treatments. Between vulnerable crops, rotations involving nonhosts are almost essential. Nematicides, whether natural or artificial, are a last resort and should not be used as a crutch to compensate for poor management. They are always costly and frequently toxic and environmentally damaging. However, their side effects can make them attractive in some situations; the oxime carbamates control a broad range of pests, until they develop resistance, while the fumigant nematicides release nitrogen, further increasing yields.

7.3.8.2 Multiple-Pest Interactions

This is actually a basis for crop loss assessment and nematode management. It is frequently not possible to confidently predict crop losses due to nematodes based solely on soil and root sample information of nematode population density, because of the uncertainty of the interactions between plant-parasitic nematodes and their environment and with other pest species. Much is known about the impact of specific pests, agronomic inputs, and environmental factors on plant growth when they are manipulated and studied separately. Less is known about the combined action of various pests and the effects interacting plant stresses have on pest populations or the rates at which these populations develop. In particular, prediction of crop loss for advisory purposes must

be able to partition and account for the interaction of multiple pests under varying agronomic practices and conditions. Crop loss information from the total pest complex forms the basis for rational or optimal farm, crop, and pest management decisions. In this way pesticide use can be most efficiently and prudently prescribed.

During development, plants are exposed to different levels and complexes of competing pests. For example, many kinds of nematodes and fungi are generally present in the soil and their populations may be assessed prior to planting. Other pests, including insects, weeds, and certain fungi and bacteria, arrive and are assessed much later in the growth of the crop. The timing of pest attacks, whether they occur simultaneously, sequentially, or any combination of the two during the development of the plant, can profoundly alter final crop yield.

Individual species of nematodes (root knot, sting, lesion, etc.) seldom occur alone but rather in a community with many other species of plant-parasitic nematodes. The presence of one species may enhance, retard, or have no obvious effect on the population dynamics of another competing nematode species when present on a particular host plant. For other host plants, soil types, and cultural, edaphic, and environmental conditions, the effects of such competitive interactions between nematode species may be very different. Therefore, one cannot extrapolate interaction predictions from one host plant to another. The interactions between nematodes or with other pests may be physical, such as simple competition for food or space, or may be functionally mediated through the plant and represented by a change in food quantity or quality, or in production of antibiotic chemicals.

7.4 Types of Interactions

Changes caused in the plant by one stress factor may indirectly influence the subsequent impact of a second stress factor. Alteration of host plant physiology in response to nematode parasitism may increase, decrease, or have no apparent effect on the susceptibility of the plant to additional pests. When two or more pests attack a plant, the interaction may be synergistic where the combined effects of the pests are greater than the sum of the effects of each pest acting alone. Multiple-pest associations that cause synergistic increases in yield losses are particularly well documented for nematodes and fungi. The best documented example is the root-knot nematode, *Meloidogyne* spp., and Fusarium wilt disease on old tomato production land. The root-knot nematode, by causing the development of root galls, provides a nutrient-rich food source which the fungi colonize rapidly. Root-knot nematodes can thus significantly enhance disease development and yield loss, elevating primary or secondary pathogens to major pest status even though population levels or pathogenic potential of the fungi was initially very low and yield losses would have been minimal in the absence of the nematode.

In other multiple-pest associations, different pests may interact negatively, so that the combined effects are less than the sum of the effects of each pest acting alone. Direct competition for feeding sites or substrates or effects on host physiology may serve to lessen the full expression of each pest's damage potential. In other cases, the presence of the two or more pests does not appear to increase or decrease yield in relation to the sum of the individual pest effects. The effects are simply the pathogenic potential of each species and the levels to which they are suppressed or enhanced. Ultimately, multiple-pest effects are dependent on a myriad of complex factors, many of which are not well understood or studied.

Periodic measurement of pest population density may also be needed to detect seasonal population changes, since affected tissues and prediction of yield as a function of pest population vary seasonally when different pests and disease-causing organisms are present. For example, highest nutsedge populations frequently occur in the field when moderate to high *Meloidogyne* populations have reduced crop growth and allowed weed development. This further reduces crop yields and increases pest control expenses. The interaction in this case is sequential and illustrates the importance of nematode management programs. Failure to account for

covariation of weed and nematode populations misrepresents the true impact of the nematode on crop productivity even though the weeds, through competition for water, light, and nutrients, caused the additional loss in yield.

7.4.1 Quantifying Nematode Stress

Many factors serve to isolate and maintain certain nematodes within particular locations of the field. As the environment of a particular field changes, so do the relative involvement and pathogenicity of the nematode and pest complex present. For example, as the coarse-particle size content of soils increases, the synergistic interaction between root-knot nematode and certain fungi generally increases. Increasing soil particle size also increases damage from the nematode and fungus alone. Preliminary sampling, which is accomplished prior to harvest or after destruction of the previous crop, is necessary to identify infested areas and ranges in nematode population levels within the field.

Typically many different species of nematodes are recovered from a soil sample submitted for nematode diagnostic and advisory purposes. To formulate a control recommendation, the damage anticipated from the most pathogenic species is first considered. Other less pathogenic species of nematodes present are then ranked and their expected effects related to the damage expected from the most pathogenic species. Their relative pathogenic ratings in terms of the most pathogenic species are then summed across species and population densities to provide a cumulative total of pathogenic equivalents. Since anticipated damage from the most pathogenic species is the benchmark, plant damage is assessed in terms of standardized units of pathogenicity for all nematode species involved.

7.5 Distributional Aspects

The ability to predict crop losses attributable to nematodes and other pests at a field level is based on accurate description of pest density, distribution, and areas where different nematode or pest species occur together. The areas of overlap are important since they form the critical areas for pest interaction. Development of nematode crop loss predictions uniquely determined for individual fields and pest species will undoubtedly await further refinements in many different areas of nematology, including sampling methods, and descriptions of nematode field distribution patterns.

If nematode field distribution were known, field estimates of crop loss including the relative involvement of the species present and recommendations for "spot" treatment could be estimated. For example, in all areas of the field where no species overlap occurs, it would be possible to apply a single damage relationship accounting for each pest by summing over the frequency and density of each pest occurring within each unit area of the field. For areas in which pests overlap, the resultant damage relationship would have to include the individual effects of each pest as well as the interaction term summed over the number of overlapping areas to arrive at an estimate of crop loss. The total loss would then be the simple addition of expected loss for each area of the field with respect to pest density and distribution. For many pest–crop systems, incorporation of the interaction term could significantly improve crop loss prediction by considering synergistic or antagonistic relationships among nematodes and with other pathogenic organisms.

Nematode management strategies may influence other pest species, which in turn can alter the incidence and severity of the disease complex or alter the susceptibility of the plant to other stress factors. In fact, much of the evidence for the involvement of nematodes in disease complexes is based on lower disease severity when nematode was controlled. Most soil fumigant nematicides, applied at specific rates and formulations, can differentially affect nematode and other soilborne pests as well as soil–nutrient relations through their effects on nontarget soil microorganisms. Justification for use of specific formulations and dosage levels of fumigant nematicides could well be based on the diversity

and levels of pests within the field, since many fumigants differentially affect the soilborne pest complex. Similarly, selection of non-fumigant insecticide/nematicides could be based on consideration of their expected effects or levels of injury for all pests that are present.

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8 Nematode Diseases of Horticultural Crops

8.1 Fruit Crops

8.1.1 Banana

About 132 species of nematodes belonging to 54 genera are reported to be associated with banana rhizosphere. In the same order, the burrowing nematode, *Radopholus similis*, followed by the lesion nematode (*Pratylenchus coffeae*), spiral nematodes (*Helicotylenchus erythrinae, H. multicinctus, H. dihystera*), root-knot nematode (*M. incognita, M. javanica*), and reniform nematode (*Rotylenchulus reniformis*), is economically important in most banana-growing areas.

8.1.1.1 Losses Due to Nematodes in Banana

Various species of nematodes infest banana roots with adverse consequences. Losses to nematodes in banana plantations average 20 % and reach 40 % or more where frequent tropical storms topple plants with rotten, nematode-infested root systems. Some localities also record these nematodes as major pests which include *Meloidogyne arenaria, H. africanus, H. punicae, Hoplolaimus* spp., *P. minutus, P. brachyurus, P. goodeyi, P. penetrans, P. scribneri, P. thornei, Paratrichodorus renifer, Scutellonema brachyurum, S.* mangiferae, *Macroposthonia ornata, Hemicriconemoides cocophilus, Hoplolaimus indicus, Rotylenchulus reniformis, Tylenchorhynchus brevidens, Trichodorus porosus, Xiphinema americanum, X. ensiculiferum, X. basiri,* and *X. insigne.*

8.1.1.2 Weeds as Hosts for Banana Nematodes

Certain weeds can be significant reservoirs of phytonematodes including *R. similis* in banana fields. This information is crucial in devising appropriate nematode management strategies for use with rotation crops or fallow before replanting banana fields with nematode-free planting material. Several weed species have been reported to act as hosts of *R. similis*, 23 were hosts of *Helicotylenchus* spp., 13 were hosts of *Pratylenchu*s spp., 13 were hosts of *Hoplolaimus seinhorsti*, 29 were hosts of *Meloidogyne* spp., and 24 were hosts of *Rotylenchulus reniformis.* The presence of the burrowing nematode was more consistently found within three families, the Euphorbiaceae, Poaceae, and Solanaceae. In some weed species such as *Caladium bicolor*, *Commelina diffusa, Echinochloa colona*, and *Phenax sonneratii*, the levels of nematodes recovered were similar to or greater than the numbers recovered from *Musa* roots.

8.1.1.3 Relationships Among Nematodes, Weevil, and Banana Cultivars

Although a wide range of phytonematodes are reported on banana, *Radopholus similis, Helicotylenchus multicinctus, Pratylenchus* species, *and M. incognita,* are predominant and economically important pests, which together are known as "nematode complex." This nematode complex destroys the suckers along with roots. Such decayed roots are later attacked by secondary

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Fig. 8.1 Infested plants

invaders including insect weevil, *Cosmopolites sordidus*. Nematode damaged suckers are more prone to secondary invasion leading to complete rotting of the suckers.

8.1.1.4 Burrowing Nematode (*Radopholus similis*)

This nematode is widespread in the tropical and subtropical banana-growing areas of the world. The disease caused by *Radopholus similis* in banana is known by different names such as toppling-over disease, *Radopholus* root rot, blackhead, and banana decline. The nematode also infects pepper, coconut, areca nut, coffee, and sugarcane and causes serious yield losses.

The nematode has migratory endoparasitic feeding habit. All the stages are vermiform in shape throughout the life cycle. Sexual dimorphism is clear and adult males are not parasitic. Preadult and adult female nematodes enter through epidermal cells by puncturing the cell along the entire length of the root system. The nematode occupies an intercellular position within the cortex. The nematodes feed on the cell contents causing cell walls to break down. The destruction of cells by continuous nematode feeding results in the formation of tunnels and cavities in the cortex. If necrosis accompanies breakdown of cells, the nematodes migrate to fresh tissue in advance of necrosis or move out of the root into the soil to reinfest the root at another site.

Symptoms

The nematode induces reddish-brown cortical lesions on infested roots. The destruction of the distal portion of the root system reduces root mass resulting in dislocated anchorage of the plant. The nematode also destroys the parenchymatous cells producing cavities in the root cortex. The nematodes do not enter the stele. The heavily infested banana plants are uprooted by high winds. The nematode causes purplish streak lesions on young roots. Infested plants develop yellowing in the outer whorls of leaves first, which gradually extend to inner whorls in 7–10 days. These leaves and bunches later wither and drop, leading to the death of the plant (Fig. 8.1). Formation of deep extensive lesions, cavities, and tunnels in the roots is a common characteristic symptom of this nematode. Attacked roots become rotten and

Fig. 8.2 Infested sucker and roots

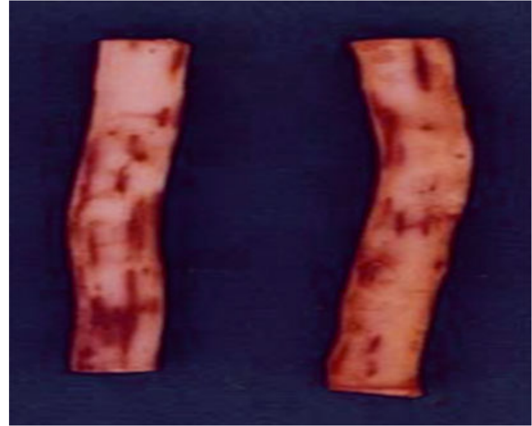

Fig. 8.3 Lesions on root

show little or no side roots. Rotten patches are also seen on the rhizomes (Fig. 8.2).

In the advanced stages, the rotting extends into the pseudostem. The infested cortical region turns brownish and decays. Nematode damage first appears in scattered patches in the first crop which later coalesce, and the infestation spreads to more areas. In about 5 years time, the population spreads to the entire fields creating substantial impact on the growth parameters of the standing crop under monoculture. The survival and multiplication of the nematode deep in the cortex of rhizomes or planting material help the faster spread to new, healthy areas. The suckers removed for transplanting from nematode-infected banana clumps and planted in new areas produce infected plants.

8.1.1.5 Root Lesion Nematode (*Pratylenchus* spp.)

Root lesion nematodes are the next major nematode pests on banana which are widespread in their distribution and cause considerable damage to banana crop. The lesions caused by lesion nematodes are almost similar to *R. similis* but less extensive than that of *R. similis* (Fig. 8.3.). The initial entry of nematode produces reddish elongated flecks in the cortex which later enlarge due to their continuous feeding. The older parts of the lesion turn black and shrink, while the advancing margin remains red. The infested roots develop black necrotic lesions with surface cracks. The colonized adventitious roots produce large cavities in the cortical parenchyma. The plants are stunted and twisted with discolored roots having no secondary roots, the discoloration extending into the cortex and stele. *P coffeae* and *P. zeae*, the two principal lesion nematodes of banana, are also reported on crops like citrus, coffee, cassia, tobacco, and rose.

8.1.1.6 Root-Knot Nematodes (*Meloidogyne* spp.)

The root-knot nematodes, *Meloidogyne incognita* and *M. javanica*, are emerging as major nematode parasites of banana in many parts of the world, although they were found frequently associated with banana rhizosphere, earlier. They penetrate into the endodermis of roots and later into the stele where they induce multinucleate giant cells. In heavily infested roots, conduction elements are blocked. The most obvious symptom is swollen roots and galls produced on primary and secondary roots, whereas the roots free from nematodes appear white in color without galls (Fig. 8.4). Profuse galling with egg masses is observed when the roots are cut longitudinally. Affected plants are stunted with poor plant development, and wilting may be seen occurring

Fig. 8.4 Knots on the roots

on hot days. Infested plants appear thirsty and sickly with drooping leaves, premature senescence, and drying of leaves. Most infected plants, in general, cannot throw out flower bunches.

8.1.1.7 Spiral Nematode (*Helicotylenchus* spp.)

The spiral nematode is found to infest all varieties of banana through the tropics and subtropics. Among the 17 major species, *H. multicinctus, H. dihystera, H. africanus*, *and H. erythrinae* are the most important damaging ones. *H. multicinctus* is a major species damaging banana. They tend to feed close to the surface of the roots than either burrowing or lesion nematodes. They are normally seen just under the epidermis of the root and do not penetrate into the cortex as seen in the case of lesions produced by *R. similis* and *P. coffeae*. The damage caused by spiral nematodes is often overshadowed by *R. similis*. The adult and larval stages penetrate into the epidermis of the root and feed directly on parenchyma cells, and in about 4 days, the nematode penetrate up to 4–6 cells deep in the cortex. The head of the nematode is oriented parallel to the long axis of the root with its posterior portion curved. Due to the feeding, the cell walls are distorted or ruptured with elongated nucleus. Evacuated cells become necrotic and discolored with brown discrete necrotic lesions. Besides banana, the nematode infests cocoa, sweet potato, citrus, sugarcane, coffee, maize, etc.

8.1.1.8 Reniform Nematode (*Rotylenchulus reniformis*)

Reniform nematode can induce lesions around the feeding site of the banana roots. *R. reniformis* penetrate into the cortex at right angles to the stele and establishes a permanent feeding site in the endodermis. It feeds on the fusion of endodermal, pericycle, and vascular parenchymal cells to form a syncytium with hypertrophied nuclei and prominent nucleoli. Sections of the banana root are infested concomitantly with the endodermal and pericycle cells at the periphery of stele and that of *M. incognita* inducing giant cells in the vascular cells of differentiating parenchyma in the central part of the stele.

8.1.1.9 Interactions with Other Microorganisms

Fungi, in association with nematodes, are known to aggravate the initial nematode damage or inhibit the nematode multiplication. Incidence and losses due to *Fusarium* wilt caused by *F. oxysporum* f.sp. *cubense* is enhanced in association with *R. similis, P. coffeae*, *and M. incognita.* In the presence of the nematodes, the severity of the pathogens increases with the host succumbing to the injury much earlier than when the fungal pathogen alone is present.

Several studies have been conducted on this complex in banana. *Fusarium oxysporum* f.sp. *cubense* could cause the “Panama” disease of banana in the presence of *R. similis*. In most investigations, *F. oxysporum* f.sp. *cubense* and *Rhizoctonia solani* were isolated from the cortical lesions of banana roots infested with *R. similis*. The interaction *of F. oxysporum* f.sp. *cubense* and *R. similis* in the formation of lesions indicated that *R. similis* was the primary pathogen of root rot, creating conditions favorable for weak fungal pathogens and thereby increasing the invasive potential of these weak fungal plant pathogens. *Fusarium oxysporum* f.sp. *cubense* failed to cause root lesions but colonized parenchyma cells of the cortex which were initially wounded either mechanically or by *R. similis*. The fungus was unable to invade living unwounded healthy cells of lateral roots of banana. Lesions formed after inoculation by both *R. similis* and *F. oxysporum*

f.sp. *cubense* were more extensively necrotic and increased their area more rapidly than when *R. similis* alone was the incitant. In the presence of *F. oxysporum* f.sp. *cubense,* the burrowing nematode migrated away from the necrotic spots usually by invading healthy peripheral cells or by moving out of the roots into the surrounding soil. *F. solani* and *Rhizoctonia solani* are the predominant fungi in nematode and non-nematode lesions of banana roots in many regions. In deep lesions of the main root caused by *R. similis, F. solani* predominated followed closely by *Rhizoctonia solani.* In shallow lesions of *H. multicinctus, R. solani* predominated whereas, in necrotic feeder roots, *R. solani* predominated, while in root tip dieback, *F. solani* was of common occurrence.

8.1.1.10 Management of Nematode Complex in Banana

The objective of nematode management is to improve plant growth and also yield. This can be achieved through a reduction of the nematode population in soil and the plant.

Nonchemical

Effective prophylactic measures through appropriate cultural practices and intercropping reduce the initial inoculum level and curb the progressive multiplication of the nematodes. Use of nematode-free planting materials is an important prophylactic measure to manage nematodes. Appropriate cultural measures are needed to reduce the losses caused by nematodes.

Disinfestations of Banana Corms/Rhizomes

Paring: It is an important practice to be done at planting time which includes shaving off the infested, discolored outer skin of the infested corm till a white, healthy portion of the corm is seen.

Pralinage: This practice involves dipping the pared corms in a mud/clay-slurry mixture/cow dung slurry, followed by sprinkling carbofuran granules (20–40 g depending on the size of the corm) over the corm and before planting.

Hot Water Treatment: Treating the suckers in hot water at 55 °C for 20 min helps to eliminate the nematodes that are present inside the banana rhizomes.

Cultural

- Crop rotation with a nonhost like paddy, sugarcane, green gram, sunn hemp, cotton, and *Panicum maximum. Phaseolus atropurpureus* and *Tagetes patula* help in reducing the nematode populations.
- Application of neem cake at 500 g/plant at planting and second application after 120 days or fallow associated with application of 25 tons of castor cake per hectare help in suppressing the nematode populations.
- Growing antagonistic plants like *Tagetes erecta*, *T. patula,* and *Crotalaria juncea* as intercrop in banana orchards drastically minimizes the nematode populations.
- Intercropping with radish, marigold, lucerne, and *Crotalaria* or sunn hemp.
- Mulching with black polythene.

  Resistant Varieties: Some varieties possess field tolerance/resistance, i.e., Kadali, Octoman, Then Kunnan, Goldfinger, Tongat, Dudhsagar, China Kurlan, Yelakki Bale, Pidi Monthan, Pisang Jari Buaya, Karpuravalli, Monthan Nattupoovan, Kunnan, Beecha, Paka, Annaikomban, Pey Kunnan, Singhlal, and Manik Champa (Parvatha Reddy et al. 1989; Ravichandra and Krishnappa 1985)

8.1.1.11 Nematode-Resistant Transgenic Bananas

Genetically modified nematode-resistant banana would overcome many of the risks associated with nematicide use and be safe for consumers.

Biological

- Soil incorporation with *Trichoderma harzianum/T. viride* at 250 g/plant along with 400 g neem cake
- *Pseudomonas fluorescens* at 20 g/plant along with 2 kg FYM at the time of planting and later at an interval of 3 months
- *Glomus fasciculatum* 100 g/plant along with neem cake at 200 g/plant

Chemical

Although several chemicals have been used to manage the nematodes infesting banana, some are widely used and have become popular.

- Bare root-dip treatment with carbofuran/fensulfothion at 750 ppm for 10 min/phorate at 50 ppm for 15–30 min.
- Main field application with Phenamiphos at 3 kg a.i./ha (Parvatha Reddy 2008).
- Phenamiphos at 36 g/stool, applied thrice in a year; 20–30 g/plant of carborfuran, ethoprop, or aldicarb for a period of 4 months from the time of application; aldicarb, carbofuran, DBCP, fensulfothion, and oxamyl, all at 4 g a.i./plant applied thrice at planting and again 4 months later; and cadusaphos at 1 g a.i./ha.
- However, carbofuran application at 30 g/plant and another dose at 30 g/plant after 3 months of planting is effective in most locations.

Integrated Approach

Various combinations have proven effective against nematodes infecting banana. Some are given below.

- Paring followed by a dip in monocrotophos solution at 0.5 % for 30 min and later dried under shade for 72 h before planting.
- For tissue culture plants, 10 g/plant with carbofuran at planting and 20 kg at third and fifth months of planting.
- Hot water treatment of pared banana suckers at 55 °C for 20 min. and application of neem cake at 1 kg/plant and carbofuran 3G at 16.6 g/plant at the time of planting reduce the population of banana nematode complex (*R. similis, Helicotylenchus multicinctus*, and *Meloidogyne incognita*).
- Paring of suckers + hot water treatment at 55 °C for 20 min + application of carbofuran 3G at 16.6 g/pit + neem cake at 1 kg/pit.

8.1.2 Citrus

Although more than ten species of phytophagous nematodes have been proved pathogenic to citrus, the citrus nematode, burrowing nematode, root-knot nematodes, and root lesion nematode are considered important. Other nematodes include *Belonolaimus longicaudatus, Hemicycliophora arenaria, Hoplolaimus indicus, Rotylenchulus reniformis, Rotylenchus orientalis, Criconemoides citri, C. parvatum, Criconemalaterale* spp., *Longidorus brevicandatus, Xiphinema* spp., *Paratrichodorus minor*, *and P. porosus.* Populations of the citrus nematode usually are higher in older orchards (6 years and older). Young trees also may be injured if planted in orchard sites where nematodes previously prevailed. Since the presence of the citrus nematode cannot be detected visually, positive diagnosis is based on external symptoms and laboratory examination of root and soil samples. Aboveground symptoms associated with nematode-infected trees include wilting, lack of vigor, poor fruit production, and poor response to watering and fertilization.

8.1.2.1 Symptoms

Most nematode species that are known pathogens of citrus do not actually kill the citrus tree but can significantly reduce tree vigor, growth, and grove productivity. Nematode-infested trees generally grow more slowly and may ultimately be of smaller size and quality. Aboveground symptoms which develop as a result of damage to roots include thinner canopies with less new foliar growth and twig dieback within the upper tree canopy. Symptoms of decline frequently increase with time and are more apparent during periods of environmental stress or when combined with other damaging soil pests (i.e., root weevils, *Phytophthora*). The young adult females penetrate the root, producing a gelatinous matrix in which the eggs are embedded. These hatch and the second-stage juveniles attack roots. *Tylenchulus semipenetrans* occurs worldwide on citrus. It is also a pest on crops like grape, lilac, olive, and persimmon.

8.1.2.2 Citrus Nematode (*Tylenchulus semipenetrans*)

T. semipenetrans induces slow decline of citrus, which is known to be prevalent in all the citrus-growing areas the world over. Most of the commercial citrus rootstocks are attacked by this nematode.

Damage

Damage caused by a citrus nematode infestation depends on the age and vigor of the tree, density

Fig. 8.5 Symptoms on citrus

of the nematode population, and susceptibility of the rootstock. Mature trees can tolerate a considerable number of these nematodes before showing lack of vigor and decline symptoms. In heavily infested sites, young trees may be stunted or fruit production may be reduced on bearing trees that have susceptible rootstocks. The damage is greater when trees are predisposed by other factors such as *Phytophthora* root rot and water stress.

Symptoms

The aboveground symptoms include the loss of plant vigor and yellowing of leaves; general symptoms of malnutrition like sparse foliage, small nonuniform fruits, and defoliated branch ends; and gradual dieback of twigs. The symptoms collectively are known as "slow wilt" (Fig. 8.5). The symptoms are particularly noticeable in the uppermost portion of the tree. The symptoms are particularly noticeable in the uppermost portion of the tree. Belowground symptoms include curing and distortion of feeder roots, soil particle adherence to infected roots (due to gelatinous matrix secreted by the female) giving a muddy and thick appearance, cortex peeled off in extensive infections exposing the central eyeliner, and necrotic strips in secondary infections by other microorganisms.

If nematode-free citrus seedlings are planted in infested soil, symptoms of decline appear as early as 5 years after planting. Conversely, infected nursery trees planted in citrus nematode-free soil grow well for several years till the nematode population attains damaging levels of approximately 40,000 per 10 g of roots. The decline symptoms appear in 12–17 years when such a level is attained.

Management

Sanitation: Once established, nematodes cannot be eradicated from groves; hence, periodic management of populations may be required. Therefore, the best method to manage plant-parasitic nematodes in new plantings is to exclude them from a grove by using only certified trees from nurseries. Use of certified trees will virtually eliminate the possibility of nematode problems in new groves planted in virgin soils or in old citrus soils never infested by nematodes, provided that care is taken to always use clean equipment in those groves. Use of certified trees also reduces damage during the early years of growth in old, previously infested groves if soil nematode populations are low. High soil nematode densities hinder the beneficial effects of the use of certified trees. Sanitation of equipment to remove soil and root debris before moving between groves is an effective means of preventing the spread of nematodes.

Cultural

- Use of healthy seedlings/cuttings, never to establish nurseries near old citrus orchards.
- Sterilization of nursery soil before planting.
- Avoid spread through tools, machinery, and irrigation water.
- Soil application of oil cakes (castor, neem, mahua, groundnut, mustard) at 20 kg/tree basin at an interval of 4 months.
- Intercropping with marigold/mustard or onion/garlic.

Proper grove management is critical to mitigate damage caused by phytonematodes. There is no value to managing nematodes if other problems like poor soil drainage, insufficient irrigation, foot rot and fibrous root rot, root weevils, improper fertilization, and poor disease control limit root function and/or reduce tree quality.

Physical

Bare root dips in hot water at 45 °C for 25 min or at 46.7 °C for 10 min.

Resistant Varieties

Use of resistant rootstocks like trifoliate orange (*Poncirus trifoliata*) and its hybrids (Citrumello) rootstocks evolved at the India Horticultural Research Institute, Bangalore, Karnataka, India, by crossing Rangpur lime (*Citrus limonia*) with *P. trifoliata*, viz., CRH-3, CRH-5, and CRH-41 are resistant ones. Swingle citrumelo is a widely planted rootstock with resistance to citrus nematode.

Biological

- *P. fluorescens* at 20 g/tree applied thrice in a year at 15 cm depth and 50 cm away from tree trunk (Parvatha Reddy 2008)
- Avermectins at 1.1 kg a.i./ha for 7 months
- *Glomus fasciculatum/G. mossae* at 50–100 g/plant

Chemical

- Nursery soil application with aldicarb/carbofuran at 4 kg a.i./ha
- Seedling bare root dip with chlorpyrifos/monocrotophos at 1,000 ppm for 45 min
- Carbofuran/aldicarb/ethoprophos/dichlofenthion/phorate at 4–6 kg a.i./ha
- Aldicarb at 33 lb/acre applied in band along the drip line on both sides of the tree row by spreading granules uniformly and immediately working into soil (preferred method) or shank 2–3 in. deep using 4–6 shanks on 12-in. centers.

8.1.2.3 Burrowing Nematode (*Radopholus citrophilus*)

The occurrence of this nematode on citrus is restricted to deep, sandy soils where it causes "spreading decline" disease resulting in reduction in yield of grapefruits and oranges.

Symptoms

Aboveground symptoms include small, sparse leaves of infected trees, with low nutrient level; branches and twigs showing slow dieback; infected groves showing the same degree of decline; and small fruits resulting in low yields. The disease is capable of spreading at an average rate of 15.2 m per year, hence the name "spreading decline." The belowground symptoms include destruction of apical meristem and arrest of terminal growth of roots leading to stubby appearance, swelling of root tips and reduction in functional feeder roots, and nematode infection sites turning brown black. An infected tree can have a reduction of functional feeder roots to an extent of 50 % or more.

8.1.2.4 Root Lesion Nematode (*Pratylenchus* spp.)

Three species are considered economically important on citrus, viz., *Pratylenchus brachyurus, P. coffeae*, and *P. vulnus.*

Symptoms

P. vulnus has been reported to cause severe stunting of *Citrus aurantium* seedlings. It causes growth reduction in citrus seedlings. Damage to young trees is more pronounced than older ones. *P. coffeae* has been reported to be pathogenic to citrus from several countries. The damage to the root system is characterized by brown spots followed by necrosis. More than 125 citrus species are known to be the hosts of *P. coffeae.*

Management

1. In the case of burrowing nematodes, specific cultural practices (avoidance of disking, frequent irrigation, and fertigation) are critical to maintain a vigorous root system in the shallow soil horizons where the nematode is much less active.
2. Resistant rootstocks are also available to manage burrowing nematodes. Milam lemon, Ridge Pineapple, and Kuharski or Carrizo citrange are all resistant to burrowing nematode. The existence of races of these nematodes capable of breaking resistance compromises their value somewhat; nevertheless, large numbers of groves are currently growing well

on resistant rootstocks in the presence of these nematodes.
3. Use of resistant lines like *Poncirus trifoliata,* four selections of *Microcitrus australis* and *M. australasica*, and Rubidoux 70-A5 trifoliate orange.

Chemicals

Application with fensulfothion/phenamiphos/carbofuran at 4.4 kg a.i./ha is effective; in young orange orchards on replant soils, up to three successive applications of cadusafos at 1.5 g/sq.m in spring-summer; and single application of cadusafos at 3.0 g/sq.m before mulching.

Biofumigation

Soil-incorporated residues of commercial *Brassica* cultivars and the weed *B. tournefortii* can reduce soil population of *T. semipenetrans* by up to 81 % as observed in various laboratory experiments. Humus rape, followed by Rangi, Arran, and Hobson rapes, and Simax hybrid were most effective. *T. semipenetrans* was undetectable in soil amended with these biofumigant crops.

8.1.2.5 Other Nematodes

Damage to citrus crops by *Belonolaimus longicaudatus, Hemicycliophora arenaria, H. nudata, Trichodrus* species, *Paratrichodorus minor, P. porosus, Xiphinema americanum, X. brevicolle*, and *X. index* has also been reported from several regions.

8.1.3 Grapes

Root-knot nematodes, root lesion nematode, and dagger nematodes are the major pathogens of grapevine in various parts of the world. Other nematodes usually associated with rhizosphere of grapes include *Tylenchulus semipenetrans, Paratrichodorus minor, P. pachydermis, Helicotylenchus dihystera, H. pseudorobustus, Rotylenchus gracilidens, Rotylenchulus reniformis, Paratylenchus hamatus, P. neoamblycephalus, Criconemella xenoplax, Longidorus attenuates*, and *L. coespiticola* (Al Banna and Gardner 1993).

Fig. 8.6 Knots on the roots

8.1.3.1 Root-Knot Nematode (*Meloidogyne* spp.)

Three species of root-knot nematodes, viz., *Meloidogyne incognita, M. javanica*, and *M. arenaria*, have been recognized as major pests of grapes, causing economic damage. These are worldwide in distribution. Other species associated with grapevine is *M. thamesi.*

Symptoms

The root-knot infestation is not manifested by typical aboveground symptoms. The visibly unthrifty growth has invariably been erroneously attributed to moisture stress, low fertility, and salinity conditions. Patches of poorly branched vines with scant foliage, pale and small leaves, and poor bearing are the symptoms. In young plants, premature decline and weak vegetative growth are also seen (Argelis 1987). The root system shows localized swellings particularly on the feeder roots, young secondary roots, and root tips (Fig. 8.6). Numerous females were found on the internodal trunk just below the ground level. *M. incognita* has been reported to stimulate the production of many new rootlets above the infection site, resulting into hairy root conditions. Varied sizes and shapes of galls are formed depending upon the grape variety. Distortion of normal root system is the damaging symptom.

In resistant varieties, dissolution of middle lamella in some cells surrounding the nematode head has been reported. Also, due to energy demand for nematode growth and reproduction, the productivity of the vine may be reduced. Normally nematode causes more damage to susceptible cultivars and fewer eggs are produced in resistant cultivar. Nematode normally does not affect the concentration of reducing sugar at nematode feeding sites on susceptible and moderately susceptible cultivars, whereas nonreducing sugars are increased in susceptible and decreases in moderately susceptible cultivar, indicating that there is more translocation of photosynthesis to the feeding sites of susceptible cultivar than resistant.

Fig. 8.7 Knots on roots

8.1.3.2 Root Lesion Nematode (*Pratylenchus* spp.)

Pratylenchus vulnus is considered to be economically important on grapevines. Other species include *P. scribneri* and *P. minyus.*

Symptoms

P. vulnus produces lesions of various sizes and color throughout the root system resulting in loss of vigor and affecting production. Lesions initially appear as brown spots turning black in the later stages and ultimately girdling the roots. Reduction in fruit production, weakening of young vines, failing to establish root system, and gradual death are some of the other major symptoms. Nematode penetration is restricted to cortical tissues only. *P. vulnus* infection has been reported to reduce the uptake of potassium and zinc.

8.1.3.3 Dagger Nematode (*Xiphinema* spp.)

X. americanum and *X. index* are the important species of this genus. Other species infecting grapevines are *X. diversicaudatum, X. mediterraneum, X. vuittenezi, X. brevicoides, X. italiae, X. elongatus, X. aurcicum*, and *X. turcicum.*

Symptoms

Feeding by dagger nematodes on grape roots results in terminal swellings and cessation of root elongation causing distortion and bending. Discoloration, decay, and enormous branching of roots occur at later stages. *X. index* rarely feeds on root tips. Epidermal and outer cortical cells collapse at feeding site and show necrosis. Multinucleate enlarged cells have been reported beneath the layer of necrotic cells (Fig. 8.7). Leftover roots of dead and old plants in vineyards serve as source of vital inoculum for the nematode year after year.

X. index is reported to transmit fanleaf virus/yellow mosaic virus/vein banding disease in grapes (GVFV-GVYMV), whereas *X. americanum* is known to be the vector of tobacco mosaic virus, yellow vein virus, tomato ring spot virus, and peach rosette mosaic virus. *X. italiae, X. diversicaudatum*, and *Longidorus attenuatus* have also been observed to transmit grapevine viruses experimentally.

8.1.3.4 Other Nematodes

Tylenchulus semipenetrans and *Paratrichodorus minor* have also been reported from several grape-growing regions.

8.1.3.5 Nematode Management in Established Vineyards

Avoidance

Movement of contaminated grape-harvesting equipment and tractors from a site infected with *X. index* should be avoided (Edwards 1991).

Biomanagement

The addition of biological agents to soil has been found effective in several instances, while they have given inconsistent results in many cases. It may be possible, however, with the advent of drip irrigation systems, to apply microbe-produced toxins directly to the soil in irrigation water.

Cultural

By reducing vine stress through more frequent irrigation, the damage caused by nematodes can be reduced. The use of grassy cover crops in vineyards infested with *X. index* should be studied. However, legume cover crops in vineyards should be monitored to assess population levels of *Mesocriconema xenoplax,* which may increase. Populations of *X. americanum* will also increase on most cover crop selections. The difficulty in choice of cover crops is that the host range of most ectoparasites is quite broad.

Nematode species with long bodies tend to be in shallow and non-disturbed sites of a vineyard, so placement of any treatment is important. Tillage and soil disturbance can reduce population levels in short periods of time, but root surface area is also reduced. Drip-irrigation-applied fertilizers that release ammonia may reduce population levels of ectoparasitic nematodes when applied repeatedly. Fifteen kilogram per hectare of nitrogen in urea salt when reapplied three to five times at 30- to 45-day intervals can reduce population levels of most ectoparasites by half. More field testing of these strategies is necessary. Since grapevines do not have a high nitrogen requirement and some vineyards already have excess nitrogen, this technique should be tested and implemented with caution.

Chemical

Organophosphate and carbamate chemicals are normally lethal to ectoparasitic nematodes when used as single treatments at high rates via drip irrigation. Treatments with currently available commercial nematicides only reduce populations about 50 % for 6–8 months after treatment. Multiple treatments with low rates of phenomiphos (1 kg/ha) are ineffective against ectoparasites. When multiple treatments are used against endoparasites for several years, population levels of ectoparasites such as *X. americanum* can be observed to increase above the nontreated population levels. In the case of ectoparasites, the value of systemic chemicals as nematicides will be minimal unless the toxicant is available during feeding or leaks out into the rhizosphere (Edwards 1991).

8.1.3.6 Management in Brief

Cultural

Use of nematode-free planting material is an effective practice. Intercropping with *Tagetes patula* also can minimize nematode population. Soil amendments with mustard oil cake (500 kg/ha) or margosa leaves (300 kg/ha) are effective against the nematode.

Physical

Hot water treatment of the planting material at 53 °C for 30 min or 52 °C for 10 min or 50 °C for 30 min helps in disinfesting the material.

Resistance

Use of resistant varieties is a popular method. *Example*: Dog Ridge (*Vitis champini*), Salt Creek (*V. champini*), 1613 (*V. solonis* X Othello), Harmony (1613 X Dog Ridge), St. George (*V. rupestris*), AXR[1] (Aroman X Ganzin), Lake Emerald, and Tompa. Varieties like Cardinal, Banquabad, Muscat, Jasbeli, Loose Perlette, and Reisling are resistant to *M. javanica*. A few commercial varieties, namely, Cardinal, Early Muscat, Banquabad, and Loose Perlette, have been recorded very resistant to *M. javanica.*

Chemical

Chemical dips in phenamiphos (1,000 ppm) or oxamyl or carbofuran for 1 h minimizes the nematode population. Single application of aldicarb at 5 kg/ha or oxamyl at 10 kg/ha or carbofuran/phorate/benfurocarb at 6 kg/ha or carbofuran at 13 g/sq.m at budbreaking stage is effective against nematode. Various chemicals that have been found effective in different regions include methyl bromide at 448 kg/ha, 1,3 D at 280 kg/ha, DBCP at 800 kg/ha, DD at 450–1,500 L/ha, Vapam at 562–843L/ha, etc.

8.1.4 Papaya

Major phytonematodes infesting papaya include root-knot nematode (*Meloidogyne incognita, M. javanica, M.* arenaria, and *M. hapla*), and reniform nematode (*Rotylenchulus reniformis*), and *R. parvus.* The root-knot nematode is an economically important plant pathogen and is distributed worldwide (Cohn and Duncan 1990).

Root knot is caused by *Meloidogyne* nematodes. The larvae of these nematodes can travel short distances in soil, finding and attacking papaya roots, usually near the tips. When female larvae feed near the water-conducting core of the roots, the plant cells increase in number and size until readily visible swellings, called galls or "knots," are formed. To confirm a diagnosis, one can cut into the galls and observe pearly looking, pear-shaped female nematodes embedded in the tissue. Leaves of papayas that are affected by root-knot nematodes appear pale green or slightly yellow and are generally unthrifty. They may be more sensitive than normal to water stress. Fruits are smaller than normal and more likely to have an off-flavor.

8.1.4.1 Symptoms

Aboveground symptoms of heavily infected plants appear as moderate to severe leaf chlorosis and plant stunting. Some wilting may occur during periods of peak transpirational stress on the plant. Papaya roots attacked by root-knot nematode show varying degrees of galling depending on the numbers of the nematode in the soil or the subsequent hatching of eggs, migration of the larvae, and reinfection of surrounding tissue (Fig. 8.8). Unlike the reniform nematode, the female root-knot nematode and most of her egg mass are usually completely embedded in the root tissue. Hence, dissection of the root is mandatory before a positive identification can be made. Root systems may be somewhat reduced because terminal infections of roots cause a slight swelling and cessation of further elongation.

Severe galling of roots and stunting of papaya due to root-knot nematodes have been observed primarily in sandy soils. Galling may be so extensive on seedlings that they may be killed as a result. In some loam or clay soils, galling is light to moderate without noticeable aboveground symptoms.

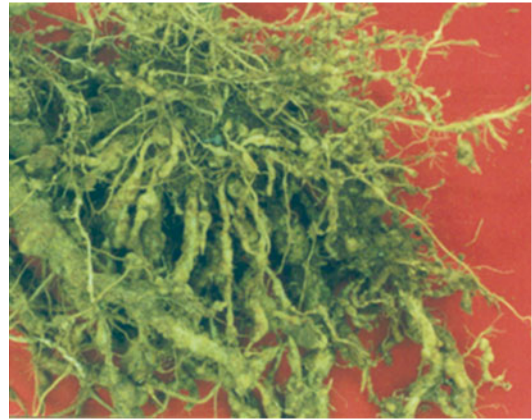

Fig. 8.8 Knots on the roots

8.1.4.2 Management

Physical

Hot water treatment of infested seedling roots at 50 °C for 10 min

Cultural

Soil application with neem cake at 2.5 t/ha

Biological

T. harzianum and *P. lilacinus* each at 5–10 g/kg soil

Resistance

Pusa 22-3, CO_2, Pusa Delicious, Pusa Dwarf, and Pusa giant are resistant, and Mukund farm, Selection No. 7, Peradeneya, Washington, Pusa 1-15, and Pusa 1-45 are moderately resistant (Parvatha Reddy 2008).

Chemical

Nursery: Carbofuran/phenamiphos at 2 kg a.i./ha 1 day before sowing

Main Field

- Carbofuran/aldicarb at 2 kg a.i./ha
- Carbofuran at 6 kg a.i./ha along with neem cake at 2.5 t/ha
- Preplant fumigation is effective against root-knot nematodes (Ayala et al. 1971). However, consideration must be given to first allow

adequate time for breakdown of heavily galled roots. Moist root tissue is a barrier to the penetration of fumigants. Roots decay readily in moist soils and very slowly in dry ones.

8.1.5 Strawberry

In strawberry production, plant growth and development are affected by a number of nematode species. Plant-parasitic nematodes are closely associated with host plants for most of their lives, and they tend to favor sandy or sandy loam soils.

8.1.5.1 Distribution of Phytonematodes

The species of nematodes which are commonly associated with strawberry are lesion (*Pratylenchus penetrans*), root knot (*Meloidogyne incognita, M. hapla*, and *M. javanica*), foliar (*Aphelenchoides fragariae, A. besseyi*), stem (*Ditylenchus dipsaci*), dagger (*Xiphinema americanum*), and needle (*Longidorus elongatus*) apart from others (Tremblay and Baker 1990). The northern root-knot nematode is found in the soil or as a sedentary endoparasite in roots. The foliar nematode is a parasite of aboveground plant parts and may be endo- or ectoparasitic. Symptoms caused by *A. fragariae* are sometimes called spring crimp, spring dwarf, or strawberry crimp. Sting nematode, *Belonolaimus longicaudatus*, may be of some importance in several locations.

8.1.5.2 Symptoms Due to Belowground Feeders

Symptoms of nematode activity are similar in many crops. Root function is impaired, resulting in poor growth, low yield, deficiency symptoms, and a poor root system often with decay. Root-knot nematodes cause roots to have very small swellings, about 2 mm in diameter, which can be seen with the unaided eye. Lesion nematodes make fine reddish-brown "scratches" 1.5–3.0 mm in length, on the main roots of young plants or on new roots from the crowns of older plants. A laboratory analysis is needed to confirm the presence of nematodes and assess if they are present at levels requiring treatment.

Fig. 8.9 Lesions on the leaf lamina

8.1.5.3 Symptoms Due to Aboveground Feeders

Plant symptoms can be indicative of a nematode problem but are not diagnostic because similar symptoms could result from other problems as well. The symptoms either may be widespread or may appear in small patches within a field. The foliar nematode is a parasite of aboveground plant parts and may be endo- or ectoparasitic. Symptoms caused by *A. fragariae* are sometimes called spring crimp, spring dwarf, or strawberry crimp.

8.1.5.4 Symptoms and Diagnosis

The two most prominent foliar nematodes are *A. ritzemabosi* called the chrysanthemum foliar nematode and *A. fragariae* called the strawberry crimp or fern nematode. Both are serious pathogens of ornamental plants both in the greenhouse and outside in the garden. However, their host ranges rarely overlap except on African violet, begonia, gloxinia, Siberian bugloss, violet, and verbena. The most prominent symptoms occur in the leaves of infected plants. Two interveinal symptom types are commonly found. The first is a development of linear lesions between the leaf veins causing the leaf to become striped in appearance (Fig. 8.9). Plants that have parallel venation are likely to display this leaf pattern. The second leaf symptom which is more common is the development of angular, water-soaked lesions occurring between the veins of netted-veined plants (Jagdale 2002). These lesions will become brown and eventually turn black and perhaps drop out of the leaf leaving a ragged, wind-tattered appearance.

Other symptoms may appear as stunting, leaf proliferation, or bunching of leaves around the crown, multicolored leaves, lack of flowering, and plant death. It is not uncommon to find new leaves emerging without symptoms while older leaves turn brown and collapse or fall. This occurs because the spread of nematodes requires free moisture. Lack of water on the leaves causes them to become localized so that they cannot move to new plant parts. Thus, under dry conditions, new leaves may appear unaffected.

8.1.5.5 Management of Foliar Nematode

Cultural

- *Sanitation:* Remove all infested debris from the plant and ground. This should be buried or burned. Floors and benches of propagation areas should be thoroughly cleaned of debris. Tools and containers should be heat treated in an oven or steamed for 30 min at 180 °F.
- *Destroy Infested Plants:* It is almost impossible to rid the plant of foliar nematodes. Therefore, it is best to destroy infested plants. Plants that are suspected of being infected should be isolated from healthy plants.
- *Select Disease-Free Plants:* Only healthy, nematode-free plants should be purchased and used for propagation stock. Contact between plants and extended periods of free moisture from overhead irrigation should be avoided. Dormant plant material can be treated with warm water (120 °F) for 15 min to eliminate the nematode infestation on valuable planting stock.

Physical

Hot water treatment of strawberry runners at 50 °C for 5 min or at 48 °C for 20 min.

Resistance

George Soltwedel, Regina, Festivalnaya, Saksonka, and Talizman are resistant varieties.

Chemical

Foliar spray with phenamiphos at 4–6 ml/3.8 L of water

Fumigation

Fumigation prior to planting is an effective way to control nematodes. With the phaseout of methyl bromide, the most effective soil fumigation is a sequential application of chloropicrin or 1,3-dichloropropene/chloropicrin followed 5–7 days later by metam sodium or metam potassium. This combination of materials can provide effective control of weeds as well as soilborne pathogens, soil insects, and nematodes.

Belonolaimus longicaudatus has subsequently become recognized as one of the most economically important nematode crop pest on strawberry. Based on evidence from other crops, parasitism by *B. longicaudatus* has also been shown to interact with other soilborne pests, causing a greater incidence and severity of certain fungal diseases, most notably *Fusarium* and *Pythium*. The sting nematode has such a preference for sandy soils that it fails to exist in significant numbers in soils containing even small amounts of silt, clay, or organic matter content. Sting nematode reproduction is greatest in sandy soil, at temperatures of 25–30 °C with constant, but moderate, moisture levels. Under suitable conditions, a life cycle is completed in about 28 days. The higher numbers and greater distribution of sting nematode are probably not only related to the predominance of fine sandy soil but also due to the warm subtropical environment. In addition, sting nematode appears to be very sensitive to sudden changes in soil conditions such as rapid drying.

8.1.5.6 Symptoms

Strawberry production problems caused by sting nematode tend to occur in more or less definite areas where transplants fail to grow off normally. Infested areas consist of spots that vary in size and shape, but the boundary between diseased and healthy plants usually is fairly well defined. Initially a field may have only a few such areas, which may then increase in size and number until the entire field becomes involved. The effect on strawberries is to cause both stunting and decline, the intensity of which is related to initial population level and the rates to which populations increase during the course of strawberry crop

Fig. 8.10 Symptoms due to sting nematode

growth. Affected plants become semidormant, with little or no new growth. Leaf edges turn brown, progressing or expanding from the edges to midrib to include the entire leaf (Fig. 8.10) (Noling 2012). Leaves seldom become chlorotic, although cases have been reported in which leaf yellowing occurs when essential nutrients are present in limited supply. Since the outer older leaves die first, the plant gradually decreases in size and eventually may be killed.

Sting nematode can be very damaging to nursery seedlings and transplants. As a general rule, most other crop plants are not killed unless subjected to other adverse conditions, but affected strawberry plants undergo progressive decline and may eventually die. Older plants that have already developed an extensive root system can still be severely affected. Under field conditions, instances are common where sting nematodes have caused only minor root system damage in the upper 3–4 in. of soil. In this soil zone, plants can develop a dense root system, but no roots are able to penetrate below this upper layer. Such plants can be easily lifted or pulled from soil and are much more susceptible to droughty conditions and injury from fertilizer salt accumulations.

Root growth abnormalities are caused, in part, by injury inflicted at the root tips, which results in little or no new root growth, plants lacking in fine feeder roots, and the development of short stubby branches. Root tips are killed, forcing the development of new lateral roots, whose root tips in turn are killed. This results in the production of a root system consisting of coarse roots with knobby tips. In addition, necrotic lesions may also be produced laterally along the sides of roots. Since sting nematode does not feed internally, the usual microscopic examination of diseased roots does not aid diagnosis since no organism is present within roots. Positive confirmation of sting nematode can only be made by soil examination.

8.1.5.7 Management of Sting Nematode

Nematode management should be viewed first and foremost as a year-round, programmatic activity requiring consideration of all cultural, chemical, and agronomic practices within the areas where strawberry plants will be grown. Because strawberry must be vegetatively propagated and transplanted into the field, growers must first pay special attention to the source of strawberry transplants to ensure they are not infested with nematodes. Growers should use only the best quality transplants available. After final harvest, the crop should be destroyed as quickly as possible to remove nematode food sources. In most cases, delays in crop destruction contribute to greater nematode population increase and greater difficulty in achieving nematode control. Fallowing for even short durations therefore, particularly when coupled with early crop destruction, generally gives significant and immediate reduction in total nematode population densities in soil. To extend the fallow period, frequent tillage of the field may be required to maintain a clean, weed-free, fallow soil condition.

As an alternative to summer fallowing, crop rotation with a poor or nonhost crop can be another effective means for reducing soil populations of sting nematode. Cover crop rotations with American joint vetch, hairy indigo, or showy crotalaria have all been shown to reduce sting nematode populations. Hairy indigo (*Indigofera hirsuta*), a vigorous growing legume and excellent soil building crop, has been

reported to suppress sting nematode and to be resistant to several *Meloidogyne* spp. Velvet bean (*Mucuna deeringiana*), another vigorous growing legume, was also observed to suppress sting and root-knot populations in field demonstration trials. Sorghum Sudan grass is a poor choice for a summer rotation on land infested with the sting nematode. To be effective, cover crop stands should be established quickly and kept as free as possible of grasses and other undesirable host weeds.

The fact that all populations of sting nematode have such a wide host range, including numerous weeds and grasses, must be considered in developing an effective crop rotation system for nematode management. Tobacco, cucumber, okra, watermelon, and possibly peanut are the only cultivated crops which have been reported as nonhosts to some sting nematode populations. Some care should be exercised however, because most of these crops, though reported as nonhosts for the sting nematode, are excellent host for other nematodes such as *Meloidogyne* spp. For these rotations to be effective, weed and grass management should be as complete as possible since they can act as excellent carry-over hosts. Once again, crop rotation systems developed for a given geographical area may be of limited value in others because some local populations of sting nematode respond differently onto different hosts. In this case, small-scale grower field evaluations should precede broadscale implementation of a specific crop rotation strategy.

8.1.5.8 Chemical Management

The sting nematode can be controlled with both fumigant and non-fumigant nematicides. It was determined that either solid fumigation treatment before bedding or row treatment as the beds were made allowed strawberry growers to produce productive crops of strawberry on land in which sting nematode had been destructive to previous crops. Strawberry acreage treated with a fumigant nematicide results in a fourfold increase in strawberry yields over that of previous nonchemical methods. Methyl bromide is being used almost exclusively for soilborne nematode, weed, and disease control. With the anticipated loss of methyl bromide however, alternative chemical control options will most likely involve individual or combined treatments of other federally registered fumigant. Use of these broad-spectrum fumigants has also been shown to effectively reduce nematode populations and to increase strawberry yields, particularly when compared with non-fumigant nematicides. Growers are encouraged to personally field validate and/or customize alternative strategies before methyl bromide is finally phased out of production and use in January.

8.2 Temperate Fruits

In general, fruits of temperate climates are almost always borne on trees or woody shrubs or herbs or lianas. They will not grow adequately in the tropics, as they need a period of cold each year before they will flower. The apple, pear, cherry, and plum are the most widely grown and eaten, owing to their adaptability. Many other fruits are important regionally but do not figure prominently in commerce.

Rosaceae Family: The family Rosaceae dominates the temperate fruits, both in numbers and in importance. The pome fruits, stone fruits, and brambles are fruits of plants in Rosaceae.

8.2.1 Apple

Of the many genera of plant-parasitic nematodes detected in soils from apple orchards, two species of root lesion nematode, four species of root-knot nematode, and the dagger nematode are believed to be the most economically important ones (Egunjobi 1968). Both species of root lesion nematode, *Pratylenchus vulnus* and *P. penetrans* are widely distributed. *Meloidogyne hapla* is found throughout the state in association with crops such as alfalfa. *Meloidogyne incognita, M. javanica, and M. arenaria* occur throughout the warmer apple-growing regions, with *M. incognita* having the most common occurrence. Dagger nematodes occur throughout the state but are believed to be more widespread in the northern part.

8.2.1.1 Damage

Root lesion nematodes penetrate into roots and cause damage by feeding and migrating through the cortical tissues. Interaction of root lesion nematodes with other soilborne organisms can increase injury to roots. They are occasionally associated with the apple replant disease, which is characterized by poor growth of young trees after transplanting. Feeding by root-knot nematodes can impair root functions such as uptake of nutrients and water. Dagger nematodes feed from outside the roots but can reach the vascular tissues with their long stylet. They are capable of suppressing growth of young trees, but the major problem caused by them is transmission of tomato ring spot virus, which causes apple union necrosis and decline on some varieties.

8.2.1.2 Symptoms

The symptoms described below are indicative of a nematode problem, but are not diagnostic as they could result from other causes as well. Damaged trees generally occur in a circular area within the orchard. Aboveground symptoms of nematode damage are lack of vigor, twig dieback, and decline in growth and yield. Infestation of older trees also results in chlorosis or yellowing of leaves, orange bark, fruit sunburn or sunscald typical in green varieties, and small fruit. Heavy infestation on young trees may result in stunting and sometimes death. Nematode infestations may occur without inducing any aboveground symptoms. Belowground symptoms include poor growth of feeder roots or main roots and soil adhering to roots. Root-knot nematode infestation will produce characteristic knots on roots.

8.2.1.3 Management

Physical

Hot water treatment of rootstocks at 50 °C for 15 min or 47 °C for 30 min.

Cultural Practices

Remove old roots and plant green manure cover crops resistant to root lesion nematodes (such as the oat cultivar Saia) for 1–2 years or fallow the site for 4 years. If *Pratylenchus vulnus* is present, maintain a poor host for this nematode, such as tall fescue, red fescue, or perennial ryegrass as orchard ground cover. These crops are hosts for *Pratylenchus penetrans* and should not be used if this species of root lesion nematode is present. Use certified rootstocks or seedlings to establish new orchards. Improve soil tilth and drainage and control other pests. Proper irrigation and fertilizer application will also reduce stress on trees.

Rootstock Selection

Most standard rootstocks and some dwarfing rootstocks are believed to have some tolerance to *P. penetrans,* the root lesion nematode species that is frequently found in apple orchard soils in northern California. However, the dwarfing rootstocks are considered to be susceptible to *P. vulnus,* which is common in the orchard soils of the San Joaquin Valley. Always use trees from nurseries, which are certified nematode-free.

Chemical

Trees planted in infested orchard sites that have been fumigated generally have improved growth and yields compared to those on non-fumigated sites.

8.2.2 Peach

Root-knot nematodes (*Meloidogyne arenaria, M. hapla, M. incognita*, *and M. javanica*), dagger nematodes (*Xiphinema americanum* and *X. rivesi*), lesion nematodes (*Pratylenchus penetrans* and *P. vulnus*), and ring nematode (*Mesocriconema xenoplax*) are commonly associated with peach. Lance, stunt, citrus, and spiral nematodes may also be associated in some regions (Pokharel and Larsen 2008).

8.2.2.1 Root-Knot Nematodes

Symptoms occur as small, spherical galls or knots on the roots of peach trees. Trees infected when very young may die from massive root destruction. Older trees that gradually become infected generally do not die but become weaker as more of the root system becomes damaged. Infected trees often show a lack of vigor and may show wilt symptoms during dry periods.

8.2.2.2 Management

Physical

Hot water treatment of seedling roots at 50–52 °C

Cultural

Use of nematode-free rootstocks for planting

Resistance

Resistant rootstocks like Nemared, Japanese Double, Elberta, China Flat, Bidbillos Early, Nemaguard, Okinawa, and Rancho can be used.

8.2.2.3 Replant Problems and the Root Lesion Nematode

Replanted fruit trees frequently have difficulty becoming reestablished, often because of interactions between nematodes and other soil microorganisms. The root lesion nematode, *Pratylenchus penetrans,* is often the cause of the problem. It is perhaps the most widespread and best-known nematode pest of fruit trees. It damages roots through feeding and intracellular migration, which destroys tissue in the root cortex. Root damage caused by this nematode promotes infection by root-rotting microorganisms. The resulting damage is greater than that caused by the nematode alone. Root lesion nematodes migrate and seek new feeding sites when roots become crowded or decayed. Although root impairment results in a loss in vigor and yield of mature trees, the role of root lesion nematodes in the development of replant problems is of greater economic importance.

Orchards affected by replant disease never reach their full production potential, and there are no remedial measures that can fully correct problems after the orchard is established. Depending on the extent of the problem, infested orchards force the grower to make tough economic decisions, such as whether to keep trees that are not highly profitable or to reestablish a new orchard at major expense and loss of several years' productivity. However, replant disease can be prevented by assessing the risk of problems with preplant nematode assays and by proper site preparation. The symptoms of orchard replant disease include stunting, yellowing of leaves, discolored and necrotic feeder roots, and in severe cases tree death within the first few years after planting. Necrotic roots may or may not show obvious lesions. Typically, affected trees show a patchy distribution, and the severity of disease may be quite variable within the orchard.

8.2.3 Plum

8.2.3.1 Damage

Ring nematodes spend their lives in soil feeding on roots. Feeding by ring nematodes, particularly on small feeder roots, predisposes trees to bacterial canker (*Pseudomonas syringae*) (Anonymous 2006.). Dagger nematodes reduce tree vigor with their feeding but mostly are important because they vector tomato ring spot viruses. *Pratylenchus vulnus* damage roots by moving through cortical tissues and feeding in these areas. *Meloidogyne* species take up a single feeding site within a root where they remain for their lifetime.

8.2.3.2 Symptoms

The symptoms described below are indicative of a nematode problem but need not be always diagnostic as they could result from other problems as well.

Belowground

Nematodes puncture and remove the contents of plant cells. This activity stunts root growth and reduces the tree's ability to take in water and nutrients. Because of this, nematode-infested trees may have poorly developed root systems. With root-knot nematodes in particular, feeding reduces the overall energy of the tree. Nematode feeding also creates entry points for other disease organisms.

Aboveground

Lack of vigor, small leaves, dieback of twigs, and yield reduction are typical symptoms of nematode damage. Nematodes are usually distributed unevenly throughout an orchard resulting in patches of low vigor trees. Orchards infested with high population levels of ring nematodes frequently exhibit symptoms associated with bacterial canker including blighted buds, blossoms,

and leaves and cankers that occur on and can result in the girdling and death of limbs and/or trees. Trees on plum rootstocks tend to host higher population levels of ring nematode than those on Nemaguard peach rootstocks.

8.2.3.3 Management

Cultural

Whenever possible, plant new orchards in land that has previously been planted with nonwoody crops for several years. Root lesion nematodes survive well within dead root tissue or soil, with 5 % lasting 5 years after tree removal.

Prevention

Using certified planting stock, cleaning soil from equipment before moving between orchards, and avoiding reusing irrigation tailwater will help to prevent spread of nematodes to uninfested fields.

Resistant Rootstocks

Nemaguard peach rootstock is resistant to root-knot nematodes but susceptible to root lesion and ring nematodes. Plum rootstocks (Marianna 2624 and Myrobalan 29C) tend to be least damaged by root lesion nematodes but are susceptible to damage by ring nematode.

8.2.3.4 Considerations in Managing Nematodes in Fruit Tree Plantings

- Purchase nematode-free planting stock from reputable dealers. Reject any whose roots have knots or galls.
- Buy varieties with nematode-resistant rootstocks when available; these are only available for peach, nectarine, and plum.
- When planting, use copious quantities of organic matter. If nematodes are suspected, remove native soil and replace with nematode-free potting soil.
- Water plants according to need; do not allow them to go through water stress.
- Fertilize plants and follow good cultural practices; this allows plants to tolerate more nematodes.
- Keep the area within the fruit tree drip line free of plants (ornamentals and weeds) which could serve as hosts to increase nematodes on fruit trees as well as compete with the fruit plants for water and nutrients.

8.3 Tree Fruits and Small Fruits

Most orchard trees suffer from nematode attacks which undergo “decline” and replant failures. “Decline” describes a situation whereby fruit trees that have previously produced profitable yields no longer grow or produce satisfactorily. Some of the important nematode problems of tree fruits and small fruits are summarized below.

8.3.1 Cherry

Lesion and root-knot nematodes are considered to be important factors in limiting plant growth. Dagger nematode can reduce growth but is more important as a virus vector. Pin nematode is common, but effects on cherry have not been studied and it is not considered a pathogen on other stone fruits. In most instances, *P. penetrans, P. pratensis*, *and P. vulnus* have all been reported as associated with decline or unthrifty growth, but *P. penetrans* causes the greatest damage and is the most prevalent. In New York State, this nematode has been reported to be the most numerous species in cherry and apple orchards and to be the primary cause of poor growth and death of young trees due to damage of small roots.

P. penetrans is primarily responsible for the cause of the replant problem. Growth of cherry seedling rootstocks was poor on untreated soil heavily infested with this nematode, but good on similar soil treated with a nematicide. Several species have proven to be vectors, but the exact nature of other nematode–virus relationships remains in question. In Europe, X. *diversicaudatum, w*hich is a known vector of arabis mosaic virus (AMV) and strawberry latent ring spot virus, is associated with a leaf enation symptom in cherry, but transmission by the nematode has not been demonstrated.

8.3.1.1 Damage

Damage caused by nematodes is likely to become evident during the first year after planting. Feeding by nematodes can impair root functions such as uptake of nutrients and water. Lesion nematodes penetrate into the roots and cause damage by feeding and tunneling through the root tissues. Dagger nematodes feed from outside the roots but can reach the vascular tissues with their long stylet and are capable of reducing vigor and yield of trees. However, *X. americanum* is more important on stone fruit trees as a vector of the cherry rasp leaf virus, which causes rasp leaf disease, and strains of tomato ring spot virus, which cause yellow bud mosaic, cherry mottle leaf, and *Prunus* stem pitting diseases. These virus diseases can reduce the productivity of the trees significantly, and infected trees may eventually die. Feeding by root-knot nematodes causes swellings of the entire root, which impairs normal root functions.

8.3.1.2 Symptoms

Symptoms described below are indicative of a nematode problem, but are not diagnostic as they could result from other causes as well. Symptoms of a nematode infestation are reduced vegetative vigor and fruit yield and unevenly sized trees. Heavily infested trees are more susceptible to moisture stress. Lesion nematodes may cause reddish-brown lesions on roots that later turn dark and ultimately black. Root-knot nematode produces characteristic knots on roots. Symptoms of dagger nematode infestation include reduced tree growth and vigor. If the tree is infected with cherry rasp leaf virus, characteristic enations, which are distorted tissue growths, develop on the underside of leaves.

Affected leaves have normal color but are deformed and initially found on the lower parts of the tree. If the strain of tomato ring spot virus that causes yellow bud mosaic is involved, infected trees produce bare limbs, small leaves, and enations adjacent to the midrib on the underside of leaves. Cherry mottle leaf infections are usually symptomless except on highly susceptible sweet cherry cultivars that exhibit chlorotic mottling, distortion, puckering of younger leaves, and small fruits that ripen late. Trees infected by the strain of tomato ring spot virus that causes stem pitting exhibit symptoms of delayed budbreak, yellow leaves, a wilted appearance early in summer, and reddish purple leaves by late summer.

8.3.1.3 Management

Cultural Practices

Before fumigating, remove old trunks and large roots brought up by ripping and fallow or plant green manure cover crops for 1–2 years (3–4 years if lesion nematodes are present). Do not use cover crops that are known hosts of nematodes that damage the rootstock you plan to plant; contact your farm advisor for additional information. Use certified nematode-free rootstocks or seedlings to establish new orchards. When the orchard is developed, use procedures that improve soil tilth and drainage to help reduce nematode damage.

Rootstock Selection

Use certified nematode-free rootstocks. Both Mazzard and Mahaleb are susceptible to cherry rasp leaf virus and to the lesion nematode, *Pratylenchus vulnus.* Colt rootstock is the most susceptible to *P. vulnus.* Mazzard is immune to *M. incognita* and resistant to *M. javanica.* (Immune rootstocks are not attacked by nematodes, whereas resistant or nonhost rootstocks may be invaded by the nematodes and show damage, but do not allow population increases.) Mahaleb is resistant to *M. incognita* and susceptible to *M. javanica.* Stockton Morello is immune to *M. incognita* and susceptible to *P. vulnus.*

Trees planted on fumigated orchard sites generally show improved growth and yields compared to those on non-fumigated sites. Preplant fumigate in fall when soils are dry and warm. Threshold levels of nematode pests for postplant treatment have not been established. Yield and vigor increases may not become evident for 6–18 months following treatment with phenamiphos. To help determine the effectiveness of postplant treatments, leave several trees or rows of trees untreated for comparison with treated trees.

8.4 Vegetable Crops

Vegetable crops are excellent hosts for a wide range of plant-parasitic nematodes. Different groups of vegetable crops are prone to several species of nematodes incurring significant damage/losses. Major earlier milestones of reports of parasitic nematodes of many groups of vegetables which are root-knot nematodes (*Meloidogyne* spp.), onion bloat/stem and bulb nematode (*Ditylenchus dipsaci*), sugar beet nematode (*Heterodera schachtii*), potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*), potato rot nematode (*D. destructor*), and pea cyst nematode (*Heterodera goettingiana*), which are now part of the history, initiated the realization of the importance of nematodes on vegetables. Later, detailed studies were concentrated on the nematode problems of vegetables. Root-knot nematodes are considered as the most potential, economic pests of vegetables everywhere.

8.4.1 Economic Loss

Estimated overall average annual yield loss of the world's major vegetable crops by nematodes is 12.3 %. Average losses for the 40 crops in developed countries were estimated to be 8.8 % compared with 14.6 % for developing countries (Sasser and Freckman 1987). *Meloidogyne, Pratylenchus, Heterodera, Ditylenchus, Globodera, Xiphinema, Radopholus, Rotylenchulus,* and *Helicotylenchus* in the same order are considered as the most damaging genera is different vegetable-growing tracts of the world (Tables 8.1 and 8.2). However, in the tropics, root-knot nematodes, *Meloidogyne* species are predominant.

Table 8.1 Global estimated annual yield losses of some vegetables due to nematodes

Crop	Loss (%)	Nematodes
Potato	12. 2	*Meloidogyne, Globodera, Pratylenchus*
Brinjal	16.9	*Meloidogyne, Ditylenchus*
Okra	20.4	*Meloidogyne, Rotylenchulus*
Pepper	12.2	*Radopholus, Rotylenchulus*
Tomato	20.6	*Meloidogyne*

Table 8.2 Estimated losses on vegetables in the tropics caused by *Meloidogyne* spp.

Crop	Loss (%)	*Meloidogyne* spp.
Tomato	29	*M. incognita*
Brinjal	23	*M. javanica, M. arenaria*
Okra	22	*M. hapla*
Beans	28	*M. incognita*
Pepper	15	*M. incognita*
Cabbage	26	*M. incognita*
Potato	24	*M. incognita*

8.4.2 Major Nematode Parasites of Vegetables

Nematodes belonging to both ectoparasitic and endoparasitic groups attack vegetables. Ectoparasites in general may be either surface or subsurface feeders. While surface feeders normally feed on epidermal cells of the root and root hairs, subsurface feeders feed on cells in the outer layers of cortex or in deeper layers close to the stele.

Example: Species of *Tylenchorhynchus, Pratylenchus, Xiphinema, Hoplolaimus, Longidorus, Paratrichodorus,* etc.

On the other hand, endoparasites enter the host tissue either completely or partly. Endoparasites partly entering the host tissue are also referred to as semi-endoparasites. Endoparasites are either sedentary (sessile in the host tissue) or migratory (moving about actively within the host tissue).

Example: Species of *Meloidogyne, Heterodera, Globodera, Pratylenchus,* and *Ditylenchus* are important endoparasites of vegetables. Females of *Rotylenchulus reniformis* which parasitize vegetables partly remain within the host tissue, and the nematode is usually designated as semi-endoparasites.

These endoparasites, particularly root-knot nematodes, predispose several soil fungi and bacteria and may result in complex diseases leading to severe crop loss in vegetables especially solanaceous group, viz., tomato and brinjal. Major fungi may include species of *Fusarium, Pythium, Phytophthora, Rhizoctonia,* and *Sclerotium*, and the most common bacterial pathogen is *Ralstonia solanacearum*.

Table 8.3 Root-knot nematodes commonly associated with vegetables

Nematode	Vegetables
M. incognita	Okra, onion, cabbage, cauliflower, turnip, tomato, brinjal, *Capsicum annuum*, cucumber, pumpkin, *Cucumis melo var. utilissimus, Cucurbita maxima, C. moschata, C. pepo*, carrot, sweet potato, potato, *Lagenaria leucantha, Luffa cylindrica*, pea, radish, *Spinacia oleracea, Cyamopsis tetragonoloba*, leafy vegetables, gherkin
M. javanica	Okra, brinjal, tomato, onion, beta vulgaris, cabbage, cauliflower, turnip, *Cucumis melo, C. sativus, Cucurbita pepo, C. moschata,* carrot, pepper, *Cyamopsis tetragonoloba, Dolichos lablab*, sweet potato, *Luffa cylindrica, L. acutangula,* potato*, Spinacia oleracea*, pea
M. arenaria	Okra, *Capsicum annuum, C. frutescens,* tomato, radish, brinjal, potato
M. incognita acrita	Okra, brinjal, tomato, potato, radish, *Capsicum frutescens, Luffa acutangula, L. cylindrica*
M. hapla	Okra, potato, *Spinacia oleracea,* tomato
M. africana	Sweet potato
M. lucknowica	*Luffa cylindrica*, brinjal
M. thamesi	Okra, brinjal

8.4.3 Endoparasitic Plant-Parasitic Nematodes

8.4.3.1 Root-Knot Nematodes (*Meloidogyne* spp.)

Every vegetable crop grown is known to be attacked by either of the four major species of *Meloidogyne*, viz., *M. incognita, M. javanica*, *M. arenaria*, and *M. hapla.* Root-knot nematodes are most important parasites of vegetables all over the world (Table 8.3) (Taylor and Sasser 1978). They are easily detectable by characteristic symptoms in the form of galls formed on roots of host crops. Galls also develop on other underground parts of plants, such as modified stems like tubers and rhizomes. Infected plants show stunted and poor growth. Chlorosis, wilting, and poor growth of the leaves are major symptoms.

About 95 % of the populations are represented by only *M. incognita, M. javanica, M. arenaria*, and *M. hapla. M. incognita* and *M. javanica* are widely distributed in tropical, subtropical, and warm temperate climates of the world. *M. arenaria* is also found in such climates but is relatively less frequent. All the three species occur in areas with an average temperature of 36 °C or lower in the warmest month. *M. hapla* occurs in temperate climates, in areas with an average temperature of −15 °C during the coldest month, but is limited to areas with an average temperature of less than 27 °C during the warmest month.

In *M. incognita* four races have been reported which are designated as race 1, race 2, race 3, race 4, and two races, race 1 and race 2, in *M. arenaria* have been distinguished. However, race 1 and race 2 in *M. incognita* and race 2 in *M. arenaria* are more widely distributed. *M. incognita, M. javanica, M. arenaria, M. acrita, M. hapla, M. africana, M, lucknowica*, and *M. thamesi* attack a wide range of vegetable crops including okra, tomato, brinjal, onion, cabbage, cauliflower, turnip, peppers, cucumber, pumpkins, long melon, squash, bottle gourd, sponge gourd, carrot, sweet potato, potato, pea, radish, spinach, and beans.

As known in root-knot nematodes, they develop feeding sites, known as giant cells which are essential for successful host parasite relationship. Tissues around the female and its feeding site undergo hyperplasia and hypertrophy and from characteristic root gall. Recent studies show that giant cells are mostly formed by repeated endomitosis without subsequent cytokinesis and that they are essentially transfer cells passing nutrients to the nematodes. Root-knot nematodes are known for their interaction with other soil pathogens on vegetables. In their interactions, the pathogenic effects of interacting pathogens on host are increased or decreased. They possess either synergistic or antagonistic relationship with other microorganisms.

Symptoms

Stunted growth, yellowing of foliage, and wilting appearance in patch areas are the most common symptoms. Medium to severe galling on the roots is the characteristic symptom (Fig. 8.11a–d)

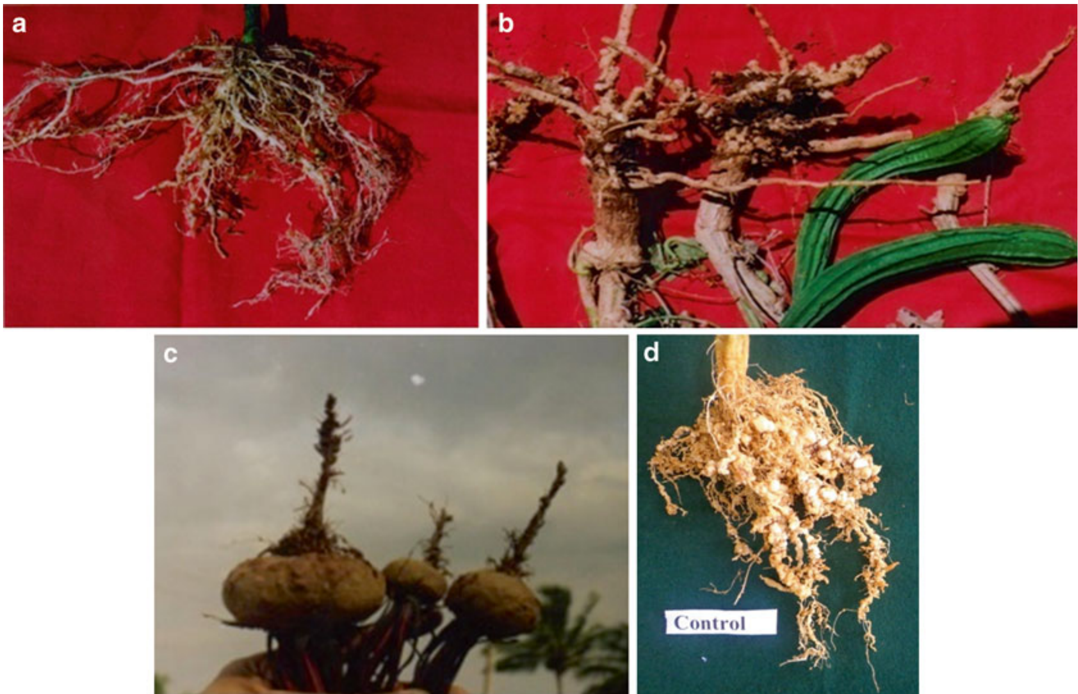

Fig. 8.11 Root-knot nematode infestation in vegetable crops. (**a**) Tomato, (**b**) Ridge gourd, (**c**) Beet root, (**d**) Okra (Bhendi)

(Ravichandra 2008). Production of galls is mainly due to hypertrophy of the pericycle and hyperplasia of cortical cells. Giant cells are produced in vascular tissues which are basically nurse cells or mother cells that have cell wall invagination to draw nutrients from adjoining cells induced by nematode. They are produced by mitosis without cytokinesis. Reduced root growth, devoid of feeder roots, is also common.

8.4.3.2 Cyst Nematodes

Many species of cyst nematode genera *Globodera* and *Heterodera* have been reported to induce economic losses in some vegetable crops. Potato cyst nematodes *Globodera rostochiensis* and *G. pallida* are most important in many parts of temperate zones. Although they are associated with crops like tomato and brinjal, they are not economically very important on these crops. Their remarkable ability to survive under unfavorable conditions, remaining dormant in soil in the absence of host plants for many years, creates great problems in way for their management. Development of resistance-breaking biotypes by the nematodes is another great obstacle in this respect. Their cysts provide enough protection to the juvenile stages against toxic effects of nematicides. Due to these factors, they are the most difficult nematodes to manage, when established in crop fields.

Major nematode pests including *G. rostochiensis* and *G. pallida* are problematic on potato. *Heterodera carotae* on carrot; *H. schachtii* and *H. cruciferae* on sugar beet, cabbage, and cauliflower; *H. trifolii* on cucumber, pumpkin, and squash; *H. galeopsidis* on tomato; and *H. cajani* and *H. goettingiana* on peas are some of the cyst nematodes associated with vegetable crops and may pose a problem in some locations.

Symptoms

Stunted growth, yellowing of the foliage, and wilting accompanied by the reduced root system with characteristic cyst formation are some important symptoms in most crops, which affect both the quantity and quality of the yield.

8.4.3.3 Golden Cyst Nematode: *Globodera* spp. on Potato

This is a serious pest of potato throughout the world and derives its name from the golden color of its cysts. There are two species, viz., *Globodera rostochiensis* which is widely distributed and *G. pallida*. Nematodes have been reported to cause about 11 % loss in potato. At present, 135 species belonging to 45 genera are reported to be associated with potato. However, root-knot and cyst nematodes are economically most important ones. Though the host range is limited, the complete elimination of cyst nematodes from soil is difficult. The cysts remain viable in the soil for 7–10 years even in the absence of host. The larvae hatch out when potato root exudates stimulate them. Further, from low infestation levels, its population in soil reaches the damaging level within 3–5 cropping seasons of potato, tomato, and brinjal which are the most preferable host crops. The parasitism has evolved so well that several pathotypes within the species occur in different areas of the world. Strict quarantine measures are being adopted throughout the world when potatoes have to be imported from a country where cyst nematode infestation occurs.

Symptoms

Disease symptoms are expressed only after the nematode population build up to appreciable levels. Low nematode infestations aren't easily detected. When the soil population is sufficiently high, small patches of poorly growing plants may appear in the field. Temporary wilting of plants occurs during hotter parts of the day. Typical symptoms of heavy infestations are stunted plants with unhealthy foliage, premature yellowing, poor development of root system, and reduction in size and number of tubers which become more pronounced in swampy areas. Characteristic cysts attached to the roots are confirmatory symptoms (Fig. 8.12). However, in evenly distributed infestation levels, the nematode causes gradual reduction in yield year after year.

8.4.3.4 Root Lesion Nematodes (*Pratylenchus*)

Being migratory endoparasites, juvenile and adult root lesion nematodes invade roots or other underground parts. The nematodes feed and reproduce in the root cortex causing necrosis of the attacked and surrounding cells. As a result brownish, irregular, longitudinal lesions appear on the infected roots. Various species of this genus are greatly influenced by climatic conditions. For instance, *P. pratensis* is restricted to cooler climates, while *P. minyus* is a species of warmer climates. Although they have a wide host range, they are not very common on vegetables. Species like *P. penetrans, P. minor*, *P. brachyurus, P. coffeae, P. vulnus, P. pratensis, P. scribneri, P. thornei,* and *P. capitatus* have been reported on tomato, cauliflower, cabbage, okra, potato, radish, pea, beans, pepper, onion, sugar beet, etc., from various regions. Lesion nematode may interact with several fungi like *Verticillium*, *Colletotrichum, Rhizoctonia, Fusarium,* and *Aphanomyces*.

Fig. 8.12 Cysts on roots

8.4.3.5 Reniform Nematode (*Rotylenchulus reniformis*)

The reniform nematode is an important semi-endoparasite that feeds on the secondary or tertiary roots producing necrotic lesions in the area of the roots around the female head.

Symptoms

Severe necrosis and destruction of feeder roots, yellowing, and wilting of aboveground parts are the major visible symptoms on severely infected plants. The young adult females are infective and penetrate the roots both close to the root tip and the entire root system. The head of the female transverses through the cortex and rests in an endodermal cell that later becomes hypertrophied

and assumes dome-shaped appearance with the adjacent cell undergoing a similar change. The other pericycle cells also become hypertrophied with thickened cell walls, enlarged nucleus, and dense granulated cytoplasm. These hypertrophied cells form the semilunar sheet surrounding the vascular region along two sides of the nematode head. The posterior part of their body remains outside the root tissue. The young females feed, assume sedentary habit, and enlarge. At maturity, the posterior exterior part of the body becomes kidney shaped, hence known as "reniform" nematode.

The nematode has a wide host range and is an important pest of tomato, cauliflower, brinjal, okra, pepper, cowpea, cucumber, lettuce, radish, turnip, bean, carrot, etc. *R. reniformis* interacts synergistically with a number of fungi like *Rhizoctonia solani, Macrophomina phaseolina, Fusarium oxysporum* f.sp. *vasinfectum*, and *Verticillium* sp. on several vegetables. This interaction effect may result in greater disease severity.

8.4.3.6 Potato Rot Nematode (*Ditylenchus destructor*)

Ditylenchus destructor is widespread in many potato-growing areas. Infestations of potato rot nematode have caused yield losses of up to 40 %. Storage of potatoes infected with rot nematode can cause additional losses of 10–20 % (Saad L. Hafez 2001). This nematode is easily spread to other fields and cannot be eradicated from a field with any conventional farm chemical or management practice.

Symptoms

Potato plants infected with rot nematode may not show any distinct recognizable aboveground symptoms. Rare cases of severe infection cause a general lack of plant vigor and some leaf deformation. Symptoms are primarily on tubers and stolons, not on roots. Tuber decay and rotting is usually unnoticed until potatoes are placed in storage. Symptoms of tuber infection at harvest are easily missed or confused with those of other potato diseases. Small (less than 1/32-in.), white or pearly spots with pin-sized holes in the center are present just beneath the tuber skin. The infected area becomes soft and may be more readily detected by touch than by sight. As the decay progresses, the tissue under the skin turns brown and forms slightly grayish brown depressions, often with a honeycombed appearance (Fig. 8.13). The tuber skin above these depressions becomes papery and frequently splits, exposing dry, chalky, crumbled, and decayed tuber tissue. The decay symptoms in the tuber flesh commonly resemble those of *Fusarium* dry rot. Surface cracks in the tuber skin are usually present.

Fig. 8.13 Potato rot nematode infestation

Infected tubers may be invaded by secondary fungi and bacteria that typically darken tuber tissues and cause greater shriveling and rotting. *Fusarium* dry rot is a common secondary infection. Infected potatoes in the field or in storage may also have soft rot that effectively masks the nematode rot symptoms. Numerous mites and saprophytic nematodes may also invade infected tubers. Tuber symptoms of potato rot nematode can be confused with those of common scab, bacterial ring rot, and late blight. Definitive diagnosis requires microscopic examination of infected tissue for the presence of potato rot nematode.

Management

Potato rot nematode cannot be eradicated from cropland with any conventional farm chemical or management practice, but sanitation and some management practices help prevent the nematode's further spread.

Plant only certified potato seed that has originated from a limited generation program. Do not plant year-out or eliminator seed. Resistance among recently released potato cultivars has not been determined, but all older cultivars are considered susceptible. Do not store potatoes if potato rot nematode is detected during harvest. The nematode can grow and develop under normal storage conditions.

Do not grow potatoes in infested fields for 4–6 years and include cereals and grasses in the rotation.

Properly clean and disinfect all storage areas and cultivation and handling equipments that have come in contact with contaminated potatoes. Avoid movement of infested soil or potatoes onto cropland. Properly dispose of infested cull potato piles. Control weeds in infested cropland. Application of a nematicide is also an effective measure for minimizing the soil populations of the potato rot nematode.

8.4.4 Ectoparasitic Nematodes

These nematodes may include species of *Tylenchorhynchus, Paratylenchus, Hoplolaimus, Trichodorus, Paratrichodorus, Longidorus, Hemicycliophora, Helicotylenchus, Rotylenchus, Dolichodorus*, and *Xiphinema* are ectoparasites on various kinds of vegetable crops, but they may not cause significant damage.

8.4.4.1 Symptoms

Affected plants, in general, lack specific symptoms on aboveground parts or on the roots. Reduced root vigor, damaged feeder roots, and poor regeneration are general symptoms appearing due to association of these nematodes. Some species of stubby-root nematodes may induce stubby roots and root proliferation. Most of the species are surface ectoparasites feeding from the surface without penetrating the roots. Others, subsurface feeders, use their long robust stylet to penetrate in the deeper layers of root cortex, or a few even penetrate a small portion of their anterior body while feeding. Estimates of crop losses caused by these nematodes are lacking. But damage potential in artificial inoculation in terms of percent reduction in plant biomass has been estimated.

Stunt nematodes (*Tylenchorhynchus* spp.) are commonly associated with vegetables including cabbage and cauliflower. *Tylenchorhynchus brassicae* and *Rhizoctonia solani* are often associated with roots of cabbage and cauliflower. Important species are *T. brassicae, T. macrurus, T. claytoni, T. martini, T. dubius, T. cylindricus*, etc. (Table 8.4).

Spiral nematode (*Rotylenchus robustus, R. uniformis, R. laurentinus*) causes severe reduction in roots and stunting of top growth in carrots. They also infect cabbage, red beet, spinach, celery, and cauliflower. The pin nematodes, *Paratylenchus* species, have a wide host range which includes several major vegetables. Important species are *P. minutus, P. hamatus*, etc.

Lance nematodes (*Hoplolaimus* species) also infect vegetables. Some species also become endoparasitic. In India, major species on vegetables are *H. indicus, H. abelmoshi, H. dubius, H. galeatus,* etc. Other parasitic genera like *Xiphinema, Longidorus, Trichodorus*, *and Paratrichodorus* are also very common which feed at root tips and along the sides of the roots inducing mechanical damage to roots. They also act as vectors of many plant viruses. Dagger nematode (*Xiphinema* species) with major species like *X. americanum, X. basiri*, and *X. vulgare* are also associated with vegetables. Needle nematodes (*Longidorus* species) affect and cause damage to host plant particularly at root tips. Several species act as vectors of viruses. In India, species like *L. brevicaudatus, L. nirulai*, *and L. pisi* have been reported on vegetables.

8.4.4.2 Management of Phytonematodes Infesting Vegetables

Physical Measures

- Rabbing of nematode-infested nursery with either paddy/bajra husk/sawdust at 7 kg/sq.m 1 week before sowing.
- Soil solarization of nursery beds is an effective method to minimize nematode population in soil. Covering the beds with 200–400 gauze LLDPE polythene sheets during hot summer

Table 8.4 Major ectoparasitic nematodes associated with vegetable crops

Nematode	Vegetable
Longidorus elongatus	Brinjal
L. brevicaudatus	Sugar beet, tomato, pea, brinjal
L. nirulai	Potato
L. pisi	Pea
Trichodorus spp.	Brinjal, tomato
T. acaudatus	Cabbage, cauliflower
T. minor	Potato
Hoplolaimus spp.	Cabbage, cauliflower
	Potato, chillies
H. obelmoshi	Okra
H. galeatus	Tomato, pea
H. indicus	Okra, onion, sugar beet, cabbage, cauliflower, turnip, peppers, cucumber, pumpkins, carrot, bean, bottle gourd, tomato, radish, brinjal, potato, spinach
H. tylenchiformis	Okra
Tylenchorhynchus spp.	Tomato
T. badliensis	Tomato, cabbage, cauliflower sugar beet
T. brassicae	Lettuce, radish, pepper, garlic, sponge gourd, carrot, onion, brinjal, spinach turnip
T. brevidens	Cabbage, pepper, cauliflower, tomato, potato
T. brevilineatus	Cauliflower, tomato, brinjal
T. curvus	Pepper
T. dactylurus	Pepper
T. digitatus	Lettuce
T. divittatus	Okra
T. dubius	Tomato
T. elegans	Tomato
T. haki	Cauliflower
T. mashhoodi	Okra, pea, radish
T. nilgiriensis	Cabbage
T. punensis	Brinjal
T. spinaceae	Spinach
T. vulgaris	Sugar beet, beans tomato, brinjal, okra, onion, cucumber, pea, potato, radish, cauliflower, cabbage, carrot, pepper, lettuce, spinach
T. zeae	Okra, sugar beet, pepper
Paratylenchus spp.	Pea
X. americanum	Cauliflower, okra
X. basiri	Okra, tomato, brinjal
X. vulgare	Pepper

Fig. 8.14 Soil solarization

months, preferably April–May, for 2 weeks can effectively minimize the nematode population (Fig. 8.14).

Cultural Measures

- Use of nematode-free site to prepare nursery beds.
- Destruction of infested plant material after harvest.
- Crop rotation with nonhosts or poor hosts, viz., sorghum, finger millet, maize, groundnut, rice, or wheat.
- Intercropping with antagonistic crops like marigold, mustard, and sesame.
- Flooding or fallowing, though considered noneconomic, can be practiced wherever possible.
- Organic amendments with neem/mahua/mustard/castor cake at 1 kg/sq.m.
- Three to four deep summer plowing can effectively destroy the juveniles, adults, and also eggs of nematodes due to exposure to heat and desiccation.

The nonhosts and poor hosts to major phytonematodes (Table 8.5) may serve as a starting point for developing effective integrated nematode management strategies (Trivedi and Barker 1986).

Cover/trap crops and antagonistic plants are useful for reducing nematode populations as well as conserving soil and often improving soil texture (Trivedi and Barker 1986). In localities where land availability permits, the use of cover crops, especially plants that serve as trap crops or offer other suppressive effects on nematode populations, should be considered (Table 8.6). Carefully selected cover crops may serve as living mulches and provide multiple pest control.

Table 8.5 List of selected nonhost plants for nematodes

Nematode species	Nonhosts or poor hosts
Belonolaimus longicaudatus	*Crotalaria* spp., *Crotalaria spectabilis*, hairy indigo, marigold, tobacco
B. gracilis	*Crotalaria* spp., tobacco, watermelon
Dolichodorus heterocephalus	*Crotalaria spectabilis*
Helicotylenchus dihystera	*Alfalfa, maize, fescue*
Heterodera glycines	Maize, cotton, cowpea, potato, small grains, grains, tobacco, most vegetables
H. schachtii	*Alfalfa, bean, clover, maize, Hesperis matronalis,* onion
H. zeae	Wide range of crops
Globodera rostochiensis	Maize, green beans, red clover
Hoplolaimus indicus	Cabbage, chilli, eggplant
Meloidogyne javanica	*Andropogon, Crotalaria* spp., cotton, groundnut, sorghum, velvet bean
M. hapla	Maize, cotton, grasses, lettuce, onion, radish
M. incognita	Fescue, orchard grass
Meloidogyne spp.	*Crotalaria spectabilis, Indigofera hirsuta,* millet, oats, wheat
Paratrichodorus minor	*Maize, Crotalaria spectabilis*
Pratylenchus leiocephalus	Groundnut
P. penetrans	Alfalfa, beet, fescue, marigold, oats, Sudan grass, rye
Pratylenchus spp.	Lettuce, onion, radish
Radopholus similis	*Crotalaria spectabilis*
Tylenchorhynchus mirzal	Wheat
T. brassicae	Potato, tomato
Xiphinema americanum	Alfalfa, maize, fescue, tobacco

Table 8.6 Trap crops for some phytonematodes

Nematode species	Trap crop
Meloidogyne spp.	*Crotalaria spectabilis,* cowpea, English pea, periwinkle
Heterodera avenae	*Oat*
H. schachtii	*Hesperis matronalis*
Globodera spp.	Potato

Biological Measures

- Nursery bed treatment with bio-agents, viz., *Trichoderma viride, T. harzianum, Pochonia chlamydosporia, Pseudomonas fluorescens,* and *Paecilomyces lilacinus* at the rate of 20–30 g/sq.m or *Glomus fasciculatum* and *G. mossae* at 30–50 g/sq.m along with organic amendments (farmyard manure/oil cakes/vermin compost) (Kirankumar et al. 2004)
- Application with 'Royal 350' (*Arthrobotrys irregularis*) at 140 g/sq.m 1 month before transplanting
- Soil application with avermectins B1 and B2a at 0.093–0.34 kg a.i./ha

Chemical

- Nursery bed treatment with carbofuran 3G at 16–20 g/sq.m.
  - Bare root-dip treatment with carbofuran or oxamyl at 1,000 ppm for 15–30 min (Parvatha Reddy and Singh 1979) or with phorate, aldicarb, and carbofuran at 500 ppm for 1 h (Parvatha Reddy and Singh 1979).
- Main field application with carbofuran, cartap hydrochloride, aldicarb, ethoprophos, and phenamiphos each at 1–2 kg a.i./ha.
  - Integration of several of these measures are useful in effective management of root-knot nematodes on vegetables.

Resistant/Tolerant Varieties: Use of tolerant/resistant varieties is an excellent alternative to manage nematodes. A wide range of varieties are available in several vegetable crops (Tables 8.7, 8.8, 8.9, and 8.10) (Kanwar and Bhatti 1990; Patnaik et al. 2004; Parvatha Reddy 2008).

8.4.4.3 Management of Phytonematodes in Potato

Prevention of Spread

The spread of the golden cyst nematode (*Globodera rostochiensis* and *G. pallida*) is generally with the movement of the infested soil with dust storm, flood, irrigation water, and the soil attached to the tubers. The soil left out in gunny bags used for storing seed tubers also serves as source of infestation. Transportation and rising of seedlings in the infested soil or any operation that carries the soil from the infested field to a new or healthy land helps in the movement of cysts. Though elimination of potato cyst nematodes is very difficult, the

Table 8.7 Some resistant cultivars of tomato

Nematode	Cultivars
M. incognita	Atkinson, Manalucei, Hisar Lalit, Keck Ruth, Patriot, Nematex, S-120, NTDR-1, Biggest, Bonus, Contess, Better Boy, Monte Carlo, Beefmaster, VFN-8, VFN-360, VFN-Bush, Peirnita, Motabo, Motella, Hessolini, Radient, Nemared, Ronita, Arka Vardan, CI 3279, CI 3104, CI B110, EC 118277
M. javanica	Leader, Atkinson, Manalucei, Nematex, S-120, SL-12, Resistant Bangalore, NTDR-1, Biggest, Bonus, Contess, Better Boy, Monte Carlo, Beefmaster, VFN-360, Peirnita, Motabo, Motella, Hessolini, CI 3279, CI 3104, CI B110, Pelican, VFN-8, Pusa 120, EC 173898 (72 T6), EC173897 (Calmart), EC 173896 (Kewa 10), CLN 363 BCIF2-167 1-0, CLN 363 BCIF2-190 1-0, CLN 363 BCIF2-344-0-0, CLN 229 BCIF2-4,1-4 1-1-0, Calmart VFN, VFN-Bush, Punjab 6NR-7
M. arenaria	Atkinson, Manalucei
M. incognita	Pelican, Rossol, Ronita, VFN-8, Karnataka Hybrid

Table 8.8 Some resistant cultivars of brinjal, okra, chilli, and *Capsicum*

Nematodes	Vegetables cultivars
	Brinjal
M. incognita	Black Beauty, Giant of Banaras, Vijaya, Mysore Green, Pusa Purple Long Black, Maroo Marvel, Gulla
M. javanica	Bhanta, Muktakeshi, Round Red, Coolie, Mathis B, Mysore Green, America Big Round, Arka Sheel, R-34, Sonepat, BR-112, Gulla
	Okra
M. incognita	Parbhani Kranti
M. javanica	Long Green Smooth, IC-9273, IC-18960
	Chilli
M. javanica	Pusa Jwala, 579, CAP 63 Suryamukhi Black, Jwala, Bull Nose, Hungarian Wax, Chinese Giant, Chilli NP-46-a, Chilli G-3 Suryamukhi, California Wonder
M. incognita race 1	Pusa Jwala, Jwala, Wonder Hot, Teja, Utkal Abha, Utkal Rashmi
M. incognita race 2	Jwala
M. incognita race 3	Pusa Jwala, Jwala
M. incognita race 4	Jwala
	Capsicum
Meloidogyne spp.	Mississippi-68, Santanka, Anaheim Chile, and Italian Pickling

Table 8.9 Some resistant cultivars of cucurbits, cauliflower, and cabbage

Nematodes	Vegetable cultivars
	Cucumber
M. javanica	S-445 (muskmelon), Improved Long Green
M. incognita	GY-5937-587
	Ridge gourd
Meloidogyne spp.	Panipati, Meerut Special
	Watermelon
Meloidogyne spp.	Shehjanpuri
	Cauliflower
M. javanica	74-6C, Superial Maghi, Dania, Pusa Late
M. incognita race *1*	Pusa Snow Ball, 74-6C, Dania
M. javanica race 2	Pusa Snow Ball, Balwan Snow Ball, Indian Snow Ball, Katki, Dania
M. incognita race 3	Early of India, Dania, Suttons, Pusi
M. incognita race 4	74-6C, Pusa Snow Ball, American White King (Vilayati). Early of India, Dania, Pusa Late
	Cabbage
M. javanica	American Special Ballhead, Suttons Eclipse Drumhead
M. incognita race 1	American Special Ballhead, Red Drumhead,
	Suttons Eclipse Drumhead
M. incognita race 2	American Special Ballhead, Glory of Enkhuizen,
	Suttons Eclipse Drumhead
M. incognita race 3	American Special Ballhead, Red Drumhead,
	Glory of Enkhuizen
M. incognita race 4	American Special Ballhead, Glory of Enkhuizen,
	Suttons Eclipse Drumhead

following precautions along with other cultural and chemical control measures will be helpful in keeping the nematode population below the damaging levels:

1. Since potato, tomato, and brinjal crops are the only important host crops, these should not be grown more than once in 3–4 years in the infested field. Nursery of these crops should not be raised in such field.
2. Potato grown in the infested fields should not be used for seed purposes. The produce should not be allowed to be carried out of the notified infested area unless it is certified and fully ensured for the absence of adhering soil and

Table 8.10 Garden crop varieties resistant to root-knot nematodes

Crop	Resistant variety
Tomato	Anahu, Atkinson, Auburn 76, Best Boy, Better Boy, Big Beef, Big Set, Bonus VFN, Bush Big Boy, Bush Early Girl, Celebrity, Champion, Empire, Ensalada, French Rose, Lemon Boy, Meltine, Monte Carlo, Nemared, Nemato, Northern Exposure, OG 50, Park's Extra Early, Park's Whopper, Patriot, Pelican, Pink Saturn, President, Red October, Resaplus, Rossol, Spring Giant, Sugar Snack, Summer Flavor 5000, Super Beefsteak, Supersteak, Supertasty, Sweet Tangerine, Terrific, Viva Italia, Winter Red
Cherry tomato	Small Fry
Lima bean	Cangreen, Nemagreen
English pea	Wando
Southern pea	California Blackeye, Floricream, Magnolia Blackeye, Mississippi Cream, Mississippi Pinkeye, Mississippi Purple, Mississippi Silver, Worthmore, Zipper Cream
Snap or pole bean	Alabama No. 1
Pimento pepper	Mississippi Nemaheart
Hot pepper	Carolina Cayenne, Charleston Hot

nematode cysts on potato tubers by the competent authorities (Evans et al. 1993).

3. Avoid the movement of irrigation and rainwater from infested fields to other fields to check the spread of cysts.

Use of Resistant Varieties

The most effective and economic way to contain the cyst nematodes is the introduction of resistance in the plants. The nematode populations usually decrease by 80 % when grown with resistant varieties. Resistant varieties like 'Maris Piper' and 'Ulster Glade' have been released in Europe. The source of resistance was from the crosses between the *S. tuberosum* and *S. tuberosum* subspecies *andigena.* A large number of germplasm collection accessions and cultures were screened for cyst nematodes, and the resistance was found in *S. varnei, S. acaule, S. microdontum, S multidissectum, S. oplocense, S. sparsiplum, S. phureja, A. fendleri, S. chacoense*, and *S. sucrense.* Complete resistance has been observed in CN-312 which is a cross between *S. tuberosum* subsp. *andigena* and *S. multidissectum.* Peconic and Wauseon are resistant to cyst nematodes, Russet Burbank is tolerant to lesion nematode, and Kufri Dewa is resistant to root-knot nematodes.

Cultural Practices

Since cysts can persist in soil for 10–15 years in the absence of host, it is difficult to eradicate this nematode completely. However, these practices are effective.

- Nonhost crops like wheat, maize, and beans can be used to minimize the population in soil.
- Crop rotation with cabbage, peas, cauliflower, carrot, etc., during autumn season brings down the population of cyst. Potato–French beans–peas = peas sequence in a 4-year rotation is effective.
- Some vegetables have been reported to bring down soil population, viz., garlic, radish, cabbage, peas, French beans, and carrot.
- Potato grown in infested soil should not be used for seed.
- Potato, tomato, and brinjal should not be grown for 3–4 years in infested fields.
- Volunteer potato plants should be removed from the infested fields.
- Movement of water and soil from infested fields should be avoided
- Crop rotation may be followed even in healthy fields so that potato is grown only once in 3–4 years.

Chemicals

1. Fensulfothion at 30 kg a.i./ha, aldicarb at 2 kg a.i./ha as spot treatment, and carbofuran at 2 kg a.i./ha have been effective to manage the cyst nematodes and to achieve increased yields.
2. Tuber disinfection by fumigation with methyl bromide for 5–6 h, formaldehyde vapor for 12 h and soaking seed tubers in 10 % formalin for 1 h, 9 % calcium hypochlorite for 30 min, 0.4–1.6 % solutions of aldicarb/aldicarb sulfone, or carbofuran for 3–60 min is effective.

8.5 Onion and Garlic

Stem and bulb nematode (*Ditylenchus dipsaci*), root-knot nematode (*Meloidogyne hapla, M. incognita, M. javanica,* and *M. chitwoodi*), and stubby-root nematode (*Paratrichodorus* sp.) are major nematode pests on these crops. Stem and bulb nematode is a potential pest on these crops.

The stem and bulb nematode lives within the plant, feeding in stems, leaves, and bulbs. It is capable of living without water and tolerates desiccation for several years. Root-knot nematodes live within the roots; the second-stage juveniles are motile and the other stages are sedentary. The stubby-root nematode lives in the soil and feeds on the roots. Lesion nematode, *Pratylenchus penetrans,* has also been reported on onions and garlic where it suppresses the growth and yield of these crops. The stem and bulb nematode penetrates the germinating clove and destroys tissue as it moves through seeking food. Nematodes sucking the cell contents and salivary secretions cause the cells to collapse. Root-knot nematodes can cause stunting and reduce a stand. Stubby-root nematode causes stunting of plants.

8.5.1 Major Symptoms

The symptoms described below may be an indication of a nematode problem, but are not diagnostic as they could result from other causes as well. Symptoms of the nematode feeding on leaf tissue include blisters, twisting and malformation of leaves, and swellings that can resemble thrips injury. Bulb damage can be mistaken for *Fusarium* basal plate rot, with bulb decay occurring at both the neck and the basal plate of the bulbs (Nicola 1993). In advanced stages, lesions caused by the nematode can become colonized by fungi and bacteria, leading to complete decay of bulbs. The common name “garlic bloat” results from the deformed growth and swelling of the bulbs.

Fig. 8.15 Irregular swelling and twisted onion leaves

8.5.2 Onion

Symptoms of bulb and stem nematode infestations on different crops vary depending on crop and population of nematodes. In onions, the base of infected seedlings appears swollen and leaves appear twisted and malformed and may bear slightly raised pimple-like spots (Fig. 8.15).

Severely infected plants eventually turn yellow and die. Scales on older infected bulbs often split and appear swollen or bloated (Fig. 8.16). Symptoms frequently look similar to damage caused by onion maggot. Infected bulbs are also very susceptible to secondary infections by bacteria and fungi.

Plants infested with the stem and bulb nematode have distorted and bloated tissue with a spongy appearance; the plants are stunted with shortened and thickened leaves, often with brown or yellowish spots. The bulb tissue begins softening at the neck and gradually proceeds downward, the scales appear pale gray, and the

Fig. 8.16 Bloated symptoms

bulbs desiccate and split at the base under dry conditions. Under moist conditions, secondary invaders such as bacteria, fungi, and onion maggots induce soft rot and decay of the bulbs. Root-knot infestation can cause stunting and uneven stands of plants and produces characteristic galls on roots. Knots induced by *M. hapla* are generally small and difficult to see, whereas galls produced by the other root-knot species are larger. Roots fed on by the stubby-root nematode are extremely short with a yellow to brownish cast.

8.5.2.1 Management

Long rotations out of onion and garlic production, sanitation, eradication, and exclusion are the primary management tools. Start with certified disease-free planting material. Prompt removal and destruction of infested plants can limit nematode damage. Chemical, physical, and cultural methods of control have been used to restrict damage, but most chemical treatments are no longer registered for use. Hot water treatment of bulbs can be effective but must be done accurately with careful monitoring to avoid heat damage to the bulbs.

Nonchemical measures include understanding the cropping history of fields to be planted with seed garlic, onion transplants, or onion sets. If the field is not known to be infested with nematode pests of onion and garlic, make sure clean, uninfested cloves are used when planting garlic. Growing nonhost crops such as carrots and lettuce for several years is helpful in reducing populations of stem and bulb nematodes, but is not usually feasible in fields with root-knot and stubby-root nematodes because of their wide host range. Avoid infesting new fields by cleaning machinery and equipment with water and preventing movement of infested soil.

Clean Seed, Sets, and Bulb

Make every effort to insure that seed; sets from onions, garlic, and shallots; and flower bulbs are clean to prevent contaminating your farm. Sets and bulbs should be examined carefully, and only plant if they appear perfectly healthy. Infected sets tend to be soft, shrunken, and discolored (dark brown) near the neck of the plant and are lighter in weight.

Hot Water Treatment of Planting Material

38 °C for 30–60 min, 49 °C for 15–30 min, followed by 18 °C for 20 min water.

Sanitation

Dispose of cull onions and carrots away from the field, where they will not contaminate irrigation canals. Clean off soil adhering to equipment before moving between fields. Remove infested plants and debris from both field and storage and then destroy them.

Rotation

Rotate all fields, including those with low populations, with a nonhost crop for 3 years.

Fumigation

Field fumigation with a registered fumigant in the fall prior to planting a susceptible crop the following spring has also been shown to effectively control these nematodes.

8.6 Sugar Beet

The sugar beet nematode, *Heterodera schachtii*, *is* the major pest on sugar beet that attacks the entire root system. Root-knot nematodes also are a problem in some regions.

8.6.1 Symptoms and Damage

Fields may be uniformly infested or have one or more localized areas of infestations in circular or oval areas exhibiting poor plant stands and growth. Seedlings may be severely injured or killed resulting in poor stands. However, the older the plant when attacked, the less damage will occur (Franklin 1972). Young plants attacked by *H. schachtii* have elongated petioles and remain stunted until harvest. Outer leaves of infected plants usually wilt during the hot period of the day. Plants that are severely attacked have pronounced yellowing of the leaves. Affected plants have small storage roots and excess fibrous roots often referred to as "bearded" or "whiskered" (Fig. 8.17). Early attack of roots often causes severe branching of storage roots. When older plants are attacked, symptoms are less noticeable. Amount of damage is determined largely by the level of parasitism and the length of favorable environmental conditions. Damage to plants is greatest in a dry summer following a wet warm spring which is favorable to the nematode.

Fig. 8.17 Root-knot-infested sugar beet

8.6.2 Management

8.6.2.1 Sanitation

Tare soil should be evenly spread over an area used for nonhost crops or used as fill in washes, barrow bits, etc. Dumping tare soil into fields can result in "hot spots" for the cyst nematode, *Rhizoctonia*, and other sugar beet diseases and pests.

8.6.2.2 Rotation with Nonhost Crops

The most widely used method of control is the rotation out of sugar beets with a nonhost crop. Rotation with nonhost crops such as wheat, barley, corn, beans, or alfalfa will reduce the soil population of *H. schachtii* through natural decline. Population reductions of 30–50 % per year are common. However, weed hosts must be controlled during the rotation. The number of years of rotation out of sugar beets will depend on the density of cysts in the soil. In a heavily infested field, a rotation of 3–4 years is minimal.

8.6.2.3 Early Planting

Planting early when soil temperatures are relatively cool (below 60 °F) greatly reduces damage from *H. schachtii*. Plants can better tolerate attack by *H. schachtii* at a later age. The younger the plant when parasitism occurs, the greater the injury and yield loss.

8.6.2.4 Nematicides

Nematicides are useful, particularly in short rotations and when egg populations are above the suggested threshold prior to planting sugar beets. Preplant soil fumigant and at-plant granular nematicides are labeled for control of *H. schachtii* on sugar beets. Soil fumigants such as Telone II (1,3-D) must be applied in the fall or preplant during the early spring. Temik 15G (aldicarb) is applied in a band at-plant and incorporated.

8.6.2.5 Trap Crops

Use of trap crops like oil radish and yellow mustard

8.6.2.6 Integrated Approach

A combination of rotation with nonhost crops, good weed control, and planting a trap crop when

available and practical will all reduce the soil population of *H. schachtii*. However, laboratory analysis of soil should be made to determine nematode density, when sugar beets can safely be planted, and the need for a nematicide application. Early spring seeding should result in better plant stands and healthier sugar beet plants.

8.7 Medicinal and Aromatic Plants

Although many species of nematodes have been reported to be associated with major aromatic and medicinal crops (Table 8.11), not much detailed information is available in this regard. Major phytonematode associated with medicinal and aromatic crops that are of economic significance includes root-knot nematodes. Major crops that are found infested by nematodes include mints, basils, henbane, geranium, davana, *Dioscorea, Coleus*, patchouli, brahmi, ashwagandha, sarpagandha, opium poppy, safed musli, and vanilla apart from few others.

8.7.1 Mints (*Mentha* spp.)

Major oil-bearing mint plants which are commonly cultivated are Japanese mint (*Mentha arvensis*), Scotch spearmint (*M. cardiaca*), Bergamot mint (*M. citrata*), peppermint (*M. piperita*), garden mint (*M. viridis*), and spearmint (*M. spicata*). These crops are of flavoring, perfumery, cosmetics, and pharmaceutical value. Root-knot and root lesion nematodes are the major, economically important pests of these crops.

8.7.1.1 Root-Knot Nematodes

M. incognita, M. javanica, and *M. hapla* are commonly associated with mint crops like Japanese mint and Bergamot mint.

Symptoms

Yellowing of the foliage which become thin and weak, scorch, and turn brown later. But veins remain green. Stunted growth with gall formation on the roots is the main symptom. Heavy infestation by the nematode has been reported to affect chlorophyll content, rate of photosynthesis, and oil content in plants.

Management

1. Soil application with carbofuran at 1.5 kg a.i./ha along with neem cake at 500 kg/ha (Pandey 1995).
2. Soil amendment with neem cake enriched with *T. harzianum* at 1 t/ha.
3. Accessions SS-5, SS-5-4, Kalka, and SS-20 recorded lowest nematode infection (Pandey and Patra 2001).

8.7.1.2 Root Lesion Nematodes

Major species like *Pratylenchus minyus, P. penetrans*, and *P. thornei* are associated with mints.

Symptoms

Severe wilting, chlorosis, and weakening of the plants along with the lesion formation on roots are the major symptoms. *P. penetrans* has been reported to increase the severity of soil fungi like *Verticillium albo-atrum.*

Management

1. Carbofuran/fensulfothion/oxamyl at 5.6 kg/ha or terbufos at 11.2 kg/ha
2. Soil amendment with neem cake at 1 t/ha

8.7.1.3 Other Nematodes

Several plant-parasitic nematodes are found associated with different mint crops. They are *Aphelenchoides ritzemabosi* on *M. piperita, A. olesistus* on *M. spicata, Paratylenchus macrophallus* on commercial peppermint, and *Longidorus elongates* on peppermint. *Tylenchorhynchus, Tylenchus, Hoplolaimus, Helicotylenchus, R. reniformis, and Xiphinema* have been frequently encountered in the rhizosphere of several mint crops.

8.7.2 Basils (*Ocimum* spp.)

Although about 60 species of *Ocimum* are being cultivated, *O. basilicum*, known as sweet basil, is the most commonly and commercially cultivated one. It yields high-quality essential oil, which

Table 8.11 Association of phytonematodes on important medicinal and aromatic plants

Sl. no.	Host plant	Nematode
1	*Abutilon indicum* (*Malvaceae*)	*Hoplolaimus* sp., *Meloidogyne incognita, M. javanica, M. incognita acrita, Tylenchorhynchus vulgaris*
2	*Allium cepa* (*Liliaceae*)	*Aphelenchoides subtenuis, A. xylophilus, Belonolaimus* sp., *Ditylenchus destructor, D. dipsaci, Helicotylenchus dihystera, Heterodera* sp., *Hoplolaimus indicus, Longidorus maximus, M. incognita, M. arenaria, M. javanica, M. hapla, Pratylenchus coffeae, P. penetrans, Trichodorus* sp., *Tylenchorhynchus brassicae, T. vulgaris*
3	*Asparagus racemosus* (*Liliaceae*)	*Helicotylenchus* sp., *Hoplolaimus* sp., *Longidorus* sp., *M. incognita*
4	*Calotropis gigantea* (*Asclepiadaceae*)	*Hirschmanniella* sp., *Longidorus* sp., *Meloidogyne* sp., *T. vulgaris*
5	*Cannabis sativa* (*Cannabinaceae*)	*D. dipsaci, Helicotylenchus erythrinae, Heterodera humuli, H. schachtii, Longidorus maximus, M. incognita, M. javanica, T. vulgaris*
6	*Cassia angustifolia* (*Fabaceae*)	*Helicotylenchus* sp., *Hoplolaimus* sp., *Heterodera* sp., *M. incognita, M. javanica, Pratylenchus* sp., *T. vulgaris*
7	*Catharanthus albus* (*Apocynaceae*)	*Longidorus* sp., *Meloidogyne* sp., *Pratylenchus* sp., *T. vulgaris*
8	*Cichorium intybus* (*Asteraceae*)	*D. dipsaci, Hoplolaimus* sp., *M. incognita, M. javanica, M. arenaria, M. hapla, R. reniformis, P. penetrans, P. pratensis*
9	*Coriandrum sativum* (*Apiaceae*)	*M. incognita, M. javanica, Heterodera oryzae, P. thornei, T. vulgaris, R. reniformis*
10	*Curcuma amada* (*Zingiberaceae*)	*M. incognita, Radopholus similis, T. vulgaris*
11	*Datura metel* (*Solanaceae*)	*M. incognita, T. vulgaris, Pratylenchus* sp.
12	*Desmodium gangeticum* (*Fabaceae*)	*Helicotylenchus* sp., *Hoplolaimus* sp., *M. incognita, M. javanica, T. vulgaris, Longidorus*
13	*Digitalis lanata* (*Scrophulariaceae*)	*Hoplolaimus* sp., *Longidorus, M. incognita, M. javanica, M. arenaria, T. vulgaris, Tylenchus* sp.
14	*Ipomoea hederacea* (*Convolvulaceae*)	*Longidorus, M. incognita, M. incognita acrita, T. vulgaris, Pratylenchus* sp.
15	*Ixora arborea, I. coccinea* (*Rubiaceae*)	*M. javanica, M. incognita, R. similis, T. vulgaris*
16	*Jatropha curcas* (*Euphorbiaceae*)	*Longidorus, T. vulgaris, Tylenchus* sp.
17	*Oxalis latifolia, O. corniculata* (*Gerianaceae*)	*M. javanica, M. incognita, M. arenaria, T. vulgaris*
18	*Piper betle, P. longum, P. nigrum* (*Piperaceae*)	*M. javanica, M. incognita, M. arenaria, R. reniformis, Hoplolaimus indicus, Helicotylenchus* sp., *Hirschmanniella* sp., *Criconemella parvula, Pratylenchus coffeae, P. zeae, X. diversicaudatum*
19	*Phyllanthus niruri* (*Euphorbiaceae*)	*Hoplolaimus* sp., *Longidorus, M.* spp., *T. vulgaris, Longidorus* sp., *Pratylenchus* sp.
20	*Punica granatum* (*Punicaceae*)	*Hemicriconemoides mangiferae, Hoplolaimus indicus, Longidorus* sp., *M. javanica, M. incognita, Pratylenchus coffeae, R. reniformis*
21	*Rauwolfia serpentina* (*Apocynaceae*)	*Helicotylenchus* sp., *M. incognita, Xiphinema* sp.
22	*Ruta graveolens* (*Rutaceae*)	*T. vulgaris, Longidorus* sp., *M.* spp.
23	*Salvia sclarea* (*Lamiaceae*)	*M. javanica, M. incognita, M. hapla*
24	*Spilanthes acmella* (*Compositae*)	*A. ritzemabosi, Hoplolaimus* sp., *Longidorus* sp., *M. incognita, Pratylenchus, T. vulgaris, Tylenchus* sp.
25	Vanilla	*Pratylenchus brachyurus*
26	*Zingiber officinale*	*M. incognita, M. javanica, M. arenaria, P. coffeae, P. pratensis, P. zeae, R. similis, R. reniformis, X. basiri*

consists of linalool (43–50 %), methyl chavicol (18–33 %), eugenol and isoeugenol (5–6 %), and traces of alpha and beta pinene, camphor, geraniol, etc. This oil is more widely used in several sectors like cosmetics, condiments, toiletory, perfumery, and confectionary industries.

Major nematodes frequently associated and of concern are *M. incognita, M. javanica*, and *A. ritzemabosi* on *O. basilicum* and *O. canum.* Root-knot nematodes are a problem also on *O. sanctum, O. gratissimum*, and *O. kilmandescharicum*. Other nematodes associated with basils are *Tylenchorhynchus, Tylenchus, Hoplolaimus, Helicotylenchus, R. reniformis*, and *Xiphinema.*

8.7.2.1 Management

1. Carbofuran or aldicarb at 4.5 kg/ha
2. Soil application with neem cake/mahua cake

8.7.3 Henbane (*Hyoscyamus* sp.)

Henbane is one of the important medicinal herbs which is a good source of alkaloids of tropane group, in specific, hyoscine, hyoscyamine, and atropine. They are of use in pharmaceutical industry due to their mydriatic, antispasmodic, and anticholinergic properties. *H. muticus* and *H. niger* are major species. Root-knot nematodes (*M. incognita* and *M. javanica*) are important pests of this crop.

8.7.3.1 Symptoms

Chlorotic and stunted growth of the plants, with few leaves and flowers, and galling on the roots are the common symptoms. Nematodes have been shown to affect total alkaloid yield, chlorophyll content, photosynthetic rate, and sodium, potassium, iron, manganese, copper, and zinc content in roots and shoot.

8.7.3.2 Management

1. Pre-sowing measures are important, viz., crop rotation, green manuring, fallowing, organic amendments, etc.
2. Crop rotation with nonhost crops like *Cymbopogon flexuosus, C. wintrianus*, and *C. martini* or marigold
3. Removal of weeds and destroying them
4. Carbofuran or aldicarb at 2 kg a.i./ha
5. Soil application with neem cake/mahua cake at 1 t/ha

8.7.4 Davana (*Artemisia pallens*)

Davana is an aromatic herb that belongs to the family Asteraceae. It is an annual crop grown for the high-quality essential oil. Oil constitutes cinnamoyl, cinnamate, fenchyl alcohol, cadinene, and about 12 phenols or acids with many sesquiterpenes, linalool, eugenol, and geraniol. Root-knot nematodes (*M. incognita and M. javanica*) are major pests on davana.

8.7.4.1 Symptoms

Stunted growth of the plants bearing less number of flower buds in a patch appearance in the field. Galls are seen on the roots, with reduced root growth. Oil yield is significantly reduced at severe infestation by the nematode.

8.7.4.2 Management

1. Carbofuran or aldicarb at 3.5 kg/ha
2. Soil application with neem cake/mahua cake at 1 t/ha

8.7.5 Yam (*Dioscorea* spp.)

Diosgenin, a steroidal sapogenin, is obtained from several species of *Dioscorea*. It is used as a precursor for synthesis of some drugs like cortisones, sex hormones, and oral contraceptives. *D. deltoidea* is the major cultivated species followed by *D. alata, D. cayenensis, D. floribunda, D. spiculiflora, D. spinosa*, *and D. rotundata.* Several nematodes like *Scutellonema bradys, S. clathricaudatum*, *M. incognita, P. coffeae, P. brachyurus, A. besseyi, Helicotylenchus pseudorobustus, H. cavenessi, R. reniformis, X. ifacolum,* and *Heterodera* sp., have been reported on yam.

However, *Scutellonema bradys*, the yam nematode, is the major nematode pest inciting “nematosis” or “dry rot” disease. Eggs are laid in

Fig. 8.18 Yam nematode-infested tubers

soil or plant tissues and hatch, and the larvae develop into adults by subsequent moltings, feeding ectoparasitically or endoparasitically on roots and tubers. More than one generation may be completed within the host.

8.7.5.1 Symptoms

The nematode attacks the primary roots and enters the developing tubers. They also enter tubers through the cracks or damaged areas in the suberized epidermis. It is characterized by the loosening of the tuber cortex, leading to rotting and decay (Fig. 8.18). Aboveground symptoms are not easily recognizable. Peeling or lifting of the skin of yam and yellowing of infected area later turning brown/black with the association of other microorganisms in older darker lesions beneath the skin are major symptoms. Soil fungi like *Fusarium solani, F. oxysporum*, *and R. solani* are associated.

The nematodes confine their activities to the tissues lying within the periderm causing extensive cell destruction. Nematodes move intracellularly and break cell walls leading to the formation of irregular spaces in large numbers around the nematode. Damage is confined to cell layers just beneath the periderm up to a depth of 2–3 mm. When cell destruction is abundant, the remaining cell walls become coated with a brown substance, which is also noticeable on the xylem elements of the vascular bundles.

Pratylenchus brachyurus, P. coffeae, Criconemoides, R. reniformis, Helicotylenchus sp., *Aphelenchoides* sp., *Aphelenchus* sp., and *Hoplolaimus* sp. have also been reported to be associated with yam tuber from various parts of the world.

P. coffeae causes cracking of the skin of the yam underlined by a brown, corky rot in the storage tissues. Rot further progresses deeper into the yam tissues following harvest and prior to planting or consumption and is generally more pronounced toward the stem and yam tubers. On heavily burned heads, stem primordia appear to be damaged or destroyed by the dry rot resulting in heads not sprouting or veins growing from them being less thrifty than those from less affected heads.

8.7.5.2 Management

- Hot water treatment of tubers at 50 °C for 30–40 min
- Soil amendments with yam molds at 1,886 kg/ ha
- Use of resistant varieties like INRA-25 and Grosses-Caille
- Exposing tubers to gamma radiation between 10 and 15 krad
- Disinfestations of tubers by dipping in oxamyl at 600 ppm for 30 min
- Preplant treatment with carbofuran/aldicarb/oxamyl at 2 kg a.i./ha

8.7.6 Patchouli (*Pogostemon cablin, P. patchouli*)

The oil of patchouli extracted from steam distillation of dried leaves is a very important essential oil for use in the perfumery industries. It is popular because of its fixative property which blends well with other essential oils like geranium, lavender, and sandalwood. The oil is also used in Ayurvedic medicine for treating diarrhea, nausea, headache, cold, etc. It is used extensively in food industries as flavor ingredient also. Most important nematodes attacking patchouli are *M. incognita, M. javanica*, and *Pratylenchus brachyurus*.

8.7.6.1 Symptoms

Typical gall formation on roots is observed which leads to stunted growth, wilting, defoliation, and yellowing of the infected plants. Nematode infection may take place even in the very early stage of the crop growth. Nematodes reach the cortex, multiply, and cause galling which is commonly called root knot. The diameter of the knots varies from 0.2 to 5.0 cm. Heavily infected plants show stunting of top growth and wilting. Initially it will be difficult to differentiate between the infested and healthy plants. Typical symptoms of an infested plant become apparent only after 8 months. In case of severe attack, the crop dwindles and the whole stand may perish during the next 2 or 3 months.

8.7.6.2 Management

- Carbofuran at 20 kg/ha (3 % a.i.) or Dasanit 150 kg/ha (5 % a.i.) is found to be very effective. First dose of the nematicide should be given as a preplanting treatment, and the second dose can be given 1 year after transplanting as pocket application to the plants. In order to get a good crop of patchouli in nematode-infested soil, it is desirable to take a crop of citronella or other nematode-resistant crop for the next few years before planting patchouli.
- The nursery should be raised from healthy mother stock under nematode-free conditions.
- Aldicarb–sulfone/phorate at 3.6 kg a.i./ha as preplant treatment.
- Postplant treatment with carbofuran/aldicarb–sulfone at 10 kg a.i./ha.
- Soil application of neem cake at 2–4 t/ha.

8.7.7 Geranium (*Pelargonium graveolens*)

Geranium is grown mainly for its essential oil used in high-grade soap, perfumery, and cosmetic industries due to its strong rosy aroma. This oil comprises 1-isomenthone, isoamyl alcohol, rhodinol, and methyl pentanol. Nematodes of importance are *M. incognita, M. hapla*, and *Helicotylenchus dihystera.*

8.7.7.1 Symptoms

Typical root-knot symptoms are seen on infected plants, viz., stunting of the plants, yellowing of leaves, and gall formation on root system. Spiral nematodes may initiate lesions on the root surface, leading to browning and decay of the roots.

8.7.7.2 Management

Phorate/aldicarb/carbofuran at 2–3 kg/ha 4 months after the transplant of the cuttings is effective in reducing the nematode population and increasing the oil yield.

8.7.8 *Coleus* (*C. forskohlii, C. aromaticus*)

It is one of the commercial medicinal plants cultivated in several parts of the world. It is a perennial aromatic herb and produces root tubers, which are used as condiments in the preparation of pickles and for extraction of the diterpenoid forskolin by drug industries. Forskolin has the unique property of activating almost all hormone-sensitive adenylate cyclase enzymes in biological systems. It is useful in the treatment of congestive heart failure, glaucoma, asthma, and cancer and in preventing immature graying of hair (Joy et al. 1998). Root-knot nematodes are one of the major constraints to the cultivation of coleus. Severely infected plants often fail to produce root tubers and yield up to 86 % less than healthy plants (Senthamarai et al. 2006a). Root-knot and fungal wilt disease complex may lead to heavy losses.

8.7.8.1 Symptoms

Major symptoms include stunted growth of plants, yellowing of the foliage, and wilting in patch areas (Fig. 8.19). Characteristic medium- to large-sized knots on the roots are important diagnostic symptoms, and the root system may be devoid of feeder roots, malformed, and nonfunctional (Fig. 8.20).

8.7.8.2 Management

Cultural

Use of trap crop like sweet potato cv. Sree Bhadra

Fig. 8.19 Root-knot-infested *Coleus* plants

Fig. 8.20 Root-knot-infested *Coleus* tuber

Biological

Soil application with *Trichoderma harzianum* or *Pseudomonas fluorescens* at 2.5 kg/ha (Senthamarai et al. 2006a, b)

Chemical

Carbofuran soil application at 12 kg/acre

Root-knot nematodes also attack other medicinal crops like ashwagandha (*Withania somnifera*), sarpagandha (*Rauvolfia serpentina*), kacholam (*Kaempferia galanga*), Chinese potato (*Solenostemon rotundifolius*), chamomile (*Matricaria recutita*), Babchi (*Psoralea corylifolia*), brahmi (*Bacopa monnieri*), *Ammi majus*, Khasi kateri (*Solanum viarum*), and safed musli (*Chlorophytum borivilianum*) (Pandey 2002; Sheela et al. 2004; Pandey and Karla 2005).

8.7.9 Vanilla (*V. planifolia*)

Pratylenchus brachyurus has been found to be associated with vanilla in several areas, apart from root-knot nematodes. But economically significant damage and losses by phytonematodes have not been reported on this crop.

8.8 Ornamental Crops

Major traditional and commercially grown ornamental crops commonly grown are crossandra, tuberose, chrysanthemum, rose, jasmine, orchids, gladiolus, carnation, gerbera, anthurium, lilies, champak, marigold, aster, etc. Although several nematodes have been reported to be associated with many ornamental crops throughout the world (Table 8.12), detailed studies are lacking on several crops.

With floriculture emerging as a major industry worldwide, nematodes form one of the major constraints in protected/polyhouse-grown crops, including ornamentals.

8.8.1 General Symptoms

8.8.1.1 Aboveground Symptoms of Nematode Damage to Ornamentals

Yellowing, slow growth, and unusual sensitivity to heat expressed as premature wilting (water stress)

Table 8.12 Major nematodes associated with important ornamental crops

Sl. no.	Crop	Nematode
I.	**Flowering plants**	
1	*Asparagus sprengeri*	*Helicotylenchus cavenessi*
2	Asters	*Aphelenchoides ritzemabosi*
3	*Begonia*	*Meloidogyne incognita*
4	*Cineraria hybrida*	*Helicotylenchus* spp.
5	*Carnation*	*R. reniformis, M. arenaria, M. hapla, M. javanica, M. incognita, Ditylenchus dipsaci, Heterodera trifolii, Criconemella curvata, Pratylenchus dianthus, Paratylenchus curvitatus, Tylenchus, Macroposthonia curvata*
6	*Chrysanthemum*	*Aphelenchoides ritzemabosi, Helicotylenchus* sp., *Meloidogyne* spp., *Pratylenchus penetrans, R. reniformis*
7	*Crossandra*	*M. incognita, Pratylenchus delattrei, P. africans*
8	Dahlia	*A. ritzenabosi, Ditylenchus destructor*
9	Gladiolus	*Meloidogyne* spp., *Helicotylenchus rotundicaudatus, Scutellonema brachyurum,*
10	Hemerocallis	*H. rotundicaudatus*
11	*Hibiscus rosa-sinensis*	*Helicotylenchus varicaudatus*
12	Hyacinth and tulip	*Ditylenchus*
13	Jasmine	*M. incognita, A. besseyi*
14	Lily	*Pratylenchus penetrans*
15	Orchids	*A. ritzemabosi*
16	Phlox	*X. vuittenezi*
17	Rose	*Pratylenchus vulnus, M. hapla, Helicotylenchus labiata, H. dihystera, H. nannus, Hoplolaimus galeatus, X. diversicaudatum, Pratylenchus penetrans, Hemicycliophora typical, Longidorus macrosoma*
18	Salvia	*A. ritzemabosi*
19	*Zinnia elegans*	*A. ritzemabosi*
II.	**Ferns**	
1	*Asplenium nidus*	*A. ritzemabosi*
2	*Aucuba japonica*	*M. arenaria*
3	*Buxus microphylla* (*Japanese boxwood*)	*M. arenaria*
4	*B. sempervirens* (*American boxwood*)	*M. arenaria, Pratylenchus vulnus, Criconemella xenoplax*
5	*Camellia japonica*	*Tylenchorhynchus claytoni*
6	*Gardenia jasminoides*	*M. arenaria*
7	*Ilex cornuta* (*Chinese holly*)	*C. xenoplax, T. claytoni*
8	*I. crenata compacta*	*M. arenaria*
9	*Juniperus conferta*	*Criconemella xenoplax*
10	*J. excelsa*	*Pratylenchus vulnus*
11	*J. horizontalis*	*P. vulnus*
12	*Photinia* and *Rhododendron indicum*	*T. claytoni*
13	*Struthiopteris orientalis*	*M. arenaria*
14	*Tuberose*	*M.*spp.
III.	**Woody plants**	
1	*Abenda grandiflora*	*M. hapla*
2	*Cacti*	*M. incognita, Cactodera cacti, Heterodera cacti*
3	*Cornus florida*	*M. hapla*
4	*Croton*	*H. cacti*
5	*Ctenatha*	*Helicotylenchus dihystera*
6	*Ficus elastica*	*Pratylenchus coffeae*
7	*Hippeastrum vittatum*	*Pratylenchus coffeae*
8	*Hoya carnosa*	*Scutellonema brachyurus*
9	*Momordica*	*P. coffeae*
10	Palm	*Helicotylenchus dihystera*

occur in irregularly shaped patterns. Woody ornamentals may suffer leaf drop and dieback of branches.

8.8.1.2 Belowground Injury by Nematodes

Root-Knot Nematodes

Plant roots have medium to large knots/galls.

Sting, Awl, and Stubby-Root Nematodes

Injurious to grasses and many ornamentals. They damage the root tips and deform the tips.

Lance, Lesion, and Burrowing Nematodes

They cause discoloration in roots. Nematode feeding and the invasion of fungi and bacteria lead to softening and darkening of the root tissue. Lesion and burrowing nematodes rarely injure grass but are serious pests of ornamentals. Lance nematodes attack both grasses and ornamentals.

8.8.2 Crossandra (*Crossandra undulaefolia* L.)

Major nematodes associated with crossandra which are of economic importance are root-knot, lesion, spiral, needle, reniform, lance, stunt, and dagger nematodes.

8.8.2.1 Root-Knot Nematode

Root-knot nematodes (*Meloidogyne incognita, M. javanica, M. arenaria*) are most commonly occurring parasites of crossandra in most regions.

Symptoms

Stunted growth of the infected plants (Fig. 8.21), often with fried peripheral branches is the major symptom. Yellowing of leaves, sometimes with chlorotic patches, is seen, with roots having galls. Infected plants will have small inflorescence. Flower yield is reduced. This nematode forms a complex disease in crossandra by associating with wilt-causing fungi like *F. solani, F. oxysporum, R. solani, R. bataticola,* and *Macrophomina phaseolina*. Such complex diseases may lead to increased damage to the crop and also may result in poor flower yield.

Fig. 8.21 Infested (*left*) and healthy (*right*) crossandra plants

Management

- Use of healthy cuttings in a healthy field
- Intercropping with antagonistic crops like marigold
- Carbofuran application at 2–3 kg a.i./ha
- Nursery application with *Paecilomyces marquandii* grown on paddy seeds at 2 g/kg soil or *T. harzianum* at 2 kg/sq.m enriched with neem cake

8.8.2.2 Lesion Nematode

Lesion nematode (*Pratylenchus delattrei*) is an important nematode parasite of crossandra all over the world. It is reported to cause loss in the flower yield.

Symptoms

Stunted growth is the common symptom with pink–purple to yellow patches on leaves. Other symptoms include reduction in the size of the inflorescence and flower, reduction in the root growth which will have characteristic brown to blackish spindle-shaped lesions due to feeding by the nematode that moves intracellularly and remains in the cortical region. Nematodes dissolve

cell walls that lead to cavity formation in the cortical region. Thickening of cell walls and denser, granular cell contents are important changes brought about by nematode feeding.

P. delattrei is found to be frequently associated with *F. solani* and *F. oxysporum* that induce severe wilting/decline of crossandra

Management

- Use of nematode-free planting material
- FYM and intercropping with marigold
- Carbofuran 3G/phorate/aldicarb at 5 g/plant or 2.5 kg a.i./ha
- Root dip for 8 h in 0.05–0.1 % solution of carbofuran/aldicarb/phenamiphos/phorate/fensulfothion
- *T. viride* at 10 g/kg soil

8.8.2.3 Needle Nematode

Needle nematode (*Longidorus africanus*) has been reported to be associated with crossandra.

Symptoms

General stunted growth of the infected plants is the major visible symptom.

This nematode induces hyperplasia of parenchymatous cells of the peripheral region that progresses toward the center. The meristem is pushed laterally that later leads to the formation of dark-stained cells.

Host Range

A wide range of hosts include tomato, fig, sugar beet, sorghum, lettuce, mint, grapes, sour orange, snap bean, lima bean, wheat, cotton, okra, cucumber, brinjal, etc.

Management

1. Intercrop cultivation or crop rotation of poor hosts like carrot, pea, onion, and spearmint and nonhosts like cabbage, cauliflower, and radish can minimize the nematode population in soil.
2. Use of nematode-free planting material.
3. Soil solarization by mulching moist soil, particularly nursery beds with clean thin polyethylene sheet, for 15 days during April–June months in India.

Fig. 8.22 Infested (*left*) and healthy (*right*) tuberose plants

8.8.3 Tuberose (*Polianthes tuberosa*)

An important nematode frequently encountered in the rhizosphere of tuberose is root-knot nematode. Bud and leaf nematode is also of importance in several areas.

8.8.3.1 Root-Knot Nematode

Root-knot nematodes (*M. incognita, M. javanica, M. arenaria*) are the major nematode pests of concern on tuberose in most areas.

Symptoms

Yellowing, drying up of leaves, and general stunted growth are commonly found in infected plants (Fig. 8.22). Side shoot emergence from the bulbs may be affected. Under high population levels, spike emergence may be affected, which affects the flower yield.

Management

Physical: Hot water treatment of bulbs at 49 °C for an hour

Cultural: Use of nematode-free planting material; soil solarization by mulching moist soil, particularly nursery beds with clean thin polyethylene sheet, for 15 days during April–June months; and neem cake application to the soil at 1 t/ha

Resistant Varieties: Cultivar Shringar (single type) is resistant and Suvasini (double type) is tolerant to *M. incognita* (Nagesh et al. 1995a).

Chemical: Aldicarb or carbofuran at 15–20 g/plant or carbofuran/fensulfothion/phorate at 1.5–2.5 kg a.i./ha

Reniform Nematode (*Rotylenchulus reniformis*) has also been reported to be associated with tuberose rhizosphere in many regions.

8.8.4 Chrysanthemum (*Chrysanthemum* spp.)

Major plant-parasitic nematodes infecting this crop are foliar/bud and leaf nematode (*Aphelenchoides ritzemabosi*) and lesion nematode (*Pratylenchus coffeae*, *P. chrysanthus*, and *P. fallax*).

8.8.4.1 Symptoms

A. ritzemabosi

Common symptoms are appearance of angular spots/blotches on leaves, which later coalesce to form patches (Fig. 8.23), and reduced number of leaves which give a shrunken look. Such leaves undergo premature falling. Reduction in the flower yield and small-sized flowers are also common. This nematode feeds on mesophyll of the leaf causing necrosis which may spread along veins ultimately leading to stunted growth, distortion, and formation of high rough feeding sites and dieback symptoms.

Fig. 8.23 Foliar symptoms

8.8.4.2 Management

Physical

Hot water treatment of stools at 46 °C for 5 min (Parvatha Reddy 2008)

Cultural

Infested plant material should be collected and burned. Selection of nematode-free site and avoiding wetting and overlapping of foliage of adjacent plants and banding the stem bases with grease

Resistant Varieties

Cultivars Amy Shoesmith, Orange Beauty, and Orange Peach Blossom are tolerant ones.

Chemical

Stool dipping in 0.03 % parathion for 20 min, foliar application with chlorpyrifos at 0.05 %, and drenching with 0.02 % thionazin at 6.8 L/sq.m.

Pratylenchus spp.

Premature yellowing along with a general stunted growth associated with drying of leaves and reduction in flower size are some of the major symptoms. Complete destruction of parenchymatous cells due to nematode feeding is seen. Normally this nematode is confined to the cortical cells. Lesion nematode has been reported to increase the incidence of diseases caused by soil fungi like *Pythium aphanidermatum,* and *R. solani*.

8.8.4.3 Management

1. Use of nematode-free planting material
2. Hot water treatment of planting material at 46 °C for 5 min
3. Soil solarization by mulching moist soil, particularly nursery beds with clean thin polyethylene sheet, for 15 days during April–June months in India
4. Soil application with aldicarb at 500 q/ha before planting
5. *T. harzianum* at 1 t/ha enriched with neem cake

Other nematodes reported to be associated are *M. arenaria, M. javanica, Belonolaimus longicaudatus, Heterodera mothi, R. reniformis*, and *Tylenchorhynchus vulgaris.*

8.8.5 Rose (*Rosa* spp.)

Although quite a good number of nematodes have been reported to be associated with rose rhizosphere, economically important parasites are lesion and root-knot nematodes. Other nematode species associated with rose are *Hoplolaimus galeatus, X. diversicaudatum, X. basiri, Helicotylenchus nannus, Tylenchorhynchus dubius, Pratylenchus* spp.*, Hemicycliophora typica, H. labiata*, etc.

8.8.5.1 Lesion Nematode

Symptoms

Major symptoms caused by lesion nematode (*Pratylenchus pratensis, P. zeae, P. penetrans, P. vulnus*) include decreased growth of the aboveground parts of the plant mainly due to the malnutrition and chlorosis of the foliage which may result in the reduction in the flower yield. The root system is severely affected with reduced growth and devoid of feeder roots.

Management

Physical: Hot water treatment of seedlings at 50 °C for 5 min or 48 °C for 10 min can minimize the population of *P. zeae.* Soil solarization by mulching moist soil, particularly nursery beds with clean thin polyethylene sheet, for 15 days during April–June months in India

Cultural: Use of nematode-free planting material. African marigold as an intercrop reduces the incidence of *P. penetrans*.

Resistant Varieties: Use of resistant varieties, viz., ‘Major’ (*Rosa indica*) against *P. penetrans* and ‘*Rosa multiflora* 60-5’ against *P. vulnus* (Ohkawa and Saigusa 1981)

Chemical: Oxamyl 25 at 500 ppm as aerial spray; carbofuran soil application 10–14 kg/acre

8.8.5.2 Root-Knot Nematodes

Most commonly reported species on rose are *M. incognita, M. arenaria, M. javanica*, and, *M. hapla.*

Symptoms

Typical root-knot symptoms are observed in the infected plants. General stunting, yellowing of the foliage, and wilting symptoms accompanied by severe reduction in the root growth and less feeder roots with normally small galls on the roots (Fig. 8.24).

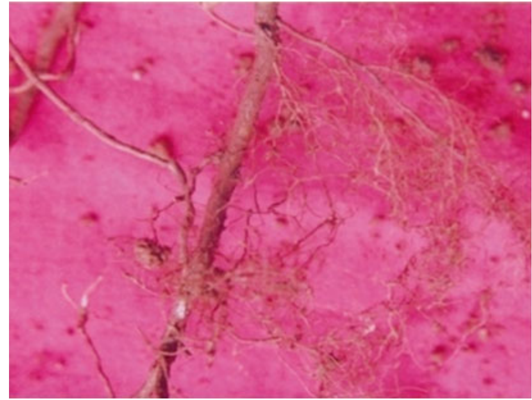

Fig. 8.24 Knots on rose roots

Management

Cultural: Use of nematode-free planting material; soil solarization by mulching moist soil, particularly nursery beds with clean thin polyethylene sheet, for 15 days during April–June months in India

Physical: Hot water treatment of roots of plants at 45.5 °C for an hour

Resistant Variety: Cultivar Mavetti is resistant to *M. hapla* (Ohkawa and Saigusa 1981).

Chemical: Bare root dipping in 0.1 % phenamiphos for 30 min

8.8.6 Gladiolus (*Gladiolus* spp.)

Root-knot nematodes are economically important nematode pests on gladiolus. *M. incognita* and *M. javanica* are the major species commonly reported from different parts of the world.

8.8.6.1 Symptoms

Stunted growth of the aboveground plant parts, yellowing of the leaves, reduction in the length of the spike, reduction in the flower yield, and reduced root growth with medium to large galls are some important symptoms. Root-knot nematodes are reported to be associated with many soilborne fungi in the rhizosphere of gladiolus.

8.8.6.2 Management

Physical

Hot water treatment of corms at 58 °C for 30 min

Cultural

Use of nematode-free planting material; soil solarization by mulching moist soil, particularly nursery beds with clean thin polyethylene sheet, for 15 days during April–June months in India; crop rotation/intercropping with marigold

Biological

Organic amendments enriched with *P. fluorescens* or neem cake enriched with *T. harzianum* at 1 t/ha

Chemical

Dipping of the rhizomes in a nematicide solution like fensulfothion at 0.5 lb/100 gal water; soil application with Vorlex at 35 gal/acre

8.8.7 Jasmine (*Jasminum* spp.)

Major nematodes affecting jasmine are root-knot nematodes. However, other species associated with this crop are *T. semipenetrans, A. besseyi*, and *R. similis.* In root-knot-infected plants, severe reduction in the plant growth, yellowing of the leaves, reduced root growth with galls, and reduced flower yield are the major symptoms. In burrowing nematode-infected roots, yellowish brown lesions on the roots, blackening at later stages, stunning, and drying of the branches with wilted leaves that leads to the dropping are important symptoms.

8.8.7.1 Management

Use of nematode-free planting material; soil solarization by mulching moist soil, particularly nursery beds with clean thin polyethylene sheet, for 15 days during April–June months in India

8.8.8 Carnation (*Dianthus caryophyllus*) and Gerbera (*Gerbera jamesonii*)

Important parasitic nematodes attacking carnations and gerbera are root-knot nematodes (*M. incognita, M. arenaria, M. hapla*, *and M. javanica*). Other nematode species associated with these crops are *D. dipsaci, Heterodera trifolii, Paratylenchus dianthus, Criconemella curvata, Tylenchus* spp., *Pratylenchus* spp., *Macroposthonia curvata*, etc.

Fig. 8.25 Infested root system

8.8.8.1 Symptoms

Characteristic symptoms are observed in these crops, viz., stunted growth, wilting, yellowing of foliage, and premature foliage dropping along with moderate to severe galling on the infested roots (Fig. 8.25). Overall growth of the plants is affected that may lead to low flower yield in terms of both quality and quantity.

8.8.8.2 Management

Cultural

Neem cake/castor cake application at 1 kg/sq.m; use of nematode-free planting material; soil solarization by mulching moist soil, particularly nursery beds with clean thin polyethylene sheet, for 15 days during April–June months in India

Biological

Paecilomyces lilacinus at 0.5 g/kg soil or *T. viride* at 100 g/sq.m along with organic amendments like neem cake at 0.5 kg/plant (Johnson 2000)

Resistant Varieties

Cultivars Kappa, Desio, Logupink, Antalia, Castelaro, Rara, and Target are resistant to *M. incognita*.

Chemical

Carbofuran 3G at 2 kg a.i./ha and preplant soil sterilization of the nursery beds with dazomet/ metam sodium at 40 g/sq.m or formalin

8.8.9 Tulips (*Tulipa* spp.)

Stem and bulb nematode (*Ditylenchus dipsaci*) is the major nematode infecting tulips.

8.8.9.1 Symptoms

Foliage and flowers are attacked by the nematode that may lead to the thickening of the flower stem and may bend below with yellow lesions which may affect the appearance of the flower. Natural color of the petals may be lost, and leaves may turn fragile with traversed and longitudinal splits.

8.8.9.2 Management

- Use of nematode-free planting material
- Hot water treatment 43.3 °C for 3 h
- Soil solarization by mulching moist soil, particularly nursery beds with clean thin polyethylene sheet, for 15 days during April–June months in India
- Thionazin spray at 21/100 gal of water

8.8.10 Lilies (*Lilium* spp.)

The bulb and leaf nematode (*A. fragariae*) and lesion nematode (*Pratylenchus pratensis* and *P. penetrans*) are important on lilies.

8.8.10.1 Symptoms

Induction of yellow blotches on foliage that later turn into brown-yellow color. At the later stages, dieback symptoms may be seen with the production of blind buds that fail to flower.

8.8.10.2 Management

1. Use of nematode-free planting material
2. Hot water treatment of bulbs at 36 °C for 6 h or at 41 °C for 1 h
3. Soil solarization by mulching moist soil, particularly nursery beds with clean thin polyethylene sheet, for 15 days during April–June months in India

8.8.11 Dahlia (*Dahlia* spp.)

The aboveground parts of the plants are infected by *A. ritzemabosi*, which is a major nematode on this crop.

8.8.11.1 Symptoms

Foliage will develop larger brownish patches that may lead to drying of leaves which later result in defoliation, stunted growth, and reduction in the flower yield.

8.8.11.2 Management

1. Use of nematode-free planting material
2. Soil solarization by mulching moist soil, particularly nursery beds with clean thin polyethylene sheet, for 15 days during April–June months in India

In most ornamental crops, it is important to follow domestic and international quarantine measures for checking spread of nematodes from infected to healthy regions.

8.8.12 China Aster (*Callistephus chinensis*)

Root-knot nematodes are predominant on this crop, which affect the quality and quantity of flowers.

8.8.12.1 Management

Use of varieties like Shashank (resistant) and Poornima (moderately resistant) (Nagesh et al. 1995a, b)

8.9 Plantation Crops

8.9.1 Coconut (*Cocos nucifera*)

Economically important nematode pests of coconut are red ring nematode, *Rhadinaphelenchus* (*Bursaphelenchus*) *cocophilus*, and the burrowing nematode, *Radopholus similis.*

8.9.1.1 Red Ring Nematode

Red ring nematode (*Rhadinaphelenchus cocophilus*) causes red ring disease of palms. Red ring disease can appear in several species of tropical

palms, including date, Canary Island date, and Cuban royal, but is most common in oil and coconut palms. The red ring nematode parasitizes the palm weevil *Rhynchophorus palmarum* L., which is attracted to fresh trunk wounds and acts as a vector for *R. cocophilus* to uninfected trees.

Symptoms

Internal damage can be seen within 2–3 weeks after *B. cocophilus* enters the tissue of a healthy palm. External symptoms can take up to 2 months to appear. The major internal symptom of red ring infection is the telltale red ring for which the disease was named. A crosswise cut through the trunk of an infected palm 1–7 ft above the soil line usually will reveal a circular, colored band approximately 3–5 cm wide, variable with the size of the tree (Fig. 8.26a, b). The surface of the cut in a healthy tree appears a solid, creamy white. The most common color of the band is bright red, although the shade can vary from light pink or cream to dark brown in infected African oil palms.

Under external symptoms, already established leaves become short and deformed and turn yellow bronze, then deep reddish brown. The color change usually begins at the tip of each leaf and starts in the older leaves before moving to the younger ones. As the leaves change color and dry up, they wilt and die. In some African oil palms and older coconut palms, infected trees begin to produce small, deformed leaves that retain their green color and are not initially necrotic. This is a sign of little-leaf disease, a chronic disease that can lead to red ring disease. New leaves often get shorter as the disease progresses, causing the central crown of the tree to resemble a funnel. Eventually, these new "little leaves" display varying degrees of necrosis. These trees often stop producing fruit. This symptom is not as common in coconut palm as it is in African oil palm (Chinchilla 1991; Giblin-Davis 2001).

In coconut palms, red ring nematodes most often attack trees between 3 and 7 years old. These young trees usually die 6–8 weeks after the appearance of symptoms. Older trees can live up to 20 weeks (Esser and Meredith 1987). In some areas, infected trees can live several more years, and in some of these trees, the disease becomes chronic, manifesting itself in the continuing production of little leaves for years. Not many palms recover from red ring disease. The few that do recover often undergo a recurrence of the disease in later years. Trees that have been affected by red ring disease for more than 3 years are noticeably stunted compared with healthy trees of a similar age (Chinchilla 1991).

Fig. 8.26 (**a**) Infested tree, (**b**) red ring internal symptom

Management

- Phytosanitation is still the best method of red ring disease management. The most useful and most important method for management for red ring nematode is the early removal and destruction of red-ring-infested palms. Infested

palms should be sprayed with an insecticide and then destroyed as soon as possible once the presence of red ring nematodes has been confirmed. Sometimes weevil larvae will remain in the tissue of palms that are killed with herbicide. These trees should be cut into sections and treated with insecticide or burned (Giblin-Davis 2001).

- Treating infested palms with nematicides is difficult because the nematicides do not easily spread throughout an infested tree and often do not penetrate the area of the trunk usually inhabited by the nematodes (Chinchilla 1991).
- Controlling the vector *R. palmarum* can help reduce red ring nematode infestation. Trees should be sprayed with an insecticide (i.e., methomyl) and killed with 100–150 ml (48.3 % a.i.) of the herbicide monosodium acid methanearsonate (MSMA) or other herbicides that are injected or placed into the trunk. Occasionally trees injected with MSMA will harbor weevil larvae. Therefore, the tree should be cut and sectioned to make sure that weevils are not present.
- Palms that are heavily infested with weevils should be cut, sectioned, and treated with an insecticide such as methomyl, trichlorfon, monocrotophos, carbofuran, carbaryl, or lindane. Injections of systemic nematicides, such as phenamiphos, oxamyl, and carbofuran into little-leaf symptomatic palms can help with palm recovery. However, because of the damage to the very young leaves in little-leaf palms, the recovery can take between 6 and 8 months.
- Mass trapping with traps baited with sugarcane and synthetic aggregation pheromone (Rhyncholure, racemic 6-methyl-2-hepten-4-ol) is also effective.

8.9.1.2 Burrowing Nematode

(*Radopholus similis*): It is another major endoparasite being encountered in coconut root samples.

Symptoms

Infested palms show general yellowing and reduced growth, vigor, and yield. Appearance of orange-colored lesions, blackening of tips of lateral and tertiary roots, and rotting of roots are conspicuous symptoms (Koshy and Sosamma 1987). Button shedding, reduction in size and number of foliage, and delayed flowering, leading to reduced yield. Tender and semihard roots may exhibit elongated orange-colored lesions, which later enlarge and coalesce to lead to extensive rotting of roots. Tender, infested roots may turn spongy with cracks on the surfaces.

Management

Cultural

- Use of nematode-free planting material.
- Soil application with neem cake (2 kg/tree), farmyard manure, or green leaf material at the base of the tree.
- Intercropping with cocoa, *Crotalaria juncea*, avoiding banana, black pepper, and areca nut, which are good hosts for this nematode.
- Soil application with FYM/cow dung/neem cake at 50 kg along with 25 kg green manure to the base of the tree.
- Intercropping with *Crotalaria juncea* as a green crop in the basins, later incorporating into the soil.

Biological: Nursery potting mixture amendment with *Paecilomyces lilacinus* or *Trichoderma viride* or *T. harzianum* along with organic material is effective.

Resistant Varieties: The dwarf cultivars Kenthali and Klappawangi and hybrids like Java Giant X Kulasekharam Dwarf Yellow, Java X Malayan Dwarf Yellow, and San Ramon X Gangabondam are some resistant/tolerant types.

Chemical

(a) *Nursery Treatment:* Soil application with phorate/phenamiphos/carbofuran at 2.5 kg a.i./ha or fensulfothion at 50 kg a.i./ha three times during September, December, and May (Parvatha Reddy 2008).

(b) *Bare Root-Dip Treatment:* Coconut seedlings bare root dip in 1,000 ppm of DBCP for 15 min

(c) *Main Field:* Soil application with carbofuran/phorate at 10 g a.i./palm or phenamiphos at 10 g a.i./palm in June–July and later in September–October; aldicarb at 0.5–1 kg a.i./ha or 10–50 g a.i./palm applied twice at 3 months interval

8.9.2 Areca Nut (*Areca catechu*)

Radopholus similis is the major nematode pest attacking areca nut. Symptoms are similar to that given under coconut.

8.9.2.1 Management

Cultural

Use of nematode-free planting material; avoidance of intercropping/mixed cropping with hosts like banana and black pepper; neem cake application at 1.5 kg/palm thrice a year during June, September, and January

Chemical

Aldicarb/fensulfothion at 1 g a.i./seedling three times a year for 3 years while in adult trees at 50 g a.i./palm; fensulfothion at 50 g a.i./palm or aldicarb at 10 g a.i./palm or DBCP at 10 ml a.i./palm three times a year in June, September, and January

Resistant Varieties

Hybrid VTL-11 X VTL-17 and cultivars Indonesia-6, Mahuva B, and Andaman-5 are tolerant to *R. similis*.

8.9.3 Coffee (*Coffea arabica, C. canephora*)

Several species of nematodes are associated with rhizosphere of coffee and many of them infect the roots. However, root lesion nematode (*Pratylenchus coffeae, P. brachyurus, P. pratensis, P. zeae*, *and P. flakkensis*) is the economically most important nematode pest of coffee all over the world. Root-knot nematode (*M. incognita, M. javanica, M. hapla, M. arenaria, M. africana, M. exigua, M. coffeicola, M. decalineata, M. megadora, M. kikuensis, M. thamesi*) also is of importance in many parts. Other parasitic nematodes associated with coffee are *R. similis, R. reniformis, Hemicriconemoides,* etc.

8.9.3.1 Coffee Lesion Nematode (*Pratylenchus coffeae*)

Host Range

Coffee root lesion nematode (*Pratylenchus coffeae*) possesses a wide host range including citrus, banana, abaca, cabbage, peas, cauliflower, chrysanthemum, tomato, groundnut, areca, cacao, cardamom, etc.

Survival and Spread

P. coffeae can survive till 12 months in the field. Dispersal is through implements and irrigation water. Nematodes may gain entry into the nursery through jungle soil and multiply there. It has four biological races, viz., coffee, cardamom, pavetts, and bamboo races. Coffee race is specific to coffee.

Symptoms

Nematodes destroy cortical parenchyma cells of roots including tap root and secondary and feeder roots. Nematodes attack both young and grown-up plants with bearing. The outer layer of roots peels off and results in the death of feeder roots, which may not absorb nutrients and moisture. Infected plants become weak, and premature defoliation is observed in many regions (Fig. 8.27). Some plants will have leaves at the tip of main stem that are chlorotic and crinkled. This gives the infected plants a “tuft” look. The main stem may become weak and tender, and plants look prematurely old that may require collar pruning or uprooting. Such pruned plants may fail to produce new suckers, or suckers may have weak, unhealthy, reduced, chlorotic, and crinkled foliage. Infected plants will have adventitious root growth at the collar region particularly during rainy season which could be easily dislodged since they are loosely supported.

Short internodes, sparse flower buds, poor setting, and production of unfilled/small-sized

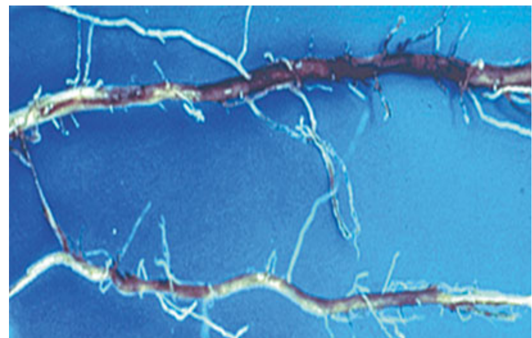

Fig. 8.27 Lesion nematode-infested coffee plants

beans are some of the important symptoms. If there is a little delay in the blossom shower, plants may produce vegetative buds instead of floral buds. Infected plants may possess a bush appearance due to Matty crisscrossing of numerous fresh vegetative growth on the primaries and secondaries. While the foliage produced during monsoon (July–October) are normal and healthy, those produced during pre-monsoon (April–June) may be crinkled, chlorotic, smaller, leathery, and linear. Till December death of leaves may continue on both sides, leaving one or two pairs of leaves at the tip during the next summer.

Management

- Raising healthy seedlings in the nursery.
- Destruction of weeds.
- Change the nursery site once in every 6–7 years.
- Avoid carrying soil from infested area to healthy ones.
- Infected plants should be uprooted and burned.
- Deep soil digging to expose to the sun for a minimum of one season during summer.
- Robusta (*Coffea canephora*) and tree coffee like Excelsa and Liberica (varieties of *C. dewevrei*) are tolerant, while all Arabica varieties are susceptible.
- Grafting of Arabica scion to Robusta rootstock in 40-day-old "topee stage" plants by using a wedge-cleft technique.
- Soil application with carbofuran at 3 g/plant every 3 months at least for 1 year to protect in the initial stages.
- Soil application with phenamiphos at 0.5–1.0 g/plant or methyl bromide at 150 cc/sq.m.

8.9.3.2 Root-Knot Nematodes (*Meloidogyne* spp.)

Symptoms

Several species of root-knot nematodes (*Meloidogyne exigua*, *M. incognita*, *and M. coffeicola*) have been found associated with coffee which induce chlorotic symptoms in the affected trees; unthriftiness; wilting and drying foliage, accompanied by the cracks in the roots; and necrotic and sloughing off of cortical tissue, and rootlets are destroyed with the nonfunctioning of root hairs. Infested roots possess small- to medium-sized knots. Trees lack vigor and may not withstand unfavorable conditions. *M. exigua-affected* roots may show small elongated knots at root tips.

Management

Cultural

- Avoid introduction and spread of the nematode into the new field.
- Growing nonhosts, mostly graminicolus hosts, may reduce the nematode population in soil (Parvatha Reddy 2008).
- Crop rotation with soybean, cotton, maize, castor, and groundnut in *M. exigua*-infested fields.
- Crop rotation with groundnut, castor, *Stizolobium* deeringiana, and *Crotalaria spectabilis* in *M. incognita*-infested fields.

Resistance: Coffea canephora (line 2258 from Costa Rica) and *C. arabica* var. Mundo Novo or Catuai Vermelho grafted on line 2258 have shown resistant reaction to the nematode.

Chemical: Soil application with carbofuran/aldicarb/phenamiphos at 1.6–6 g a.i./plant, three times in a year, first being during rainy season and later at 3 months interval (Campos et al. 1990).

8.9.4 Tea (*Camellia sinensis*)

Several nematode species have been reported to associate/attack tea. Throughout the world, around 47 species belonging to 22 genera have been so far recorded, although very few are economically important and need attention. Some of the important genera are *Aphelenchoides, Boledorus, Ditylenchus, Helicotylenchus, Hemicricone-moides, Hoplolaimus, Longidorus, Meloidodera, Meloidogyne, Paratylenchus, Pratylenchus, Paratylenchoides, Radopholus, Rotylenchus, Scutellonema, Trophotylenchus, Tylenchorhynchus, Xiphinema*, and others.

Among all, lesion and root-knot nematodes are found to be widely distributed in most tea-growing countries and are potential pathogens on tea.

8.9.4.1 Root-Knot Nematodes

In most tea-growing regions, root-knot nematodes (*Meloidogyne incognita* and *M. brevicauda*) are a major problem. Both young (*M. incognita*) and mature (*M. brevicauda*) tea plantations are infested.

Symptoms

Young Seedlings: M. incognita is the most common pest on tea seedlings (apart from *M. javanica* and *M. hapla* reported from some regions). Nematodes attack both seedlings and vegetatively propagated clonal plants (Parvatha Reddy 2008). Normally tap roots and lateral roots are affected. Roots will be destroyed with the formation of knots.

Mature Plants: Infested tea bushes look unhealthy and weak, with severe galling leading to the growth decline. *M. brevicauda* has been reported from South India and Sri Lanka. Foliage are affected in terms of their size, color, and brightness, while roots may also exhibit swelling and pitting, a typical symptom due to *M. brevicauda.*

Management

Physical: Soil solarization of nursery beds can avoid nematodes. It is useful to moist heat the nursery soil mixture by spreading on galvanized iron sheets with frequent water sprinkling and stirring to 60–62 °C for 5 min. Soil forking breaks the hardpan, improving soil aeration and growth of feeder roots, thus promoting microbial population that may antagonize nematodes (Mohotti 1998).

Cultural

- Nursery beds should be raised in nematode-free soil.
- Organic matter incorporation into the soil, viz., FYM, oil cakes, and green leaf manure.
- Soil application with mustard cake at 15 g/seedling.
- Neem cake incorporation at 5 g along with carbofuran or phorate at 1.5 g/seedling.
- Uprooting of old tea roots.
- Guatemala grass as nonhost can suppress nematodes.
- Intercropping with marigold or as a preplant crop.
- Intercropping with black pepper cultivar, PW-14, which is resistant to *R. similis*.

Resistance: Tea clone Yunkang 14, TRFK 303/577, and DT 1 are resistant varieties reported from various countries.

Chemical

- Carbofuran 3G at 1.5 g/seedling + neem cake at 5 g/seedling
- Aldicarb at 0.5 and 11.0 g/seedling
- Phenamiphos at 10 g/plant after pruning

8.9.4.2 Lesion Nematode

Symptoms

Unthrifty plants, pale yellowish foliage, premature flowering and fruiting, and destruction of feeder roots are the primary symptoms due to lesion nematodes (*Pratylenchus coffeae* and *P. loosi*), which later may become brownish and necrotic with severe galling (Gnanapragasam and Manuelpillai 1990). Severe reduction in quality leaves is the ultimate damage.

Management

- Mulching with Guatemala grass at 20–30 t/ha.
- Potassium-enriched fertilizer application to soil may help plants to tolerate nematode parasitism and reduce the nematode population increase (Gnanapragasam and Manuelpillai 1990).
- Green leaf incorporation from plants like marigold and *Tephrosia*.
- Intercropping of preplant cultivation of marigold reduces nematode population.

8.9.5 Betel Vine (*Piper betle* L.)

Root-knot nematodes (*M. incognita*), burrowing nematodes, and reniform nematodes have been reported on betel vine. However, root-knot nematodes are economically important pests on this crop, which are widely distributed in most betel vine-growing regions and pose problems when present in high population levels.

Fig. 8.28 Infested vines

8.9.5.1 Symptoms

The most common and visual symptoms due to root-knot nematode infestation are blackening and drooping of the growing tips and yellowing of foliage. Roots exhibit knots of different sizes which may lead to destruction of roots and decay (Figs. 8.28 and 8.29).

8.9.5.2 Management

Physical

Mulching the nursery beds with white polythene for 2 weeks increases soil temperature which leads to reduction in nematode population (Sivakumar and Marimuthu 1987).

Cultural

- Use of nematode-free planting material
- Crop rotation with nonhosts like groundnut, sesame, and rice
- Soil amendments with neem cake/castor cake at 1 t/ha or FYM at 25 t/ha before planting
- Crop rotation with rice, sesame, corn, or groundnut
- Intercropping with marigold around betel vine
- Soil application with neem cake at 1 t/ha or sawdust at 2 t/ha (Jagadale et al. 1985)

Fig. 8.29 Knots on the roots

Biological

Soil application with *Paecilomyces lilacinus* along with neem cake as substrate at 10 g/vine at 2 months' interval (Jonathan et al. 2000).

Resistant Varieties

Cultivars Kuljedu, Bangla, Karapaku, Kakair, Gachipan, Aswani Pan, and Behrampuri are quite tolerant to root-knot nematode.

Chemical

Aldicarb/carbofuran application to the soil at 0.75 kg a.i./ha; furrow application with aldicarb/carbofuran/benfurocarb at 1.5, 3.0, and 5.0 kg a.i./ha, respectively

8.9.6 Cocoa (*Theobroma cacao* L.)

Among various phytonematodes associated with cocoa, root-knot nematodes (*M. incognita* and *M. javanica*) are of much significance as they are responsible to cause considerable damage and crop loss in this crop.

8.9.6.1 Symptoms

Major characteristic symptoms due to root-knot nematode infestation include wilting, dwarfing, yellowing of the foliage, dieback symptoms, and reduction in the size of the foliage. Moderate to severe galling on infested roots is a common feature. In some instances, the tips and margins of the foliage may turn brownish, which later get dried up. Defoliation may occur and the sudden death of the plants is also noticed.

8.9.6.2 Management

Cultural

- Use of nematode-free seedlings for fresh planting.
- Selection of nematode-free areas.
- Select the shade plants which are poor hosts/nonhosts for root-knot nematodes. Avoid banana and *Leucaena glauca* (Sosamma et al. 1980).

Chemical

Soil application with aldicarb/carbofuran/phenamiphos at 50 g/plant; field application with fensulfothion/ethoprophos at 34 kg/ha or phenamiphos at 22 kg/ha

8.10 Spices and Condiments

Nematodes are major pests of many crops grown for spices and condiments purpose. They affect the quality and quantity of these like black pepper, cardamom, ginger, and turmeric.

8.10.1 Black Pepper (*Piper nigrum* L.)

Phytoparasitic nematodes belonging to 29 genera and 48 species have been found associated with black pepper (Koshy and Bridge 1990), of which most important are *Meloidogyne* species and *Radopholus similis*. They also are responsible to induce "slow wilt/slow decline" in this crop. However, R. similis is the primary incitant of slow decline in black pepper (Mohandas and Ramana 1991).

8.10.1.1 Root-Knot Nematode

M. incognita, M. javanica, and *M. arenaria* are the major species affecting black pepper.

Symptoms

Major symptoms are unthrifty growth and yellowing of foliage, yellowing of interveinal area of the foliage, dark green leaf veins, and formation of galls on the roots (Figs. 8.30 and 8.31). It has been reported that nematode alters the levels of amino acids, organic acids, and sugars in the infected plant.

This nematode is normally found associated with *F. solani, Phytophthora*, and other soilborne wilt-inducing fungi in the plantations resulting in severe yield losses.

Management

Cultural: Earthling up to 50-cm radius at the base of the vines and mulching the base of the vines with leaves help in reducing the yellowing.

Resistant Varieties: Use of live resistant standards like *Garuga pinnata, Tamarindus indica, Artocarpus heterophyllus, A. hirsutus*, and *Ailanthus malabarica* (Parvatha Reddy 2008). IISR Pournami is also resistant to root-knot nematode (*Meloidogyne incognita*) (Ravindran et al. 1992).

Biological: Paecilomyces lilacinus/Pochonia chlamydosporia at 20–50 g/vine

Chemicals: Aldicarb or carbofuran at 1 g a.i./vine two times a year (May/June and October/November) in two equal split doses; aldicarb sulfone or fensulfothion at 8 a.i. kg/ha

Fig. 8.30 Healthy and infested vines

Fig. 8.31 Knots on the roots

8.10.1.2 Burrowing Nematode

This nematode causes "yellows disease" in pepper and "slow wilt" in southern Karnataka, India, a highly devastating disease.

Host Range

Coconut, areca nut, banana, ginger, turmeric, etc.

Symptoms

Slow wilt symptoms include appearance of a few, pale, yellow drooping of the foliage, increasing in their number gradually, leading to wilting of the entire foliage within a span of 1 or 2 years. Later, shedding of leaves and cessation of growth and dieback symptoms appear. Both stages of the crop, either young or old, are infected by the nematode. The symptoms may disappear in the early stage with the onset of southwest monsoon. Entire foliage are shed within 3–5 years of the initiation of yellowing, leading to the death of the vine. Spike shedding is also seen. Root systems also show symptoms like orange to purple lesions on the white, tender, feeder roots. Advanced stages may result in rotting along with the main roots devoid of feeder roots. Matured roots may develop extensive necrosis.

R. similis has been reported to plug the xylem vessels with a "gumlike compound" and is found in inter- and intracellular positions within the cortex. It does not enter the stelar region. This nematode enters the root within a day after inculcation, and the cells around the site of penetration turn brown.

Management

Cultural

- Planting nematode-free rooted cuttings.
- Destruction of the infected vines and replanting after a year.
- Soil solarization of nursery beds/potting mixture (Venkitesan 1976).
- Mulching with Guatemala grass.
- Use of nonliving supports.
- Avoid other hosts of *R. similis* like banana, turmeric, and ginger as intercrops.
- Organic amendments with neem cake at 100–200 g/vine, green manuring at 2–4 kg/vine, or FYM application at 1 kg/vine.

Fig. 8.32 Knots on the cardamom roots

Resistant Varieties

- Peringamala, a local variety from Kerala, India, is resistant.
- *Piper hymenophyllum, P. colubrium, P. attenuatum,* and several accessions like HP 39 and C-820 are resistant (Eapen 2006).
- PW14 from Sri Lanka is immune (Gnanapragasam 1989).

Chemical: Aldicarb sulfone at 8 kg a.i./ha/ aldicarb or phorate or carbofuran at 3 g a.i./ vine applied once in May/June and again in September/October helps in preventing yellowing of the foliage and reduction in nematode population (Ramana 1992). Carbofuran at 114 g/vine or phenamiphos at 1 % a.i. and phorate with systemic fungicides like Ridomil MZ and Akomin have also been reported to be effective.

8.10.2 Cardamom (*Elettaria cardamomum*)

Root-knot nematodes (*Meloidogyne incognita* and *M. javanica*) are the major nematodes affecting this crop.

8.10.2.1 Host Range

Several annual weeds and commonly grown shade trees like *Erythrina indica* and *E. lithosperma* are hosts for the nematode.

8.10.2.2 Symptoms

Stunted growth, yellowing, reduced tillering, and delay in flowering are the major symptoms followed by dropping of fruits, resulting in yield reduction. Excessive branching of the roots is a major symptom along with galling. The nematode also affects germination in the nursery. Galling is seen on roots with yellowing and stunting symptoms (Fig. 8.32). Interaction of *M. incognita* with *R. solani* (rhizome rot and damping-off pathogen) and other soilborne fungi has been observed in nurseries.

8.10.2.3 Management

Physical Methods

Soil solarization of nursery with 400-gauze transparent LDPE for 40 days

Nursery Treatment

- Disinfesting nursery beds with fumigants like methyl bromide at 500 g/10 sq.m.
- Drenching with 2 % formalin to a depth of 20–30 cm and covering with a polythene sheet for 3–7 days and sowing seeds 2 weeks later
- Aldicarb application at 5 kg a.i./ha thrice a year
- Carbofuran at 5 g/phorate at 10 g a.i./plant

Cultural

- Neem cake application at 1 kg/plant two times in a year (Ali 1984)
- Removal of weeds/shade trees

- Removal and destruction of infested crop residues
- Mulching with dead leaves
- Pruning of infested root tips before planting

Biological

Soil application with *T. harzianum* multiplied on decomposed coffee husks of 7 days old at the time of sowing at 2.5 kg/bed of 4.5 m × 1.0 m dimension (Khan and Jairajpuri 2010).

Chemical

- Nursery treatment with carbofuran/aldicarb/phorate at 5 kg a.i./ha after 10 days of germination and this is repeated after 3 months. In secondary nurseries, the plants may be treated with carbofuran at 10 kg a.i./ha after transplanting and every 3 months thereafter.
- Fumigation of primary and secondary nursery beds with methyl bromide is another effective method for the control of nematodes in the nursery. Methyl bromide at 500 g/10 sq.m. The treated area has to be kept covered with polythene sheet for 2–3 days.

8.10.3 Ginger (*Zingiber officinale*)

Although several species have been reported to be associated with ginger, root-knot and burrowing nematodes are of economic importance.

8.10.3.1 Symptoms of *M. incognita*

As in other root-knot-infected plants, stunted growth, chlorosis, and marginal necrosis of leaves along with galling and rotting of the roots and underground rhizomes are the primary symptoms. Medium- to large-sized knots on the roots are the characteristic symptoms (Fig. 8.33). Normally fleshy roots are invaded along the entire length, but in fibrous roots it is in the area of differentiation. Infested rhizomes develop brown, water-soaked area in the outer tissues in particular, in angles between shoots.

Most infected tissues with the exception of rhizome meristem possess abnormal xylem and hyperplasic parenchyma. Lignified wall thickening of the endodermis and pericycle is observed.

Fig. 8.33 Knots on the ginger roots

Fig. 8.34 Infested rhizomes with lesions

8.10.3.2 Symptoms of *R. similis* Infestation

Infected plants show stunted growth, reduced vigor, and tillering, and they mature fast. Infected plants die faster than the healthy ones. Normally top leaves show chlorotic symptoms with scorched tips. Rhizomes, when infected, exhibit small, shallow, sunken, water-soaked lesions (Fig. 8.34). Infected planting material serves as a source for further spread of the nematode. It forms large channels/galleries/tunnels within the rhizomes.

8.10.3.3 Management

Physical

- For fresh planting, use of nematode-free rhizomes is very useful.
- Soil solarization of nursery beds for a minimum of 40 days during hot summer months.
- Hot water treatment of rhizome at 50–55 °C for 10 min or 45 °C for 30 min.

Cultural

- Before planting, a thorough washing of the seed material free of soil and drying in the shade (Parvatha Reddy 2008)
- Crop rotation with nonhosts like cereals or millets for a minimum of 3 years
- Intercropping with either corn or capsicum
- Deep summer plowing with furrow turner
- Organic amendments with FYM at 25–30 t/ha or neem cake at 2 t/ha at planting and again during crop growth
- Mulching with green leaves at 10–12 t/ha at planting and again during the crop growth

Biological

- Soil treatment with *Trichoderma harzianum* + neem cake at 1 t/ha
- Seed treatment with *Pseudomonas fluorescens* at 10 g/kg (Srinivasan et al. 2001)

Chemical

- Pre-sowing dip of rhizomes in 0.04 % phenamiphos or 0.18 % mancozeb for 30 min
- Soil application with carbofuran at 4 kg a.i./ha or phenamiphos at 3 kg a.i/ha (Kaur 1987)
- Basal application with carbofuran at 1 kg a.i./ha and later 45 days after planting
- Aldicarb at 2 kg a.i./ha
- Preplant fumigation with ethylene dibromide and postplant sprays with phenamiphos (Khan and Jairajpuri 2010)

8.10.4 Turmeric (*Curcuma longa*)

Most important nematode pests of turmeric are *Meloidogyne* spp., *R. similis*, and *Pratylenchus coffeae.*

Fig. 8.35 Knots on the roots

8.10.4.1 Root-Knot Nematode

M. incognita is the major species infecting this crop.

Symptoms

Typical symptoms of root-knot nematodes are produced like stunted growth, yellowing, and marginal and tip drying of foliage with reduced tillering. The root system shows severe galling and rotting (Fig. 8.35). Infested rhizomes may lose the bright yellow color, thus affecting the market value.

Management

Physical: Hot water treatment of rhizomes at 50–55 °C for 10 min or 45 °C for 30 min

Cultural

- Use nematode-free rhizomes for planting
- Deep summer plowing and application with well-decomposed FYM/compost at 25–30 t/ha or neem cake at 2 t/ha (Kaur 1987)
- Green leaf mulching at 10–12 t/ha at planting and later during the growth period
- Crop rotation with nonhosts like cereals or millets for a minimum of 3 years

Resistant Varieties: Kodur, Chayapasupu, 5363-6-3, 5379-1-2, 5335-1-7, 5335-2-7, Duggirala, Guntur-1 and Guntur-9, Rajampet, Sugandham, and Appalapadu are found resistant (Gunasekharan et al. 1987).

Biological: T. harzianum with neem cake at 1 t/ha

Fig. 8.36 Discoloration and rotting of roots

Chemical: Carbofuran or aldicarb at 1 kg a.i./ha; phenamiphos at 2.5 kg a.i./ha a day prior to planting; row application with carbofuran at 4 kg a.i./ha to 4-month-old plants

8.10.4.2 Burrowing Nematode

It is major pest which is disseminated through infected planting material.

Symptoms

Shallow water-soaked lesions on the rhizomes, loss of the golden yellow color, and rotting of roots with decay (Fig. 8.36) and without cortex and stelar portions are common symptoms. Nematodes may also be found in scale leaves. Drying of infected plants is seen.

Management

- Avoid monocropping.
- Avoid intercrops like coconut, areca nut, and banana.
- Carbofuran or aldicarb at 1 kg a.i./ha.

8.10.5 Other Spices

Other spices like nutmeg, cinnamon, clove, cumin, coriander, and fenugreek have been reported to be associated with nematodes like root-knot, spiral, lance, lesion, reniform, and burrowing nematodes. But detailed investigations/information is lacking.

8.11 Tuber Crops

8.11.1 Sweet Potato (*Ipomea batatus*)

Root-knot and reniform nematodes are of importance in this crop. Typical symptoms described in earlier crops are found in sweet potato with similar management practices.

8.11.1.1 Root-Knot Nematodes

Management

Physical

Hot water treatment of tubers at 50 °C for 5 min

Hot air treatment of roots (5–7 cm diameter) at 50 °C for 4–6 h

Cultural

Rotation with either maize or groundnut is effective.

Transplanting of the clean “slips” into nematode-free soil.

Resistant Varieties

Nemagold, Heartgold, Jerssy types, Redmar, and Kyushu No. 52 are resistant to the nematode, while Jasper is moderately resistant and Painter is a tolerant variety (Parvatha Reddy 2008).

8.11.1.2 Reniform Nematode

Physical

Hot water treatment at 50 °C for 3–5 min (Martin 1970)

Resistant Varieties

Goldrush is tolerant to reniform, while it is susceptible to root-knot nematodes

8.11.2 Yam (*Dioscorea alata*)

Yam nematode (*Scutellonema bradys*) and root-knot nematode (*Meloidogyne* spp.) are the major pests on yam.

8.11.2.1 Symptoms of Yam Nematode Infestation

In the early stages, small, yellow lesions are seen below the skin, which later turn dark brown to form a continuous dark, dry rot layer under the surface of the tuber. It is known as "dry rot." However, externally, tubers develop deep, malformed cracks in the skin; the skin flakes off exposing dark brown, dry tissue below. During storage, severe damage may take place due to a large number of nematode population buildup.

8.11.2.2 Management

Physical

Hot water treatment of tubers at 50–55 °C for 40 min

Cultural

- Use of nematode-free tubers for planting.
- The distal portions of the tuber normally will have less nematode population and hence can be preferred.
- Pre-sowing mixing of yam mounds with cow dung at 1.5 kg/mound, i.e., 1,886.3 kg/ha.
- Fallow for 6 and more months may reduce nematode population in soil.
- Crop rotation with nonhosts like tobacco, cotton, corn, sorghum, and groundnut
- Growing sweet potato cv. Sree Bhadra as a trap crop

Resistant Varieties

Cultivar Florido of *Diascorea alata* is resistant to yam nematode (Ayala and Acosta 1971).

Chemicals

- Bare root-dip treatment of infested tubers with thionazin/fensulfothion at 1,250 ppm for 15 min
- Immersing infested yams in a suspension of carbofuran or oxamyl at 1,000 ppm for 30 min
- Soil application with carbofuran/oxamyl/aldicarb at 2 kg a.i./ha 2 weeks after planting

8.11.2.3 Management of Root-Knot Nematode on Yam

Physical

Hot water treatment of tubers at 51 °C for 30 min

Cultural

Use of trap crop sweet potato cv. Sree Bhadra

Chemical

Soil application with carbofuran at 3 kg a.i./ha

Resistant Variety

Cultivars Sree Latha and Sree Kirthi are resistant to the root-knot nematode. A species of cluster yam*, D. dumentorum*, is resistant to *M. incognita.*

8.11.3 Cassava (*Manihot esculenta*)

Root-knot (*Meloidogyne incognita, M. javanica*, *and M. arenaria*) and lesion nematodes (*Pratylenchus brachyurus*) are major pests on cassava.

8.11.3.1 Management of Root-Knot Nematode

Cultural

Deep summer plowing

Resistant Varieties

Use of root-knot-resistant cultivars, viz., Sree Sahya, Manidocol, and Narayaniyakappa

8.11.3.2 Symptoms Due to Lesion Nematode Infestation

The presence of lesions on roots is followed by rotting of roots, while infested plants start exhibiting yellowing and dieback symptoms on twigs with less number of tubers. Defoliation may also be seen.

Management

1. *Chemical:* Soil application with DBCP at 50 L/ha
2. *Resistant Varieties:* Use of lesion nematode-resistant cultivars like Agba Tiega, Agba Boquia, Atitogen, Ba Pou-II, and Sodjievi.

8.11.4 Colocasia (*Colocasia esculenta*)

Root-knot nematodes, *M. incognita* and *M. javanica*, are the major pests on this crop.

8.11.4.1 Management

Physical

Dipping corms in hot water at 50 °C

Cultural

Cultivation of *Colocasia* in wet or flooded condition may avoid nematode infestation.

Use of nematode-free planting material

Chemical

Soil application with DBCP at 3.6 L/ha

Resistant Varieties

Cultivars Sree Reshmi, Dodare, and C-9 are resistant to nematode.

Root-knot nematodes are also associated with other tuber crops like *Amorphophallus* in some regions.

8.12 Polyhouse Crops/Crops Grown Under Protected Condition

Protected cultivation involves the modification of natural environment to achieve optimum plant growth. Modifications can be made to both the aerial and root environments to increase crop yields, extend the growing season, and permit plant growth during periods of the year not commonly used to grow open field areas. Phytonematodes are major constraint in protected crops mainly because of highly preferable crops; congenial environmental condition, particularly temperature and relative humidity; lack of awareness/knowledge of nematode problems; and favorable conditions for association with other microbes. Main sources of nematode infection are infested soil and infected planting material.

Polyhouse Crops: These are mainly grouped as vegetable and ornamental/flower crops.

(a) *Vegetable Crops:* Tomato, capsicum, okra, cucumber, muskmelon, watermelon, broccoli, Brussels sprout, etc.
(b) *Ornamental/Flower Crops:* Rose, carnation, gerbera, gladiolus, chrysanthemum, tuberose, orchids, anthurium, etc.

8.12.1 Prominent Phytonematodes Associated with Vegetable Crops

1. *Tomato: Meloidogyne incognita, M. arenaria, M. javanica, Globodera rostochiensis, Heterodera rostochiensis, Ditylenchus dipsaci, Pratylenchus brachyurus, Rotylenchulus reniformis*, and *Helicotylenchus microlobus*
2. *Capsicum: Helicotylenchus* spp., *Meloidogyne incognita, Heterodera pseudorobustus, H. microcephalus, H. cavenessi*, and *Scutellonema clathricaudatum*
3. *Melons: Meloidogyne incognita*
4. *Okra: Meloidogyne incognita*

8.12.2 Prominent Phytonematodes Associated with Flower Crops

1. *Rose: Meloidogyne hapla, Meloidogyne incognita, Trichodorus christiei*, and *Pratylenchus vulnus*
2. *Carnation: Meloidogyne incognita, Meloidogyne javanica, Ditylenchus dipsaci, Heterodera trifolii, Paratylenchus dianthus*, and *Criconemoides curvatum*
3. *Chrysanthemum: Meloidogyne incognita, Pratylenchus penetrans, Aphelenchoides ritzemabosi*, and *Belonolaimus longicaudatus*
4. *Tuberose: M. incognita, M. javanica*, and *Aphelenchoides besseyi*
5. *Gladiolus: Meloidogyne incognita* and *Trichodorus* spp.
6. *Anthurium: Meloidogyne* spp. and *Radopholus similis*
   Gerbera: Meloidogyne incognita (Nagesh and Parvatha Reddy 2000).
7. *Orchids: Aphelenchoides ritzemabosi*

Fig. 8.37 Root-knot-infested okra plants

8.12.2.1 Symptoms

While aboveground symptoms of most phytonematode infestation are stunting, chlorosis/yellowing, and wilting of plants or foliage, belowground symptoms depend on the nematode species and its type of feeding.

8.12.3 Belowground Feeders

Belowground feeders produce symptoms on the roots. It may be knots, lesions, cysts, abnormalities, stubby roots, loss of feeder roots, and cavities/tunnels as seen in genera *Meloidogyne, Pratylenchus, Heterodera, Ditylenchus, Trichodorus, Belonolaimus,* and *Radopholus*, respectively.

8.12.3.1 Root-Knot Nematodes

Root-knot nematodes (*Meloidogyne* spp.) damage plants by devitalizing root tips and causing the formation of swellings of the roots. These effects not only deprive plants of nutrients but also disfigure and reduce the market value of many root crops. When susceptible plants are infected at the seedling stage, losses are heavy and may result in complete destruction of the crop. Nearly 95 % of the damage is done by these nematodes.

Symptoms

Aboveground Symptoms: Aboveground symptoms are reduced growth and fewer, small, pale green, or yellowish leaves that tend to wilt in warm weather (Figs. 8.37 and 8.38). Blossoms and fruits are few and of poor quality.

Belowground Symptoms: Typical root-knot galls on the roots are the characteristic symptoms (Figs. 8.39 and 8.40). Several such infections may give a rough, clubbed appearance. Tubers or other fleshy parts produce small swellings over their surface, which become quite prominent and cause distortion or cracking.

8.12.3.2 Others

(a) *Pratylenchus*: Necrotic lesions on the roots leading to destruction of the root system
(b) *Helicotylenchus*: Lesions on the roots
(c) *Trichodorus*: Produces stubby-root system
(d) *Belonolaimus*: Exhibits coarse root system

8.12.4 Aboveground Feeders

Phytonematodes which feed on aboveground/aerial parts of plants exhibit different kinds of symptoms. The most common pest is chrysanthemum

Fig. 8.38 Root-knot-infested carnation plants

Fig. 8.39 Healthy and root-knot-infested roots of okra

Fig. 8.40 Infested root system of carnation

foliar nematode, *Aphelenchoides ritzemabosi.* Major symptoms are production of crippled flowers, yellow buds, and blighted racemes. However, general symptoms include yellow and dwarf plants and small and discolored flowers.

8.12.4.1 Management

Belowground Feeders

Before Planting: Soil flooding (Thomas 1953), heat treatment of soil (Ratan 1986), soil solarization, and soil fumigation

Soil Fumigation: It is done before 3 weeks of planting. Use DD at 450 kg/ha or EDB at 225 kg/ha or methyl bromide at 50–100 g/sq.m (Newhall 1947) or formalin at 20 ml/L.

During/After Planting

- Application of organic manure, hot water treatment of planting material at 50 °C/5–10 min, application of carbofuran 3G and use of bio-agents like *Paecilomyces lilacinus, T. harzianum*, *or T. viride* at 20 g/sq.m.
- Incorporation of aqueous spore suspensions of *P. lilacinus* and *P. chlamydosporia* to the beds at 1 L/sq.m (2×10^{12} spores) after the application of neem cake at the rate of 1 kg/sq.m bed.
- Steam sterilization of soil to at least 30-cm depth or application with metam sodium/dazomet (40 g/sq.m on light and 50 g/sq.m on heavy soil) or aldicarb 10G (5 g/sq.m).
- Dazomet as a preplant treatment applied at 40 g/sq.m 30 days before planting or carbofuran 3G (10 g/sq.m).

- Carbosulfan (2 L of 0.03 %/sq.m) and chlorpyrifos (2 L of 0.03 %/sq.m) application to the beds separately, 30 days after planting as a postplant treatment. The application of postplant chemicals needs to be repeated 6 months after planting.
- Soil sterilant such as dazomet, followed by establishment of antagonistic potential in the substratum soil/media using fungi like *P. lilacinus* and *P. chlamydosporia* (Nagesh and Parvatha Reddy 2005).

Resistant Varieties

Tomato: Pusa Ruby, NTDR-1, and Nematex to *M. incognita* and Resistance Bangalore and Punjab 6NR-1 to *M. javanica*

Brinjal: Black Beauty and Mysore Green to *M. incognita* and Bhanta nad Muktakeshi to *M. javanica*

Chilli: Pusa Jwala and Jwala to M. incognita and Bull Nose and Surya Mukhi to M. javanica

Cucumber: Improved Long Green to *M. incognita* and GY-5937 to *M. javanica*

Aboveground Feeders: Most common practices for the management of aerial feeders include aerial spraying on the affected plant parts with Multiguard at 425 kg/ha or with oxamyl.

General Precautions to Be Taken: Avoid monoculture, use certified seeds, and follow crop rotation with nonhosts. Soil solarization is suitable in greenhouse.

8.12.4.2 Chrysanthemum Foliar Nematode

The foliar nematode of chrysanthemum (*Aphelenchoides ritzemabosi*) is widespread that leads to severe losses. Foliar nematode also attacks several other plants, including aster, dahlia, zinnia, and strawberry.

Symptoms

Affected buds or growing points sometimes do not grow but turn brown, or they produce short, bushy-looking plants with small and distorted leaves. As the season progresses, first the lower and then the upper leaves show small yellowish spots that later turn brownish black, coalesce, and form large blotches. At first the blotches are contained between the larger leaf veins but eventually the entire leaf is covered with spots or blotches, shrinks, becomes brittle, and falls to the ground. Defoliation, like infection, progresses from the lower to the upper leaves. Affected ray flowers fail to develop. Severely infected plants die without producing much normal foliage or many marketable flowers.

Management

- Several sanitary practices are quite important in managing the foliar nematode.
- The foliage and stems should be kept dry to prevent spreading of the nematodes.
- Cuttings should be taken only from the tops of long, vigorous branches.
- Suspected dormant cuttings or stools may be disinfested by dipping in hot water at 50 °C for 5 min or at 44 °C for 30 min.
- Treating plants with appropriate nematicides as sprays or drenches, for instance, Thiozinon at 80 ppm.

8.12.4.3 General Nematode Management Practices in Polyhouse

Exclusion

- Preventing introduction and their spread into the greenhouse
- Use of nematode-free planting material
- Use of transplants produced from soilless culture without soil

Sanitation

Remove and destroy the nematode-infested material/debris

Eradication

- Removal of nematodes from soil or plant material within the greenhouses, which is not usually feasible from natural soil-based greenhouse systems.
- Steam sterilization of soil at 100 °C.
- Steam pasteurization of soil at 60 °C for 30 min.
- Hot water treatment: Treat the plant material in hot water to destroy nematodes in roots or foliar tissues.
- Soil fumigation to be done before 3 weeks of planting. Use DD at 450 kg/ha or EDB at 225 kg/ha or methyl bromide at 50–100 g/sq.m (Newhall 1947) or formalin at 20 ml/L.

- Drenching the soil with 0.5 % formalin at 100 ml/kg soil followed by covering with polythene tarps for 2 weeks before sowing/planting (Ramakrishnan and Devrajan 2013).
- Growing trap crop like cowpea (for root-knot nematode) closely to the main crop and removing the plants out of the polyhouse at 45 days after sowing.
- Raising antagonistic crops like marigold and incorporating in situ after plucking flowers.
- Adding well-decomposed farmyard manure at rate of 20 t/ha to enrich soil.
- Incorporating neem cake at 250 kg/ha soil 2 weeks before sowing/planting. Filtering irrigation water using mesh sieves before usage.
- Applying *Pseudomonas fluorescens/Trichoderma viride* at 2.5 kg/ha mixed with 50 kg farmyard manure 10 days before sowing/planting and using chemical pesticide like carbofuran 3G at rate of 1 kg/ha.

8.13 Landscape Plants

Dozens of nematode species are associated with landscape ornamentals, but relatively few of these cause most of the serious problems. The root-knot nematodes (*Meloidogyne* spp.) are by far the most important (Dreistadt et al. 1994) (Table 8.13). Their easily recognized galls on roots make their presence obvious. Galls result from the growth of plant tissues around juvenile nematodes that feed near the center of the root and secrete plant growth hormones that stimulate cell growth.

8.13.1 Symptoms

Aboveground symptoms are similar to those resulting from many kinds of root injury. Foliage loses its luster and wilts; plant yellows and eventually loses leaves from prolonged root stress; new flushes of growth are weak, with fewer and smaller leaves than healthy plants; and damage is spotty since nematodes are rarely distributed evenly in the soil. Root symptoms vary widely. Some kinds of nematodes produce distorted growth of roots or stop growth completely; others kill the cells on which they feed, leaving patches of dead tissue as they move on. Fungi and bacteria which cause root rots, wilts, and other plant diseases often infect nematode-damaged roots earlier and more severely than uninjured roots. Therefore, depending on the kind of nematodes involved, nematode damage may include galls, stunting, and decay of roots; roots are often darker in color than healthy roots.

8.13.2 Management

Use of resistant plants and provision of optimal care are two of the most important management practices. With proper care, it is possible to establish and maintain an attractive landscape, despite nematodes (Angel 2006).

8.13.2.1 Proper Preparation of New Planting Sites

Give plants the best chance to become rapidly established. Native soil in which annuals are to be planted should be prepared well, including removal of any old roots, debris, etc., from the site. Water- and nutrient-holding capacity of the soil and activity of natural enemies of nematodes are improved by incorporating organic soil amendments into the soil before planting.

8.13.2.2 Soil Treatment

If nematodes build up to high levels on the preceding plant(s) or if plants that especially favor nematodes were recently removed from the site, soil solarization may give the new plant(s) a better chance. Soil solarization is a nonchemical way to reduce soil pest populations, but it takes a lot of work and the area must be left bare 6–8 weeks during the summer. Clear polyethylene is used to cover moist soil that is well tilled and ready to be planted, so the heat generated by sunlight hitting the soil will be trapped in it and raise the soil temperature high enough to kill many nematodes, fungi, and weed seeds in the upper

Table 8.13 Landscape, fruit, nut, and vegetable plants infested by phytonematodes

Host plant	Nematodes	Host plant	Nematodes
Landscape plants		Fruit and nut trees	
Albizzia	Root knot	Almond	Root knot, root lesion, ring
Alder	Root knot	Apple	Root knot, root lesion
Azalea	Stunt	Apricot	Root knot, root lesion, ring
Boxwood	Root knot	Avocado	Root lesion
Cactus	Root knot, cyst	Cherry	Root lesion
Catalpa	Root knot	Citrus	Root lesion, citrus
Cedar	Root knot, pinewood	Olive	Root lesion, citrus
Euonymus	Root knot	Peach, nectarine	Root knot, root lesion, ring
Fir	Dagger	Pear	Root lesion
Ginkgo	Root knot	Plum, prune	Root lesion, ring, pin
Hibiscus	Root knot	Walnut	Root knot, root lesion
Hydrangea	Root knot		
Juniper	Root knot	*Vegetables*	
Larch	Pinewood	Beans	Root knot, root lesion
Lilac	Citrus	Beets	Root knot, cyst
Mulberry	Root knot	Carrots	Root knot
Oak	Root knot	Celery	Root knot
Palm	Root knot	Cole crops	Root knot, cyst
Pine	Pinewood	Corn	Root lesion
Pittosporum	Root knot	Cucumbers	Root knot
Poinsettia	Root knot	Eggplant	Root knot
Rose	Root knot, root lesion	Lettuce	Root knot
Spruce	Pinewood	Melons	Root knot
Tamarisk	Root knot	Onions, garlic	Stem and bulb
		Peas	Root knot, root lesion, cyst
Grapes and small fruits		Peppers	Root knot
Grape	Root knot, root lesion, ring, citrus, dagger, stubby root	Potatoes (Irish)	Root knot, root lesion
Blackberry, raspberry	Root lesion, dagger	Potatoes (sweet)	Root knot
Strawberry	Root knot, root lesion, foliar	Potatoes (sweet)	Root knot
		Radish	Root knot, cyst
		Spinach	Root knot, cyst
		Squash	Root knot
			Root knot
		Turnips	Root knot, cyst

few inches of the bed. Solarization works best in hot climates and sandy soils.

8.13.2.3 Replacement of Nematode-Infested Soil

For small areas it may be simpler to remove all soil or planting mix from an annual bed and replace it with new nematode-free planting medium. Nematodes eventually will invade the new medium, and bedding plant roots can grow out of the new medium into the infested native soil, but infection will be delayed. Be sure to dispose of the removed soil in an area where it will not be used for future plantings.

8.13.2.4 Use of Nematode-Free Stock

No matter how perfect and pest-free the planting site may be, a nematode infection already started in roots of transplants is right where it must be to do the most damage. Buy only top-quality plants. Reject any that have clear evidence of nematodes or other hard-to-control pests.

8.13.2.5 Selection of Plants That Are Well Adapted

Plant suitability to the location is important at all levels: region (climate), soil type, shade, drainage, etc. Plants that are "out of place" are more likely than well-adapted ones to suffer environmental stress. Moreover, a plant species that is "well adapted" to an area probably has some degree of tolerance or resistance to locally common pests, such as nematodes.

8.13.2.6 Avoid Nematode-Susceptible Plants

It is better not to use plants that are very susceptible to nematodes known to occur in a planting site. Plants vary widely in their susceptibility to different nematodes. There are many attractive plants that could be planted into a particular site without serious damage or immediate losses due to nematodes.

8.13.2.7 Proper Maintenance

Give the plants optimum care from the start and for as long as you want them to perform well. "Optimum" does not mean "maximum." Fertilize as needed to maintain steady, healthy growth rather than excessive, succulent growth that invites attack by nematodes and other pests. Water deeply to encourage development of a deep root system that can exploit large volume of soil for water and nutrients. Frequent shallow watering causes plants to develop shallow root systems that are less able to withstand nematode attack. Sudden dry periods or pest outbreaks can weaken plants in an incredibly short time. Even under normal conditions, erratic or inadequate watering can weaken a plant so that it can no longer tolerate a modest nematode population that had existed for years. Keep the plant root zone mulched to keep roots cool in hot weather, and minimize evaporation of water from the soil surface. Organic mulches also contribute organic matter to the soil, thus enhancing the capacity of the soil to retain water and nutrients. Mulches reduce stress on the plant as a whole and the root system specifically, improving the plants chances to do well despite some nematode damage to roots. Greater soil organic matter content also stimulates activity of natural enemies such as certain fungi, predatory nematodes, etc., that apparently help suppress nematode populations.

8.13.2.8 Chemical

Conspicuously lacking is any recommendation of a nematicide to treat nematode problems of plants after they are established in the landscape. There is presently no effective nematicide that may be applied legally to ornamentals already planted in the landscape.

8.13.2.9 Soil Solarization

Soilborne pathogens such as nematodes can be killed in the upper layers of the soil by soil solarization (Elmore et al. 1997). This process traps the heat from the sun shining through plastic and kills the plant pathogens. Soil solarization needs to be done during the summer when air temperatures and solar radiation are high and in an area where no plants are growing. Moist soil improves efficiency of the kill. Cover the area with thin polyethylene film and leave it in place for at least 3 months. To be effective, soil temperatures should be maintained between 98 and 126 °F for several weeks.

8.13.2.10 Planting and Harvesting Dates

Most nematode species are active during the warm summer months and cannot penetrate roots at soil temperatures below 64 °F. Therefore, you can reduce nematode injury to fall-planted crops such as carrots, lettuce, spinach, and peas by waiting until soil temperatures have dropped below 64 °F. Plant summer vegetables as early as possible in spring before nematodes become active. Plants with larger root systems, even though nematode infested, may be able to remain productive longer. It is also helpful to remove annual vegetables (including their roots) as soon as harvest is over, to prevent nematodes from feeding and breeding on root systems.

8.13.2.11 Nematode-Suppressive Plants

Certain marigolds (*Tagetes*) suppress root-knot and lesion nematodes. French marigolds (varieties

include 'Nemagold,' 'Petite Blanc,' 'Queen Sophia,' and 'Tangerine') are most effective. Signet marigolds (*Tagetes signata* or *tenuifolia*) should be avoided because nematodes will feed and reproduce on these. Marigolds do not work well against the northern root-knot nematode (*Meloidogyne hapla*), a species common in areas with cool winters. The effect of marigolds is greatest when they are grown as a solid planting for an entire season. When grown along with annual vegetables or under trees or vines (intercropping), nematode control is usually not very good. To prevent marigold seed from getting in the soil, cut or mow the plants before the flowers open. As with other cultural control methods, nematode populations will rapidly increase as soon as susceptible crops are grown.

8.13.2.12 Soil Amendments and Irrigation

Various organic amendments can be added to the soil to reduce the impact of nematodes on crop plants. The amendments, which include peat, manure, and composts, are useful for increasing the water- and nutrient-holding capacity of the soil, especially sandy soils. Because plants that are water stressed are more readily damaged by nematodes, increasing the soil's capacity to hold water can lessen the effects of nematode injury. Likewise, more frequent irrigation can help reduce the damage caused by nematodes. In either case, there will be just as many nematodes in the soil, but they will cause less damage.

8.13.3 Foliar Nematodes

These are an emerging problem on a host of landscape plants. Unlike many other plant pathogens that have narrow host ranges, foliar nematodes, particularly *Aphelenchoides fragariae,* have broad host ranges and are capable of infecting hundreds of species of plants. In addition to broad host range, there are few effective nematicides labeled for home use. This can create "mini-epidemics" in the home landscape and leave the homeowner frustrated.

8.13.3.1 Symptoms

Symptoms of foliar nematode damage are due to the feeding by nematodes on the foliage, stems, and buds. This feeding usually causes a "V"-shaped necrosis. In hosta, the wedge-shaped lesion is delimited by the veins. In broad-leafed plants and ferns, the lesions appear patch-like. It is quite normal to see healthy tissue right next to the diseased portions of the tissue. Lesion delimitation by the veins should make you suspicious of nematode; however, examination with a 10× hand lens is essential.

Unlike most phytonematodes, foliar nematodes live in and feed upon the aerial portion of the plant. After the eggs hatch, there are four larval stages prior to the mature adult stage. The entire life cycle can be completed in 2–4 weeks, even sooner if the temperatures are higher. Any infected leaf will contain multiple generations of nematodes. Foliar nematodes spread by contact between plants in the presence of water. They move through the surface of the plant and enter via the stomata. Because of the broad host range of this pathogen, it is recommended that plants be well spaced to allow foliage to dry between watering. Drip irrigation, which minimizes foliar wetness, is recommended for gardeners who have this problem. Controlling leaf wetness isn't enough, though; foliar nematodes are very tolerant of dry conditions and can remain viable for several years in decaying plant material.

8.13.3.2 Management

- Minimize foliar wetness to reduce the spread of the nematodes between plants.
- Remove and destroy infected leaves.
- Remove all dried leaves and stems during fall cleanup.
- Insecticidal soap or ZeroTol (a concentrated solution of hydrogen peroxide) should be applied when symptoms become evident. This is a "contact kill" and has no residual benefits.
- Foliar nematodes are easily killed by heat. Remove any dead leaves, and soak infected plants in hot water (120–140°) for up to 10 min (you may wish to divide plants up and

soak 4, 7, and 10 min intervals). Continuous monitoring of the temperature is important. Use a timer or stopwatch. Immediately following the hot water treatment, the plants are plunged in a bucket of cold water (as cold as possible from the faucet). Do not leave plants in the cold bath more than about 5 min – just enough time for the tissue to cool. Drain and pot the plants immediately. Plants do not store at all well after this dipping regime, so it is best to treat plants about the time they would be breaking dormancy. Unfortunately, because the nematodes can survive saprophytically in the soil, the long-term efficacy of this approach is questionable.

- Although there are nematicides labeled for nursery use, these products are extremely toxic, especially to fish and wildlife, and are not advisable for use.

8.14 Nursery Crops

Foliage ornamentals, floral crops, landscape ornamentals, and fruit and nut trees are normally produced in nurseries; most may be produced in containers, in ground beds or fields, or by some combination of both growing sites. Principles of nematode management for nursery crops apply equally to all ornamentals. Pesticide labels often group herbaceous (foliage and floral crops) ornamentals, woody ornamentals, and nonbearing fruit/nut trees together for pesticide registration restrictions. Therefore, these groups of plants are not treated separately here. However, it is the grower's responsibility to determine from the label that his/her crop, method, and application site are legal for the pesticide he/she intends to use.

8.14.1 Important Phytonematodes of Nurseries

Major nematodes of nurseries include root-knot (*Meloidogyne* spp.), lesion (*Pratylenchus* spp.), foliar (*Aphelenchoides* spp.), and stunt (*Tylenchorynchus* spp.) nematodes. Burrowing (*Radopholus* spp.) and reniform (*Rotylenchulus* spp.) nematodes can injure nursery crops (Fig. 8.41). Citrus nematode (*Tylenchulus semipenetrans*) disqualifies a site for use as a citrus nursery.

Nematodes are spread easily in any manner by which infested soil or plant materials are moved within growing areas. Equipment, water, hands, shoes, clothing, transplants, and seeds can help spread them. Even wind-borne dust sometimes carries nematodes. Nematodes sometimes may be avoided by careful cultural practices and strict

Fig. 8.41 Root-knot-infested nursery seedlings

sanitation procedures. However, nematicides are often needed to control established infestations.

8.14.1.1 Management

Plant injury and losses to nematodes are most effectively reduced by an integrated program of preventive measures, sanitation, and chemical nematicides. For greatest energy and financial efficiency, consider all practical management techniques to select the combination that best fits each growing situation. Sanitation and prevention of stock plant infestation are the best defenses against nematodes in nursery crops. Knowledge of visual symptoms and nematode testing services offer the means to monitor these situations during the life of a crop. If a nematode infestation develops despite preventive measures, appropriate nematicides often may be applied with a good prospect of success if the infestation is detected early (Anonymous 2007).

8.14.2 Sanitation and Preventive Maintenance

- Plant only in pest- and pathogen-free soils or planting mixtures; heat-treat or fumigate if necessary to assure that planting mix is clean.
- Disinfest all plant containers, bins, benches, and other equipments. A surface disinfestant such as sodium hypochlorite can be used for this purpose.
- Keep plant containers, flats, and planting soil clean. Store clean containers, sand, peat, and other potting medium components or completed media on concrete slabs, in concrete bins, or in other surfaces or containers that prevent contamination by runoff water, casual soil contact, etc.
- Do not move soil, plant material, pots, flats, or any other materials from areas known to be infested to uninfested areas. A color code can be used as an aid to restrict movement.
- Wash hands and disinfect tools frequently when working in planting stock. Do this especially when moving from one area to another. Clean heavy machinery before it is used to move clean medium or medium components.
- Use nematode-free propagating stock; this may often be obtained from unrooted cuttings that have never been in contact with soil or other source of infestation. Source plants of some species which have been infested may be treated with hot water, thus enabling production of more propagating material aboveground, but nematode eradication is never assured.
- Use raised benches if possible; do not allow hose nozzles or tools to touch the ground. Quarantine regulations of some markets, such as California, may specify the height of raised benches.
- Where maximum greenhouse sanitation is desired, reduce the level of windblown soil that enters the structure, especially in windy weather, by maintaining continuous vegetative cover such as mowed turf around them. Paved roadways running near the structures also help. The value of some crops, particularly plugs from tissue culture, may merit such precautions.

8.14.2.1 Chemicals

Preplant Fumigation: Soil Fumigation

There are several broad-spectrum soil fumigants that may be used to disinfest potting media and/or soil in field nurseries before planting (Tables 8.14, 8.15, 8.16, and 8.17). Methyl bromide is most effective. Metam sodium (Vapam, Busan, Nemasol, and several other trade names) is injected into soil as a liquid and turns into a gas following application. It is fairly effective against weeds and moderately effective against fungi and nematodes. Dazomet (Basamid) is incorporated with soil as a granular material but releases gases as it breaks down in soil. It is fairly effective against weeds and moderately effective against fungi and nematodes. Basamid is labeled for use on both potting media and field soil. 1,3-Dichloropropene is mixed with chloropicrin in Telone C-17 to get a broad spectrum of activity. 1,3-Dichloropropene is fairly effective against nematodes and chloropicrin is fairly effective against fungi. Neither chemical has much activity against weeds. Telone C-17 is labeled only for use on field soil.

Table 8.14 Preplant treatment for potting media

Material	Rate	Directions
1. Aerated steam	140 °F for 30 min	Inject steam into medium under a cover. Soil temperature at the coolest point should be maintained at 140 °F for 30 min. Excessive or low soil moisture or tightly compacted soil will reduce effectiveness
2. Live steam	180 °F for 30 min	Same as for aerated steam except that soil temperature should be maintained at 180 °F for 30 min
3. Chloropicrin (Chlor-O-Pic)	10 cc/cu ft	Inject 6–8 in. deep into soil, in a grid pattern with injection points not more than 12 in. apart. Soil should be no more than 12 in. deep on plastic and be covered with plastic sheeting for at least 24 h after treating
4. Methyl bromide (several trade names)	1.0 lb actual/cu yd	Decomposed compost, mulching materials, potting media, manure, and top soil. Place materials to be treated on a cement floor, 4-mil polyethylene or other non-soil gastight surface, and level to not more than 18 in. deep. Make holes on 12-in. centers in the mass with a broom handle. The material should be loose, 60–85 °F, and have sufficient moisture for good seed germination. Cover airtight with 4-mil polyethylene and release methyl bromide at the top of the pile with special applicators. Expose to fumigant 24–48 h. Aeration before planting: 72 h before seeding, 6–10 days before setting plants. If soil dries, irrigate 2 or 3 days prior to seeding or planting
Warning: Poor growth of some ornamental crops has sometimes occurred after methyl bromide treatment of soil. Crops known to be sensitive include carnations, conifers, delphiniums, holly, multiflora rose, salvia, and snapdragons		
5. Metam sodium (several trade names)	1.0 fl oz/2 cu ft	Potting soil. It may be applied as a drench (1 pt metam sodium in 5 gal water sprinkled over 100 sq ft of soil spread out in a layer 4 in. deep) or in a cement mixer or soil shredder. Pile medium on concrete slab or plastic tarp and cover with plastic tarp, or enclose in tightly sealed bin for at least 48 h. Uncover and allow to aerate for 5 days, and then mix to encourage escape of fumes. Total wait after treatment should be at least 4 weeks, agitating soil weekly to enhance aeration. Do not use until odor is gone. If in doubt of crop safety, transplant several test plants in small samples of soil and observe 24 h for injury before planting the major crop
6. Dazomet (Basamid Granular)	1.0–1.75 oz/sq yd	Application: Spread moist soil 8–10 in. deep on solid surface (preferably with plastic tarp under it); spread Basamid evenly on the surface, and then mix thoroughly (e.g., with tiller or large-scale medium mixing equipment). Treated soil can be piled up to 1 yard high after mixing. Cover with plastic tarp. Check label for temperature-dependent waiting period (10–30 days) before soil is safe to use

8.15 Noncommercial Vegetable Garden

Phytonematodes are one of the major constraints in the successful cultivation of vegetable gardens. Root-knot nematodes are the major pests followed by ectoparasites like the sting, awl, and stubby-root nematodes

8.15.1 Damage and Symptoms by Nematodes

As plant-parasitic nematodes feed, they damage the root system and reduce the ability of the plant to obtain water and nutrients from the soil. When nematode population densities get high and/or when environmental stresses occur, aboveground

Table 8.15 Preplant treatments in raised benches, ground beds, and propagation benches

Soil treatment	Rate/acre	Directions
1. Aerated steam	140 °F for 30 min	Inject steam into soil under a cover. Measure soil temperature 6 in. below surface. Soil temperature at the coolest point should be 140 °F for 30 min. It may be planted as soon as cool. Excessive or low soil moisture or tightly compacted soil will reduce effectiveness
2. Live steam	180 °F for 30 min	Same as for aerated steam except that soil temperature should be maintained at 180 °F for 30 min
3. Chloropicrin (Chlor-O-Pic)	35 gal (480 lb actual) (0.8 gal/1,000 sq ft)	Application method: Inject 6–8 in. deep with chisels or Fumigun spaced 10–12 in. Cover with gastight plastic. Exposure period: 24 h
		Aeration before planting: 14 days
4. Terr-O-Gas 67	250 lb actual (8 lb/1,000 sq ft)	Application method: Inject 6–8 in. deep with chisels spaced 10–12 in. apart. Cover with gastight plastic. Exposure time: 48 h
		Aeration before planting: 14 days
5. Methyl bromide (several trade names)	872 lb actual (20 lb/1,000 sq ft)	Application method: Release with special applicator under plastic or inject 6–8 in. deep with chisels spaced 10–12 in. apart. Cover with gastight plastic. Exposure period: 48 h
		Aeration before planting: 14 days
6. Metam sodium (several trade names)	100 gal (2.3 gal/ 1,000 sq ft)	Application method: Apply as a drench in water or inject through chisels 5 in. apart. Follow label instructions carefully. Treated beds should be sealed with plastic tarp for at least 48 h after treatment and aerated with weekly cultivation for a total of at least 4 weeks after treatment. Testing with a few plants before planting the entire crop is suggested to check for complete absence of metam sodium from treated soil
7. Dazomet (Basamid Granular)	8 lb/1,000 sq ft = 350 lb/acre	Application method: Apply granules as evenly as possible, incorporate to depth desired, preferably with tiller with L-shaped tines, roll surface, and then seal by either wetting soil or covering with plastic tarp. See label for rate variations, exposure times, aeration, and testing for complete escape of fumes

Table 8.16 Surface disinfestant for containers, bins, benches, and equipment

Material	Rate	Use and directions
Sodium hypochlorite (household bleach)	1 part common bleach in 5 parts water	Surfaces: Thoroughly drench solution over the surfaces of benches, bins, and containers. Use a scrub brush to remove soil, algae, and other debris before final drench in disinfestant

symptoms may become evident. Aboveground nematode symptoms often resemble nutrient deficiencies or drought stress. Symptoms include yellowing, wilting, stunting, or dying. Nematode damage usually occurs in irregularly shaped patches that may enlarge slowly over time. Be aware that similar conditions may be caused by other factors such as localized soil conditions, fungal diseases, or insects.

Table 8.17 Hot water treatment of caladium tubers for nematode control

Material	Rate	Directions
1. Hot water	122 °F (50 °C)	Caladium tubers only. Soak in vat of hot water for 30 min and transfer to cold to avoid heat injury to tubers. Heat treatment may work well with some other plant materials, but the best combination of temperature and period of exposure will differ for each plant species and size of tissue mass to be treated

8.15.2 Root-Knot Nematodes

Root-knot nematodes (*Meloidogyne incognita*, *M. arenaria*, and *M. javanica*) cause knots or galls on the roots. These galls are the only nematode

symptoms that are easily recognized. Root-knot nematodes cause extensive damage and changes in the root system. These changes allow fungi and bacteria to get into the plant. Some of these secondary organisms cause rotting of the root systems, and others cause vascular wilts. Sometimes the damage caused by the nematodes and the other organisms together is worse than that caused by both organisms separately. High infestations can kill many types of vegetables. Garden vegetables that are commonly damaged by root-knot nematodes are tomato, potato, okra, beans, pepper, eggplant, peas, cucumber, carrot, field peas, squash, and melons. Sweet corn is tolerant to root-knot nematodes and is not normally damaged by them.

8.15.3 Ectoparasites

The most destructive of these are the sting, awl, and stubby-root nematodes. Feeding by these nematodes usually causes a stunted or stubby-looking root system. Sting nematodes are found in sandy soil and are common. Awl nematodes are usually found in wet habitats such as near ditches, ponds, or poorly drained areas. Other ectoparasitic nematodes that parasitize some vegetables are spiral, stunt, and lance nematodes. Reniform and cyst nematodes are sedentary endoparasites that occasionally damage vegetables. These nematodes do not cause galls like root-knot nematodes. Root symptoms are generally unthrifty root systems. Reniform nematodes are limited to soils with a high silt content. Reniform nematodes cause damage to garden crops such as beans, field peas, tomato, pineapple, and sweet potato. Lesion nematodes are migratory endoparasites that are common in some regions. These nematodes usually cause dark sunken areas called lesions on roots. Feeding by lesion nematodes can make many vegetables susceptible to root-rot and vascular wilt diseases.

8.15.3.1 Management

Solarization

Solarization is a process of using heat from the sun to kill nematodes and other pests. The soil should be worked with a hoe or rototiller to break up clods. Remove all sticks, roots, and clumps. The soil should be moist, but not wet. Cover the soil with a clear plastic tarp and bury the edges of the plastic. Leave the plastic on the soil for at least 4–6 weeks. Do not remove the plastic until you are ready to plant.

Flooding

Flooding may be used to disinfest soil from plant-parasitic nematodes. This needs to be done in areas with a hardpan or clay layer below the soil surface. High areas with deep sand are not conducive to flooding as the water simply sinks down to the water table. The area should be flooded three times in 2 week cycles with 2 weeks flooded and 2 weeks dry.

Cover Crops

A cover crop is a crop that is not harvested. Instead it is planted in the season between harvestable crops. Because most vegetables are grown in the spring or fall, cover crops are usually grown in the summer. Cover crops have many benefits including improving the soil, preventing erosion, and increasing fertility. Cover crops may also be used to decrease soil pests. Because the nematodes cannot feed on the cover crop, their population densities will decline over time. Nematode damage to the vegetables will be less severe due to the lower population densities. *Examples:* Bermuda grass, *Cynodon dactylon;* French marigold, *Tagetes patula;* hairy indigo, *Indigofera hirsuta;* Pangola digit grass, *Digitaria decumbens;* showy crotalaria, *Crotalaria spectabilis;* sunn hemp, *Crotalaria juncea;* velvet bean, *Mucuna pruriens*.

Organic Amendments

Organic amendments can be added to soil as compost, manure, green manure, or other materials. Organic matter can help prevent nematode damage in several ways. The organic matter increases the ability of the soil to hold water and nutrients and improves soil structure. This makes a better environment for most plants and can help the plants survive in spite of the nematodes. Organic amendments can also increase natural enemies of nematodes that suppress the nematode

populations. Some organic amendments can release chemicals or gases that are toxic to the nematodes.

Soil Tillage and Root Destruction

Roots left in the soil can continue to live and support nematode reproduction. Endoparasitic nematode eggs are attached to roots and will continue to hatch. Therefore, as soon as the crop is harvested, pull up all plants and get rid of them. Till the soil with a rototiller or hoe, and remove all roots that might harbor nematodes. Exposure to sunlight and drying kills nematodes, so working the soil several times can help reduce nematode populations.

8.16 Home Lawns

Nematodes are major pests of lawns. Nematodes injure lawns by feeding on plant root cells. The root system becomes damaged to the point where the lawn cannot properly absorb water and nutrients. The lawn becomes thin and weak, making it much more susceptible to other stresses, such as drought.

8.16.1 Symptoms

Nematode damage to lawns may appear very similar to symptoms caused by other stresses, so a close examination of the site and a soil test are important. Accurate diagnosis of nematode damage can be made when the following types of evidence are considered:

8.16.1.1 Aboveground Symptoms

The lawn may appear yellow, weak, and slow to grow. Areas of the lawn may begin to thin, allowing weeds to invade easily. During periods of drought or mild stress, the lawn may wilt. Affected areas may appear irregular in size and shape, since the numbers of nematodes can vary greatly within a few feet.

8.16.1.2 Belowground Symptoms

Grass roots that are short and stunted or have knots and swollen areas on them may indicate a nematode problem. The root system may also appear shallow with areas that are dead or branched excessively.

All types of lawns can be affected by nematodes. The most damage occurs to lawns grown in the sandy coarse-textured soils of our state. Sting nematodes are generally limited to coarse-textured soils that are high in sand content. They can cause serious damage to any type of lawn that is grown in the region. Ring nematodes also commonly occur in sandy regions. Centipede grass is very susceptible to their damage. Infested lawns typically have poor growth, become thin, and are easily invaded by weeds (Anonymous 2007).

8.16.2 Management

- Maintaining a healthy lawn is the best way to manage nematode damage in home lawns. There are no chemicals available to control nematodes in the home lawn, even if applied by a certified pesticide applicator. The method that can reduce the effects of a nematode problem is by improving the overall health of the lawn.
- Irrigate the lawn during periods of drought and keep fertility levels adequate. Use deep and infrequent irrigation to encourage deep root growth. Keep the lawn free from insect and disease problems. A healthy lawn with a root system slightly damaged by nematodes may be able to survive if other stresses are kept to a minimum.
- Selection of a different species of turfgrass may provide a solution to certain nematode infestations. For instance, substituting St. Augustine grass for centipede grass in areas heavily infested with ring nematodes has been successful in some instances. Choose a substitute grass only after careful consideration of the site and maintenance requirements of the turfgrass in question.

Use of organic/biological products is effective. Soil amendments with neem cake/castor cake/bio-agents can help the grass tolerate nematode damage or possibly suppress nematode

population densities. Improving root health is a better option. Colloidal phosphate incorporated into fine sand has been shown to help Bermuda grass withstand attack by certain nematodes.

8.17 Bedding Plants

Bedding plants can be annuals, biennials, or perennials. Annuals are plants which are grown from seed, produce flowers and seed, and die in one growing season. Biennials complete their life span within 2 years, and perennials last for 3 years or longer. However, certain plants can be annuals, biennials, or perennials depending on the locality or purpose for which they are grown. Many of them bloom during winter months, contributing splendidly to a colorful landscape and producing flowers for home decorations. Others grow and flower during the trying months of June, July, August, and September, persistently blooming through the heat and heavy summer rains.

These plants are massed with others to produce the maximum in visual appeal. With an eye to the five basic elements of landscape design (color, scale, line, form, and texture), a landscape designer skillfully arranges each bedding plant in relation to the accompanying annuals, perennials, shrubs, and trees. By browsing the pictures in the following resource, one can learn more about how to use the five basic elements of landscape design: Bedding plants are really all plants that, irrespective of their growing habits, are used to make a temporary show, for example, hardy bulbs (hyacinths and tulips), hardy and half-hardy perennials (chrysanthemums), and even tender shrubs (castor oil plant) (Black 2006).

But “bedding” is usually taken to mean those half-hardy annuals or half-hardy perennials planted out to make a splash of color in the summer. For example, petunias, begonias, pelargoniums, and calceolarias. Bedding plants with their seemingly infinite variety of flower color and plant form fit into almost any landscape situation. They provide that necessary touch of color to an often drab landscape. Bedding plants can be grown in containers to add a splash of color to a porch, deck, or patio area. They are also enjoyed as fresh and dry cut flowers and can be a very rewarding hobby.

Petunias, pansies, and snapdragons that grow well and flower under cool night temperatures (45–65 °F) should be planted in the fall, winter, and early spring. Bedding plants such as marigold, gazania, amaranthus, celosia, crossandra, impatiens, vinca, and coleus that can tolerate high temperatures and humidity should be planted in late spring or early summer. Some plants such as wax begonias and salvias grow relatively well during both hot and cool seasons and can be planted year-round.

Major phytonematodes include root-knot nematodes, apart from which ectoparasites like sting, awl, and stubby-root nematodes are of importance. Feeding by these ectoparasitic nematodes usually causes stunted or stubby-looking roots. Sting nematodes are found in sandy soil and are common. Awl nematodes usually are found in wet habitats such as near ditches, ponds, or poorly drained areas. Other ectoparasitic nematodes that may parasitize bedding plants are stubby-root, lance, spiral, stunt, ring, sheath, sheathoid, dagger, and needle nematodes.

Four species of *Meloidogyne* are commonly associated with bedding plants, viz., *Meloidogyne incognita, M. arenaria, M. javanica*, and *M. mayaguensis*. The nematodes inject hormones into the roots that cause knots or galls to form. These galls are the only nematode symptoms that are easily recognized. Root-knot nematodes cause extensive damage and changes in the root system. These changes allow fungi and bacteria to get into the plant. Some of these organisms cause rotting of the root systems, while others cause vascular wilts. Sometimes the damage caused by the nematodes and the other organisms together is worse than that caused by both organisms separately.

8.17.1 Management

8.17.1.1 Resistance

Ageratum ‘Blue Mink’, alyssum ‘Rosie O’Day’, marigold ‘Dwarf Primrose’, periwinkle ‘Little Bright Eye’, and salvia ‘Bonfire’ are some bedding

plants which can resist root-knot nematode infestation.

Different types of marigolds are resistant to different species of root-knot nematodes. African marigold, French marigold, *Coreopsis*, evening primrose, *Argemone, Gaillardia, Rudbeckia*, and *Ageratum* are resistant to nematodes. *Vinca* (periwinkle) is a plant that does not support populations of certain root-knot nematodes even though it may have light galling.

8.17.1.2 Solarization

Solarization is a process of using heat from the sun to kill nematodes and other pests. The soil should be worked with a hoe or rototiller to break up clods. Remove all sticks, roots, and clumps. The soil should be moist, but not wet. Cover the soil with a clear plastic tarp and bury the edges of the plastic. Leave the plastic on the soil for at least 4–6 weeks. Do not remove the plastic until you are ready to plant. Long-term exposure to high temperature kills nematodes, as well as many weeds, fungi, and insect pests. The disinfested zone is usually 6–8 in. deep. Do not till or mix the soil after solarization, since that may reinfest the soil you just solarized with nematodes from below the treated zone. Because it depends on sunlight and heat, solarization works best during the summer months. It does not work well in areas that get shade. So, only solarize planting beds in full sun.

The benefits of solarization may be enhanced by using a second layer of plastic suspended by wire hoops. An air gap is created between the two layers of plastic giving extra insulation. Also, weeds that penetrate the first layer may then be killed by heat trapped between the plastic layers.

8.17.1.3 Organic Amendments

Organic matter can help prevent nematode damage in several ways. The organic matter increases the ability of the soil to hold water and nutrients and improves soil structure. This makes a better environment for most plants and can help the plants survive in spite of the nematodes. Organic amendments can also increase natural enemies of nematodes that suppress the nematode populations. Some organic amendments can release chemicals or gases that are toxic to the nematodes.

8.17.1.4 Planting

Nematode activity slows as soil temperatures drop. Therefore, annual plants grown in cooler months do not suffer as much from nematodes as those grown in warmer months. It is better to get familiar with the temperature requirements of the plant and grow as early as possible in the spring or as late as possible in the fall. Older transplants generally are more resistant to nematodes than younger transplants. So, older transplants should be used in nematode-infested soil. Inspect the roots of all landscape plants before purchase for root-knot galls or other root-related problems. Occasionally nematodes can be introduced with contaminated plants.

8.17.1.5 Sanitation

Roots left in the soil can continue to live and support nematode reproduction. Root-knot nematode eggs are attached to roots and will continue to hatch even after the plant appears to be dead. Therefore, as soon as it is clear that a plant is dying, it is best to pull it up. Make sure to dig up as many roots as possible and destroy them. Always be aware that when soil is moved pathogens can be moved with it. After digging up sick plants, make sure that any shovels or other equipment are thoroughly cleaned after use. It is best to rinse the equipment with a solution of 1 part bleach in 9 parts water to disinfest the surface. This should remove any plant-parasitic nematodes or other pathogens in adhering soil.

8.17.1.6 Removal of Infested Soil

In annual planting beds, it is sometimes practical to replace infested soil and start over. Nematodes may reinfest the soil later, but by then the plants should have a good root system established. The better developed the root system is, the more tolerant the plant will be to nematodes. Reinfestation can be slowed by using root barrier fabric. This fabric is laid down and then the new soil is placed

on top. The barrier will prevent plant roots from growing into the infested soil below. It also may prevent infested roots from outside growing into the non-infested soil.

8.17.1.7 Container Growing

Growing plants in containers off the ground can avoid having to deal with nematode problems. Make sure to inspect the plants for root-knot galls. It is better to use clean potting media, and the potting media should not be mixed with native soil to avoid contamination.

8.18 Mushrooms

Various types of nematodes are associated with mushroom cropping beds, viz., myceliophagous, saprophagous, predatory, animal parasites, and phytoparasitic. Among these, myceliophagous and saprophagous are destructive to mushrooms.

Major myceliophagous nematodes that attack mushrooms include a tylenchid, *Ditylenchus myceliophagous*, and aphelenchids like *Aphelenchoides agarici, A. composticola, A. myceliophagous, A. neocomposticola, A. sacchari, A. swarupi, A. dactylocerus, A. minor*, and *A. asterocaudatus. Paraphelenchus*, and *Seinura* also have been reported to be associated with mushrooms (Sharma and Seth 1993).

8.18.1 Symptoms

Growth of the mycelia is sparse and patchy and the mycelia may turn stingy. Sinking of the compost surface; whitening of spawn, which later may turn to brown; delayed and poor sporophore flushes; variation in the yield, mostly reduced; reduction in the yield of sporophore; and least or no mushroom yield are some of the major symptoms due to nematode attack. From the composting stage to the cropping stage, mushroom can be prone to nematodes. Components of composting, casting material, platform soil, used trays, handling implements, and even some arthropods like sphaerocerid flies may act as the source of nematode infestation.

8.18.2 Management

8.18.2.1 Physical

- Maintenance of air and bed temperature at 60 °C for a minimum of 2 h during peak heating of compost
- Steam pasteurization of casting soil for 4 h
- Used trays should be treated with boiling water for 1–2 min or disinfested with formalin (Sharma and Seth 1993)

8.18.2.2 Cultural

Oil cake incorporation (neem/coconut/castor/pongamia/groundnut) at 2 kg/100 kg of compost during the first or 280-second turning (Parvatha Reddy 2008).

8.18.2.3 Resistance

Agaricus edulis is resistant to *D. myceliophagous*, whereas *Pleurotus sajor-caju* and *Stropharia rugosoannulata* are resistant to *A. sacchari* and *D. myceliophagous* (Sharma and Chandel 1984).

8.18.2.4 Chemical

Usage of chemicals in mushroom cultivation is to be done with care. Toxic chemicals are not recommended, as mushroom is a short duration crop and is consumed immediately after harvest. Thionazin at 80 ppm is recommended for the nematode management. This compound does not have any residual problems (Sharma and Seth 1993).

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9 Nematode Disease Complexes

In the soil environment, plants are constantly exposed to a range of microorganisms which are likely to influence one another, as they occupy the same habitat. Phytonematodes are often considered as pathogens in their own right and are capable of producing a single, recognizable disease. Apart from this, they also get associated with other soil pathogens that result in the complex diseases, which are more devastating and cause huge crop losses. Phytonematodes are major predisposing factors for other potential soil pathogens, which deserve more attention. In the case of soilborne pathogens, further opportunities exist for interactions with other microorganisms occupying the same ecological niche. The significant role of nematodes in the development of diseases caused by soilborne pathogens has been demonstrated in many crops throughout the world. In many cases, such nematode–fungus disease complexes involve root-knot nematodes (*Meloidogyne* spp.), although several other endoparasitic (*Globodera* spp., *Heterodera* spp., *Rotylenchulus* spp., *Pratylenchus* spp.) and ectoparasitic (*Xiphinema* spp., *Longidorus* spp.) nematodes have been associated with diseases caused by soilborne fungal pathogens (Back et al. 2002).

As Fawcett (1931) quoted, "nature does not work with pure cultures," and several plant diseases are influenced by the associated microorganisms. A disease complex is produced through a synergistic interaction between two organisms. Synergistic interactions can be summarized as being positive where an association between nematode and pathogen results in plant damage exceeding the sum of individual damage by pest and pathogen ($1+1>2$). Conversely, where an association between nematode and fungus results in plant damage less than that expected from the sum of the individual organisms, the interaction may be described as antagonistic ($1+1<2$).

Most nematodes either influence or influenced by associated soil organisms as their common habitat is soil. It thus seems appropriate to consider interrelationships among such organisms and the ultimate effects of these complexes upon host plants. It is not realistic to assume that a plant although infected with one pathogen will not be affected by another. In fact, it seems much more appropriate to assume that because a host is infected by one pathogen, its response to additional invaders will be altered. These alterations may have significant influences upon disease development within a particular host, epidemiologically of all pathogens involved and ultimately on disease management. Infection by nematodes also may alter the host response to subsequent infection by another.

Under normal growing conditions, rhizosphere biotic and abiotic factors are constantly subjected interrelationships in different degrees of equilibrium. However, under conditions where significant alterations in this equilibrium occur, an interaction between the several factors may result in disease complex etiology and synergistic interactions. In general, disease complexes are mostly the result of interactions involving significant alteration in the abiotic soil climate that leads to variation in

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this balance and not by complex interrelationships of disease-inducing organisms. It has long been understood that the development of disease symptoms is not solely determined by the pathogen responsible, but is dependent on the complex interrelationship between host, pathogen, and prevailing environmental conditions (Back et al. 2002). In addition, in nature plants are rarely, if ever, subject to the influence of only one potential pathogen. This is especially true of soilborne pathogens, where there is tremendous scope for interaction with other microorganisms occupying the same ecological niche.

Ectoparasitic nematodes such as *Belonolaimus* and *Trichodorus* spp. are rarely recorded to have a role in synergistic interactions with fungi, probably because their feeding behavior causes only minor tissue damage to plant roots (Back et al. 2002). In comparison, ectoparasites such as *Xiphinema* (dagger nematode) and *Longidorus* (needle nematode) have longer stylets for feeding in the vicinity of the vascular cylinder and are recognized as important vectors of plant viruses. The life cycles of endoparasitic nematodes are far more complex compared with those of ectoparasites and involve closer associations with their plant hosts. This means that plants infested with endoparasites are usually subject to various nematode-induced modifications. These can vary from localized forms of damage caused during invasion and feeding to overall systemic effects such as retarded plant growth. It is these changes which influence infections by soilborne pathogens. The endoparasites *Globodera, Heterodera, Meloidogyne, Rotylenchulus*, and *Pratylenchus* are the genera most commonly reported to be involved in disease complexes with fungal pathogens. These typically interact with the wilt fungi *Fusarium* and *Verticillium* and the root-rot pathogens *Pythium, Phytophthora*, and *Rhizoctonia.*

There is a greater chance for soilborne pathogens to interact with other microorganisms occupying the same ecological niche. The significant role of nematodes in the development of diseases caused by other soilborne pathogens has been well documented in a range of crops across the globe. It has been estimated that a gram of field surface soil consists of 10^6–10^8 bacterial cells, 10^6–10^7 actinomycete cells, 10^4–10^6 fungal colony-forming units, 10^5–10^6 protozoa, and 10^4–10^5 algae (Gottlieb 1976). However, Richards (1976) reported that 1 m of fertile soil may consist of 1×10^7 nematodes. Although several of these organisms are saprophytic, having little, if any, effect on cultivated crops, the moist soil environment is favorable for the activities of phytonematodes and for the growth and multiplication of pathogenic fungi. Therefore, a variety of interrelationships between these are sure to take place. Powell et al. (1971) cautioned that the failure of disease management practices in several instances could be due to incomplete diagnosis of disease complexes, resulting in inappropriate management.

The association of phytonematode with other soil microorganisms may be synergistic, antagonistic, symbiotic, or neutral. The well-recognized associations among nematodes and other microorganisms include etiological interactions which may lead to disease complexes in cultivated crop plants; competitive association between nematodes and soil fungi, bacteria, mycorrhizal fungi and other nematodes; nematodes as vectors of plant viruses and other soil microorganisms; nematodes as parasites and predators; other organisms as nematode vectors and nematodes as host or prey of other parasites and predators (Khan 2008). Among these varied types of associations, however, from the point of view of plant disease complexes, etiological associations are of much significance. All nematodes may not all have effects on host plants. Nematodes that feed shallowly on the root cortex or epidermis (e.g., *Helicotylenchus, Paratylenchus*) usually have far less effect on plant productivity and energetics than vascular parasites (e.g., *Meloidogyne, Heterodera*) (Bernard 1992). On the other hand the plant species and their ages affect the infection. For example, seedlings are particularly exposed to damage by nematodes because their tissues are more susceptible to attack by parasites and more favorable for nematode development.

Phytonematodes induce physiological, biochemical, and structural changes in their hosts. On the other hand, many abiotic and biotic factors can predispose plants to diseases that would

otherwise occur to a lesser extent (Lockwood 1988). Most nematodes are capable of elevating a normally minor pathogen to major status. Plant-parasitic nematodes favor the establishment of secondary pathogens, viz., fungi, bacteria, and virus. They alter the host in such a way that the host tissue becomes suitable for colonization by the secondary pathogens. Even though the nematodes themselves are capable of causing considerable damage to the crops, their association with other organisms aggravates the disease. The nematodes cause mechanical wound on host surfaces, which favor the entry of microorganisms. In some cases, the association of nematode and pathogen breaks the disease resistance in resistant cultivators of crop plants. Plant-parasitic nematodes commonly interact in a wide range of soil microorganisms. However, the association of phytonematodes with fungi, bacteria, viruses, mycorrhizae, and rhizobial bacteria is of much significance.

On the other hand, free-living types are nonparasitic; they live free in the soil and do not obligatorily need plants to complete their life cycle. Most of them feed on fungi and bacteria in the rhizosphere. They are usually dominant over their plant-feeding counterparts in the soils and are also commonly found inside plant roots as secondary feeders (Desaeger et al. 2004).

Although free-living nematodes may cause reductions in the populations of pathogenic bacteria and fungi, they may also aid in the dispersal of the same fungi and bacteria, as well as of mycorrhizae and rhizobia (Desaeger et al. 2004). As they can also disrupt plant health by interfering with symbionts, some of them need to be considered as facultative parasites. Not much is known about the role played by free-living bacterial- and fungal-feeding nematodes in disease epidemiology (Duponnois et al. 2000). Some fungivorous nematodes have been observed as suppressing ectomycorrhizae on pines and endomycorrhizae on many plants, and some bacterivorous nematodes have been observed to inhibit nitrogen fixation. On the other hand, these nonparasitic nematodes may compete with parasitic nematodes for habitable niches both in the soil and roots (Stirling 1991).

Free-living nematodes play notable roles in the soil ecosystem. The presence and feeding of bacterivorous and fungivorous nematodes accelerate the decomposition process. They affect organic matter decomposition in several ways: Their feeding recycles minerals and other nutrients from bacteria, fungi, and other substrates into inorganic ions and returns them to the soil where they are accessible to plant roots. Nematodes can also disseminate dispersing microorganisms throughout the soil and water (Ikonen 2001), which advances the colonization of substrates and mineralization of nutrients (Wang and McSorley 2005). Furthermore, they can serve as prey and a source of nutrients for fauna and microflora such as soil nematophagous fungi, and they affect the distribution and function of plant symbionts. Predators prey on these bacterivorous and fungivorous nematodes, improving nutrient cycling and allowing more nutrients to be released. The accumulation of plant litter can support microflora, which was indicated by the development of abundant populations of free-living nematodes. Bouwman et al. (1996) estimated that the annually produced bacterivorous nematodes consume 50 kg carbon and 10 kg nitrogen per ha, per year, in the upper, plowed 25 cm of arable soil.

9.1 Interactions in the Soil Ecosystem

The study of soil ecosystems is extremely complex, involving determination of interactions between microbial, chemical, physical, and plant host variables (Villenave and Duponnois 2002). Many processes in the rhizosphere mediated by soil microorganisms are essential to plant production. With the exception of damaging pathogens, plants may benefit from rhizosphere microorganism interactions either directly, as in the case of symbionts such as mycorrhizal fungi, or indirectly by the antagonistic activity of beneficial microorganisms toward plant pathogens (Diedhiou et al. 2003). An understanding of the interactions which occur among different groups of soil ecosystem flora and fauna will help in manipulating them in a manner which achieves favorable effects

(Desaeger et al. 2004). It is also essential for the successful development of biological control.

Most knowledge of the interactions between microorganisms and nematodes in the rhizosphere has been derived from research with rhizobia, mycorrhiza, and plant pathogens. Such research has clearly demonstrated complex tritrophic interactions in the rhizosphere, in which nematodes and microorganisms act in competitive, additive, antagonistic, or synergistic associations to affect the plant host. Direct interactions occur when microorganisms compete for space or nutrients, or when one group antagonizes another by producing toxic metabolites. Indirect interactions are mediated through the root system; for instance, one pathogen may increase or decrease the susceptibility of the host plant to another pathogen (Khan 1993). For example, endoparasitic nematodes tend to increase diseases caused by vascular wilt fungi, while ectoparasitic nematodes increase infection by cortical rot pathogens.

9.2 Mechanisms Involved in Interaction Between Nematodes and Other Microorganisms

Major mechanisms involved in the interaction between phytonematodes and soil fungi can be listed as host modifiers, vectors of fungal pathogens, mechanical wound agents, rhizosphere modifiers, and resistance breakers.

9.2.1 Host Modifiers

Various changes are brought about by the plant-parasitic nematodes in their host tissues as part of the feeding process and in order to derive nourishment from it. Root-knot nematodes induce syncytia, primarily in the stele, which are highly specialized structures in which there is an accumulation of certain chemicals resulting from the nematode-induced cellular modification. Increased quantities of organic and inorganic substances including calcium, magnesium, and phosphorus in the galled tissue of affected crops are a common feature. The feeding sites of sedentary endoparasitic nematodes (giant cells or syncytia) are zones of high metabolic activity, having a large number of Golgi apparatus and mitochondria, while the cytoplasm is dense and contains many ribosomes (Jones 1981). It is therefore no surprise that these nutrient-rich cells should become the substrate for fungal colonization. Nematode-infected plant tissue may be actively selected by certain plant pathogens.

Differences have been observed in the constitution of giant cell walls compared to those of healthy tissues, i.e., giant cell walls contain cellulose and pectin but not lignin, suberin, or starch because of the absence of arabinose. It may be concluded that nematode infection had induced a significant change in pentose metabolism. In general, biochemical changes induced by the nematode may be favorable to the fungi resulting from a nutritionally improved substrate, the destruction of certain chemicals antagonistic to the pathogen, or the obstruction of defense reactions by which the host would normally fend off invaders. In experiments designed to study the role of nematodes in the production of root necrosis in crops like celery, *M. hapla* caused more damage in the presence of a natural soil microflora than in a sterilized soil.

Nematodes may influence the host tissues in order to maintain them in a juvenile state, which improve nutritional improvement and would increase infection by several fungi. However, in cotton, seedlings infected by root-knot nematodes delayed their maturation and remained susceptible to damping-off by *Rhizoctonia solani* and *Pythium debaryanum* for much longer than did healthy seedlings. Cyst nematodes also form nutrient-rich syncytia for the purpose of development. Histological studies of *H. schachtii-infested* sugar beet seedlings exposed to *R. solani* indicated that syncytia were a more favorable substrate to the fungus than normal cells (Polychronopoulos et al. 1969). Syncytia or giant cells contain higher levels of total protein, amino acids, lipids, DNA, and sugars (Abawi and Chen 1998), which would be beneficial to many fungi. This would support the suggestion that nematode infection enhances the nutritional composition of portions of plants to fungi, but the relationship remains unproven.

Phytonematodes can elevate a normally minor pathogen to major one. *R. solani* can colonize healthy tobacco roots earlier infected with root-knot nematodes. Greatest metabolic activity and physiological changes occur in the giant cells and galled tissues when the nematode development is at the stage of molting to the adult and at the commencement of egg laying, which is normally 3–4 weeks after initial infestation by the juveniles. During this phase, there will be a considerable increase in proteins, nucleic acids, and DNA, together with increase in sugars, various inorganic ions, etc. They may stimulate the growth of fungal pathogens. Amino acids, in general, stimulate the growth of *Fusarium.* An increase in sugars in xylem sap from infected plants is considered to be possibly due to intensified photosynthesis of infected plants resulting in excessive sugar translocation to galled roots. This increases as the nematode inoculum raise at 2, 4, and 6 weeks after inoculation. The increase in total sugars and decease in amino acids of xylem sap in nematode-infected plants and the similar variations in root exudates are possible mechanisms by which tomato plants may be predisposed to *Fusarium* wilt.

9.2.2 Vectors of Fungal Pathogens

Several nematodes may carry spores of fungi on their external surface, and this increases the mobility of the pathogens, but with a few exceptions, the association is nonspecific. Nematode invasion sites and tracts are regarded as inconsequential in the etiology of fungal diseases (Taylor 1990). However, there are a number of reports which clearly illustrate that nematode damage has a role in the establishment and development of disease caused by soilborne pathogens. Histological studies appear to be the key to unraveling the association between fungal pathogens and the injuries caused to plants by phytonematodes. The invasion process of *Heterodera schachtii* (beet cyst nematode) was found to facilitate the infection of sugar beet (*Beta vulgaris*) by the damping-off fungus *Rhizoctonia solani* (Polychronopoulos et al. 1969).

In combination with *H. schachtii*, the hyphae of *R. solani* were found to grow vigorously through the epidermis and cortex. Closer examination showed that hyphal colonization frequently followed tracts made by invading nematode juveniles. On the epidermal surfaces of the seedlings, the pathogen was found to produce fewer infection cushions in the presence of nematodes than when it was present alone. In such instances, infection cushion synthesis could have been hindered in some way by the invading nematodes. However, nematode invasion sites may provide *R. solani* with the necessary portals for penetration and entry, consequently reducing the need for developing more sophisticated infection structures such as infection cushions.

The hypothesis that phytonematode-induced wounds facilitate the invasion process of some fungal pathogens seems the most likely explanation behind a synergistic interaction, although there are relatively few reports that demonstrate this mechanism, and positive quantitative data coupled with convincing histological evidence are required to validate this hypothesis. Future work in this area might benefit from using equipment such as time-lapse and video-enhanced light microscopy together with some form of image analysis system. Video-enhanced light microscopy has previously been used for studying nematodes (Wyss and Zunke 1992) and fungi (McCabe et al. 1999). The application of image analysis systems could help overcome the problems of quantifying the density of fungal pathogens in regions of nematode damage. *Meloidogyne* spp. has been frequently observed to transmit spores of several soil fungi. *Anguina funesta* carries the fungus *Dilophospora alopecuri* on its cuticle. As the nematode penetrates the plant, it can take with it either the fungus or the bacterium. Conidia of the fungus attach to the cuticle of the nematode by means of extracellular appendages.

9.2.3 Mechanical Wounding Agents

It is well known that all phytonematodes elicit a wound of some sort in the host plant during their feeding, either by a simple micropuncture or

by rupturing or separating cells. For instance, a root-knot nematode juvenile usually penetrates near the root cap with subsequent penetration into the meristematic zone of the root apex. The evidence of entry can be observed by dot-like penetration marks, but once within the plant, they move intracellularly without inducing necrosis in host cells surrounding the nematode body. Nematodes provide avenues of entry for fungal pathogens, and the severity of fungal disease will be greater when the nematode is present in the host plant few weeks prior to the exposure to the fungus than when the host is exposed to both nematode and fungus, simultaneously.

If the role of phytonematodes in disease complexes with fungi is limited just to wounding agents, the fungal invasion of the plant tissues would begin soon after nematode infection; nematodes have to induce drastic structural, biochemical, and/or physiological changes in the roots to render them suitable to fungal penetration and development. It is important to note that the delay in predisposition of host plants to several fungal diseases, mostly by species of *Meloidogyne*, suggests that the nematodes are not just wounding agents facilitating the penetration of the fungi within the roots. It is well established that pre-inoculation with phytonematodes causes a significant increase in fungal infection. The 2–4-week pre-inoculation/predisposition normally favors the fungal pathogen.

Phytonematodes which produce similar structural changes or wounds in the same host may induce different physiological or biochemical modifications resulting in a different degree of promotion of the infection and development of the same fungus. *Fusarium* wilt symptoms may appear earlier and in more severe status in chrysanthemum *Fusarium*-susceptible cultivar 'Yellow Delaware' when infected with *Meloidogyne javanica* than with *M. hapla* and *M. incognita*. A particular nematode species may interact in a different manner with two fungi on the same host plant. For instance, *Verticillium* wilt may be more severe in tomato cultivar 'Bonny Best' infected with *Heterodera tabacum* than in uninfected plants, while *Fusarium* wilt may be less severe in the presence of the nematode than in its absence (Miller 1975). Fungal invasion and colonization is normally more extensive in galled than in non-galled tissue. The mycelium present in galled tissue is more vigorous and hyphae larger than in non-galled areas. The fungus penetrates the galled tissue either directly or through openings created by the mature female nematodes to lay their eggs. After penetration the fungus showed a marked trophic intercellular growth toward the giant cells, and physiological changes appeared in these cells prior to physical contact by the fungus. Giant cells are sensitive to a translocatable physiological factor produced by the fungus.

The alterations induced by one species of nematode may predispose its host to infection by one fungus and not another. Usually, the predisposition effect on plants to fungal diseases by root-knot nematodes reaches its maximum 2–4 weeks after nematode infection. For example, *Meloidogyne incognita* maximally predisposes tobacco plants to *Fusarium* wilt and to root decay caused by *Pythium ultimum* when the nematode is inoculated 4 weeks prior to fungus inoculations. However, when *M. incognita* inoculation preceded *Rhizoctonia solani* inoculation by at least 10 days, the nematode-susceptible tobacco cultivars 'Dixie Bright 101' and 'Coker 316' may exhibit more severe root rot than when nematode and fungus are inoculated simultaneously.

Sometimes the contradictory results may indicate that the nematodes may have two effects favoring fungal infection of their hosts, viz., a "localized effect," where the fungus penetration and initial development in the host are enhanced by the modifications induced by the nematodes at their feeding sites, and a "systemic effect" where inhibition of host resistance mechanisms occurs, resulting in a stimulation of the fungal development in tissues not infected by the nematodes.

9.2.4 Rhizosphere Modifiers

It is a known fact that a wide range of organic compounds exude from intact plant roots which may influence the soil fauna and flora. The exudates may either be attractants for the motile stage of the plant pathogens or may be a source of nutrients

for the microflora or may be a stimulus for the germination of dormant spores.

Depending on specific life cycles, phytonematodes are able to cause a variety of types of wound on host plant roots while entering or feeding. For example, ectoparasitic nematodes such as *Trichodorus* spp. and *Tylenchorhynchus* spp. feed on root epidermal cells, leaving behind simple micropuncture-type wounds. In contrast, endoparasitic nematodes are far more disruptive to their hosts' roots. The root lesion nematode *Pratylenchus* spp. is a migratory endoparasite that travels intracellularly through the cortex of roots by cutting through cell walls with its stylet to create a path. The sedentary endoparasites *Meloidogyne* spp., *Globodera* spp., and *Heterodera* spp. have highly specialized feeding strategies together with elaborate life cycles. Vermiform juvenile nematodes (J2) select penetration sites behind growing root tips (Doncaster and Seymour 1973) and migrate either intracellularly (*Globodera* and *Heterodera* spp.) or intercellularly (*Meloidogyne* spp.) to the vascular cylinder, where specialized "nurse cell systems" are initiated.

When in combination with *H. schachtii*, the hyphae of *R. solani* were found to grow vigorously through the epidermis and cortex. Closer examination showed that hyphal colonization frequently followed tracts made by invading nematode juveniles. On the epidermal surfaces of the seedlings, the pathogen was found to produce fewer infection cushions in the presence of nematodes than when it was present alone. Infection cushion synthesis could have been hindered in some way by the invading nematodes. However, nematode invasion sites may provide *R. solani* with the necessary portals for penetration and entry, consequently reducing the need for developing more sophisticated infection structures such as infection cushions. In a similar way, *R. solani* is known to exploit natural openings on the outer surfaces of plants such as stomata (Chand et al. 1985) and lenticels on potato tubers (Ramsey 1917) to invade underlying tissue.

In order for female cyst and root-knot nematodes to reproduce, the females/cysts must rupture through the root cortex to allow the vermiform males to fertilize them. This event often produces a number of cracks and crevices where the swollen female has emerged. These openings might be used by opportunistic pathogens to reach the underlying tissue of roots more easily (Evans and Haydock 1993).

Phytonematodes may directly affect the release of exudates in a quantitative way by rupturing the root cell membrane during feeding, penetration, and migration within the root. The levels of electrolytes leaking from nematode-infected roots increase progressively following infection. Minerals and amino acids are the major electrolytes detected on root-gall exudates. The concept of nematode-induced changes in root exudates relates to the effect on the pathogen in the rhizosphere. The modification of the rhizosphere resulting from changes in root exudates has a general influence on the rhizosphere microflora, with the possibility of a chain of reaction in the nematode–fungus disease complex. In the case of root rot caused by *Rhizoctonia solani* on tomato, the delay may be due to a modification in the root exudates which occurs 3–4 weeks after nematode infection when a high concentration of nitrogenous compounds in nematode-infecting root leachates is favorable for maximum virulence of the fungi (Van Gundy et al. 1977). In several instances, variations in the total sugar concentration in the xylem sap which reaches maximum concentration 4 weeks after nematode inoculation may contribute to the enhancement of *Fusarium* wilt incidence in tomato infected with *Meloidogyne incognita*. Sclerotia of *Rhizoctonia solani* have been observed only in galled roots on tomato infested by *Meloidogyne incognita*.

Weinhold et al. (1972) observed that *M. incognita-infested* plants produced some form of attractant for *R. solani*. When the properties of exudates emanating from the nematode-infested roots were examined, they were found to have elevated levels of 14C metabolites. During the time of sclerotial development, 14–21 days following nematode invasion, the major constituents of the 14C-labeled metabolites were nitrogenous compounds such as amino acids and proteins; such nitrogenous compounds are important in the virulence of *R. solani*.

In some instances, *Pythium polymorphon* colonized preferentially and earlier galled areas of celery roots infected with *M. hapla* than non-galled root segments, which suggests that factors attractive to the fungus originate from galls caused by the nematode. Liberation of root leachates through wounds produced by *M. javanica* may stimulate the penetration of hyphae of *Macrophomina phaseolina* within the roots. It is a common observation that a number of actinomycetes are normally reduced and a number of *Fusarium* propagules are increased in the rhizospheric soil around the tomato roots inoculated simultaneously with *M. javanica* and *F. oxysporum* f.sp. *lycopersici* compared to those observed when the fungus was inoculated alone. The reduction in number of actinomycetes antagonistic to *Fusarium* in the rhizosphere of nematode-infected plants may be partly responsible for the stimulation of the pathogenic effect of the fungus.

Root exudates are rich in carbohydrates, amino acids, organic acids, vitamins, nucleotides, flavanones, enzymes, etc. These are subjected to variation due to several factors. For example, temporary wilting increases the release of amino acids, and high light intensity and temperature increases exudation, particularly during initial few weeks of plant growth. The patterns of exudation are altered by other organisms including nematodes.

9.2.5 Resistance Breakers

Several evidences on nematode–fungus interactions are available which illustrate the breakdown of resistant cultivars, which has led to the necessity of breeding plants resistant to both pathogens. The nematodes may cause some host response that lowers the natural resistance of the plants to fungal infection, as in the case of the interaction of *Meloidogyne* spp. with *Fusarium* wilts on tomato. There may be two types of resistance to *Fusarium* wilt. In the first, a qualitative high resistance is dependent on a basic incompatibility of the host and the fungal pathogen that is unaffected by nematode infection. The second type is regarded as quantitative which is readily influenced by nematode infection. This type of breakdown in resistance is considered as a shift in the host–parasite equilibrium due to improved nutrition for the fungus, which is "making a poor host a good host."

Root-knot nematodes greatly decrease the resistance of tomato cultivars to *Fusarium* wilt. Monogenic resistance (I gene) of tomato to *F. oxysporum* f.sp. *lycopersici* is rendered ineffective by infection with *M. hapla* race 1. However, *M. incognita* race 1 can induce wilting in *Fusarium* wilt-resistant tomato cultivar Chesapeake. The loss of *Fusarium* resistance in tomato cultivar Craigella GCR 161 when infected with *M. incognita* is associated with the lack of rishitin, an antifungal substance that is found in healthy plants but absent in nematode-infested plants. Such result may vary depending upon the cultivars and species of nematodes. In general, inconsistencies in the results may be due to differences in experimental conditions, cultivar, nematode species associations, environmental and especially edaphic factors, and population levels of pathogens.

Marley and Hillocks (1994) observed that nematode-induced loss of resistance to *Fusarium udum* in pigeon pea (*Cajanus cajan*) was associated with reduced levels of the isoflavonoid phytoalexin cajanol. They had determined that the rapid accumulation of cajanol in some pigeon pea cultivars was responsible for conferring resistance to the pathogen. However, cajanol content was 62 % lower and resistance was lost during the combined infections of *F. udum*, *M. incognita*, and *M. javanica* where wilt disease incidence and severity were significantly higher than in plants inoculated with *F. udum* alone. Similar phenomena may also occur in several horticultural crops.

Polygenic resistance is comparatively less stable than monogenic resistance (Sugawara et al. 1997). Plants with polygenic resistance to fungal pathogens are frequently found to become susceptible to fungal attack during nematode infestations, whereas plants with a single dominant gene for resistance are rarely affected. This was observed by Abawi and Barker (1984) on tomatoes, where

resistance to *F. oxysporum* f.sp. *lycopersici* was disrupted by infestations of *M. incognita* on cultivars with polygenic resistance, but not on those where resistance was expressed by a dominant single I gene. Transgenic plants involving quantitative trait loci may have a greater capacity for providing durable resistance in the presence of interacting fungal pathogens and nematodes. It is important to note that there are also other abiotic factors, such as soil type and temperature, which have been shown to affect interactions (Uma Maheswari et al. 1997) and which may have varied between individual studies on specific disease complexes.

Wajid Khan (1993) postulated that resistant plants are rendered vulnerable to pathogens via physiological alterations made by the nematode, which have no effect on the gene(s) responsible for encoding resistance. For example, the process of invasion by PPN may provide soilborne pathogens with portals through a previously impenetrable physical barrier selected for in a plant breeding program. As with nearly all investigations in science, many reports on disease complexes contradict one another. While some of these disparities might be explained by experimental procedure and accuracy, there are findings that highlight the specificity of certain disease complexes and the influence of biotic and abiotic factors on them. This is exemplified by studies on the *V. dahliae–Pratylenchus* complex of potato, where it has been found that the interaction between these organisms varies among different nematode species (Riedel et al. 1985) and populations, as well as fungal genotypes (Botseas and Rowe 1994).

Fluctuating environmental parameters are often found to affect one or more of the interacting organisms in a disease complex. Temperature has been found to be critical in some nematode–fungus interactions (Walker et al. 2000), but not in others (Uma Maheswari et al. 1997). Soil type has been shown to have no influence over disease complexes involving *M. hapla* and *Phytophthora megasperma* f.sp. *medicaginis* on alfalfa and *R. solani* and *M. javanica* on soybean (Agu and Ogbuji 2000). Conversely, Uma Maheswari et al. (1997) stated that soil type can affect interactions between *F. oxysporum* f.sp. *ciceris* and *M. javanica*. It is also interesting to note that as nematode activity can increase the severity of diseases caused by fungal pathogens, so nematode populations can be elevated during concomitant infections with root-infecting pathogens (Taheri et al. 1994). Faulkner and Skotland (1965) observed that *Pratylenchus minyus* reached its reproductive peak at the same time as the maximum expression of wilt disease (*V. dahliae* f.sp. *menthae*) on peppermint plants (*Mentha piperita*). The authors suggested that *V. dahliae* may produce root growth-promoting substances such as indole-3-acetic acid resulting in an enlarged root system, releasing greater volumes of root exudate and thereby attracting more phytonematodes.

Nematode penetration is increased in plant roots previously subjected to the enzymes of fungal pathogens (Nordmeyer and Sikora 1983). Also, fungal infections cause a deterioration or breakdown of plant resistance to nematode attack (Hasan 1985). The interaction between these organisms varies among different nematode species and populations, as well as fungal genotypes (Back et al. 2002). Bowers et al. (1996) observed that potato early dying disease (*V. dahliae*) was enhanced by populations of *P. penetrans* but not by *P. crenatus* or *P. scribneri*. Fluctuating environmental parameters are often found to affect one or more of the interacting organisms in a disease complex.

Applications involving remote sensing and digital image analysis are currently being refined and used for the determination of spatial distributions of both plant pathogens and nematodes (Heath et al. 2000) in crops. Further development of this type of technology is likely to be invaluable for the prediction of disease complexes. It would also be interesting to know whether there are any correlating patterns in spatial population densities between interacting organisms. The specificity of some of the disease complexes mentioned indicates the need to use appropriate diagnostic measures to determine whether management strategies are suitable. Marker analysis could be employed to identify different strains of these pathogens.

9.3 Lectin-Mediated Nematode–Fungus Interaction

Lectins are carbohydrate-specific proteins that are key players in many recognition events at the molecular or cellular level (Wimmerova et al. 2003). Fungi, either mushrooms or filamentous fungi, often depend on host association (symbiosis or parasitism) and appear to use lectins for host recognition and/or adhesion. One of the first examples of a lectin-mediated interaction between a fungus and its host was discovered in the nematode-trapping fungus *Arthrobotrys oligospora*. In higher fungi, lectins are involved in molecular recognition during the early stage of mycorrhization. An example is their role in the high specificity of the *Lactarius* mushroom/tree symbiotic association. A role of lectins in mycoparasitism has been proposed for a number of human pathogens such as *Candida albicans*, the agent causing oral candidosis, and *Aspergillus fumigatus*, which is a major life-threatening pathogen in hospital environments, responsible for invasive pulmonary aspergillosis in immunodeficient patients. Lectin-mediated recognition is also involved in plant mycoparasitism.

Many nematode-trapping fungi capture nematodes using an adhesive present on specific capture organs. Until recently, the mechanism of adhesion was completely unknown. In the case of *Arthrobotrys oligospora*, one of the most common nematophagous fungi, nematodes are trapped in three-dimensional structures of the adhesive network type. When a suspension of nematodes is added to an agar culture of the fungus, nematodes are immediately captured and firmly held by the traps (Hertz and Mattiasson 1979). The nematode cuticle is lysed at the point of contact and penetrated by a hypha within 1 h. An increased secretion by the fungus of a mucilaginous substance in the presence of prey has been shown by scanning and transmission electron microscopy. The firmness of attachment to the traps despite the struggle of the nematode is due to a series of events, beginning with an interaction between complementary molecular configurations on the nematode and fungal surfaces (Riley 1994). The presence of a lectin on the traps of *A. oligospora* binds to a carbohydrate on the nematode surface.

In general, nematodes seem to favor all stages of fungal infection and development. By modifying the composition of the root leachates, they can promote the growth of fungi in the rhizosphere and favor their pathogenic development. Moreover, these modifications of the rhizospheric environment may limit the development of organisms antagonistic to the pathogenic fungi and feeding sites, and the cells they modify, especially the giant cells induced by root-knot nematodes, may serve as a favorable substrate which helps the fungi to establish within the plant and promote their development. Nematode-induced or nematode-produced factors appear to be translocated from the nematode feeding sites to other parts of their host. These factors seem to modify the resistance of the host to the fungi and/or directly stimulate fungal growth.

Fruiting body lectins are ubiquitous in higher fungi and characterized by being synthesized in the cytoplasm and upregulated during sexual development (Bleuler-Martinez et al. 2011). The function of these lectins is unclear. A lack of phenotype in sexual development upon inactivation of the respective genes argues against a function in this process. Testing of a series of characterized fruiting body lectins from different fungi for toxicity toward the nematode *Caenorhabditis elegans* revealed that most of the fungal lectins were found to be toxic toward nematode. By altering either the fungal lectin or the glycans of the nematode or by including soluble carbohydrate ligands as competitors, it was demonstrated that the observed toxicity is dependent on the interaction between the fungal lectins and specific glycans in the nematode. The toxicity was found to be dose dependent such that low levels of lectin were no longer toxic but still led to food avoidance by *C. elegans*. In an ecologically more relevant scenario, it was observed that challenging the vegetative mycelium of *Coprinopsis cinerea* with the fungal-feeding nematode, *Aphelenchus avenae*, induced the expression of the nematotoxic fruiting body lectins CGL1 and CGL2. It was proposed that filamentous fungi possess an inducible resistance

against predators and parasites mediated by lectins that are specific for glycans of nematode.

Wharton and Murray (1990) in a study noticed that removal of the sheath of the ensheathed infective juvenile of *Trichostrongylus colubriformis* prevented capture by the nematophagous fungus *Arthrobotrys oligospora.* Exposure of the trap hyphae to a variety of saccharides, which may block a recognition system based on lectin/carbohydrate binding, failed to prevent capture, but some saccharides did inhibit penetration and invasion by the fungus. Capture and penetration appeared to be two distinct processes with capture being less specific than penetration. Carbohydrate residues were absent from the outer surface of the cuticle and the sheath but were present on the inner surface of the sheath. The limited accessibility of these lectin-binding sites may explain the slow process of infection of the infective juvenile by the fungus. The sheath did not protect the infective juvenile against attack by this nematophagous fungus.

9.4 Indirect Effects of Complexes on Associated Organisms

Direct antagonistic interactions, which involve the parasitism of nematodes by soilborne fungi, have been extensively studied and reviewed (Kerry 2000). The indirect effects that fungi exert on nematodes in disease complexes are less well known, yet remain important in terms of future nematode multiplication. Successful assemblage and maintenance of giant cells is vital for the growth and reproduction of root-knot nematodes and that destruction of large proportions of giant cells will result in the premature death of female nematodes. Consequently, these types of disturbance are likely to affect the development of subsequent nematode populations.

In addition to fungal disruption of nematode feeding sites, plants affected by disease complexes may be more prone to early senescence and death (Griffin et al. 1993) which, in turn, may prevent nematodes from completing their life cycles. Competition for nutrients or root space may be responsible for the decline of nematode populations, although these concepts appear to be difficult to demonstrate. Phytonematodes can induce systemic changes within their host plants to render them susceptible to fungal attack (Back et al. 2002). The reverse of this effect is that nematode invasion activates some form of hypersensitive response or triggers a mechanism of SAR. These types of plant response are widely recognized for the protection they provide plants against subsequent attacks (Sticher et al. 1997), but to date no studies have been undertaken that demonstrate SAR to pathogen infection induced by nematode invasion.

9.5 Nematode–Fungal Interactions

Investigations on disease complexes involving nematodes and fungi, especially *Meloidogyne* spp. and wilt-inducing fungi on crops like tomato, tobacco, and cotton, have been carried out frequently and their significance is appreciated. The presence or absence of a synergistic interrelationship is based on initial population densities and plant cultivars.

Plant-parasitic nematodes interact with fungi in a variety of ways to cause plant disease complexes. Some phytonematodes are able to carry fungal spores internally which not only increases their mobility but also protects them from fungicides. They frequently wound plants in the process of penetration and feeding. These wounds serve as avenues for the easy entry of fungal pathogens into the host tissues. Some nematodes modify plant tissue in such a way that it becomes a better substrate for the fungus and thus increases their growth and reproduction to the detriment of the host. Quantitative and qualitative changes in root exudates which are induced by certain nematodes stimulate the germination, growth, and reproduction of fungal propagules in the rhizosphere. These exudates may also indirectly inhibit components of the rhizosphere microflora like actinomycetes, which are antagonistic to some plant pathogens. Depending on the species of nematode and fungus, concomitant infections may stimulate nematode reproduction

(*Pratylenchus–Verticillium*) or inhibit reproduction (*Heterodera–Fusarium*) (Bergeson 1972).

Much of the literature on associations of phytonematodes with the rhizosphere microorganisms is concerned with nematodes and fungi. As early as 1892, Atkinson reported the increased wilt incidence due to *Fusarium* wilt pathogen in cotton in the presence of root-knot nematodes. Since then *Meloidogyne* and *Fusarium* association has been reported by several workers on a wide range of crops. Nematode interactions with fungi are obviously more complex. The ultimate result of this interaction includes increased fungal disease following nematode invasion, increase in nematode growth or pathogenicity following fungal invasion, diminished nematode pathogenicity following fungal invasion, or limiting of fungal invasion following nematode attack (Parvatha Reddy 2008). Table 9.1 provides a list of important nematode–fungal associations on major hosts.

Soil factors like soil texture play an important role in the development of the pathogens. For instance, *Verticillium* wilt of cotton is more common in heavy soils and not sandy loam soils where *Fusarium* is generally prevalent. The population density of nematodes also decides the complex diseases. Host plant predisposition to fungal diseases by phytonematodes needs a minimum level of nematode infestation. For instance, the addition of 1,000–2,000 *Meloidogyne incognita* juveniles per plant significantly increases the number of plants of cotton cultivars 'Deltapine Smooth Leaf' and 'Pima S-2' infected with *Verticillium albo-atrum*. However, addition of 250 and 500 juveniles per plant may not give a similar result. The level of nematode infestation is influenced by incubation period, incidence, and symptom expression of some fungal diseases. The incubation period of *Cylindrocladium* black rot of peanut may be shortened to 3 weeks when peanut plants are inoculated with 1–4 *Meloidogyne hapla* eggs compared to 4 weeks when 1–3 eggs per plant were used. An increase in incidence of a fungal disease related to an increase in nematode number which infects the plant may reflect the role of nematodes as wounding agents allowing the penetration of the fungus.

The chances of infection by the fungus increase with the increasing number of wounds (Powell 1971). The shortening of the incubation period and an increase in severity of a fungal disease when a number of nematodes infecting the plant increase may also be related to a wounding action of the nematodes. However, it is also suggested that the nematodes have to induce a certain level of physiological changes to modify the reaction of their host to the fungus. Interactions between host plant, nematodes, and fungi are specific that depends on the exact combination of these three. Sometimes, nematode species/cultivars with different biology and feeding habits may alter the capacity to predispose the same host to diseases like *Fusarium* wilt. In several instances, root-knot nematode infection on both cotton and tomato cultivars normally resistant to fungal wilt can predispose these plants to severe *Fusarium* wilt infection. The joint infection of *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *vasinfectum* can severely damage Acala cotton. Galled okra and tomato roots infected with *M. incognita* are highly susceptible to infection by *Rhizoctonia solani*, a disease complex that results in root decay caused by the fungus within about 1 month after nematode infection. Similar associations between *Verticillium* species and *Pratylenchus penetrans* have been reported on crops like brinjal, tomato, and other crops.

9.6 Nematode–Bacterial Interactions

The association between nematodes and bacteria may involve the following types: Nematodes may predispose plants to bacterial disease and may inhibit the development of bacterial disease; nematodes and bacteria together may result in a different disease than that caused by either pathogen alone and may result in production of a toxin that kills mammals. The number of complex associations between plant-parasitic nematodes and pathogenic bacteria in causing plant diseases has been demonstrated in the last seven decades. The role of nematodes in these interactions is complex, and each disease complex is distinct

Table 9.1 Major nematode–fungal interactions

Nematode	Fungus	Host
Meloidogyne incognita and *javanica*	*Rhizoctonia solani, Fusarium oxysporum* f.sp. *lycopersici*	Tomato
M. incognita	*Pythium aphanidermatum, Verticillium albo-atrum, Sclerotium rolfsii*	Tomato
M. javanica	*V. dahliae*	Tomato
Meloidogyne incognita acrita and *M. hapla*	*Fusarium oxysporum* f.sp. *lycopersici*	Tomato
Pratylenchus penetrans	*Verticillium albo-atrum*	Tomato
Trichodorus christiei	*V. albo-atrum*	Tomato
Globodera tabacum	*V. albo-atrum*	Tomato
Trichodorus capitatus	*V. albo-atrum*	Tomato
Meloidogyne javanica	*Fusarium oxysporum* f.sp. *lentis*	Lentil
Pratylenchus penetrans, P. thornei, P. neglectus	*Verticillium dahlia*	Potato
Globodera rostochiensis/G. pallida	*V. dahliae*	Potato
M. incognita	*V. dahliae*	Potato
M. hapla	*V. albo-atrum*	Potato
M. incognita	*Macrophomina phaseolina*	Brinjal
M. incognita	*Fusarium oxysporum, R. solani, R. bataticola*	Brinjal
Pratylenchus penetrans	*V. albo-atrum*	Brinjal
Globodera rostochiensis	*Rhizoctonia solani*	Potato
Globodera rostochiensis	*Verticillium dahliae*	Potato
Ditylenchus destructor	*Phytophthora infestans*	Potato
Pratylenchus neglectus	*Verticillium dahliae*	Potato
M. incognita	*R. solani*	Potato
M. incognita	*Corticium rolfsii*	*Solanum khasianum*
M. incognita	*P. aphanidermatum, R. solani*	Chilli
Meloidogyne arabicida	*Fusarium oxysporum* f.sp. *coffeae*	Coffee
Pratylenchus penetrans	*Verticillium dahliae, V. albo-atrum*	Mint
Pratylenchus minyus	*V. dahliae* f.sp. *menthae*	Peppermint
Pratylenchus neglectus	*V. dahliae*	Peppermint
Rotylenchulus reniformis	*Fusarium oxysporum* f.sp. *pisi*	Pea
M. incognita acrita	*Fusarium oxysporum* f.sp. *pisi*	Pea
Pratylenchus penetrans	*Fusarium oxysporum* f.sp. *pisi*	Pea
M. incognita	*Fusarium oxysporum* f.sp. *pisi*	Pea
Hoplolaimus uniformis	*Fusarium oxysporum* f.sp. *pisi* –race 2	Pea
Hoplolaimus uniformis	*Fusarium oxysporum* f.sp. *pisi* –race 2	Pea
Tylenchorhynchus martini	*Aphanomyces euteiches*	Pea
Rotylenchulus reniformis	*Phytophthora palmivora*	Betel vine
Radopholus similis	*Fusarium oxysporum* f.sp. *cubense*	Banana
Radopholus similis	*Rhizoctonia solani*	Banana
M. incognita acrita	*Fusarium oxysporum* f.sp. *cubense*	Banana
Meloidogyne spp.	*Rhizoctonia solani*	Banana
Meloidogyne spp.	*Fusarium* spp.	Papaya
Heterodera schachtii	*Rhizoctonia solani*	Sugar beet
Meloidogyne trifoliophila	*Drechslera halodes*	White clover
Heterodera daverti	*Fusarium avenaceum*	Clover

(continued)

Table 9.1 (continued)

Nematode	Fungus	Host
M. incognita	*F. oxysporum* f.sp. *phaseoli*	Bean
M. incognita	*R. solani*	Bean
M. javanica	*F. oxysporum* f.sp. *phaseoli*	Bean
M. incognita	*Macrophomina phaseolina*	Bean
M. javanica	*F. oxysporum*	Cowpea
M. hapla	*Pythium polymorphon*	Celery
M. hapla	*Phytophthora megasperma* f.sp. *medicaginis*	Alfalfa
Ditylenchus dipsaci	*Botrytis allii*	Onion
Pratylenchus penetrans	*Cylindrocarpon radicicola*	Tulip, narcissus
M. incognita	*F. oxysporum, F. solani*	Crossandra
Pratylenchus delattrei	*F. oxysporum, F. solani*	Crossandra
Meloidogyne spp.	*F. oxysporum*	Chrysanthemum
Belonolaimus longicaudatus, M. incognita	*P. aphanidermatum*	Chrysanthemum
Pratylenchus coffeae	*R. solani, P. aphanidermatum*	Chrysanthemum
Meloidogyne spp.	*F. oxysporum* f.sp. *dianthi*	Carnation
M. incognita	*F. oxysporum* f.sp. *dianthi*	Tube rose
Criconemella curvata, M. hapla	*F. oxysporum* f.sp. *dianthi*	Tube rose
Paratylenchus dianthus	*F. oxysporum* f.sp. *dianthi*	Tube rose
M. arenaria	*V. dahliae*	Gerbera
M. arenaria	*Phytophthora cryptogea*	Gerbera
Pratylenchus penetrans	*Verticillium* spp.	Balsam
M. incognita	*R. solani, R. bataticola*	Okra
M. javanica	*Sclerotium rolfsii*	Okra
M. incognita	*F. oxysporum* f.sp.	Ginger
M. incognita	*Pythium myriotylum, P. aphanidermatum*	
M. incognita	*S. rolfsii*	Betel vine
Meloidogyne spp.	*Phytophthora* spp.	Betel vine
Radopholus similis	*Cylindrocarpon obtussisporum*	Areca nut
M. incognita	*R. solani*	Cardamom
Helicotylenchus dihystera	*F. oxysporum* f.sp.	Guava
M. javanica	*Verticullium dahliae*	Olive
M. incognita	*F. oxysporum* f.sp. *niveum*	Watermelon
M. arenaria	*F. oxysporum*	Watermelon
M. incognita	*F. oxysporum*	Muskmelon
Pratylenchus penetrans	*Verticillium dahliae*	Strawberry
Meloidogyne spp.	*Verticillium dahliae*	Cherry
M. incognita	*F. oxysporum* f.sp. *cucumerinum*	Cucumber
M. incognita	*F. oxysporum*	Squash
M. incognita acrita	*F. oxysporum*	Cabbage
M. incognita	*F. oxysporum* f.sp. *conglutinans*	Cauliflower
Tylenchorhynchus brassicae	*R. solani*	Cauliflower
M. incognita	*F. oxysporum* f.sp. *coffeae*	Coffee
M. incognita	*M. phaseolina*	*Coleus forskohlii*

from one another and largely dependent on the type of nematode parasitism involved (Siddiqui et al. 2012). Nematodes greatly increase the development of plant disease caused by bacteria. Nematodes participate in disease complexes in different ways such as predisposing agent, increasing the susceptibility of host by modifying physiology of host tissues, breaking host resistance to bacterial pathogens, acting as vectors of bacterial pathogens, and changing the rhizosphere microflora.

Generally, in nematode–bacterium disease complexes, the nematode becomes established more readily in the presence of bacterium, but at the later stage of infection, nematode suffers some inhibition. Moreover, prior occupancy of host tissues by nematode or bacterium has different influence on the disease severity. Sometimes, the presence of both the nematode and the bacterial pathogen is necessary for the production of certain types of symptoms; for example, interaction of *Aphelenchoides fragariae*, or *A. fragariae*, and *Corynebacterium fascians* is necessary to produce "cauliflower" disease of strawberry, while neither pathogen inoculated separately reproduced the disease. The disease complexes involving plant-parasitic nematodes and pathogenic bacteria have major economic hazards. Bacteria which induce wilting in the host are actively involved in associations with nematodes. However, nematode–bacterium interactions are comparatively fewer than the nematode–fungal interactions. Nematodes may predispose plants to bacterial disease or may inhibit the development of bacterial disease. Nematodes and bacteria together may result in a different disease than that caused by either pathogen alone. Nematodes and bacteria together may result in production of a toxin that kills mammals. In general, stress predisposes trees to bacterial cancer, usually associated with younger trees and sandy soils.

The bacterial wilt of several host plants caused by *Ralstonia solanacearum* is one of the best-known diseases which have been commonly reported to be associated with nematodes. In the presence of nematodes, the incidence of this wilt will be higher. In tomato, both *M. hapla* and *Helicotylenchus nannus* contribute to an increase in wilt development, while *Rotylenchus* sp., a nonparasite on tomato, does not influence the rate or severity of wilt symptoms. *M. incognita* influences bacterial wilt development in tobacco. Plants exposed to nematodes 3 or 4 weeks before exposure to *R. solanacearum* develop more severe wilt symptoms earlier than when plants were exposed to only bacteria or to both pathogens simultaneously. Giant cells in jointly infected roots harbor bacteria-like inclusions and degenerate rapidly.

The bacterial wilt of alfalfa (*Corynebacterium insidiosum*) increases in the presence of the stem nematode, *Ditylenchus dipsaci*. The varieties which have high resistance to wilt become diseased in the presence of *D. dipsaci.* On the other hand varieties with nematode resistance remain relatively free from wilt when exposed to both pathogens. Hairy roots of roses caused by *Agrobacterium rhizogenes* are usually of minor importance but turn serious when the plants are infected with *Pratylenchus vulnus.* The crown gall of peach caused by *A. tumefaciens* is increased by high populations of *M. javanica*. At low nematode population levels, however, crown gall symptoms are no more severe than those occurring in plants inoculated with bacteria alone after wounding.

The foliage and meristem disorder in strawberry known as "cauliflower complex" involving *Aphelenchoides ritzemabosi* and *Corynebacterium fascians* is another striking example of nematode–bacterium complex (Parvatha Reddy 2008). This complex requires both the pathogens for the expression of the complete disease syndrome. Some examples of nematode bacterial associations are presented in the Table 9.2.

Based on genome-to-genome analyses of gene sequences obtained from plant-parasitic, root-knot nematodes (*Meloidogyne* spp.), it seems likely that certain genes have been derived from bacteria by horizontal gene transfer (Bird et al. 2003). Strikingly, a common theme underpinning the function of these genes is their apparent direct relationship to the nematode's parasitic lifestyle.

Table 9.2 Nematode–bacteria interactions

Crop	Nematode	Bacterium
Tomato	*M. hapla, M. incognita*	*Ralstonia solanacearum*
	Helicotylenchus nannus	*R. solanacearum*
	M. incognita	*Clavibacter michiganensis* var *michiganense*
Brinjal	*M. incognita, M. javanica*	*R. solanacearum*
Capsicum	*M. incognita*	*R. solanacearum*
Potato	*Meloidogyne incognita acrita*	*R. solanacearum*
Potato	*Globodera rostochiensis, G. pallida*	*R. solanacearum*
Beans	*M. incognita*	*Curtobacterium (Corynebacterium) flaccumfaciens*
French bean	*M. incognita*	*Rhizobium* spp.
Cowpea	*Heterodera cajani*	*Rhizobium* spp.
Garlic	*Ditylenchus dipsaci*	*Pseudomonas fluorescens*
Ginger	*M. incognita*	*R. solanacearum*
Rhubarb	*D. destructor*	*Clavibacter michiganensis* subsp. *insidiosum*
Lucerne	*Ditylenchus dipsaci*	*C. insidiosum*
Raspberry	*M. hapla*	*Agrobacterium tumefaciens*
Strawberry	*Aphelenchoides ritzemabosi*	*C. fascians*
Peach	*M. javanica*	*A. tumefaciens*
Peach and plum	*Criconemella xenoplax*	*P. syringae*
Begonia	*A. fragariae*	*Xanthomonas begonia*
Carnation	*Meloidogyne* spp. and *H. dihystera*	*P. caryophylli*
Carnation	*H. nannus*	*P. caryophylli*
Carnation	*Ditylenchus* spp.	*P. caryophylli*
Rose	*Pratylenchus vulnus*	*A. rhizogenes*
Gladiolus	*M. javanica*	*P. marginata*
Stone fruits and nut trees	*Criconemella*	*P. syringae*
Grapevine	*Rotylenchulus reniformis*	*A. tumefaciens*

Phylogenetic analyses implicate rhizobacteria as the predominant group of “gene donor” bacteria. Root-knot nematodes and rhizobia occupy similar niches in the soil and in roots, and thus the opportunity for genetic exchange may be omnipresent. Further, both organisms establish intimate developmental interactions with host plants, and mounting evidence suggests that the mechanisms for these interactions are shared too. It has been proposed that the origin of parasitism in *Meloidogyne* may have been facilitated by acquisition of genetic material from soil bacteria through horizontal transfer and that such events represented key steps in speciation of plant-parasitic nematodes.

9.7 Nematode–Virus Interaction

Virus–nematode vector relationships are a significant aspect of plant pathology that has raised curiosity in possible relationships between nematodes and viruses. These pathogens may interact in complexes that are manifested in other ways. There are 22 longidorid and 14 trichodorid nematodes that have been reported as vectors of plant viruses. Among Longidoridae, 11 species belong to the genus *Xiphinema*, ten to the genus *Longidorus,* and one to *Paralongidorus*. Of the Trichodoridae, five species belong to the genus *Trichodorus* and nine to *Paratrichodorus* (Table 9.3).

Table 9.3 Nematode vectors of viruses

Viruses	Nematodes
NEPO viruses	
Arabic mosaic	*Xiphinema diversicaudatum, X. bakeri*
Grapevine fanleaf, grapevine chrome mosaic, arabis mosaic	*X. index*
Grapevine fanleaf	*X. italiae*
Tomato ring spot	*X. rivesi*
Cherry leaf roll	*X. vuittenezi*
Strawberry latent ring spot, brome mosaic, arabis mosaic, cherry leaf roll, raspberry ring spot	*X. diversicaudatum*
Tobacco ring spot, tomato ring spot, peach rosette mosaic, cherry rasp leaf, peach yellow bud mosaic	*X. americanum*
Cowpea mosaic	*X. basiri*
Brome mosaic, arabis mosaic, cherry leaf roll, tobacco ring spot	*X. coxi*
Cowpea mosaic	*X. ifacolum*
Tomato ring spot, grapevine yellow vein strain	*X. californicum*
Arabis mosaic, raspberry ring spot, strawberry latent ring spot	*Paralongidorus maximus*
Tomato black ring, beet ring spot, artichoke Italian latent, raspberry ring spot (Scottish strain), peach rosette mosaic	*Longidorus elongatus*
Artichoke Italian latent	*L. apulus*
Artichoke Italian latent (Greek strain)	*L. fasciatus*
Raspberry ring spot, cherry rasp leaf	*L. leptocephalus*
Raspberry ring spot, cherry rasp leaf, brome mosaic, carnation ring spot, prunus necrotic ring spot	*L. macrosoma*
Arabis mosaic, raspberry ring spot	*L. caespiticola*
Mulberry ring spot	*L. martini*
Peach rosette mosaic	*L. diadecturus*
Tomato black ring, lettuce ring spot	*L. attenuatus*
Raspberry ring spot	*L. profundorum*
Arabis mosaic, raspberry ring spot, strawberry latent ring spot, cherry leaf roll	*L. maximus*

(continued)

Table 9.3 (continued)

Viruses	Nematodes
NETU viruses	
Tobacco rattle virus	*Paratrichodorus pachydermus*
	P. allius, P. nanus
	P. porosus, P. tunisiensis
	Trichodorus christiei, T. hooperi
	T. cylindiricus, T. hooperi, T. viruliferus
	T. similism, T. minor, T. primitivus, T. similis
Pea early browning	*P. anemones, P. pachydermus*
	P. teres, T. viruliferus, T. primitivus

Nematode–virus complexes have been identified by Hewitt, Raski, and Goheen (1958) who observed that *Xiphinema index* was the vector of grapevine fanleaf virus. *Xiphinema* spp., *Longidorus* spp., and *Paralongidorus* spp. transmit the ring spot viruses which are called NEPO virus derived from nematode-transmitting polyhedral-shaped particles which have isometric particles ca. 30 nm diameter (Table 9.3). *Trichodorus* spp. and *Paratrichodorus* spp. transmit the rattle viruses and called NETU derived from nematode-transmitted tubular-shaped particles which have tubular particles of predominantly two lengths, 190 nm and 45–115 nm, depending on the isolate (Parvatha Reddy 2008). All these nematodes have modified bottle-shaped esophagus with glands connected by short ducts directly to the lumen of the esophagus. This actually helps in the transmission of viruses which is different in other genera of nematodes (Lamberti and Roca 1987).

It has been observed that soybeans infected by both root-knot nematodes and tobacco ring spot virus, a strain of which causes a bud blight diseases in tobacco, suffer from extensive galling and greatly reduced root systems. Plant roots infected by the nematodes alone are galled but not reduced in size, and the virus alone has no obvious effects on the roots. Histopathological variations occur in infected plants. These phenomena occur in this complex although infection

by each pathogen takes place singly without a vector relationship. More nematode larvae enter the roots of tobacco ring spot-infected bean plants than virus-free plants, but there is no difference in the rate of nematode development, nor is there evidence of synergism between the two pathogens on root development. Root-knot nematodes grow more rapidly in roots of tomato plants infected with tobacco mosaic virus than in virus-free roots. In the latter case, the virus has no observable effect on the number of nematodes entering the root. No vector relationship has been reported in either instance.

Tobacco plants infected with tobacco mosaic virus revealed decreased populations of both *A. ritzemabosi* and *D. dipsaci*, and high growth retardation of plant under combined attack was observed. This clearly indicates that the damage by a combination of the virus and nematode pathogens is greater than would be evident when either pathogen is present alone. Even in the absence of a vector relationship, interactions between nematode and virus seem to occur. It is inconceivable that pathogens such as these would not have noticeable effects on host physiology. Accordingly, both pathogens, being obligate parasites, must be influenced by any physiological change in the host.

The ability of different nematode species of the same or different genera to transmit the same virus or, on the other hand, the capacity of two different viruses to be transmitted by the same nematode species indicates that the vector specificity is less developed in trichodorids than in longidorids. The factors affecting the efficiency of trichodorids indicate that 15 °C is the optimum temperature for the transmission of tobacco ring spot virus (TRV) and that below 10 % soil moisture content, virus transmission is unlikely. Trichodorid nematodes can retain the virus as long as 10 months and exceptionally even a year, and a single nematode has been shown to transmit TRV to several plants when allowed to feed for 1 day on the roots of each plant separately. The distribution of these nematodes in the soil is very erratic and affected by several physical and chemical components. But even low numbers of trichodorids can efficiently transmit viruses. Establishment and/or reproduction of trichodorids in the soil is more difficult than for many longidorid nematodes, but the virus vector–plant relationship is more stable among trichodorids than among longidorids.

There are often changes in root-knot nematode reproduction rates when plants are jointly infected with a virus and nematode. These changes are usually reflected as increases in the number of nematode eggs in roots of plants inoculated with both pathogens in combination. Roberts and McKenry (1985) observed that all stages of *X. index* could transmit fanleaf virus and the nematode was able to acquire the virus within 24 h of exposure. *Xiphinema index* was found to retain the virus for 12 weeks when allowed to feed on virus-immune fig roots. Nematode-transmitted viruses do not persist through a nematode molt and do not pass through nematode eggs. Nematode vectoring of fanleaf virus is important in spreading virus infection from vine to vine within an infested vineyard; however, a wide-scale transmission of virus disease is generally a result of movement of infected propagative stock. The limited distribution of the fanleaf virus remains a tribute to the diligence of quarantine officials and industry. *Xiphinema americanum* is a vector of peach yellow bud mosaic virus and grape yellow vein virus. Stubby-root nematode species, such as *P. minor, P. allius,* and *P. porosus*, are known vectors of tobacco rattle virus.

An ability by viruses and nematodes, which are relatively immobile, to utilize an extensive range of potential hosts provides a distinct advantage for survival. Nepo- and tobraviruses are transmitted through seed and in some instances through pollen, but the existence of a vector provides the only pathway for a virus to be exposed to potential new host species (Brown and Trudgill 1989). The nematode-transmitted viruses and their vector species are natural pathogens and parasites, respectively, of wild (uncultivated) plants and usually have extensive host ranges. While the presence of a vector is advantageous for a virus, there is no information

available to suggest that a virus, when present in a host, provides any advantage for its vector.

9.7.1 The Transmission Process

Transmission of a virus by a vector nematode involves several discrete but interrelated processes. However, these processes are poorly defined, and the terms used to describe them are frequently used interchangeably and thus may be misleading (Brown and Weisher 1998).

9.7.1.1 Ingestion

Any plant-parasitic nematode feeding on a virus-infected plant has the potential to ingest virus particles. Ingested virus particles may be adsorbed, but most are likely to enter directly into the nematode gut where they can remain infective. Virus ingestion simply may be defined as "the intake of virus particles during feeding." Infective tobra- and nepoviruses have been recovered from comminuted bodies of their associated vector nematodes, for example, TRV from *Trichodorus* sp., *Paratrichodorus pachydermus*, and *P. allius*. Arabis mosaic and strawberry latent ring spot nepoviruses were not recovered from *X. diversicaudatum* but were recovered from *Longidorus elongatus,* although this species is not a vector of either of these viruses.

9.7.1.2 Acquisition

A plant-parasitic nematode feeding on a virus-infected plant will passively ingest virus particles which pass through the esophagus into the intestine where they may be digested or simply defecated and thereby lost for transmission. With virus vector species, a proportion of ingested virus particles are retained in the feeding apparatus, and thus there is a potential for them to be transmitted. Virus acquisition may be defined as "the act of ingesting virus particles, some or all of which are retained in the feeding apparatus." Unattached virus particles have been observed in the stoma of some species of *Longidorus* and *Xiphinema*, and it was suggested that nonspecific transmission of these particles might occasionally occur, especially if the nematodes were transferred quickly between virus-infected and healthy plants.

9.7.1.3 Adsorption

Virus particles may be actively or passively retained during acquisition in the feeding apparatus of the nematode. Virus adsorption may be defined as "the active process by which virus particles adhere to specific sites of retention in the nematode feeding apparatus." In vector nematodes, particles of the associated viruses are specifically adsorbed to discrete parts of the esophagus. In *Longidorus* and *Paralongidorus*, they associate with the inner surface of the esophageal guiding sheath and the interior surface of the odontostyle.

9.7.1.4 Retention/Persistence

After acquisition and adsorption by the nematode, virus particles are retained in the vector. Retention may be defined as "the period during which specifically adsorbed virus particles remain attached to the site of retention in the nematode feeding apparatus." Although little information is available of the retention period for actively feeding nematodes, it probably is shorter than with specimens stored in plant-free soil, where vector nematodes can apparently retain virus for weeks, months, and even years. In contrast, a diminution of retained virus particles can occur at each occasion a nematode feeds. However, during a prolonged feeding cycle on an individual root, the nematode will initially only transfer virus, but subsequently, after virus establishment and replication in the host, the nematode will both ingest and transfer virus particles while feeding. Substantial differences have been reported between retention times for the vector genera *Longidorus, Paratrichodorus*, and *Xiphinema* when the nematodes have been denied access to a host plant.

9.7.1.5 Release

For transmission to occur, virus particles retained by a vector must dissociate from the specific site of retention in the nematode feeding apparatus. Release may be defined as "the dissociation of virus particles from the specific sites of retention in

the nematode feeding apparatus." The dissociation of virus particles from their sites of retention within the vector nematode is believed to occur during feeding when secretions from the esophageal glands in the basal bulb of the nematode pass anteriorly through the esophagus and feeding apparatus into the plant cell. It has been speculated that the gland secretions modify the pH within the lumen and alter the surface charge of the virus particles or that the dissociation may be mediated by an enzymic effect of the gland secretions on the bonding of the virus particles to the cuticular surface of the nematode (Taylor and Brown 1997).

9.7.1.6 Transfer

Transfer refers to the active relocation of virus particles from point "A" to point "B." Such a simplistic definition is not appropriate in relation to the transmission process. When referring to nematode transmission of viruses, dissociated virus particles released from the specific sites of retention have to move forward through the nematode's feeding apparatus to the external environment and then must enter a living plant cell for transmission to be concluded. Transfer may be defined as "the placement of virus particles in a live plant cell."

9.7.1.7 Establishment

It refers to the transfer of virus particles into a live plant cell. It can result in the non-propagation of the virus or successful disassembly, reassembly, and replication of the virus. Thereafter, the virus may colonize new cells, thus successfully infecting the plant. Establishment is defined as "the successful colonization (infection) of the plant by a virus following transfer (transmitted virus)."

9.7.2 The Nematode–Virus–Plant Interaction

Virus transmission involves acquisition of the virus by the nematode ingesting it from plant cells, thereafter followed by adsorption, retention, release, transfer, and finally establishment of the virus in a new plant host. Interruption to any of these processes will prevent transmission. The ingestion of virus involves an interaction between the nematode, virus, and plant; acquisition, adsorption, retention, and release probably only involve an interaction between the nematode and virus; transfer involves a nematode–virus–plant interaction; and establishment involves a virus–plant interaction. Various experiments, involving specialized techniques, rely on the recovery of virus from bait plants as the principal evidence of transmission having occurred. These experiments are used to describe "frequency" or "efficiency" or "rates of transmission" by the vector nematode. However, these terms are frequently used interchangeably and without clear definition.

9.7.2.1 Frequency

Frequency is defined as "the number of bait plants from which virus is recovered as a proportion of the total number of bait plants." Laboratory experiments used to investigate transmission of viruses by nematodes involve allowing nematodes access to virus-infected plants, subsequently recovering the nematodes, and then giving groups of variable numbers of nematodes access to bait plants. The results of these experiments are primarily expressed as the proportion of bait plants from which virus is recovered.

9.7.2.2 Efficiency

Efficiency is defined as the "relation between number of nematodes given access to a bait plant and the number of bait plants from which virus is recovered." Transmission efficiency can be described in terms of numbers of feeds on virus source plants, number of feeds on bait plants, and duration of individual feeds on virus source and/or bait plants. However, the final results are invariably based on the number of nematodes given access to bait plants and the proportion of bait plants from which virus is recovered. Application of the maximum likelihood equation provides an estimate of the probability that a single nematode transmits virus. The probability can be calculated in experiments in which groups of nematodes are added to pots containing bait plants. This probability can be referred to as the

efficiency of transmission of the nematode population or species.

9.7.2.3 Rate of Transmission

Rate of acquisition is defined as "the proportion of individual nematodes estimated to have acquired virus during a given period of access to a virus source plant." The number of bait plants infected with virus or the proportion of individual nematodes calculated to have transmitted virus have each been used to describe the rate of transmission by a vector. However, a rate involves both time and quantity, and this term should only be used when referring to aspects of the transmission process involving both these elements. Rate of retention is defined as "the proportion of individual nematodes estimated to have retained virus particles in a given period of time from when the nematodes are removed from the virus source plant to the time they are given access to a bait plant."

Rate of transfer refers to "the proportion of individual nematodes estimated to have transferred virus during a given period of access to a bait plant." Rate of transmission is "the proportion of individual nematodes estimated to have acquired, retained, and transferred virus during a given period from when the nematodes were given access to a virus source plant to their removal from the bait plant."

The distinction between efficiency and rate of transmission is that the former is the proportion of individual nematodes calculated to have transmitted virus, whereas the latter is the proportion of individual nematodes calculated to have acquired, retained, and transmitted virus in a given period of time.

9.7.2.4 Effectiveness

Effectiveness refers to the ability of a vector to successfully provide a pathway for its associated virus to infect new host species. For example, *Xiphinema diversicaudatum,* which has an extensive natural host range, transmits ArMV and SLRSV to a wide range of cultivated and uncultivated plant species, whereas *X. index*, which has a very restricted host range, transmits GFLV only to grapevine. Thus, *X. diversicaudatum* is regarded as a very effective vector species as it is capable of exposing its associated viruses to a wide range of potential hosts for the viruses. Conversely, *X. index* may be regarded as being an ineffective vector as it does not expose its associated virus to many potential hosts.

9.7.3 Specificity of Transmission

Specificity of transmission refers to "the specific relationship between a plant virus and its vector nematode, likely a recognition event between the virus and the site of retention in the vector."

9.7.3.1 The Nematodes

Harrison et al. (1961) observed that the degree of similarity between different nematode-transmitted viruses resembled the degree of systematic relationship between their vectors and concluded by stating that "this apparent specificity still needs confirmation by experiment." This initial observation was expanded later that different viruses have different vectors; it was also apparent that serologically distinct strains of the same virus are transmitted by different but closely related species of the same genus. The positively charged virus particles ingested by *Longidorus* are attracted to the negatively charged surface of the odontostyle. Different strains of one virus could then have different surface charge densities, thus requiring different vector species. Correspondingly, two different viruses transmitted by the same vector would then have similar charge densities, e.g., RRSV and TBRV, both transmitted by *L. elongatus.*

9.7.3.2 The Viruses

The nematode-transmitted plant viruses, both tobra- and nepoviruses, have bipartite genomes with the RNA-2 segment containing the genetic determinants conferring vector transmissibility. The RNA-2 of RRSV contains the determinants for transmissibility by *L. elongatus.* The transmissibility of TBRV by *L. elongatus* was determined by the RNA-2 segment of the virus. Differences in polypeptide sequences adjacent to the *N*-terminal of the coat proteins could also account for some

aspects of specific association between nepoviruses and their longidorid vectors. In tobraviruses “fingerlike” structures at the end of the coat protein subunits of particles of tobacco rattle tobravirus could be involved in the specific association with the vector (Legorboru 1993).

9.7.3.3 Vector and Virus Exclusivity and Complementarity

“Vector and virus exclusivity refers to the case where a nematode species transmits one virus or one serologically distinct virus strain and the virus/virus strain has only a single vector.” Exclusivity and complementarity are reflections of the specificity of the association between vectors and viruses. On the other hand, vector and virus complementarity may be defined as the case where a nematode species transmits two or more viruses or serologically distinct strains of a virus and where two or more viruses/virus strains share the same vector species.

9.7.4 Factors Affecting Specificity, Exclusivity, and Complementarity in Vector Transmission

Successful transmission of a virus by a nematode involves numerous complex and subtle interactions between the nematode, virus, plant host, and the environment. Nepo- and cobra viruses have developed highly specific relationships with the nematode species which function as their vectors. Nematode species occur as localized populations, usually in restricted geographical areas. Their limited mobility imposes prolonged and continuous association with their host plants, which provides the opportunity for developing specific associations with plant pathogens such as viruses.

Also, this is a dynamic process as under these conditions, their isolated occurrence will result in a reduced gene flow so that homozygosity increases which can result in speciation. Similarly, the nematode-transmitted viruses have restricted geographical distributions, usually reflecting that of their vector, and they too can mutate to form new virus strains or viruses. Thus, specific associations between nematodes and viruses are constantly evolving possibly resulting in some viruses losing their vector transmissibility and some vectors losing their ability to transmit viruses, while concurrently new virus and vector associations are becoming established.

Several pathogenic viruses from plants rely on nematode vectors for their transmission from host to host. In plants, *grapevine fanleaf virus* (GFLV), a major pathogen of grapes worldwide and its specific vector, the dagger nematode *Xiphinema index*, provides a well-established model illustrating this specificity (Schellenberger et al. 2011). The high-resolution structures of two GFLV isolates were determined that differ in their transmissibility and showed that this difference is due to a single mutation in a region exposed at the outer surface of the viral particles. This mutation does not alter the conformation of the particles but modifies the distribution of charges within a positively charged pocket at the outer surface of virions which likely affects particle retention by *X. index* and thereby also transmission efficiency. This pocket was involved in the specific recognition of GFLV by its nematode vector, and this work paved the way toward the characterization of the specific compound(s) within the nematodes that triggers vector specificity and provides novel perspectives to interfere with virus transmission.

The vector specificity, in general, is less developed in trichodorids than in longidorids since various nematode species of the same or different genera can transmit the same virus or two different viruses can be transmitted by the same nematode species. With regard to various factors which influence the vector efficiency, temperature and moisture seem to play a major role, for example, 15 °C is optimum temperature for transmission of tobacco rattle virus (Hoof 1975), whereas transmission may not occur when soil moisture is less than 10 %. Trichodorids can retain viruses as long as 10 months. However, the distribution of trichodorids in soil is erratic and influenced by various physical and chemical components, but this group is more efficient in virus transmission even when present in low numbers.

9.8 Nematode–Rhizobia Interaction

Plant root exudates play a vital role both in the survival and colonization of rhizobia on the rhizoplane. It is a known fact that phytonematodes alter the root exudates of host plants both in terms of quantity and quality. Consequently, nematodes and rhizobia exhibit a sort of association, and it has been reported that nematode infection affects the establishment of rhizobia on or in the host roots. Biotic stresses of various kinds including phytonematodes on either host tissues or the nodule bacteria would interrupt the nodulation process (Tables 9.5 and 9.6).

Phytonematodes may affect the establishment of rhizobia in rhizosphere and rhizoplane, rhizobial penetration into roots of legume hosts, nodule formation, and nitrogen fixation (Huang 1987). *Rhizobium* and *Bradyrhizobium* are the most common genera of rhizobial bacteria. The former is a fast growing one, while the latter is slow growing type.

9.8.1 Nematodes Versus Rhizobial Population and Infection

Root exudates influence the rhizosphere survival and also its successful colonization on the rhizoplane, which are influenced by phytonematodes and their activity in root zone (Ingham and Coleman 1983). Infection by nematodes exerts effect on the establishment of rhizobia on or around the roots (Tables 9.4 and 9.5). In several instances, nematodes may not possess any effect on the population of certain species of *Bradyrhizobium*, viz., *B. japonicum* in the rhizoplane of soybean with the presence of *Heterodera glycines*. Bohlool and Schmidt (1974) observed the major role played by lectin in binding *Bradyrhizobium* to soybean root surface. Phytonematodes like *H. glycines* may alter the binding sites on the root surface, thus restricting the penetration and establishment of bacteria.

Symbiosis between legume species and rhizobia results in the sequestration of atmospheric nitrogen into ammonium, and the early mechanisms involved in this symbiosis have become a model for plant–microbe interactions and thus highly amenable for agricultural/horticultural applications (Horiuchi et al. 2005). The working model for this interaction states that the symbiosis is the outcome of a chemical/molecular dialogue initiated by flavonoids produced by the roots of legumes and released into the soil as exudates, which specifically induce the synthesis of nodulation factors in rhizobia that initiate the nodulation process. The authors opine that other organisms, such as the soil nematode, *Caenorhabditis elegans,* also mediate the interaction between roots and rhizobia in a positive way, leading to nodulation. We report that *C. elegans* transfers the rhizobium species *Sinorhizobium meliloti* to the roots of the legume *Medicago truncatula* in response to the plant-released volatiles that attract nematodes.

Many nematodes are known to interfere with the symbiotic association between rhizobia and legumes and mycorrhizal associations (Williamson and Gleason 2003). However, some plant-pathogenic nematodes like *Meloidogyne* spp. can have positive effects on the legume–rhizobial association by enhancing the number of nodules and the amount of nitrogen fixed by unknown mechanism. Besides the plant-parasitic forms, there are a large group of saprophytic nematodes found in the soil. Laboratory experiments and field studies have demonstrated that saprophytic nematodes play a critical role in influencing the turnover of the soil microbial biomass and thus enhance the availability of plant nutrients (Yeates 2003).

In the rhizosphere, the interaction between the plant and other organisms including microorganisms and soil invertebrates like nematodes is mostly mediated by chemicals present in the root exudates (Horiuchi et al. 2005). Several aboveground interactions between plants and other organisms are mediated by volatile organic compounds that are released by the plant in response to biotic and abiotic cues; these cues either attract or repel other organisms. El-Bahrawy and Salem (1989) observed that the population of *Meloidogyne javanica* was high in a nematode + *Rhizobium*

Table 9.4 Effect of nematodes on nodulation and nitrogen fixation in *Rhizobium*–legume interactions

Leguminous host	*Rhizobium* species	Nematode species	Effect	
			Nodulation	N_2 fixation
White clover (*Trifolium* spp.)	*Rhizobium trifolii*	*M. javanica*	–	–
		M. hapla	–	–
		Heterodera trifolii	–	–
		M. incognita	–	–
Hairy vetch (*Vicia villosa*)	*R. leguminosarum*	*M. hapla*	–	–
		M. javanica	–	
		Trichodorus christiei	–	
		Criconemoides curvatum	–	
Alfalfa (*Medicago sativa*)	*R. meliloti*	*M. javanica*	–	
Cowpea (*Vigna sinensis*)	*R. leguminosarum*	*M. incognita*	–	–
		M. javanica	=	
		R. reniformis	–	
		Heterodera cajani	–	–
		M. javanica	–	–
Broad bean (*Vicia faba*)	*Rhizobium* sp.	*M. incognita*	–	–
Lupine (*Lupinus termis*)	*R. lupini*	*M. incognita*	–	–
Pea (*Pisum sativum*)	*R. leguminosarum*	*M. incognita*	–	–
		P. penetrans	–	
		B. longicaudatus	–	
		Acrobeloides butschlii	=	–

'Equal mark' referes to **no effect** while 'En-dash' refers to **suppression**

Table 9.5 Interaction of plant-parasitic nematodes with *Rhizobium*

Leguminous host	*Rhizobium* species	Nematode species
White clover (*Trifolium* spp.)	*Rhizobium trifolii*	*Meloidogyne javanica, M. hapla, H. trifolii*
		M. incognita
Hairy vetch (*Vicia villosa*)	*R. leguminosarum*	*M. hapla, M. javanica, Trichodorus christiei, Criconemoides curvatum*
Alfalfa (*Medicago sativa*)	*R. meliloti*	*M. javanica*
Cowpea (*Vigna sinensis*)	*R. leguminosarum*	*M. incognita, M. javanica*
		Rotylenchulus renifirmi, H. cajani
Broad bean (*Vicia faba*)	*Rhizobium* sp.	*M. incognita*
Lupine (Lupinus termis*)*	*R. lupini*	*M. incognita*
Pea (*Pisum sativum*)	*R. leguminosarum*	*M. incognita*
		Pratylenchulus penetrans
		B. longicaudatus
		Acrobeloides butschlii

treatment. Nematode egg masses were noticed only in the same treatment. While no nematode infection was observed by nematode + Vydate 10 G treatment, this infection occurred with nematode treatment alone. In addition, the nematode population was highly decreased at nematode + *Rhizobium* + Vydate 10 G treatment. Whereas the average numbers of *Rhizobium* nodules were highly noticed by *Rhizobium* alone and *Rhizobium* + Vydate 10 G treatment, the lowest ones were detected with nematode + *Rhizobium* treatment. Intermediate status between the two mentioned levels was observed in a *Rhizobium* + nematode + Vydate 10 G treatment.

The most common phytonematode *Meloidogyne* has been observed to share an ecological niche with rhizobia in the soil (Bird and Koltai 2000). Symbiotic and parasitic interactions between species and especially their molecular cross talk are fascinating features in nature. It is even more intriguing that organisms such as rhizobia and nematodes can take control of their interacting partners and are able to induce new structures in their hosts' root systems. Sedentary endoparasitic nematodes induce highly specialized feeding sites in infected plant roots from which they withdraw nutrients. In order to establish these new root structures, these organisms utilize and manipulate the endogenous molecular and physiological pathways of their hosts (Grunewald et al. 2009). The involvement of the plant hormone auxin is well established. Plant-associated microbes can actively modify auxin transport in their hosts.

9.9 Nematode–Mycorrhiza Interactions

The beneficial role of fungal mycelia colonizing plant roots was brought out for the first time in 1842 by Vittadini, and the meaning for mycorrhiza as "fungus root" was given by Frank in 1885. The mycorrhizal fungus derives its carbohydrate requirements from the plant roots and in turn helps to draw more water and nutrients from the soil by reducing the pathogenic activity. The endomycorrhizal fungi form a symbiotic relationship with their hosts by colonizing the cortical region of feeder roots both inter- and intracellularly. A good number of literature is available on the management of phytonematodes due to the successful colonization by chlamydospores of *Glomus fasciculatum* and *G. mosseae* in nematode-infested plant roots (Fig. 9.1).

These mycorrhizae produce specialized structures vesicles and arbuscules within the root cortical cells and thus were earlier referred to as "vesicular–arbuscular mycorrhiza (VAM)"; the presence of the vesicles and arbuscules is the diagnostic feature for identifying the VAM fungus. In recent years, some mycorrhizae have been reported to lack vesicles. Hence, the term AMF (arbuscular mycorrhizal fungi) is conveniently being used (Ravichandra 2010).

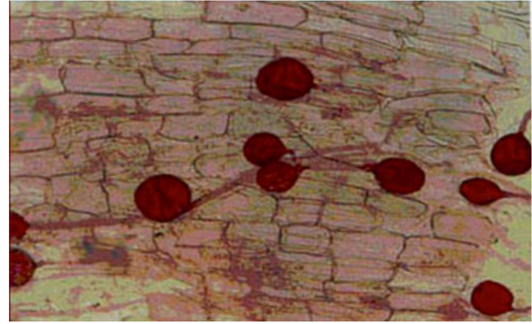

Fig 9.1 *Glomus fasciculatum* colonization in banana roots

Vesicles are usually oval or irregularly lobed. They are formed between the cortical cells or occasionally inside them. They contain oil, sometimes as a large single globule as in *Glomus*, and are believed to function as storage organs. In older roots, they develop a thick wall and presumably function as resting spores when the root decays. Arbuscules are believed to function in bidirectional transfer of nutrients; essentially this transfer involves carbohydrates from plant to fungus and minerals especially phosphate from fungus to plant. Arbuscules literally means "little trees." The arbuscules last for 2–3 weeks and then develop a granular appearance as they degenerate. A new arbuscule can replace the degenerated one in the same cell. Young infected roots often seem to be almost full of arbuscules. These arbuscules are borne on side branches of the distributive hyphae.

9.9.1 Beneficial Effects of AMF

- AMF offset the yield loss normally caused by nematodes by enhancing the uptake of phosphorus and other nutrients, thereby improving plant vigor and growth (Hamel and Strulla 2006).
- Physiologically alter or reduce the root exudates responsible for stimulating hatching of eggs or the chemotactic attraction of nematodes to roots.

Table 9.6 Interactions of major phytonematodes with AM fungi in horticultural crops

Host	Fungus	Nematode
Alfalfa	*Glomus fasciculatum, G. mossae, G. tenue*, and *Gigaspora margarita*	*Meloidogyne hapla*
Banana	*G. fasciculatum, G. mosseae*	*Radopholus similis*
Bean	*Glomus etunicatum*	*M. javanica*
Brinjal	*G. fasciculatum*	*M. incognita*
Broad bean	*G. fasciculatum*	*M. incognita*
Clover	*Glomus fasciculatum, G. mossae, G. tenue*, and *Gigaspora margarita*	*M. hapla*
Cowpea	*G. fasciculatum, G. versiforme, G. etunicatum*	*M. incognita*
Onion	*Glomus fasciculatum*	*M. hapla*
Potato	*Glomus etunicatum*	*Globodera rostochiensis*
Tamarillo	*Glomus fasciculatum, G. mossae, G. tenue*, and *Gigaspora margarita*	*M. incognita*
Tomato	*Glomus margarita*	*M. incognita*
	Glomus mosseae	*R. reniformis*
	Glomus fasciculatum	
	Glomus fasciculatum, G. mossae, G. tenue, and *Gigaspora margarita*	*M. hapla*

- May parasitize female nematodes and their eggs (Kantharaju et al. 2005).
- Retard nematode development or reproduction within or near root tissue either by production of nematostatic compound or by competition for space or host photosynthates.

AM fungi and plant-parasitic nematodes occur together in the roots or rhizosphere of the same plant each having a characteristic but opposite effect on plant vigor. AM fungi increase the supply of nutrients particularly phosphorus (P) to crop by better exploration of the soil beyond depletion zone and by receiving the fixed carbon from the plants (Table 9.6). Thus, they act as obligate symbionts, whereas nematodes as obligate parasite take out vital nutrients from the roots making reduced plant growth. But the plants that are heavily colonized with mycorrhizal fungi are able to grow well in spite of the presence of damaging levels of plant-parasitic nematodes.

AM fungi have been shown to affect the root growth, root exudation, nutrient absorption, and host physiological response to environmental stress. However, AM fungi have not been shown to interact directly with pathogens through antagonism, antibiosis, or predation. More likely they indirectly affect the host–pathogen relationship by physiologically altering the host. In addition, mycorrhizae have been shown to enhance water transport to plants and reduce the vulnerability to disease caused by root-infecting fungi. AM fungi were shown to promote plant tolerance to parasitic nematodes, viz., *Meloidogyne incognita* in tobacco (Subhashini and Ramakrishnan 2013, who observed an improved resistance in tobacco to this nematode). Histochemical analysis of banana roots inoculated with mycorrhiza revealed increased accumulation of total phosphorus, total amino acids, total phenols, more insoluble polysaccharides, and total proteins and nucleic acids in mycorrhizal plants than the non-mycorrhizal plants. Histopathological studies of tomato plants inoculated with indigenous isolate showed increase in size of epidermal cells and compactness in cortex. However, the number and size of giant cells were reduced compared to plants inoculated with nematode alone.

The root exudate experiments and *in vitro* chemotaxis bioassay point toward a reduced attraction of the burrowing nematode to the mycorrhizal banana plant roots; Christine Vos et al. (2012) opined that a water-soluble compound in mycorrhizal root exudates is at least partially responsible for the mycorrhiza-induced resistance at the pre-infectional level of *Radopholus similis* infection. Jalaluddin et al. (2008) reported the interaction among these biological systems, which can be exploited in the management of these pests. When there is no change or none of the components are affected, the interaction is categorized as neutral, and interaction is rated positive when there is significant reduction in nematode population density.

The effect of AM fungus *G. fasciculatum* on the nutrient uptake has been well documented on tomato (Shreenivasa et al. 2007). Inoculation with *Glomus intraradices* significantly increased growth of citrus plants in low P soil and was more

effective than P fertilization at increasing top-plant development. In the presence of the *Pratylenchus vulnus*, mycorrhizal plants achieved higher values in all growth parameters measured. *P. vulnus* caused a significant decrease in the percentage of root length colonized by *G. intraradices*, and fewer internal vesicles were formed within the host roots. Enhanced root mass production accounted for the twofold increase in final nematode population recovered from plants with combined inoculations of pathogen and symbiont. Low levels of Al, Fe, Mn, and Zn were found in non-mycorrhizal nematode-infected plants in low P soil. *G. intraradices*-inoculated plants reached the highest foliar levels of N, Ca, Mg, Mn, Cu, and Zn. Mycorrhizal plants infected with *P. vulnus* maintained normal to high levels of Mn, Cu, and Zn. Inoculation with mycorrhiza favors plant growth and confers protection against *P. vulnus* by improving plant nutrition. Heald et al. (1989) observed various physiological variations in the host plant due to *G. intraradices*, viz., amino acid, sugar, lignin, phenol synthesis, and ethylene production.

The increased tolerance in the host plant due to mycorrhiza was attributed to the changes in the root physiology that reduced the nematode penetration and/or inhibited the adult nematode development (Grandison and Cooper 1986). Banana plants which received *Glomus fasciculatum* 7 days prior to burrowing nematode (*Radopholus similis)* inoculation were able to offset the effect caused by nematode and increased root length and fresh and dry weights of roots. Although root colonization by mycorrhiza was reduced in plants inoculated with nematodes, the nematode number in both roots and soil was significantly lower. The mycorrhizal inoculation of banana roots infected with *R. similis* increased the insoluble polysaccharides, proteins, nucleic acids, amino acids, phosphorus, and phenols which impart resistance to the nematode infection. Tomato roots inoculated with indigenous isolates of AM fungi reduced giant cell formation and decreased the accumulation of insoluble polysaccharides, nucleic acids, and proteins which might have hindered the feeding and development of the root-knot nematode.

9.9.2 Mechanisms Adopted During Interaction

9.9.2.1 Increased Root Growth and Function

AM fungi through an increase in phosphorus nutrition enhance root growth and expand the absorptive capacity of the root system for nutrients and water (Wallace 1983). These mycorrhiza-induced compensatory process may explain the increased tolerance of mycorrhizal and P-fertilized plants. Increased root growth and nutrient uptake may lead to increased tolerance in mycorrhiza plants grown in P-deficit soils.

9.9.2.2 Nutritional Effects Other than P

In addition to P, AM fungi have been shown to enhance the uptake of K, Ca, Cu, Mn, and Zn and increase the sugar, amino acids phenylalanine and serine, and chlorophyll synthesis and water uptake. It has been observed that plants damaged by phytonematodes exhibit impaired water conductance through roots and deficiencies of N, Fe, Mg, B, and Zn (Good 1968).

9.9.2.3 Effects of Moisture

Lower moisture levels in the range of 40–70 % were found suitable for AM fungal spore production and mycorrhizal colonization and also reduced root-knot nematode, *M. incognita* population and *H. cajani* cyst population in cowpea.

9.9.2.4 Root Exudate Modification

AM fungi may inhibit nematode activities by altering the root physiology in ways unrelated to P nutrition and enhanced root growth. Root exudation is affected by the colonization of roots by mycorrhiza. These changes could alter chemotactic attraction of roots which affect those species which require a hatching stimulus or directly retard nematode development within root tissues (Harley and Smith 1983).

9.9.2.5 Parasitism of Nematode Eggs

In several instances, eggs have been shown to be parasitized by mycorrhizal fungi. Eggs of *Heterodera glycines* have been parasitized by *Glomus fasciculatum* (Francl and Dropkin 1985).

However, Harley and Smith (1983) opined that mycorrhizal fungi may colonize stressed or weakened eggs of nematodes since these fungi may possess limited saprophytic capabilities.

9.9.2.6 Production of Nematostatic Compounds

Several compounds inherent to mycorrhizal symbiosis may be nematostatic and unfavorable to the nematode (Hayman 1982). Mycorrhizal symbiosis is associated with variations in amino acids, hormones, root cell membrane permeability etc., which may inhibit several activities of nematodes by altering root physiology.

9.9.2.7 Competition for Host Photosynthates

AM fungi almost totally depend on soluble carbohydrates produced by the host for their carbon source. Mechanism affecting nematode activities in a mycorrhizal system is conversion of carbohydrates received from the host into forms that cannot be used by the nematodes. Phosphorus in the fungus may restrict the availability of P for nematode development and reproduction. It has been estimated that mycorrhiza symbiosis utilizes up to 15 % of host photosynthates (Harley and Smith 1983). Nematode activities normally are affected by overcrowding or lack of root infection sites when demands for host resources exceed supply (Wallace 1973). Both nematodes and mycorrhiza may have to compete for the photosynthates.

9.9.2.8 Competition for Space or Infection Site

Because AM fungi are soilborne fungi that colonize similar root tissues attacked by plant-parasitic nematodes, direct competition for space has been postulated as a mechanism of pathogen inhibition by AM fungi. Some reports, however, say that nematode activity inhibition on mycorrhizal roots occurs when only 40–60 % of the root system is colonized by the mycorrhiza, leaving ample space available for the penetration and infection by the nematode (Zambolin and Oliveira 1986). Apart from this, according to Wallace (1973), mycorrhizae do not colonize the root tip elongation zone, which is a preferred site for entry by several nematode species. Root knots have been frequently located on mycorrhiza-colonized roots (Hussey and Roncadori 1982).

9.9.2.9 Time of Inoculation

Earlier introduction of *G. fasciculatum* by 15 days adversely affected *H. cajani* root penetration to a greater extent in cowpea than simultaneous inoculations. Over 60 % colonization of root system by AMF considerably hampered root invasion. The interaction of *R. similis* with *G. fasciculatum* in banana revealed that mycorrhizal plants contained fewer nematodes, supported lower number of nematodes in soil, and had fewer nematode-induced root lesions than non-mycorrhizal plants if *G. fasciculatum* was added simultaneously or 7 days before with *R. similis*. Inoculation with *G. fasciculatum* 15 and 20 days earlier than the nematode had controlled the nematode population and also increased the biomass production. AMF was affected when the nematodes were inoculated earlier. When the *G. fasciculatum* was inoculated 15 days earlier than *M. incognita* on tomato, AMF prevented the multiplication of the nematode and offset the deleterious effects of nematode on the plant growth.

M. incognita infection and development were less in the plants Tamarillo pre- infected with mycorrhizal fungi. Mycorrhizal inoculation stimulated the growth of citrus, jambhiri seedlings while the nematode decreased growth in pot experiment. When the two organisms were inoculated simultaneously, the adverse effects of the nematode were partly neutralized, and the fungus limited the development of the nematode.

9.9.2.10 Inoculation Method

Application of AMF as inoculum mixed with soil and inoculum as a layer under seed gave better results in improving plant growth and reduction in nematode population. In case of nursery inoculation, incorporation of AMF into nursery beds of tomato allowing the fungus to colonize the roots before it was transplanted to the main field prevents the penetration and development of the nematode in the AMF-infected plants. Thus, AMF was able to offset the adverse effects of

nematodes and increased the yield by 91.3 % over control. With AMF as biofertilizer applied at the ragi (finger millet) nursery, the plants were able to offset the ill effects caused by *Rotylenchulus reniformis* and gave higher yield compared to control.

9.9.2.11 Biochemical Analysis

Disease incidence was observed to be greater when the level of sugars in host plants was low. It was noticed that resistant plants had higher total and reducing sugar content. Mycorrhizal plants had high concentrations of phenylalanine and serine which are known to reduce the growth and reproduction of the root-knot nematode. Root exudation of amino acids and reducing sugars were greater from mycorrhizal Sudan grass grown in phosphorous-deficient soils than from non-mycorrhizal plants. Mycorrhizal plants had increased quantities of phosphorus, potassium, calcium, and amino acids phenylalanine and serine than non-mycorrhizal plants. Total phenol, total sugar, total free amino acid, and acid phosphate activity was found to be higher in AMF-inoculated plants. Preoccupation of tomato cv. Pusa Ruby roots with *G. fasciculatum* coupled with biochemical changes such as increase in lignins and phenols made Pusa Ruby resistant to root-knot nematode, *M. incognita.*

Lignin and phenols have been found significantly more in the mycorrhizal roots. Both the chemicals are known for their role in host resistance. The elevated levels of amino acids and sugars observed in AM fungal roots are associated with increased plant resistance, each singly or collectively playing a role in suppressing nematode development. Significant difference was observed in total phenol contents in mycorrhizal and non-mycorrhizal plants. In nematode-infested plants, the phenolic content was less. Some phenols are known to form complexes of amino acid and chlorogenic acid which are highly toxic to the parasite.

The presence of nematicidal substances which have been reported in AM fungi-colonized roots may result from the improved plant vigor owing to enhanced P uptake or increased concentrations of phenylalanine and/or serine which are known to be nematicidal. Thickening of the cell walls through lignifications and production of their polysaccharides in mycorrhizal plants prevents the penetration by the nematodes. This phenomenon is the result of increased phenol synthesis in the plants brought about by an increase in phenyl propane, which is a lignin precursor.

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10 Genetics of Nematode Parasitism

It is well known that various genetic factors influence the host plant and either turn it into a resistant to the nematode pest or enable the nematode to overcome the resistance of the host plant (Sidhu and Webster 1981). Most notably, all phytonematodes are equipped with a stylet to pierce cell walls and allow solute exchange between plant and parasite. Furthermore, plant-parasitic nematodes have well-developed secretory gland cells associated with their esophagus that produce secretions released through the stylet into host tissues. Interestingly, the development of enlarged secretory cells associated with the esophagus also exists in nematode parasites of animals but is notably absent from microbivorous nematodes like *C. elegans*. In the case of the root-knot nematodes and cyst nematodes, as is the case with the other tylenchid phytonematodes, there are three esophageal glands, one dorsal and two subventral glands.

The success of nematodes as phytoparasites is measured by their capacity to develop and reproduce on host plants and rarely on ability to cause disease. Parasitism in nematodes, in most studies, is being measured in terms of rates of development and reproduction but not in terms of virulence (Triantaphyllou 1986). Physiological races and biotypes along with nematode–host interactions are the major concepts to consider. Nematodes possess genes for parasitism but not genes for virulence. Parasitism genes simply are the ones which enable nematodes to overcome the effects of genes for resistance. Substitution of parasitism for virulence in nematology will not change the widely used concepts of "virulence versus aggressiveness." However, aggressiveness may be a suitable term to use with regard to nematodes.

"Parasitism" may be defined in several ways, and unfortunately, a lack of consensus about the meanings of host–parasite (pathogen) terminology still exists in plant pathology. The most common definition of a parasite is "an organism living in or on another living organism, obtaining from it part or all of its organic nutriment and commonly exhibiting some degree of adaptive structural modification." This broad definition encompasses a wide range of potential nematode parasitism genes that have evolved specifically or perhaps were "procured" and modified from other successful parasitic organisms, to promote parasitism in a host. Nematodes should be considered first as parasites, and if disease results in the host, the parasites become pathogens. The products of nematode parasitism genes may be manifested as morphological structures that provide access to parasitism of a particular host, or they may play critical physiological roles in the interaction of the nematode with its host.

10.1 The Role of Various Secretions in Parasitism

It is well known that in phytonematodes, stylet penetrates the wall of a plant cell, injects gland secretions into the cell, and withdraws nutrients from the cytoplasm. Migratory-feeding nematodes

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remove cytoplasm from the host cell, frequently causing cell death, and then move to another cell to repeat the feeding process. Evolutionarily more advanced nematode species become sedentary and feed from a single cell or a group of cells for prolonged periods of time. For this sustained feeding, the sedentary parasites dramatically modify root cells of susceptible hosts into elaborate feeding cells, including modulating complex changes in plant cell gene expression, physiology, morphology, and function (Gheysen and Fenoll 2002). The drastic phenotypic changes of root cells during feeding cell formation are the result of nematode-mediated changes, directly or indirectly, in the developmental program of the parasitized cells. An understanding of the molecular signaling events in this process will not only provide fundamental knowledge of nematode parasitism and regulation of plant gene expression, but it will also suggest vulnerable points in the parasitic process that can be interfered with to achieve nematode control to limit nematode-induced yield losses in crops (Hussey et al. 2002a, b).

The evolutionary adaptations of nematodes for plant parasitism led to the development of the protrusible stylet as well as marked morphological and physiological modifications of the esophagus (Bird 1971). Secretory gland cells in the nematode esophagus are the principal sources of secretions involved in plant parasitism, and these gland cells enlarged considerably as nematodes evolved from microbial-feeding nematodes to become parasites of higher plants. Likewise the function of the secretions produced by the esophageal gland cells also evolved to enable nematodes to feed on plant cells and modify them into complex feeding cells. Recent discoveries also suggest that some genes encoding esophageal gland secretions of plant-parasitic nematodes may have been acquired via horizontal gene transfer from prokaryotic microbes. This treatise focuses primarily on discoveries made in identifying parasitism genes in cyst and root-knot nematodes because these nematodes induce the most dramatic and evolutionarily advanced changes observed in host cell phenotype. Cyst and root-knot nematodes have evolved to alter gene expression in specific root cells to modify them into very specialized and metabolically active feeding cells, called syncytia or giant cells, respectively (Hussey et al. 2002a, b). Cell fusion following cell wall degradation gives rise to the syncytia, whereas abnormal cell growth following repeated mitosis uncoupled from cytokinesis produces the giant cells. Major roles of nematode secretions include egg hatching, penetration and migration in plant tissue, induction and maintenance of feeding site, feeding tube formation, and digestion of host cell contents.

10.1.1 Amphidial Secretions

The amphids are the primary chemosensory organs in the head of the nematode. Antibodies directed against amphidial secretions hamper host finding (Perry 2001), indicating that those organs may be involved in the early steps of host–parasite recognition. They also capture and transport chemotactic stimuli to the sensillar membrane. Secreted proteins include annexin (Gp-nex-1), calcium-dependent phospholipid-binding protein, putative collagen, and gene in J2 amphids and hypodermis of adult female. The amphidial glands are the largest and most complex of the anterior sensory organs of nematodes. A gene coding for a putative avirulence protein (MAP-1) was isolated after AFLP fingerprinting of near-isogenic lines of *M. incognita*. The putative protein has no homologues in the database, but polyclonal antibodies against a synthetic peptide of MAP-1 clearly labeled the amphidial secretions. The dendritic nerve extensions of the amphidial neurons are surrounded by secretions of the amphidial gland cells (Aumann 1993). The secretions may protect the nerve dendrites against microbial attack. The amphidial secretions of the plant-parasitic nematode *Heterodera schachtii* are composed of glycoproteins with terminal galactose units (Aumann 1989). Several lectins with different carbohydrate specificities bind to the amphidial and "excretory" system secretions of this (Aumann and Wyss 1989) and other nematode species. The carbohydrates may be bound to the protein backbone either N-glycosidically via

N-acetylglucosamine and asparagines or O-glycosidically via N-acetylga-lactosamine and serine or threonine.

Dendritic processes project into the amphidial cavity and are bathed in secretions produced by the amphidial sheath cell (Duncan 1995). These secretions are highly glycosylated. Initial studies indicating the presence of carbohydrate residues in amphidial secretions came from the observation that the adhesion of some nematophagous fungi appeared to occur exclusively to chemosensory structures. It was shown that this was mediated by a lectin–carbohydrate interaction (Nordbring-Hertz and Mattiasson 1979; Jansson and Nordbring-Hertz 1983), with the carbohydrate being located in the amphidial secretions. Concurrent work using lectin binding carried out on the closely related *Heterodera schachtii* indicated that its amphidial secretions are composed exclusively of O-glycans (Aumann 1994). O-glycan linkages are known to be the major constituents of mucus, although they are also found in some cell membrane-associated molecules. Amphidial secretions were very resistant to proteolytic attack, often a consequence of O-glycosylation due to the relative resistance of the glycosylated regions to protease degradation. It is thought that this resistance is due to the attached carbohydrate residues blocking access to the peptide core as the removal of the carbohydrate allows subsequent protease digestion. Another effect of O-glycosylation may be to extend the functional domain of a molecule out from the cell surface, thus allowing interactions with extracellular molecules.

Secretions collected from *G. pallida* using the two different methods were analyzed using SDS-PAGE electrophoresis (Duncan 1995). Secretions were also used for antiserum production, giving two antisera, Luffness antiserum and ES antiserum. These were subsequently used for immunoblotting and indirect immunofluorescence studies. Indirect immunofluorescence studies indicated that the two antisera recognized different nematode components. This was further confirmed by immunoblotting studies which revealed that Luffness antiserum recognized a number of nematode proteins and was capable of differentiating both between and with species of *G. pallida* and *G. rostochiensis*. In contrast, ES antiserum recognized only two proteins which appeared to be conserved between the two species. Observations also indicated that presence of a nematode lectin component present in amphidial secretions with apparent specificity for N-acetylgalactosamine. Experiments were also performed to examine different methods of inducing secretions. Previous research had shown that the serotonin agonist 5-methoxy dimethyltryptamine (DMT) is an effective inducer of nematode esophageal secretions. Comparison of DMT-induced secretions with ES secretions using SDS-PAGE electrophoresis revealed that the protein profiles were similar, although some proteins were more abundant following induction with DMT. Treatment of *G. pallida* with DMT followed by indirect immunofluorescence with Luffness antiserum revealed an increased and altered distribution of antibody binding on the nematode surface.

It has been postulated that carbohydrate residues may have important functions in transduction of a chemosensory signal. Low concentrations of nematicides can impair responses to chemoattractants with no effect on motility. Exposure to the carbamoyloxime nematicide, aldicarb, resulted in the hypertrophy of the internal dendrite terminals within the amphidial sheath cell, a reduction in surface volume of the dendritic processes, and the appearance of electron-lucent granules in the cytoplasm of the amphidial sheath cell. Interestingly, these neuroanatomical effects were restricted to the amphids and not observed in the sheath cells or dendrites of the labial or cephalic sensilla. It was therefore suggested that aldicarb may have an effect via disruption of cholinesterase activity that has been reported. Amphidial secretions may be involved in initiation and/or maintenance of the host–parasite relationship. Amphidial secretions have a function in pathogenesis or the establishment of infection. It has been postulated that the feeding plug which is secreted by cyst nematodes once the feeding site is established may originate from the amphids. However, later studies suggest that feeding plug material may in fact be secreted through the cuticle.

10.1.2 Esophageal Glands Secretions

These stylet secretions have a direct role in infection and parasitism of plants, and developmental changes in the secreted proteins occur during the parasitic cycle (Davis et al. 2000a, b). Herein, the secreted products of the parasitism genes expressed in the nematode's esophageal gland cells are considered collectively as the "parasitome," a subset of the secretome (secreted proteins) of a parasite that mediates parasitism (based upon the nomenclature in Greenbaum et al. 2001). These stylet secretions may function in nematode penetration and migration through root tissue, modification and maintenance of root cells as feeding cells, formation of feeding tubes, and/or digestion of host cell cytoplasm to facilitate nutrient acquisition by the nematode (Hussey 1989). The secretions from sedentary endoparasites are particularly intriguing because of the complex changes in phenotype, function, and gene expression that they modulate in the parasitized plant cells. During parasitism of a plant cell, the nematode's stylet penetrates the cell wall but does not pierce the plasma membrane, which becomes invaginated around the stylet tip to provide an opening exclusively at the stylet orifice.

Esophageal gland cell secretions injected through the stylet by sedentary parasites transform root cells in susceptible plants into metabolically active feeding cells. These gland secretions modify, directly or indirectly, gene expression to induce profound morphological, physiological, and molecular changes in the recipient cells to enable them to function as a continuous source of nutrients for the nematode parasitic stages. The removal of the nematode at any point during the parasitic interaction results in degeneration of the feeding cells, suggesting the need for a constant and specific stimulus from the nematode to maintain the modifications in the parasitized cell. The gland secretions may be deposited outside the plasma membrane or injected directly into the cytoplasm of the recipient cell through the stylet orifice. In either case, specific molecules in the secretions could bind to plant cell receptors to elicit a signal transduction cascade to modulate gene expression in the cell. Alternatively, the secretions could enter the nucleus to directly modify gene expression in the recipient plant cell.

The development of monoclonal antibodies that bind to secretory antigens within the esophageal gland cells has been critical in the study of secreted proteins from cyst and root-knot nematodes (Hussey and Grundler 1998). The monoclonal antibodies have been used to monitor the developmental expression of different esophageal antigens at various stages of nematode development (Smant et al. 1998). During feeding, sedentary endoparasitic nematode species, viz., *Globodera, Meloidogyne, Heterodera*, and *Rotylenchulus*, also inject dorsal gland secretions that form unique tubelike structures called feeding tubes within the cytoplasm of the feeding cell. Feeding tubes function in the selective and efficient removal of nutrients from the cytoplasm of the large modified cells by the feeding nematode. Microinjection studies with fluorescent probes of different molecular weights showed that the walls of feeding tubes serve as a molecular sieve during nutrient uptake by the parasite. Dorsal gland (DG) secretions induce feeding site, produce feeding tube and modify the cytoplasm- syncytia, whereas subventral gland (SVG) contains cell wall-degrading enzymes like cellulase and proteolytic enzymes, chorismate mutase (aromatic amino acid synthesis) and SVG contains proteins (induces feeding cell).

10.1.3 Cuticular Secretions

The cuticle protects nematodes from plant defense response. The cuticle is a multifunctional exoskeleton. It is a highly impervious barrier between the animal and its environment. It is essential for the maintenance of body morphology and integrity and has a critical role in locomotion via attachments to body-wall muscles. It includes a peroxiredoxin that catalyzes the breakdown of hydrogen peroxidase; retinol protein; fatty acid-binding protein, which bind to linolenic and linoleic acid; precursors of plant defense compounds; and jasmonic acid signaling.

Parasitism gene products in nematodes include cellulase or endo-ß-1,4-glucanase, pectate lyase,

polygalacturonase, xylanase, expansins, chorismate mutase, chitinase, annexin, calreticulin, and small bioactive peptides. Feeding behavioral sequence in phytonematodes includes exploration, insertion of the stylet into host cells, injection of secretion, ingestion of host cytoplasm, and retraction of the stylet from host cell. Nematode parasitism is a complex and dynamic interaction with major activities like hatching stimuli, attraction to the host, penetration, recognition of tissue, feeding site formation, modification of host tissue, and an active response from the host. Parasite specificity in nematodes depends on the body adaptations, diverse habitats, and diverse niches.

The change in nematode morphology is accompanied by biochemical and ultrastructural changes in the surface cuticle (SC). The cuticle is a complex structure that is involved in the motility, maintenance of morphology, and interactions with the external environment. Molecules expressed at the SC of these parasitic nematodes represent the primary host–parasite interface and together with secreted–excreted products are probably the first signals perceived by the host. Nematode surfaces have a coat which contains different carbohydrates probably in the form of glycoproteins. Among the nematode's secretory products, stylet secretions are believed to play a role in the penetration and migration through root tissue, modification and maintenance of root cells as feeding sites, formation of feeding tubes, and digestion of host cell contents to facilitate nutrient acquisition by the nematode (Hussey 1989). These secretions are produced by two subventral and one dorsal esophageal gland cells and are secreted through the stylet into the plant tissue during parasitism.

Molecules expressed at the surface cuticle (SC) of plant-parasitic nematodes represent the primary plant–nematode interface and together with secreted–excreted (S-E) products are probably the first signals perceived by the host (Lima et al. 2005). These molecules, which are released into plant tissue, probably play important roles in the host–parasite interactions. They characterized these antigens that help in the identification of nematode targets useful for novel control strategies, which interfere with the nematode infection of plants. Three monoclonal (MAbs) and three polyclonal (PAbs) antibodies produced to S-E products of *Meloidogyne* spp. and *Heterodera avenae* were used to examine their reactivity toward *M. incognita* and/or *M. arenaria* second-stage juveniles and adult females. The three PAbs showed cross-reactivity with *M. incognita* and *M. arenaria.* Antibody Roth-PC 373 strongly recognized molecules present in the SC, amphids, and intestine, antibody Roth-PC 389 recognized the nematode amphids and metacorpus, while antibody Roth-PC 419 bound to molecules present in the subventral glands. Reactivity of the MAbs was only tested against *M. arenaria*. Monoclonal antibody Roth-MAb T116C1.1 showed intense reactivity with molecules present in the amphidial and phasmidial glands. Monoclonal antibodies Roth-MAb T46.2 and T42D.2 labeled the nematode amphids and molecules present in the nematode esophagus (metacorpus), respectively.

10.2 Niches Occupied by Phytonematodes

1. *Aerial*: Several nematodes feed on the aerial plant parts like stem, foliage, and flowers, for example, *Aphelenchus, Bursaphelenchus,* and *Anguina*.
2. *Subterranean*: Those nematodes which feed on the underground plant parts including roots, tubers, corms, suckers, etc., for example, M*eloidogyne, Heterodera, Xiphinema,* and *Longidorus*.

10.3 Convergent Specializations of Feeding Modes

Specializations in plant-parasitic lifestyles include migratory ectoparasitism and burrowing endoparasitism as well as various types of independently evolved, sedentary ecto- and endoparasitism and a full range of intermediates. These lifestyles relate to divergent parasite-specific host reactions, and in each case, the major pathology is the result of secretion products of pharyngeal glands injected into the host cell (Hussey 1989). Sedentary parasites

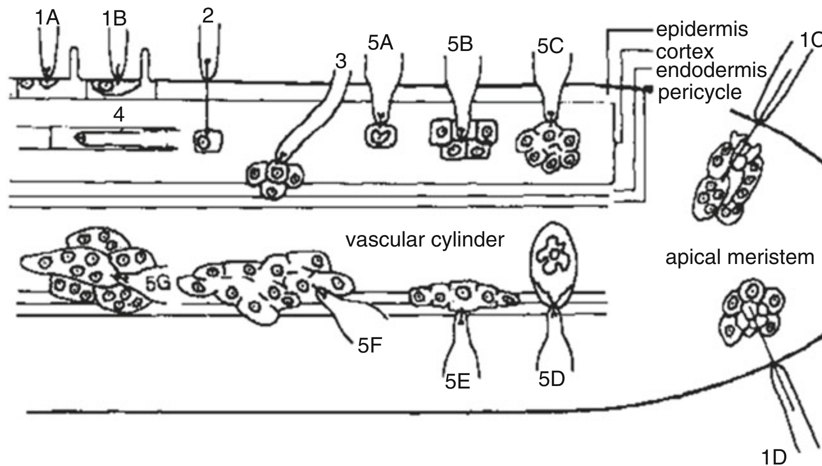


Fig. 10.1 Feeding site phytonematodes: 1A, *Tylenchorhynchus*; 1B, *Trichodorus*; 1C, *Xiphinema*; 1D, *Longidorus*; 2, *Criconemella*; 3, *Helicotylenchus*; 4, *Pratylenchus*; 5A, *Trophotylenchulus*; 5B, *Tylenchulus*; 5C, *Verutus*; 5D, *Cryphodera*; 5E, *Rotylenchulus*; 5F, *Heterodera*; 5G, *Meloidogyne*

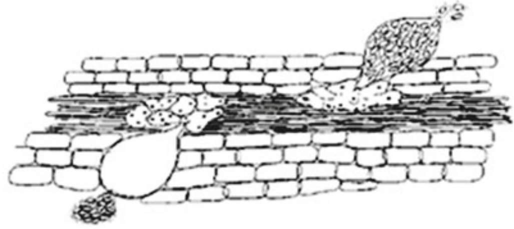
are associated with modifying and regulating host cell function to yield feeding sites acting as a metabolic sink and sustaining the parasite through its life. These feeding sites include various types of nurse cells (Mundo-Ocampo and Baldwin 1992), often specific to the nematode species. Nurse cells include single uninucleate giant cells as in *Sarisodera* sp., some *Meloidodera* (Heteroderinae) and *Rotylenchulus* sp. (Hoplolaimidae), multinucleate giant cells as in *Meloidogyne* or multinucleate syncytia as in many other Heteroderidae, and some other *Rotylenchulus.*

Host responses specific to particular nematode taxa have proven useful as characters in phylogenetic analysis of Heteroderinae, but a broader understanding of the evolution of the mode and direction of plant parasitism has been largely speculative. For example, a common perspective is that feeding sites among sedentary root-knot and cyst nematodes reflect the most elaborate and putative derived adaptations known among plant parasites (Davis et al. 2000a, b), a hypothesis testable in the context of phylogenetic trees. While acknowledging the limitations of preliminary data (taxon sampling, alignment issues, information content), some clades of plant parasites emerge with enough support to allow us to address several taxonomic and evolutionary hypotheses with a modicum of confidence. Emerging understanding of the patterns of these clades also provides a framework for mapping modes of parasitism, including the evolution of sedentary endoparasitism. Host responses specific to particular nematode taxa have proven useful as characters.

Nematodes mechanically injure plants (rubbing and probing) and bring about several physiological changes in the host, create openings for the entry of other microorganisms (interaction with other pathogens), transmit other disease-producing agents, and increase the plant's susceptibility to environmental stress.

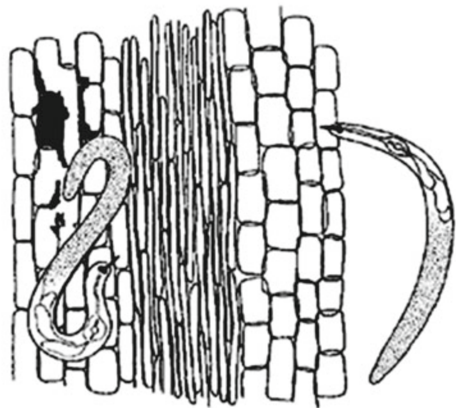
Phytonematodes are well-known obligate parasites. Some species have evolved rather simple feeding strategies, while other nematode species are highly adapted for more sophisticated parasitic relationships with host plants. A majority of research has focused upon plant response to nematode parasitism, primarily the complex modifications that some plant-parasitic nematodes induce in host plant cells and plant resistance to nematode challenge. Recent research is now providing insights into the molecular and genetic basis of the "nematode side" of plant–nematode interactions. Nematode parasitism genes may be active in any or all parts of the parasitic cycle of plant nematodes (Fig. 10.1), including "preparasitic" life stages (before invasion of the plant) and "parasitic" life stages (after invasion of the plant).

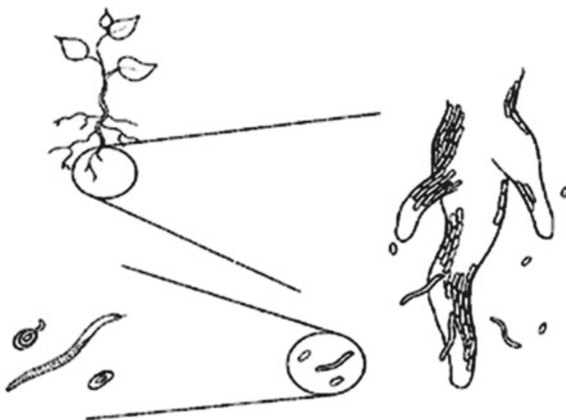
- Active feeding by nematode via the stylet
- Feeding cells serve as a nutrient sink for nematode
- Nematode stimulus maintains feeding site
- Feeding tubes aid ingestion of nutrients
- Successful nematode growth and reproduction



- Nematode signals trigger feeding site formation
- Esophageal gland secretions released through stylet
- Interaction of plant and nematode signals
- Gene expression is modified in parasitized cells
- Avirulent nematodes elicit defense in resistant genotypes

- Ectoparasites feed externally by inserting stylet
- Endoparasites enter roots to feed
- Mechanical and/or enzyme-aided migration within roots
- Nematodes select specific cells for feeding
- Resistant response to avirulent nematodes





- Egg hatch is influenced by root exudates
- Motile nematodes active in soil environment
- Nematodes respond to root signals
- Soil microbial activity affects nematodes
- Nematodes recongnize specific root tissues

Fig. 10.2 Progressive stages of parasitism by phytonematodes (from *bottom*)

Plant-parasitic nematodes have evolved diverse parasitic strategies and feeding relationships with their host plants to obtain nutrients that are necessary for development and reproduction. The vast majority of plant-parasitic nematode species are soil dwelling and feed from plant roots (Fig. 10.2). In highly specialized pathogens like root-knot nematodes, second-stage juveniles (J2) invade the roots of plants at the growing tip. They migrate between the cells and establish a permanent feeding site close to the developing vascular cylinder. There they molt three times, without

feeding between molts, to become adults. At the feeding site, they induce the formation of multinucleate "giant cells," of which they feed. Eggs are pushed to the surface of the root. The first molt takes place within the eggshell, and the second-stage juveniles hatch and disperse in the soil to search for a host.

These biotrophic parasites, depending upon species, feed from the cytoplasm of unmodified living plant cells or have evolved to modify plant cells into elaborate discrete feeding cells. Plant-parasitic nematodes use a hollow, protrusible feeding structure, called a stylet, to penetrate the wall of a plant cell, inject gland secretions into the cell, and withdraw nutrients from the cytoplasm. Migratory-feeding nematodes remove cytoplasm from the parasitized cell, frequently causing cell death, and then move to another cell to repeat the feeding process. Other nematodes become sedentary and feed from a single cell or a group of cells for prolonged periods of time. For this sustained feeding, the sedentary parasites dramatically modify root cells of susceptible hosts into elaborate feeding cells, including modulating complex changes in cell morphology, function, and gene expression. These feeding cells become the sole source of nutrients for sedentary endoparasites such as *Meloidogyne* (root-knot nematode) or *Heterodera* and *Globodera* (cyst nematode) species. Similarly, in sedentary ectoparasites such as the ring nematode, *Criconemella xenoplax*, a single feeding cell is utilized as a nutrient source for several days before the nematode moves on to establish another feeding site.

10.4 Cellular Changes

The root-knot nematodes, *Meloidogyne* spp., and the cyst nematodes, *Heterodera* and *Globodera* spp., are sedentary parasites of roots of many crop plant species that collectively incite billions of dollars in annual crop losses around the world. While both nematode groups use very similar parasitic strategies to complete their life cycles, they employ different mechanisms to carry out their strategies. In each group, the motile juvenile molts to the second stage (J2) and hatches from the egg in soil. The infective J2 follows environmental and host cues in soil to locate tissues near the plant root tip that it will penetrate. Infective juveniles of root-knot nematodes and cyst nematodes differ somewhat in their means of migration and apparent preference for feeding location near the vascular tissue of host plant roots, which shall not be revisited here (Davis et al. 2004). More substantial differences become obvious once feeding commences. If initiation of feeding is successful, the sedentary parasitic phase ensues, leading to nematode growth and three subsequent molts to the reproductive adult stage. Both root-knot nematodes and cyst nematodes transform initial feeding cells into elaborate feeding sites that share a dense cytoplasm, altered cell walls, duplication of their genetic material, and increased metabolic activity. However, root-knot nematode and cyst nematode feeding sites differ in ontogeny and appearance.

The root-knot nematode induces substantial enlargement and changes in a small group of initial feeding cells around the nematode head and turns each of them into a discreet "giant cell" from which the nematode feeds in sequence (Fig. 10.3a). In each giant cell, the nucleus undergoes repeated divisions resulting in a multinucleate state. A cyst nematode, on the other hand, induces changes in a single initial feeding cell, which then are reciprocated in neighboring cells, including cells that are not necessarily in direct contact with the nematode. These changes culminate in the fusion of many modified cells, sometimes involving over 200 cells, to form one large multinucleate cytoplasm called a syncytium (Fig. 10.3b). Nuclei of syncytial cells undergo endoreduplication of their DNA content but do not divide.

The elaborate changes in morphology of both syncytia and giant cells are accompanied by dramatic alteration in gene expression in the affected plant cells (De Meutter et al. 2003). Interestingly, root-knot nematodes and cyst nematodes in general also differ in the fact that most root-knot nematode species have broad host ranges, whereas cyst nematodes have much

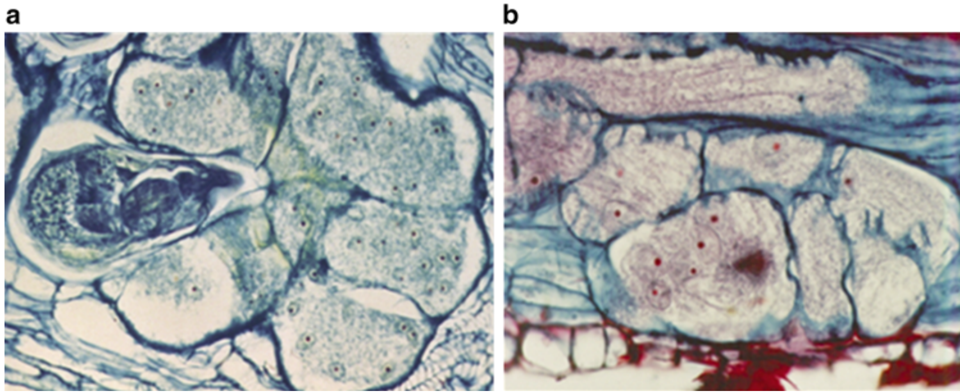


Fig. 10.3 Cross sections of feeding cells induced by sedentary endoparasitic nematodes in plant roots. (**a**) Multinucleate giant cells. (**b**) Multinucleate syncytium

smaller groups of host plants. A current hypothesis is that both nematodes use different strategies to induce their respective feeding sites and that giant cell induction by the root-knot nematode targets a plant mechanism that is widely conserved among plant species, thereby allowing parasitism of many host plants. On the contrary, for the formation of syncytia, cyst nematodes may target molecular plant mechanisms that are divergent among different plants, and therefore, individual cyst nematode taxa can only infect relatively small groups of plants (Baum et al. 2007).

Interrelationship of the genetic factors makes a host plant resistant to a parasite. Interrelationship involves a susceptible host plant on which a parasite can develop and reproduce freely. Genetics of nematode parasitism occur due to physiological variation and nematode–host interaction.

10.5 Physiological Variation

It mainly refers to the intraspecific variation, which means variation among populations of the same species. Knowledge about the species status of a given organism, particularly whether it comprises a biological species, is important before its physiological variation is recognized. The usage of some terms for identifying intraspecific variation includes the following.

10.5.1 Pathotype

An intra-subspecific classification of a pathogen distinguished from others of the species by its pathogenicity on a specific host(s). It is more preferred for potato cyst nematode, *Globodera rostochiensis* and *G. pallida*. This term is more appropriate to equivalent populations of amphimictic nematodes (Sidhu and Webster 1981). Pathotypes are differentiated based on the different breeding lines of the same plant species (Stone 1985). However, Sturhan (1985) reported that since resistance genes are often transferred through introgressive hybridization from one plant species to another, application of this definition may be ambiguous. This term should be used to individuals within a population that exhibit the same phenotype, i.e., possess the same host range. This way, a particular field population may represent one race only, which may comprise one or more pathotypes.

10.5.2 Biotype

"A group of genetically identical individuals sharing a common biological feature" is the definition of a biotype. It can be used to recognize intraspecific variation in parasitic capabilities of nematodes. In a complex genetic system, a single individual contains more than one biotype. It is

more preferred for stem and bulb nematode, *Ditylenchus dipsaci*. This term may be used to refer to parthenogenetic nematode populations with different host preferences and to identify intraspecific variation in parasitic capabilities of nematodes.

10.5.3 Race

It refers to a subdivision of a pathogen, distinguished from other members of the species by specialization for pathogenicity to different cultivars of a host. The varieties of a host species used to identify physiological races of a pathogen are known as differential hosts or host testers. Differential hosts are chosen on the basis of differences in their resistances to the pathogen, but the genes for resistance present in them are usually not known. Ideally, each of the differential hosts should posses a single resistance gene different from those present in others; such a set of differentials is known as ideal differentials. This term, implying host race, is used mostly for the soybean cyst nematode, *Heterodera glycines*, and for root-knot nematodes, *Meloidogyne* spp. (Table 10.1) (Dong et al. 1997*)*. Host races are differentiated based on the genes for resistance from various plant species. This term should be applied to phyletically related populations, for instance, those that share several common characters apart from possessing the same host range (Sturhan 1985).

Physiological variation is very commonly seen in soybean cyst nematode, potato cyst nematode, cereal cyst nematode, stem and bulb nematode, and root-knot nematode.

Table 10.1 Races of *H. glycines* on standard soybean differentials

Differential host					
Race	Pickett	Peking	PI90763	PI88788	Lee
Group I					
3	R	R	R	R	S
6	S	R	R	R	S
9	S	S	R	R	S
14	S	S	S	R	S
Group II					
1	R	R	R	S	S
2	S	S	R	S	S
4	S	S	S	S	S
5	S	R	R	S	S

Group I and group II are separated based on parasitic ability on PI88788

R resistant or female index <10 %, *S* susceptible or female index >10 %

10.5.4 Nematode–Host Interaction

It deals primarily with inheritance of plant resistance through introgressive hybridization and a very few with inheritance of nematode parasitism. Nematodes possess balanced type of parasitism presumably through coevolution with their hosts. Gene-for-gene relationship occurs in host–pathogen interaction (Ellingboe 1984). It occurs when plant-parasitic genes behave as dominant and complimentary genes for parasitism behave as recessive, and vice versa. Major and minor genes for resistance are matched with major and minor genes for parasitism, respectively, though this may not always be true (Triantaphyllou 1986). It is normally more complex when a given gene for resistance interacts with more than one gene for parasitism (phenotypes show immune/resistance/susceptible response). The genetics of parasitism in most instances may be extrapolated from the knowledge about the inheritance of resistance.

One of the efficient ways to investigate the inheritance of resistance or parasitism is to test the progeny of appropriate crosses against genetically homogenous nematode or plant populations. The host or the parasite later simplifies the genetic system of the interaction and allows genetic analyses which may give confirmed results.

10.6 Genetic Models of Plant Parasitism by Nematodes

Analysis of mutants has been an extremely powerful approach to unravel complex biological mechanisms in organisms such as *C. elegans*, *Arabidopsis thaliana*, and *Drosophila melanogaster*. Unfortunately, the artificial generation of

phytonematode mutants altered in their parasitic behavior is still technically challenging and, in most cases, presumably lethal. Thus, plant nematologists have to confine their studies to the genetic variation offered by nature. A well-known group of naturally occurring variants among plant-parasitic nematodes is those revealed by their (in)ability to reproduce on host plants that carry major resistance genes. Most of the reported variants in nematode virulence can be explained by gene-for-gene relationships with their hosts, similar to what is observed with many microbial plant pathogens (Davis 2000). For one nematode/plant combination, a gene-for-gene relationship has been confirmed by genetic analyses of both interacting partners. Virulence tests of 15 F2 lines, obtained by selfing of the F1 of a cross between a virulent and avirulent line, showed that virulence in *G. rostochiensis* toward the *H1* gene in potato is controlled by a single recessive gene. Although Mendelian proof for both interacting partners remains scarce, evidence is accumulating that such gene-for-gene mechanisms are common among plant/nematode interactions.

The most evolutionary advanced adaptations for plant parasitism by nematodes are the products of parasitism genes expressed in their esophageal gland cells and secreted through their stylet into the host tissue to control the complex process of parasitism (Hussey et al. 2002a, b). Molecular analyses of nematode parasitism genes are revealing the complexity of the tools that enable the nematode to attack plants, and the results paint a more elaborate picture of host cellular events under specific control by the parasite than previously hypothesized. Interestingly, the majority of the parasitism genes discovered encodes proteins unique to plant-parasitic nematodes. Identifying the nematode parasitome, i.e., the complete profile of parasitism gene products secreted through the nematode stylet during the parasitic cycle, is the key to understanding the molecular basis of nematode parasitism of plants. Such knowledge will identify vulnerable points in the parasitic process that can be interfered with to achieve nematode control to limit nematode-induced yield losses in crops.

Phytonematodes deploy a broad spectrum of feeding strategies, ranging from simple grazing to the establishment of complex cellular structures, including galls in host tissues. Various models of feeding site formation have been proposed, and a role for phytohormones has long been speculated by Bird and Koltai (2000) although whether they perform a primary or secondary function was unclear. On the basis of recent molecular evidence, they presented several scenarios involving phytohormones in the induction of giant cells by root-knot nematode and presented the models for horizontal gene transfer. Also discussed is the origin of parasitism by nematodes, including the acquisition of genes to synthesize or modulate phytohormones.

At present more than 25 major resistance genes (R genes) against nematodes have been mapped. With the exception of the first nematode R gene identified, *Hs1pro-1*, the other cloned nematode R genes share various structural features with other plant disease resistance genes that operate in gene-for-gene relationships. Several nematode R genes are members of a family characterized by a nucleotide-binding site (NBS) and leucine-rich repeats (LRRs). Recent cloning of the potato cyst nematode resistance gene *Gpa2* also revealed NBS and LRR domains. Interestingly, the *Gpa2* gene has a remarkably high homology with the virus resistance gene *Rx*. Various studies have shown that *Rx*-mediated resistance against potato virus X is a gene-for-gene mechanism in which the R gene encodes a putative receptor that recognizes the viral coat protein as an avirulence gene product. A major challenge in plant nematology is to identify the avirulence gene products of parasitic nematodes. To reach this goal, various research groups have conducted selections of virulent and avirulent nematode lines. Such lines have been established for *H. schachtii*, *M. incognita*, *H. glycines*, and *G. rostochiensis*. Root-knot nematodes have been subjected to rigorous selection experiments to generate parasitic variants in these asexual nematode species. Selection experiments with *M. incognita* against the *Mi* resistance gene of tomato showed a slow but progressive increase

in the proportion of virulent nematodes after each generation, suggesting a polygenic inheritance.

10.7 Parasitism Genes

Although it is currently not possible to predict the number of members of the parasitome of plant-parasitic nematodes, only a small fraction of the estimated 15,000–20,000 genes (based on the ~ 19,000 genes of *Caenorhabditis elegans*) of a plant-parasitic nematode should be expected to encode proteins that have a direct role in parasitism. The first members of a parasitome to be cloned from plant-parasitic nematodes were ß-1,4-endoglucanases (cellulases) developmentally expressed in the two subventral gland cells of *Heterodera glycines* and *Globodera rostochiensis* (Smant et al. 1998). A smaller cellulase cDNA in *G. rostochiensis* (Gr-eng-2) lacks the CBD, and one (Hg-eng-2) from *H. glycines* is missing both the peptide linker and CBD. The presence of a CBD presumably enhances cellulase activity toward crystalline cellulose. mRNA in situ hybridization and immunolocalization with anti-ENG polyclonal sera confirmed that eng-1 and eng-2 were expressed exclusively within the subventral esophageal gland cells of both nematode species.

Differential screening of gene expression has been the most widely used method to clone parasitism genes expressed within the esophageal gland cells of plan parasitic nematodes. Esophageal gland regions from second-stage juveniles of *M. javanica* were excised, and cDNA was prepared from this tissue by reverse transcriptase-polymerase chain reaction (RT-PCR). The cDNA pool was differentially screened against cDNA from the nematode tail region to isolate genes that are upregulated or expressed specifically in the esophageal gland region. A full-length cDNA clone that had homology to a bacterial chorismate mutase was obtained with this screening strategy (Lambert et al. 1999). Expression of Mj-cm-1 is localized within the subventral esophageal gland cells of parasitic *M. javanica* by mRNA in situ hybridization and with antisera generated to the product of Mj-cm-1. Chorismate mutase initiates the conversion of chorismate, the end product of the shikimate pathway, to the aromatic amino acids, phenylalanine and tyrosine. The secretion of Mj-cm-1 into the cytosol of a plant cell could potentially alter the spectrum of chorismate-dependent compounds, which, among other functions, are involved in cell wall formation, hormone biosynthesis, and synthesis of defense compounds in plants. Alternatively, these compounds (tyrosine) could be used by the nematode in cuticle formation. RNA fingerprinting has been used to analyze differential gene expression between preparasitic and parasitic stages of *M. incognita*. A cDNA encoding for a secretory cellulose-binding protein (Mi-cbp-1) was isolated using this method (Ding et al. 1998). Mi-cbp-1 is specifically expressed in the subventral gland cells of *M. incognita*, and in vitro analysis confirmed the secretion of Mi-cbp-1 through the nematode stylet. The N-terminal region of the predicted peptide has no similarity to known proteins, but the C-terminus has strong homology to a CBD. Two of the candidate parasitism genes identified share homology with Ran-binding proteins and are hypothesized to be involved in feeding cell induction (Qin et al. 2002).

Proteinaceous stylet secretions from nematodes that are synthesized in the esophageal gland cells are considered as primary signaling molecules at the plant–nematode interface because the morphology, contents, and activity of the gland cells change in relation to nematode migration within plant tissues, feeding cell formation, and nematode feeding activity (Hussey 1989). The genes encoding these secretions have been termed parasitism genes. The first phytonematode parasitism genes identified encoded cellulases (endoglucanases) synthesized in the esophageal gland cells of cyst nematodes that were expressed and secreted only during nematode migration within roots. These were the first endogenous endoglucanase genes cloned from an animal and phylogenetic analysis, which indicated strong similarity to cellulase genes of soil bacteria, suggesting the potential for ancient horizontal gene transfer as a mechanism of gene acquisition in nematodes (Davis et al. 2000a, b).

A number of nematode parasitism genes encoding other cell wall-modifying proteins, including

the first non-plant expansin (Qin 2004), have since been identified that are expressed in the esophageal gland cells during nematode migration in plant tissues. Beyond cell wall modifications, phytoparasitic nematodes appear to be armed with a suite of stylet secretions to modulate many of the features observed in nematode feeding cells. Genes encoding secreted chorismate mutase (CM) that are most similar to bacterial CM have been isolated from root-knot and soybean cyst nematodes. Chorismate mutase is a pivotal enzyme in the shikimic acid pathway that modulates synthesis of "Phe" and "Tyr," having pleiotropic effects on cellular metabolism and auxin synthesis and as precursors of plant defense compounds. Expression of nematode CM in tissues affected the vascular tissue differentiation and was indirectly related to local indole-3-acetic acid concentrations and cellular partitioning of chorismate (Doyle and Lambert 2002).

Whole nematode expressed sequence tag (EST) analysis also has been used to identify gland-expressed genes. However, this approach has limited potential because it predominately identifies only parasitism genes whose translation products are obviously related to parasitism, like cell wall-digesting enzymes (Dautova et al. 2001). Analysis of ESTs from a preparasitic second-stage juvenile cDNA library of *G. rostochiensis* identified a full-length cDNA that encoded a predicted protein with a signal peptide at its amino terminus that had strong homology to class III pectate lyases of bacteria and fungi (Popeijus et al. 2000). Localization of transcripts of the pectate lyases to the subventral esophageal gland cells in nematodes indicates the potential for secretion of a pectate lyase from the nematode stylet during the early stages of plant parasitism.

The signal peptide-selection, microarray, and SSH analyses of gland cell cDNA libraries provided a sampling of parasitism genes expressed within *H. glycines*, but the apparent complexity of the libraries suggested that a more comprehensive approach was necessary to obtain a complete profile of the nematode parasitome (Hussey et al. 2002a, b). The *H. glycines* gland cell library generated by LD-PCR was macroarrayed on nylon membranes for indexing, and ESTs of 3,711 cDNA clones were analyzed. The presence of the signal peptide identified these gland cell proteins as candidates for being secreted through the nematode's stylet and potentially having a biological function in *H. glycines* parasitism of soybean.

In EST analyses of parasitism genes in root-knot nematodes, 37 unique clones from a gland cell-specific cDNA library were expressed within the subventral (13 clones) or dorsal (24 clones) esophageal gland cells of *M. incognita* (Huang et al. 2002). In BLASTP analyses, 73 % of the predicted proteins were novel proteins, and those with similarities to known proteins included a pectate lyase, acid phosphatase, and hypothetical proteins from other organisms. Molecular analysis of genes expressed in the esophageal gland cells is proving to be the most direct and efficient approach for identifying nematode parasitism genes. These direct molecular studies are providing for the first time new and surprising information on the complexity and dynamics of the parasitome of a multicellular parasite (Hussey et al. 2002a, b). Obtaining a comprehensive profile of the parasitome is critical for dissecting the molecular signaling events and regulatory mechanisms involved in nematode parasitism of crops by these economically important pathogens.

Another group of candidate secreted nematode parasitism gene products that may also augment host cellular metabolism includes members of the proteasome (Skp-1, RING-H2, and ubiquitin extension protein) with significant similarity to plant genes involved in selective host cell protein degradation. Several proteins have been identified in nematode secretions, and in some cases, their roles in parasitism have been determined (Table 10.2).

Root-knot and cyst nematode genes with known putative functions in parasitism, mostly based on similarities to characterized proteins in other organisms, are known. In addition to the parasitism proteins with similarity to characterized proteins, there are an even larger number of parasitism genes from root-knot and cyst nematodes for which no similarities to characterized proteins in other organisms exist.

Table 10.2 Some gene products secreted from the esophageal glands of phytonematodes

Gene product	Species in which identified	Organisms with close homologues	Possible function
β-1,4-Endoglucanase (cellulase)	*Globodera rostochiensis*	Bacteria	Cell wall degradation
	G. tabacum		
	Heterodera glycines		
	Heterodera schachtii		
	Meloidogyne incognita		
Pectate lyase	*Meloidogyne javanica*	Bacteria and fungi	Cell wall degradation
	G. rostochiensis		
	H. glycines		
Polygalacturonase	*M. incognita*	Bacteria	Cell wall degradation
Chorismate mutase	*H. glycines*	Bacteria	Alter auxin balance
	M. javanica		Feeding cell formation
	G. rostochiensis		
Thioredoxin peroxidase	*G. rostochiensis*	Animal-parasitic nematodes	Breakdown of H_2O_2, protect against host defenses
Venom allergen-like protein	*M. incognita*	Animal-parasitic nematodes,	Early parasitism?
	H. glycines	*C. elegans*	
Calreticulin	*M. incognita*	Animal-parasitic nematodes	Early parasitism?

10.8 Genetic Analysis of Nematode Parasitism

A parasite must reproduce to successfully complete its life cycle. In this sense, the ability of a *H. glycines* individual to parasitize a soybean plant is measured by reproduction. In general, resistant hosts do not support female nematode development to reproductive maturity. Thus, parasitism of a particular host genotype is a qualitative trait that the individual nematode either possesses or does not. Nematode populations may be additionally described quantitatively by their level of reproduction on a given host plant. Field populations of *H. glycines* are mixtures of many genotypes, some of which may confer the ability to overcome host resistance genes. Selection pressure from growing resistant cultivars can alter the frequency of alleles in the population for reproducing on a resistant host. Because of the importance of soybean cyst nematode as a pathogen, and also the identification and utilization of host resistance by soybean breeders, a considerable body of literature exists on the genetic basis of parasitism in *H. glycines* (Dong et al. 1997). It is generally believed that both major and minor genes (including dominant, partially dominant, and recessive alleles) are all involved to some degree in conferring resistance to *H. glycines* (Triantaphyllou 1987), although it is not clear which genes are essential and which are specific to certain nematode genotypes, if any. Interpretation is complicated by the use of *H. glycines* field populations to evaluate resistant soybean; field populations are highly heterogeneous, both among and within isolates. Results from population measurements usually are biased by this genetic variability, and the frequency of certain genes for parasitism (nematode genes necessary to overcome host resistance) may affect phenotypic designation of either parasitism or the levels of reproduction. Therefore, it is believed that the previous results are not accurate indications of the genetic basis of soybean parasitism in *H. glycines* (Opperman and Bird 1998).

Several parasitism genes are essential genes; however, the converse is not the case. Johnsen and Baillie (1997) estimated that 15–30 % of *C. elegans* genes are essential and this is the largest single class in *C. elegans*. Although mutations at

many other loci can give drastic phenotypes, the functions encoded by these genes appear to be dispensable for reproduction per se, so they are not classified as essential (Opperman and Bird 1998). This assignment is, however, to a large degree, an artifact of the way *C. elegans* is maintained in the laboratory. For example, the second largest class of genes in *C. elegans* is that in which mutation gives an uncoordinated (Unc) phenotype. Because coordinated movement is dispensable for a free-living nematode lying on a Petri plate in a sea of bacteria, the Unc loci are considered to be nonessential. In contrast, the equivalent genes (and many others) are almost certainly essential for obligate parasites such as *H. glycines*. For these nematodes to reproduce, they must locate a host, invade, and select and establish a feeding site, events that certainly require coordinated movement and behavior. Thus, correct interpretation of genetic ablation experiments requires an assay that accurately scores disruption of the specific parasitic interaction being tested. Being able to phenocopy a previously characterized genetic phenotype by reverse genetics would be a powerful confirmation of equivalent function and underscores the power of classical genetics to study parasitism.

Significant progress on the genetics of parasitism in nematodes has been made in plant-parasitic species, particularly *Globodera rostochiensis* and *H. glycines* (Opperman and Bird 1998). This is partly because these nematodes are sexually dimorphic, obligate amphimictic species, making them genetically tractable, but also because plants are experimentally more amenable as hosts than are many animals, especially in the numbers required for classic genetics. Importantly, it has proven possible to score for parasitism traits that enable particular nematode genotypes to evade host defense responses. A gene-for-gene relationship appears to be in operation in the case of the golden potato cyst nematode–potato interaction. Potatoes carrying the dominant H1 gene are resistant to certain pathotypes of *G. rostochiensis*. Pure parasitic and nonparasitic lines of *G. rostochiensis* have been selected, and crosses using these lines have revealed that parasitism is inherited at a single locus in a recessive manner (Janssen et al. 1991). However, results from reciprocal crosses suggested that there is no evidence for sex-linked inheritance of parasitism.

Dong et al. (1997) developed pure lines of *H. glycines* that carry single genes for parasitic ability on soybeans and were used to demonstrate that *H. glycines* contains unlinked dominant and recessive genes for parasitism of various host genotypes; parasitism genes in *H. glycines* were analyzed by crossing two highly inbred lines (>29 generations). A nonparasitic *H. glycines* line, which fails to reproduce on the resistant soybean lines PI88788 and PI90763, was used as the female and recurrent parent and was crossed to a parasitic line that does reproduce on these resistant hosts. The segregation ratio of the progeny lines developed by single female inoculation revealed that parasitism to these soybean lines is controlled by independent, single genes in the nematode. In accord with genetic nomenclature rules for parasitic nematodes, these loci were named ror for reproduction on a resistant host (Dong et al. 1997). In the inbred lines, ror-1(kr1) confers the ability to reproduce on PI88788 and is dominant. The recessive gene, ror-2(kr2), controls reproduction on PI90763. A second recessive gene, ror-3(kr5), controls the ability to parasitize the soybean line Peking. Although not verified, it is an intriguing possibility that some genes controlling parasitism may be acting additively. Examination of F1 data from controlled crosses revealed that the presence of two ror genes results in twice as many females being formed on PI88788 as when only one of these genes is present. This may explain varying levels of aggressiveness between different nematode populations on the same host genotype. It is particularly significant to note that these loci are entirely independent and do not appear to interact; that is to say, no novel host ranges are detected when combinations of ror genes are present in a particular nematode line. In addition to alleles for parasitism of resistant soybeans, there are SCN lines that have been selected to reproduce on tomato (Opperman and Bird 1998). The genes controlling this host acquisition remain to be characterized, either at the genetic or at the molecular level.

10.8.1 Cell Wall-Digesting Enzyme

The major structural component of the plant cell wall is cellulose, the most abundant biopolymer in the world (Davis et al. 2011). Cellulose is composed of successive glucose residues which are inverted 180°, forming a flat ribbon with cellobiose as the repeating unit. These (1,4)-β-linked glucan chains are able to form extensive hydrogen bounds to adjacent glucan chains. Approximately 36 of these crystalline chains are arranged in parallel in 3-nm-thick microfibrils forming insoluble cable-like structures. Cellulose microfibrils are among the longest molecules known in nature, since they are believed to consist of 8,000 (primary cell wall) to 15,000 (secondary cell wall) glucose molecules. Glycoside hydrolases are enzymes that catalyze the hydrolysis of the glycosidic bonds in sugar polymers. These glycosyl hydrolases are classified into different families according to their sequence similarity (Henrissat and Bairoch 1996). Cellulases or endo-1,4-β-glucanases, for example, are capable of degrading cellulose by hydrolyzing the (1,4)-β bonds. Several endoglucanases (or cellulases, EC 3.2.1.4) belonging to different glycosyl hydrolase families have been found in nematodes, facilitating the penetration and migration of the nematode through the plant cell wall.

The sclerotized, protrusible stylet of phytoparasitic nematodes provides a tool to mechanically breach the host plant cell wall. Such stylet activity can be readily observed for nematodes grown in monoxenic plant root culture and has been documented for both ectoparasitic and endoparasitic nematodes in video microscopy (Davis et al. 2011). An early body of evidence suggested that nematodes also secrete hydrolytic cell wall-degrading enzymes to assist in this process. Protein extracts and exudates from a number of phytoparasitic and fungal-feeding nematode species contained cellulolytic, amylolytic, chitinolytic, and pectolytic enzyme activity, suggesting the potential for endogenous production and secretion of cell wall-degrading enzymes from nematodes.

Most of the identified endoglucanases in nematodes belong to glycosyl hydrolase family 5 (GHF5). GHF5 endoglucanases were found in several nematodes belonging to the superfamily of the Tylenchoidea (order Rhabditida, suborder Tylenchina, infraorder Tylenchomorpha) (De Ley and Blaxter 2002). The majority belongs to the well-studied sedentary nematode genera *Heterodera*, *Globodera*, and *Meloidogyne*. Besides these sedentary nematodes, GHF5 endoglucanases have also been identified in the migratory nematodes *Radopholus similis*, *Ditylenchus africanus*, and *Pratylenchus* species. The GHF5 endoglucanases consist of several domains. They all have a signal peptide, which is required to secrete the protein, and a catalytic domain with the actual enzyme activity. Some endoglucanases have an additional carbohydrate-binding module (CBM) at the C-terminal end of the protein, which is thought to aid the enzyme in binding to its substrate.

The root-knot nematodes and cyst nematodes use a mixture of enzymes to soften root-cell walls, which should aid in penetration through the root epidermis as well as migration within root tissues (Yan et al. 1998). To date, there have been cellulase and pectinase genes described for root-knot nematode and cyst nematode species. The discovery of cellulase genes in the soybean and potato cyst nematodes represented the first major breakthrough in parasitism gene discovery. Hewezi et al. (2008) reported that phytocyst nematodes secrete a complex of cell wall-digesting enzymes, which helps in root penetration and migration. *Heterodera glycines* also produces a secretory cellulose-binding protein (Hg CBP). To determine the function of CBP, an orthologous cDNA clone (Hs CBP) was isolated from the sugar beet cyst nematode *H. schachtii*, which is able to infect *Arabidopsis thaliana*. CBP is expressed only in the early phases of feeding cell formation and not during the migratory phase. Transgenic *Arabidopsis* expressing Hs CBP developed longer roots and exhibited enhanced susceptibility to *H. schachtii*. A yeast two-hybrid screen identified *Arabidopsis* pectin methylesterase protein 3 (PME3) as strongly and specifically interacting with Hs CBP. Transgenic plants overexpressing PME3 also produced longer roots and exhibited increased susceptibility to *H. schachtii*,

while a pme3 knockout mutant showed opposite phenotypes. Moreover, CBP overexpression increases PME3 activity in plants. Localization studies supported the mode of action of PME3 as a cell wall-modifying enzyme. Expression of CBP in the pme3 knockout mutant revealed that PME3 is required but not the sole mechanism for CBP overexpression phenotype. They concluded that CBP directly interacts with PME3, thereby activating and potentially targeting this enzyme to aid cyst nematode parasitism.

The identification of endogenous genes encoding multiple types of cell wall-degrading enzymes in phytoparasitic nematodes has confirmed early physiological evidence for their expression and potential roles in plant parasitism. Since the initial identification of endoglucanase genes in cyst nematodes, both a candidate gene approach and extensive EST analyses have been the primary means of gene identification. The genome sequences of both *M. incognita* and *M. hapla* have not only confirmed the presence of multiple cell wall-modifying genes that were found in expressed sequence analyses, but they have revealed how unexpectedly large some of these gene families are, most notably the genes encoding pectolytic enzymes and expansin-like proteins (Opperman et al. 2008). The existence of gene families that encode cell wall-modifying enzymes in nematodes presents the potential for functional redundancy, although the biological significance of this potential remains unclear. The expression of nematode cell wall-modifying enzymes is almost exclusively localized within the esophageal gland secretory cells and developmentally consistent with the putative functional role of these secretions in migratory life stages of phytoparasitic nematodes.

10.8.2 Expansins

Expansins are extracellular, cell wall-loosening proteins involved in growth and cell wall disassembly. They mediate pH-dependent extension of the plant cell wall and growth of the cell. In many plants, they were found to be involved in a variety of growth processes including cell expansion, cell differentiation, and cell wall disassembly and breakdown (e.g., softening of fruits). Expansins belong to relatively conserved protein subfamilies, the alpha-, beta-, and gamma-expansins. Currently, 12 expansins in *Lycopersicum* are known. Alpha-expansins are involved in the auxin- and ethylene-mediated expansion and ripening of tomato fruits. These processes are similar to the syncytium (NFS – nematode feeding site) formation by cyst nematodes (*Heterodera schachtii*, Hs; *Globodera rostochiensis*, Gr), especially in the expansion and ripening phase.

Expansins are known to play an important role in cell wall formation and modification. Therefore it can be anticipated that they are involved in plant–pathogen interactions that go along with major structural changes in cell wall architecture, such as the formation of hypertrophic and hyperplastic tissues (Wieczorek et al. 2006). Expansins were first identified more than a decade ago as the key cell wall factors responsible for “acid growth.” Characteristically, expansions induce cell wall extension at an acidic pH optimum in vitro and enhance stress relaxation of isolated cell walls over a broad time range. They comprise two major gene families: a-expansins (EXPA) and b-expansins (EXPB). EXPA proteins bind tightly to cellulose and hemicellulose, but they have no hydrolytic activity against these major polysaccharides of the cell wall. Expansins disrupt non-covalent bonding between cellulose microfibrils and matrix glucans, thereby allowing turgor-driven slippage of microfibrils relative to one another. Comparable studies of EXPB binding and hydrolytic activity have not yet been published, but their wall-loosening action is similar to that of EXPA.

Nematodes secrete proteins with sequence similarity to expansins (Qin 2004). Nematode secretions containing these and other cell wall-loosening proteins may assist the rapid penetration of the nematode into the root tissues. However, the highly orchestrated patterns of altered cell growth and syncytium formation would seem to require more subtle spatial and temporal control of cell wall loosening and growth processes that could not be achieved through nematode secretion alone. In addition to the ability to break down

covalent bonds found in plant cell walls through cellulases and pectinases, there is evidence that the potato cyst nematode also secretes a protein having the ability to break non-covalent bonds (Qin 2004). This activity is accomplished by an expansin-like protein discovered in the potato cyst nematode. Expansins soften cell walls by breaking non-covalent bonds between cell wall fibrils, thereby allowing a sliding of fibrils past each other. Wieczorek et al. (2006) analyzed whether members of the expansin gene family are specifically and developmentally regulated during syncytium formation in the roots of *Arabidopsis thaliana*. PCR was used to screen a cDNA library of 5–7-day-old syncytia for expansin transcripts with primers differentiating between 26 alpha- and three beta-expansin cDNAs. AtEXPA1, AtEXPA3, AtEXPA4, AtEXPA6, AtEXPA8, AtEXPA10, AtEXPA15, AtEXPA16, AtEXPA20, and AtEXPB3 could be amplified from the library. In a semiquantitative RT-PCR and a Genechip analysis, AtEXPA3, AtEXPA6, AtEXPA8, AtEXPA10, and AtEXPA16 were found to be upregulated specifically in syncytia, but not to be transcribed in surrounding root tissue. Histological analyses were performed with the aid of promoter:GUS lines and in situ RT-PCR. Results from both approaches supported the specific expression pattern. Among the specifically expressed genes, AtEXPA3 and AtEXPA16 turned out to be of special interest as they are shoot specific in uninfected plants. It was concluded that syncytium formation involves the specific regulation of expansin genes, indicating that the encoded expansins take part in cell growth and cell wall disassembly during syncytium formation.

Griesser and Grundler (2013) investigated gene expression patterns and localization of expansins in a comparative analysis. Expansins are cell wall-loosening proteins involved in growth and cell wall disassembly. The expression of expansins in syncytia of *G. rostochiensis* in tomato and in syncytia and galls induced in *Arabidopsis thaliana* has already been described. They provided additional information on the expression of 10 tomato expansin isoforms, namely, *LeEXPA1*, *LeEXPA2*, *LeEXPA3*, *LeEXPA4*, *LeEXPA5*, *LeEXPA8*, *LeEXPA9*, *LeEXPA10*, *LeEXPA*11, and *LeEXPA18* in 5- and 10-day-old galls of *M. incognita* with sqRT-PCR. They also determined the quantitative expression of seven differentially regulated tomato expansins in syncytia and galls at different developmental stages. They observed a very high induction of *LeEXPA2*, *LeEXPA5*, and *LeEXPA11* with maxima in 10-day-old syncytia and 5-day-old galls. Other members of the gene family were slightly induced in syncytia, whereas in galls only *LeEXPA2*, *LeEXPA5*, and *LeEXPA11* were found to be upregulated. Previous results on the expression of *LeEXPA5* in galls were confirmed, and new detailed information on expansin expression in nematode feeding site was provided. *LeEXPA4* and *LeEXPA5* were localized in syncytia recently, and these results were confirmed with in situ RT-PCR. *LeEXPA1*, *LeEXPA2*, *LeEXPA9*, *LeEXPA11*, and *LeEXPA18* were also detected in 5- and 10-day-old syncytia and neighboring cells. Especially the expression pattern of *LeEXPA2* and *LeEXPA5* was of interest, because of their low expression in uninfected roots but their high induction in nematode feeding sites. These results confirmed that expansins are differentially regulated during the formation of both syncytia and galls and indicate that these genes are involved in cell wall-modifying processes during plant–nematode interactions.

During syncytium development, extensive cell wall modifications take place. Cell wall dissolution occurs during cell wall opening formation, cell walls expand during hypertrophy of syncytial elements, and local cell wall synthesis leads to the thickening of syncytial cell wall and the formation of cell wall ingrowths. Numerous studies revealed that nematodes change expression of plant genes encoding cell wall-modifying proteins including expansins. Expansins poses unique abilities to induce cell wall extension in acidic pH. Fudali et al. (2008) demonstrated that two α-expansin genes *LeEXPA4* and *LeEXPA5* were upregulated in tomato roots infected with *Globodera rostochiensis*. They also presented the most recent results concerning the involvement of plant cell wall-modifying genes in syncytium development and discussed possible practical

applications of this knowledge for developing plants with resistance against nematodes.

10.8.3 Metabolic Enzymes

These enzymes catalyze the conversion of the shikimate pathway product chorismate to prephenate. This process represents a key regulatory mechanism determining the ratio of the aromatic amino acids phenylalanine and tyrosine on one hand and tryptophan on the other. Consequently, this regulatory activity influences the production of the metabolites that have these amino acids as precursors, among which auxin and salicylic acid are of particular interest in plant–parasite interactions. The plant shikimate pathway is found in the plastids from where chorismate also is translocated to the plant cytoplasm. According to the current understanding of chorismate mutase function, nematode-secreted chorismate mutases will deplete the cytoplasmic chorismate pool leading to an increased translocation of chorismate from the plastids, effectively decreasing synthesis of plastid-produced chorismate-dependent metabolites like auxin or salicylic acid. A lack of salicylic acid production in response to nematode chorismate mutase injection could result in a downregulation of plant defenses.

10.8.4 Ubiquitination/Proteasome Functions

The proteasome is a multicatalytic proteinase complex which is characterized by its ability to cleave peptides with Arg, Phe, Tyr, Leu, and Glu adjacent to the leaving group at neutral or slightly basic pH. The proteasome has an ATP-dependent proteolytic activity. The proteasome is a protein-destroying apparatus involved in many essential cellular functions, such as the regulation of cell cycle, cell differentiation, signal transduction pathways, antigen processing for appropriate immune responses, stress signaling, inflammatory responses, and apoptosis (Hirano et al. 2005). It is capable of degrading a variety of cellular proteins in a rapid and timely fashion, and most substrate proteins are modified by ubiquitin before their degradation by the proteasome. The proteasome is a large protein complex consisting of a proteolytic core called the 20S particle and ancillary factors that regulate its activity in various ways.

The most common form is the 26S proteasome containing one 20S core particle and two 19S regulatory particles that enable the proteasome to degrade ubiquitinated proteins by an ATP-dependent mechanism. Another form is the immunoproteasome containing two 11S regulatory particles, PA28 alpha and PA28 beta, which are induced by interferon gamma under the conditions of intensified immune response. Other regulatory particles include PA28 gamma and PA200. Although PA28 gamma also belongs to a family of activators of the 20S proteasome, it is localized within the nucleus and forms a homoheptamer. PA28 gamma has been implicated in the regulation of cell cycle progression and apoptosis. PA200 has been identified as a large nuclear protein that stimulates proteasomal hydrolysis of peptides. The proteasome is in the final common step of protein degradation and is part of a pathway called the ubiquitin–proteasome pathway. Ubiquitin effectively tags proteins and marks them for presentation to the proteasome, where the protein is digested, and ubiquitin is actually recycled in the cell. Ubiquitin is the marking agent to covalently link the protein and present it to the proteasome structure.

Targeted and timed protein degradation is a final and powerful means to regulate gene expression. Cyst nematodes apparently use this mechanism to alter gene expression in parasitized plant cells since these nematodes appear to secrete proteins involved in polyubiquitination, i.e., the process that specifically decorates proteins with ubiquitin protein molecules, thereby targeting these proteins for degradation.

10.8.5 Venom Allergen Proteins

The venom allergen-like proteins form a family of effectors that seems to be conserved among all parasitic nematodes of plants and animals studied to date. The venom allergen-like protein

of *Globodera rostochiensis* Gr-VAP1 interacts with the apoplastic cysteine papain-like proteases Rcr3pim of *Solanum pimpinellifolium* (Lozano Torres et al. 2013). They reported that Gr-VAP1 and Rcr3pim are both required to activate defense-related programmed cell death and resistance to nematodes mediated by the extracellular plant immune receptor Cf-2 in tomato. Thus, Gr-VAP1 is able to trigger defense responses in a host plant of *G. rostochiensis*, but the virulence function of Gr-VAP1 or of any other venom allergen-like protein of a phytonematode is not known. A specific knockdown of Gr-VAP1 expression in *G. rostochiensis* showed that the effector is indeed important for virulence of infective juveniles in host plants. Similarly, the ectopic expression of venom allergen-like proteins in transgenic plants alters their response to nematodes and other plant pathogens. RNAseq analysis of these transgenic plants has shed light on the molecular mechanisms underlying the virulence function of venom allergen-like protein of plant-parasitic nematodes in plants.

Animal and phytonematodes have the capability to remain within the host for a long time. To do so, they have evolved immunoevasive and immunosuppressive strategies. Secretory proteins produced in the esophageal glands of parasitic nematodes likely include suppressors of plant innate immunity (Lozano et al. 2009). A venom allergen protein from *Globodera rostochiensis* (Gr-vap1) was identified, by cDNA-AFLP, as being strongly upregulated in invasive second-stage juveniles. In situ hybridization microscopy showed specific expression of Gr-vap1 in the subventral esophageal glands. Gr-vap1 codes for a secretory protein, including a single SCP/CAP domain. Temporal expression analysis of Gr-vap1 in different developmental stages revealed upregulation in the motile J2s and adult males. Knocking down Gr-vap1 expression, by RNA interference, significantly reduced the infectivity of nematodes on host plants. Protein interaction studies using Gr-vap1 and a tomato root cDNA library, in a yeast two-hybrid screening, resulted in the identification of various interacting host proteins associated with plant immunity. A pull-down assay confirmed the physical interaction of Gr-vap1 with Rcr3, an extracellular cathepsin-like cysteine protease from tomato. Others showed that Rrc3 is required for disease resistance to fungi and oomycetes in plants. However, heterologous expression of nematode VAPs in *Arabidopsis thaliana* caused enhanced susceptibility toward diverse plant pathogens. It was hypothesized that VAPs are important modulators of innate immunity and as such interfere with different host defense response pathways.

These parasitism protein candidates are similar to known proteins whose functions, however, are still unknown or too diverse. This intriguing group of parasitism proteins contains representatives from root-knot nematodes and cyst nematodes that are collectively called “venom allergen proteins” (vaps). Gene sequences for these venom proteins were first described from hymenopteran insects, and vaps were also identified as secreted proteins (ASP) in the animal-parasitic nematode, *Ancylostoma caninum*. Secretory proteins encoded by genes expressed in the esophageal gland cells of phytonematodes have key roles in nematode parasitism of plants (Gao et al. 2001b). Two venom allergen-like protein cDNAs (designated hg-vap-1 and hg-vap-2) were isolated from *Heterodera glycines* gland cell cDNA libraries. Both cDNAs hybridized to genomic DNA of *H. glycines* in Southern blots. The hg-vap-1 cDNA contained an open reading frame encoding 215 amino acids with the first 25 amino acids being a putative secretion signal. The hg-vap-2 cDNA contained an open reading frame encoding 212 amino acids with the first 19 amino acids being a putative secretion signal. Genes of hg-vap-1 and hg-vap-2 contained four introns, which ranged in size from 44 to 574 bp, and five exons ranging in size from 43 to 279 bp. In situ hybridization analyses showed that mRNAs of both vap genes accumulated specifically in the subventral gland cells of *H. glycines* during parasitism. The gland cell-specific expression and the presence of predicted secretion signal peptides in both VAPs suggest that these proteins are secreted from the nematode and may play a role in the infection of host plants by this parasite.

Secretions from the esophageal glands of *Bursaphelenchus xylophilus* play an important

role in pathogenicity (Shifeng Lin et al. 2011). A cluster of three venom allergen-like protein genes and one pseudogene, BxVap-1, BxVap-2, and BxVap-3 and BxVap-P, were identified within a 3.7-kb region. Additionally, three putative modification, transport, and regulatory protein genes were also detected in the same flanking region of the BxVap gene cluster. Genes vap-1, vap-2, and vap-3 were functional and encoded three major allelic variants of PWN venom allergen-like proteins. But BxVap-P was an untranscribed pseudogene. Genes vap-1, vap-2, and vap-3 produced predicted products of 204, 206, and 203 amino acid residues, respectively, including the putative signal peptide sequence at the amino termini. In situ mRNA hybridization analysis showed that the transcripts of genes vap-1, vap-2, and vap-3 accumulated exclusively within the esophageal gland cells of *B. xylophilus.*

Of the three genes encoding the venom allergen-like protein in *B. xylophilus*, BxVap-1 showed the highest transcript levels at the pine-grown propagative stage (Kang et al. 2012). In addition, Western blot and immunohistochemical analyses using anti-BxVap-1 polyclonal antibody verified a specific increase in BxVap-1 expression levels at the pine-grown propagative stage. Using immunohistochemistry, BxVap-1 was detected around the putative esophageal glands and metacarpus, suggesting that BxVap-1 is secreted into the host pine tree and is involved in the parasitic mechanism. To explain the parasitic role of BxVap-1, the migration rate inside pine seedlings of *B. xylophilus* was measured either with or without BxVap-1 knockdown by RNA interference. BxVap-1 knockdown resulted in a significantly lower migration rate in the >6-cm region compared with the control *B. xylophilus*. These results suggest that BxVap-1 is involved in *B. xylophilus* migration, perhaps by suppressing the pine tree defense mechanism.

Venom allergen-like proteins are members of the SCP/Tpx-1/Ag5/PR-1/Sc7 family of eukaryotic secreted proteins. Lu Shunwen et al. (2013) identified a VAP gene (designated GrVAP-1) from *Globodera rostochiensis*. The GrVAP-1 gene contains an open reading frame (660 bp) encoding a putative secreted protein that contains a SCP-like domain and a cysteine-rich C-terminus. Southern blot analysis indicated the presence of multiple copies of the GrVAP-1 gene in the *G. rostochiensis* genome. The GrVAP-1 genomic DNA contains three introns with sizes ranging from 48 to149 bp. In situ mRNA hybridization showed the transcript of GrVAP-1 accumulated exclusively within the subventral esophageal gland cells of both preparasitic second-stage juvenile and parasitic stages of *G. rostochiensis*. RT-PCR analysis revealed that the GrVAP-1 gene was highly expressed in both preparasitic J2 and parasitic stages, but its expression was low in the egg stage.

Secretory proteins encoded by genes expressed in the esophageal gland cells of phytonematodes play key roles in nematode parasitism of plants. Two venom allergen-like protein cDNAs (designated hg-vap-1 and hg-vap-2) were isolated from Heterodera glycines gland cell cDNA libraries (Gao et al. 2001a). Both cDNAs hybridized to genomic DNA of *H. glycines* in Southern blots. The hg-vap-1 cDNA contained an open reading frame encoding 215 amino acids with the first 25 amino acids being a putative secretion signal. The hg-vap-2 cDNA contained an open reading frame encoding 212 amino acids with the first 19 amino acids being a putative secretion signal. Genes of hg-vap-1 and hg-vap-2 contained four introns, which ranged in size from 44 to 574 bp, and five exons ranging in size from 43 to 279 bp. In situ hybridization analyses showed that mRNAs of both vap genes accumulated specifically in the subventral gland cells of *H. glycines* during parasitism.

10.8.6 Calreticulin

Calreticulin, also known as calregulin, CRP55, CaBP3, calsequestrin-like protein, and endoplasmic reticulum resident protein 60 (ERp60), is a protein that binds to misfolded proteins and prevents them from being exported from the endoplasmic reticulum to the Golgi bodies. Esophageal secretions from endoparasitic sedentary nematodes have key roles throughout plant parasitism, in particular, during the invasion of the root tissue

and the initiation and maintenance of the nematode feeding site essential for nematode development (Jaubert et al. 2005). Calreticulin-like proteins are secreted from other parasitic nematodes and, therefore, are good candidates for being involved in parasite–host interactions. A calreticulin-like protein preceded by a signal peptide was identified as being produced in the subventral glands of a root-knot nematode.

Root-knot nematodes are obligate biotrophic parasites that settle close to the vascular tissues in roots, where they induce the differentiation of specialized feeding cells and maintain a compatible interaction for 3–8 weeks (Jaouannet et al. 2013). Transcriptome analyses of the plant response to parasitic infection showed that plant defenses are strictly controlled during the interaction. This suggests that, similar to other pathogens, this nematode secretes effectors that suppress host defenses. It was showed that Mi-CRT, a calreticulin (CRT) secreted by the nematode into the apoplasm of infected tissues, played an important role in infection success, because Mi-CRT knockdown by RNA interference affected the ability of the nematodes to infect plants. Stably transformed *Arabidopsis thaliana* plants producing the secreted form of Mi-CRT were more susceptible to nematode infection than wild-type plants. They were also more susceptible to infection with another root pathogen, the oomycete, *Phytophthora parasitica*. Mi-CRT overexpression in *A. thaliana* suppressed the induction of defense marker genes and callose deposition after treatment with the pathogen-associated molecular pattern elf18. These findings showed that Mi-CRT secreted in the apoplasm by the nematode has a role in the suppression of plant basal defenses during the interaction.

The secretion in plants of esophageal cell wall-degrading enzymes by migratory juveniles has been shown, suggesting a role for these enzymes in the invasion phase. Nevertheless, the secretion of an esophageal gland protein into the nematode feeding site by nematode sedentary stages has never been demonstrated. The calreticulin Mi-CRT is a protein synthesized in the esophageal glands of the root-knot nematode *Meloidogyne incognita*. After three-dimensional modeling of the Mi-CRT protein, a surface peptide was selected to raise specific antibodies. In plants, immunolocalization showed that Mi-CRT is secreted by migratory and sedentary stage nematodes, suggesting a role for Mi-CRT throughout parasitism. During the maintenance of the nematode feeding site, the secreted Mi-CRT was localized outside the nematode at the tip of the stylet. In addition, Mi-CRT accumulation was observed along the cell wall of the giant cells that compose the feeding site, providing evidence for a nematode esophageal protein secretion into the nematode feeding site.

10.8.7 Annexin

Annexin is a common name for a group of cellular proteins. The annexins are a class of calcium-dependent, phospholipid-binding proteins that are presumed to underlie a number of calcium-regulated activities on membrane surfaces or between interacting membranes. The mRNA for a secretory isoform of an annexin-like protein was identified as being expressed in the dorsal gland of the soybean cyst nematode. Annexin genes represent a large family coding for calcium-dependent phospholipid-binding proteins with a wide range of reported functions. An annexin gene also had been identified from the potato cyst nematode *G. pallida*. This gene coded for a protein that was immunodetected in the excretory/secretory products of this nematode despite the fact that the protein did not contain a signal peptide and was not present in the esophageal glands.

Nematode parasitism genes encode secreted effector proteins that play a role in host infection (Patel et al. 2010). A homologue of the expressed Hg4F01 gene of *Heterodera glycines,* encoding an annexin-like effector, was isolated by these authors in the related *Heterodera schachtii* to facilitate the use of *Arabidopsis thaliana* as a model host. Hs4F01 and its protein product were exclusively expressed within the dorsal esophageal gland secretory cell in the parasitic stages of *H. schachtii.* Hs4F01 had a 41 % predicted amino acid sequence identity to the nex-1 annexin of *C.*

elegans and 33 % identity to annexin-1 (annAt1) of Arabidopsis, it contained four conserved domains typical of the annexin family of calcium- and phospholipid-binding proteins, and it had a predicted signal peptide for secretion that was present in nematode annexins of only *Heterodera* spp. Constitutive expression of Hs4F01 in wild-type *Arabidopsis* promoted hypersusceptibility to *H. schachtii* infection. Complementation of an AnnAt1 mutant by constitutive expression of Hs4F01 reverted mutant sensitivity to 75-mM NaCl, suggesting a similar function of the Hs4F01 annexin-like effector in the stress response by plant cells. Yeast two-hybrid assays confirmed a specific interaction between Hs4F01 and an *Arabidopsis* oxidoreductase member of the 2OG-Fe(II) oxygenase family, a type of plant enzyme demonstrated to promote susceptibility to oomycete pathogens. RNA interference assays that expressed double-stranded RNA complementary to Hs4F01 in transgenic *Arabidopsis* specifically decreased parasitic nematode Hs4F01 transcript levels and significantly reduced nematode infection levels. The combined data suggested that nematode secretion of an Hs4F01 annexin-like effector into host root cells may mimic plant annexin function during the parasitic interaction.

The recent characterization of an annexin, nex-1, from the nematode, *C. elegans,* suggested that this annexin was associated with collagen secretion and/or deposition, membrane trafficking during autophagocytosis of yolk granules, and with the coordinated folding and unfolding of cell surface membranes during the opening and closing of the spermathecal valve (Creutz et al. 1996). These activities parallel a number of activities the annexins have been postulated to underlie in mammalian cells, such as cartilage formation and mineralization, and the chaperoning of membrane interactions in endocytosis and exocytosis. However, the initial isolation and localization of the major nematode annexin left several important issues unresolved (Daigle and Creutz 1999). First, progress in the sequencing of the nematode genome had revealed the presence of two additional nematode annexin genes (nex-2 and nex-3; Creutz et al. 1996), and more recently, a fourth has been encountered for which we propose the name nex-4 (GenBank acquisition number U88315). Since only a single annexin protein, nex-1, was isolated, it has not been clear whether the other annexin genes are expressed. It is important to know whether these genes are active in order to design and interpret annexin gene knockout experiments since the different annexins may be redundant in function. Evidence was provided for active transcription of mRNA from the nex-2 and nex-3 genes.

A second area that was not addressed in the initial description of the nematode nex-1 annexin was the characterization of this protein as a lipid-binding and aggregating protein regulated by calcium. Different members of the mammalian annexin protein family have characteristic calcium sensitivities and abilities to promote membrane aggregation. Since the nex-1 annexin is 39–42 % identical in sequence to all mammalian annexins, it is not possible to speculate to which mammalian annexin the nex-1 annexin is most closely related on the basis of sequence data alone. They demonstrated that recombinant nex-1 protein produced in a yeast expression system can bind and aggregate biological membranes in a calcium-dependent manner. A third important unresolved issue concerns the identity of the annexin(s) that was localized previously in the nematode using an antiserum directed against nex-1. Because of sequence similarities among the annexins, it is possible that the antiserum reacted with other annexins as well. The specific nex-1 promoter was used to drive the expression of GFP to permit localization of cells that actively express the nex-1 gene. This has led to the discovery of a new site of nex-1 expression, the hypodermal cells of the body wall. A fourth outstanding issue was whether the nex-1 annexin is an intracellular or an extracellular protein.

Although often presumed to be intracellular proteins as a family, some annexins are also released from cells. In some areas of the nematode, particularly the grinder and the spermathecal valve, the nex-1 annexin is highly enriched on the cell surface membrane. However, even at the electron microscope level, it was not possible to resolve whether the protein may be partially

exposed to the extracellular space (Creutz et al. 1996). Daigle and Creutz (1999) injected fluorescently labeled antibodies into non-permeabilized nematodes to determine if any nex-1 is exposed on the extracellular surfaces of nematode tissues.

The nematode, like higher animals and green plants, expresses a diverse family of annexins (Daigle and Creutz 1999). The major annexin, nex-1, is localized to an array of different cell types and may underlie a multiplicity of functions or a common function in an assortment of cells. The most evocative localization is on the intracellular faces of the folds of the membranes in the spermathecal valve where it is likely the annexin functions in the coordinated, calcium-regulated folding of these membranes. Whether this will prove to be a representative model for the role of other annexins in membrane trafficking events will hopefully be revealed by annexin gene knockout experiments in this and other model organisms.

10.8.8 Avirulence Genes

Single pathogen genes that are required for R gene-mediated resistance have been identified in bacteria, viruses, and fungi. There is genetic evidence for avirulence genes in *Globodera rostochiensis* that correspond to the resistance gene *H1*. Genetic analyses of inbred strains of soybean cyst nematodes have identified dominant and recessive determinants of parasitism on different soybean lines. The root-knot species against which *Mi* is effective does not reproduce sexually, making Mendelian analysis of its avirulence and pathogenicity genes impossible. Nearly isogenic strains of root-knot nematodes that differ in virulence in the presence of *Mi* have been used to investigate pathogenicity. Differential-marker analysis identified a polymorphic band that was present in avirulent strains but absent from closely related virulent strains of *M. incognita.* The corresponding gene, *Meloidogyne* avirulence protein-1 (map-1), was cloned and found to encode a protein that localized to nematode amphidial secretions. Secretions from the virulent and avirulent nematodes were not compared, however, and functional analysis of map-1 has not yet been carried out. A transcript that is present in avirulent but lacking in virulent *Meloidogyne javanica* has also been identified. However, this gene does not resemble map-1, suggesting that there may be more than one gene that can mediate nematode recognition in tomato plants that have the *Mi* gene.

Although it is currently not possible to predict the number of members of the parasitome of plant-parasitic nematodes, only a small fraction of the estimated 15,000–20,000 genes (based on the ~19,000 genes of *Caenorhabditis elegans*) of a plant-parasitic nematode should be expected to encode proteins that have a direct role in parasitism. The first members of a parasitome to be cloned from plant-parasitic nematodes were ß-1,4-endoglucanases (cellulases) developmentally expressed in the two subventral gland cells of *Heterodera glycines* and *Globodera rostochiensis* (Yan et al. 1998). Two cellulase cDNAs in each cyst nematode species (Hg-eng-1 and Gr-eng-1) encode a predicted secretion signal peptide, cellulase catalytic domain, small peptide linker, and a cellulose-binding domain (CBD). A smaller cellulase cDNA in *G. rostochiensis* (Gr-eng-2) lacks the CBD, and one (Hg-eng-2) from *H. glycines* is missing both the peptide linker and CBD. The presence of a CBD presumably enhances cellulase activity toward crystalline cellulose. mRNA in situ hybridization and immunolocalization with anti-ENG polyclonal sera confirmed that eng-1 and eng-2 were expressed exclusively within the subventral esophageal gland cells of both nematode species (Smant et al. 1998).

Expression of Mj-cm-1 is localized within the subventral esophageal gland cells of parasitic *M. javanica* by mRNA in situ hybridization and with antisera generated to the product of Mj-cm-1 (Hussey et al. 2002a, b). Chorismate mutase initiates the conversion of chorismate, the end product of the shikimate pathway, to the aromatic amino acids, phenylalanine and tyrosine. The secretion of Mj-cm-1 into the cytosol of a plant cell could potentially alter the spectrum

of chorismate-dependent compounds, which, among other functions, are involved in cell wall formation, hormone biosynthesis, and synthesis of defense compounds in plants. Alternatively, these compounds (tyrosine) could be used by the nematode in cuticle formation.

The most evolutionary advanced adaptations for plant parasitism by nematodes are the products of parasitism genes expressed in their esophageal gland cells and secreted through their stylet into the host tissue to control the complex process of parasitism. Molecular analyses of nematode parasitism genes are revealing the complexity of the tools a nematode possesses that enable it to attack plants (Baum et al. 2007). RNA fingerprinting has been used to analyze differential gene expression between preparasitic and parasitic stages of *M. incognita*. A cDNA encoding for a secretory cellulose-binding protein (Mi-cbp-1) was isolated using this method (Ding et al. 1998).

Nematode esophageal gland cell secretions are released through valves within ampulla for transport out of the stylet (feeding spear) into host tissues. Cell wall-modifying proteins (endoglucanases, pectinases, hemicellulases, and expansin) may be secreted to aid the migration of infective juveniles through host plant tissues. Other nematode gland cell secretions might have multiple roles in the formation of specialized feeding cells by the nematode, including effects on host cell metabolism by secreted chorismate mutase; signaling by secreted nematode peptides such as homologues to plant CLAVATA/ESR-related peptides; selective degradation of host proteins through the ubiquitin–proteasome pathway by UBQ, S-phase kinase-associated protein 1 (Skp-1), and RING-H2 secreted from the nematode; and potential effects of secreted nematode proteins that contain nuclear localization signals within the host cell nucleus.

The first members of a parasitome to be cloned from plant-parasitic nematodes were ß-1,4-endoglucanases (cellulases) in *Heterodera glycines* and *Globodera rostochiensis* (Dong and Opperman 1997). Parasitism gene may be active in any or all part of the parasitic cycle. The ability of nematode to live on plant hosts involves multiple parasitism genes. Root-knot and cyst nematodes have evolved to alter gene expression in specific root cell to modify them into specialized cells. Genes for resistance or parasitism are altered by modifier genes present in each genome and are influenced by various environmental factors, which may be biotic or abiotic.

With *C. elegans*, the phylum Nematoda contains one of the best-studied model organisms for genetics and with this an excellent baseline for comparative genetic studies. Genetic work requires methods to induce, isolate, cross, and characterize mutants and, as probably the most challenging element, ways of physically identifying the genes that carry the mutations isolated based on their phenotypes. In *C. elegans*, this has been traditionally achieved by a process called positional cloning. This approach requires a dense, high-quality genetic map for accurate genetic mapping and a physical map, ideally a full genome sequence that is highly interlinked with the genetic map. In addition, transgenic technology is required to narrow down genomic regions during mapping and for gene verification after final identification.

A genetic analysis of parasitic ability in *Heterodera glycines* was performed by Ke Dong and Charles H. Opperman (1997). To identify and characterize genes involved in parasitism, they developed three highly inbred *H. glycines* lines, OP20, OP25, and OP50, for use as parents for controlled crosses. Through these crosses, they have identified genes obtained in the inbred parents that control the reproduction of the nematode on hosts that carry resistance genes. These genes, designated as ror-* for reproduction on a resistant host, segregate in a normal Mendelian fashion as independent loci. Host range tests of F(1) generation progeny indicated that at least one parasitism gene in both the OP20 and OP50 lines for host PI 88788 was dominant. Parasitism genes in OP50 for hosts "Peking" and PI 90763 were recessive. Two types of single female descent populations, a single backcrossed BC(1) F(2) derived and a double backcrossed BC(2) F(1) derived, were established on the susceptible

soybean cultivar "Lee 68." Host range tests for parasitism in these lines demonstrated the presence of two independent genes in OP50, one for host PI 88788 designated ror-1 and one for host PI 90763 designated ror-2. OP20 carries two independent genes for parasitism on PI 88788, designated as alleles kr3 and kr4.

One of the most surprising and interesting findings in the discovery of nematode parasitism genes is the large number of candidate parasitism genes that encode novel proteins (Hussey et al. 2002a, b). Remarkably, over 70 % of the parasitism genes have no homology with functionally annotated genes in the databases. These pioneer parasitism genes seem to represent genes unique for nematode parasitism of plants, a hypothesis supported by the unique and complex interactions that sedentary endoparasites have with their host plants. These parasitism genes may have evolved from "basal" nematode genes, while other parasitism genes, e.g., cell wall-degrading enzymes and chorismate mutase, may have been acquired by horizontal gene transfer from prokaryotic microbes. Identifying the complete profile of parasitism genes expressed throughout the parasitic cycle of a nematode is the key to understanding the molecular basis of nematode parasitism of plants and defining what makes a nematode a plant parasite. The nematode parasitism genes being discovered are revealing the complexity of the tools a nematode possesses that enable it to attack plants and paint a more elaborate picture of host cellular events under specific control by the parasite than previously hypothesized. Interspecific and intraspecific comparison of the structure of parasitism genes encoding stylet secretions that induce feeding cell formation will also provide the knowledge that should lead to establishing a genetic basis for host range specificity among nematode species or races. Understanding this genetic variability will have an important positive effect on the development and deployment of sustainable nematode management strategies.

Full tool sets for the isolation and systematic study of mutations in known genes generated by forward and reverse genetics are currently available only for two nematode species other than *C. elegans*, namely *Caenorhabditis briggsae* and *P. pacificus*. For both species, relatively dense genetic maps, which are well anchored in the genome, allow the positional cloning of genes (Koboldt et al. 2010). Mutations in molecularly defined genes have been isolated by polymerase chain reaction-based screening for small deletions. Finally, transgenic techniques are available for both species (Schlager et al. 2009). Comparative genetic work in these species has concentrated on developmental processes that are very well understood in *C. elegans*. These studies have offered interesting insights into how the genetic control of development can change during evolution. The induction of the vulva in *C. elegans* is one of the best-studied genetic processes in animal development.

The switch of the signaling system involves a novel regulatory linkage of Wnt signaling, which is unknown from other organisms (Wang and Sommer 2011). In *C. elegans* vulva development, Wnt signaling is also required but for different processes. It acts prior to induction to maintain the competence of vulval precursor cells to respond to the inductive signal, and it is used again after the induction for the correct specification of tissue polarity. Secretory proteins encoded by genes expressed in the esophageal gland cells of plant-parasitic nematodes have key roles in nematode parasitism of plants (Gao et al. 2001a). Two venom allergen-like protein cDNAs (designated hg-vap-1 and hg-vap-2) were isolated from *Heterodera glycines* gland cell cDNA libraries. Both cDNAs hybridized to genomic DNA of *H. glycines* in Southern blots. The hg-vap-1 cDNA contained an open reading frame encoding 215 amino acids with the first 25 amino acids being a putative secretion signal. The hg-vap-2 cDNA contained an open reading frame encoding 212 amino acids with the first 19 amino acids being a putative secretion signal. Genes of hgvap- 1 and hg-vap-2 contained four introns, which ranged in size from 44 to 574 bp, and five exons ranging in size from 43 to 279 bp. In situ hybridization analyses showed that mRNAs of both vap genes accumulated specifically in the subventral gland cells of *H. glycines* during parasitism. The gland cell-specific expression and presence of predicted

secretion signal peptides in both VAPs suggest that these proteins are secreted from the nematode and may play a role in the infection of host plants by this parasite.

Root-knot nematodes alter plant cell growth and development by inducing the formation of giant cells for feeding. Nematodes inject secretions from their esophageal glands through their stylet and into plant cells to induce giant cell formation. *Meloidogyne javanica* chorismate mutase 1 (Mj-cm-1) is one such esophageal gland protein likely to be secreted from the nematode as giant cells form (Doyle and Lambert 2003). Mj-cm-1 has two domains, an N-terminal chorismate mutase (CM) domain and a C-terminal region of unknown function. It is the N-terminal CM domain of the protein that is the predominant form produced in root-knot nematodes. Transgenic expression of Mj-cm-1 in soybean hairy roots resulted in a phenotype of reduced and aborted lateral roots. Histological studies demonstrated the absence of vascular tissue in hairy roots expressing Mj-cm-1. The phenotype of Mj-cm-1 expressed at low levels can be rescued by the addition of indole-3-acetic acid (IAA), indicating Mj-cm-1 overexpression reduces IAA biosynthesis. It was proposed that Mj-cm-1 lowers IAA by causing a competition for chorismate, resulting in an alteration of chorismate-derived metabolites and, ultimately, in plant cell development. It was hypothesized that Mj-cm-1 was involved in allowing nematodes to establish a parasitic relationship with the host plant.

Genetic variation in *Meloidogyne incognita* virulence against the tomato *Mi* resistance gene was investigated by Castagnone-Sereno et al. (1994). Resistance to the parthenogenetic *Meloidogyne incognita* is controlled in tomato by the single dominant gene *Mi,* against which virulent pathotypes are able to develop. Isofemale lines (i.e., families) were established from a natural avirulent isolate of *M. incognita* in order to study the genetic variability and inheritance of the nematode virulence. From the progeny of individual females, the production of egg masses on the root system of the Mi-resistant tomato “Piersol” was analyzed in artificial selection experiments. A family analysis revealed, after two successive generations, a strongly significant variation between the 63 isofemale lines tested, and the results obtained for the mothers and their daughters were also significantly correlated. These results together clearly demonstrated the existence of a genetic variability and inheritance for this character. In a second experiment, a four-generation selection was performed on 31 other isofemale lines. The results revealed a significant response to selection apparently limited only to the two families able to produce, in first generation, a significant minimal egg mass number on the resistant cultivar.

Richard Janssen et al. (1991) crossed a virulent and an avirulent inbred line of *G. rostochiensis* to determine the genetics of virulence to the resistance gene HI of *Solanum tuberosum* ssp. *andigena* CPC 1673. The 3:1 segregation in avirulent and virulent larvae of the FZ generation, obtained by selfing the FI, showed that virulence to the HI gene is controlled by a single major recessive gene. The virulence percentages of the FI generations agreed with this finding. Reciprocal crosses showed no evidence of sex-linked inheritance of virulence. The cloning parasitism genes encoding secretory proteins expressed in the esophageal gland cells are the key to understanding the molecular basis of nematode parasitism of plants (Gao et al. 2001b). Suppression subtractive hybridization (SSH) with the microaspirated contents from *Heterodera glycines* esophageal gland cells and intestinal region was used to isolate genes expressed preferentially in the gland cells of parasitic stages. Twenty-three unique cDNA sequences from a SSH cDNA library were identified and hybridized to the genomic DNA of *H. glycines* in Southern blots. Full-length cDNAs of 21 clones were obtained by screening a gland cell long-distance polymerase chain reaction cDNA library. Deduced proteins of ten clones were preceded by a signal peptide for secretion, and PSORT II computer analysis predicted eight proteins as extracellular, one as nuclear, and one as plasmalemma localized. In situ hybridization showed that four of the predicted extracellular clones were expressed specifically in the dorsal gland cell, one in the subventral gland cells and three in the intestine in *H. glycines.* The predicted

nuclear clone and the plasmalemma-localized clone were expressed in the subventral gland cells and the dorsal gland cell, respectively. SSH is an efficient method for cloning putative parasitism genes encoding esophageal gland cell secretory proteins that may have a role in *H. glycines* parasitism of soybean.

Several resistance genes have been identified and genetically mapped for *H. glycines*; however, resistance levels in many soybean cultivars are not durable. Some older cultivars are no longer resistant to certain *H. glycines* populations in many production areas, especially if a soybean monoculture has been practiced. Past soybean registration reports showed that all resistant cultivars developed in public institutions from the mid-1960s to the present were derived from five plant introductions. This narrow genetic background is fragile. To further complicate the issue, soybean–*H. glycines* genetic interactions are complex and poorly understood (Re doong et al. 1997). Studies to identify soybean resistance genes sometimes have overlapped, and the same genes may have been reported several times and designated by different names. Nevertheless, many potential resistance genes in existing germplasm resources have not yet been characterized. Clearly, it is necessary to identify new resistance genes, develop more precise selection methods, and integrate these resistance genes into new cultivars. Rational deployment of resistant cultivars is critical to future sustained soybean production.

Within the genus *Caenorhabditis,* hermaphroditism has evolved multiple times. Baldi et al. (2009) showed that reducing the activity of only two genes is required to transform *C. remanei* females into self-fertile hermaphrodites. Lowering, but not eliminating, the activity of *tra-2*, a key component of somatic and germ line sex determination in *C. elegans*, was sufficient to allow spermatogenesis to occur in addition to oogenesis in *C. remanei*. However, the sperm formed was not activated. Sperm activation and subsequent self-fertilization were achieved by reducing the activity of *swm-1*, a gene known to prevent premature sperm activation in *C. elegans*.

The genetic analysis of parasitic nematodes, so far, is rather rudimentary because of technical constrains. Usually, the sexually reproducing worms are within their hosts, rendering them difficult to access and manipulate, except for a few cases where in addition to parasitic adults, free-living adults also occur, e.g., *Strongyloides* spp. (Grant et al. 2006). Nevertheless, efforts to make parasitic nematodes amenable to genetic analysis have been made and some groundwork laid out. Strategies to perform controlled crosses between defined isolates or even individuals have been developed for a few parasitic nematodes and molecular genetic markers were isolated (Eberhardt et al. 2007). Although genetic maps are published for three phytonematodes, viz., *Meloidogyne hapla* and *Heterodera glycines* (Atibalentja et al. 2005) and *Globodera rostochiensis* (Rouppe van der Voort et al. 1999), these methods and tools are not yet sufficient for mutational analysis and positional cloning of genes but have already been used successfully to elucidate modes of inheritance and to characterize reproductive strategies.

Protocols for the experimental induction of mutations have been reported for very few parasitic or parasitoid nematodes (Viney et al. 2002; Zioni Cohen-Nissan et al. 1992). In addition, spontaneous mutants were found and characterized genetically in several parasitic nematodes, i.e., worms that are virulent for otherwise resistant hosts or resistant against certain nematicidal drugs and pose an enormous economic and medical problem. The main reason why more effort has not so far been made to isolate mutants is probably because there was no straightforward way to identify the gene in which the mutation occurred made to isolate mutants in parasitic nematodes.

Amplified fragment length polymorphism fingerprinting of three pairs of *Meloidogyne incognita* near-isogenic lines was used to identify markers differential between nematode genotypes avirulent or virulent against the tomato *Mi* resistance gene (Semblat et al. 2001). One of these sequences, present only in the avirulent lines, was used as a probe to screen a cDNA library from second-stage juveniles and allowed cloning of a cDNA encoding a secretory protein. The putative full-length cDNA, named *map-1*, encoded a 458-amino acid protein containing a

predictive N-terminal secretion signal peptide. The MAP-1 sequence did not show any significant similarity to proteins deposited in databases. The internal part of the protein, however, was characterized by highly conserved repetitive motives of 58 or 13 aa. Reverse transcription-polymerase chain reaction experiments confirmed that *map-1* expression was different between avirulent and virulent near-isogenic lines. In PCR reactions, *map-1*-related sequences were amplified only in nematode populations belonging to the three species against which the *Mi* gene confers resistance: *M. arenaria*, *M. incognita*, and *M. javanica*. Polyclonal antibodies raised against a synthetic peptide deduced from the MAP-1 sequence strongly labeled J2 amphidial secretions in immunofluorescence microscopy assays, suggesting that MAP-1 may be involved in the early steps of recognition between (resistant) plants and (avirulent) nematodes.

10.9 Defense Signaling

Complex defense signaling pathways, controlled by different hormones, are involved in the reaction of plants to a wide range of biotic and abiotic stress factors. Kamrun Nahar et al. (2011) studied the ability of salicylic acid, jasmonate (JA), and ethylene (ET) to induce systemic defense in rice (*Oryza sativa*) against *Meloidogyne graminicola*. Exogenous ET (ethephon) and JA (methyl jasmonate) supply on the shoots induced a strong systemic defense response in the roots, exemplified by a major upregulation of pathogenesis-related genes *OsPR1a* and*OsPR1b*, while the salicylic acid analog BTH (benzo-1,2,3-thiadiazole-7-carbothioic acid *S*-methyl ester) was a less potent systemic defense inducer from shoot to root. Experiments with JA biosynthesis mutants and ET-insensitive transgenics showed that ET-induced defense requires an intact JA pathway, while JA-induced defense was still functional when ET signaling was impaired. Pharmacological inhibition of JA and ET biosynthesis confirmed that JA biosynthesis is needed for ET-induced systemic defense, and quantitative real-time reverse transcription-polymerase chain reaction data revealed that ET application onto the shoots strongly activates JA biosynthesis and signaling genes in the roots. It was observed that the JA pathway plays a pivotal role in rice defense against root-knot nematodes. The expression of defense-related genes was monitored in root galls caused by *M. graminicola*. Different analyzed defense genes were attenuated in root galls caused by the nematode at early time points after infection. However, when the exogenous defense inducers ethephon and methyl jasmonate were supplied to the plant, the nematode was less effective in counteracting root defense pathways, hence making the plant more resistant to nematode infection.

Interaction between the Avr gene product and the R gene product triggers a series of signaling responses. These result in the biosynthesis of salicylic acid which acts as a central signaling intermediate in plant defense. Salicylic acid in turn triggers both local and systemic responses. These include programmed cell death at the site of infection, local resistance to the pathogen. Salicylic acid is part of the Mi-1-mediated defense response to root-knot nematode in tomato. Mi-1 gene of tomato confers resistance against three species of root-knot nematode in tomato. Transformation of tomato carrying Mi-1 with a construct expressing NahG, which encodes salicylate hydroxylase, a bacterial enzyme that degrades salicylic acid (SA) to catechol, results in partial loss of resistance to root-knot nematodes. These results indicate that SA is an important component of the signaling that leads to nematode resistance and the associated hypersensitive response (Branch et al. 2004).

10.10 Molecular Basis for Nematode Resistance

According to gene-for-gene model, for each resistance gene in the host, there is a corresponding gene for avirulence in the pathogen, and for each virulence in the pathogen, there is a gene for susceptibility in host plant. A loss or alteration to either the plant resistance (R) gene or the pathogen avirulence (Avr) gene leads to disease

(compatibility) interactions involved in R gene and Avr gene incompatibility.

10.10.1 Gene-for-Gene Hypothesis

For resistance (incompatibility) to occur, complementary pairs of dominant genes must be present in the host and pathogen. These genes are referred to as resistance (host) and avirulence (pathogen). Altering either of these genes leads to compatibility (disease). The mechanisms of resistance most likely involve interaction between the Avr protein (an elicitor) and the R gene product (the receptor). This theory proposes that HR will occur when product of plant resistance gene (*R*) interacts with product of pathogen virulence or avirulence gene (*Avr*).

10.10.2 Steps Involved

Major steps include the following: pathogen enters plant cell via wounds or connection with infected cells; protein and other molecules are released by the pathogen; R gene products bind to certain molecules from pathogens (Avr gene products); binding activates R gene product and triggers protective hypersensitivity response; and when R and Avr gene products do not match, no hypersensitivity response occurs and plant is susceptible to disease.

Disease resistance requires a dominant resistance (R) gene in the plant and a corresponding avirulence (Avr) gene in the pathogen. R genes are presumed to enable plants to detect Avr gene-specified pathogen molecules or initiate signal transduction to activate defenses and possess the capacity to evolve new R gene specificities rapidly.

10.11 Identification of Resistance (R) Genes

Most R genes are dominant, as are their cognate pathogen avirulence (Avr) genes. Plants possess many R genes active against many different pathogens. R genes are often found clustered on chromosomal loci. Plant breeders have successfully introduced resistance through introgression of foreign R genes.

In general, plant disease R genes include one or more of the following structural motifs, i.e., nucleotide-binding site (NBS), leucine-rich repeat (LRRs), or serine/threonine protein kinase domains. At present the largest number of known resistance proteins belongs to a class that contains both NBS and LRR motifs. Members of this group confer resistance to a number of nematodes (Ellis and Jones 1998). Most encode proteins that carry a structural motif with a repeating pattern of 20–30 amino acids called a leucine-rich repeat (LRR). LRR motifs participate in protein–protein interactions in a wide range of organisms (Kobe and Deisenhofer 1995).

The tomato *Mi*-1 gene confers resistance against root-knot nematodes and a biotype of the potato aphid (*Macrosiphum euphorbiae*). Four mutagenized Mi-1/Mi-1 tomato populations were generated and screened for altered root-knot nematode resistance. Four independent mutants belonging to two phenotypic classes were isolated. One mutant was chosen for further analyses; rme1 (for resistance to *Meloidogyne*) exhibited levels of infection comparable with those found on susceptible controls. Molecular and genetic data confirmed that rme1 has a single recessive mutation in a locus different from Mi-1. Cross sections through galls formed by feeding nematodes on rme1 roots were identical to sections from galls of susceptible tomato roots. In addition to nematode susceptibility, infestation of rme1 plants with the potato aphid showed that this mutation also abolished aphid resistance. A study was conducted to determine whether Rme1 functions in a general disease-resistance pathway, the response against *Fusarium oxysporum f.* sp. *lycopersici* race 2, mediated by the I-2 resistance gene (De Ilarduya et al. 2001). Both rme1 and the wild-type plants were equally resistant to the fungal pathogen. These results indicated that Rme1 does not play a general role in disease resistance but may be specific for *Mi*-1-mediated resistance.

Mi was introduced into cultivated tomato, *Lycopersicon esculentum*, from its wild relative *L. peruvianum* in the early 1940s (Smith 1944). With the assistance of linked markers, beginning with the isozyme marker *Aps-1* and more recently with DNA markers such as *Rex-1*, *Mi* has been incorporated into many modern tomato cultivars (Williamson et al. 1994).

10.12 Identified R Genes Against Nematodes

Gene from the wild relative of sugar beet, *Beta procumbens*, has been used in the isolation of resistant genes. The *Hs1^{pro-1}* locus confers resistance to the beet cyst nematode (*Heterodera schachtii*), a major pest in the cultivation of sugar beet (*Beta vulgaris*). Gpa-2 gene confers resistance against *Globodera pallida.* The *Hs1^{pro-1}* gene was cloned with the use of genome-specific satellite markers and chromosomal markers (Cai et al. 1997). Rk gene of wild species of cowpea is resistant against *Meloidogyne arenaria* and *M. incognita*, with moderate resistance against *M. javanica*. Multiple sites in broad-based resistance would increase the effectiveness of host plant resistance. Improved cultivars are being developed that carry the broad-based resistance gene Rk2 (Ehlers et al. 2000).

Root-knot nematode resistance of F1 progeny of an intraspecific hybrid (*Lycopersicon peruvianum* var. *glandulosum* Acc. No. 126443 × *L. peruvianum* Acc. No. 270435), *L. esculentum* cv. Piersol (possessing resistance gene *Mi*), and *L. esculentum* cv. St. Pierre (susceptible) was compared. Resistance to (1) isolates of two *Meloidogyne incognita* populations artificially selected for parasitism on tomato plants possessing the *Mi* gene, (2) the wild-type parent populations, (3) four naturally occurring resistance (*Mi* gene)-breaking populations of *M. incognita* and *M. arenaria* and two undesignated *Meloidogyne* spp., and (4) a population of *M. hapla* was indexed by numbers of egg masses produced on root systems in a greenhouse experiment. Artificially selected *M. incognita* isolates reproduced abundantly on Piersol, but not ($P = 0.01$) on resistant F1 hybrids. Thus, the gene(s) for resistance in the F1 hybrid differs from the *Mi* gene in Piersol. Four naturally occurring resistance-breaking populations reproduced extensively on Piersol and on the F1 hybrid, demonstrating ability to circumvent both types of resistance. *Meloidogyne hapla* reproduced on F1 hybrid plants, but at significantly ($P = 0.01$) lower levels than on Piersol (Roberts et al. 1990).

The gene of wild species of tomato, *Solanum lycopersicum*, has been exploited for the resistance. *Mi-1* confers resistance to root-knot nematodes (*Meloidogyne* spp.) and also confers resistance to sweet potato whitefly (*Bemisia tabaci*). Allele *Sgt1, Rar1*, and *Hsp90* are known to participate early in resistance gene signaling pathways (Kishor et al. 2007). Resistance can be either broad (effective against several nematode species) or narrow (controlling only specific biotypes of a species, also variously referred to as races or pathotypes). For other sources of resistance, inheritance may be controlled by single gene called monogenic (vertical resistance) or controlled by many genes called polygenic (horizontal resistance). Several dominant or semidominant resistance genes have been identified and mapped to chromosomal locations or linkage groups (Tables 10.3 and 10.4) (Williamson and Kumar 2006).

10.13 Origin of Parasitism Genes

The origin of parasitism genes is from nematode ancestors. Genes evolved from nematode ancestors of contemporary species are of one likely origin of nematode parasitism genes. In this regard, the value of the information generated in the *C. elegans* genome sequencing project to identify genes basic to the biology of plant-parasitic nematodes cannot be overemphasized. It is already clear that some *C. elegans* genes match those identified in plant-parasitic nematodes (Davis 2000). In other instances, however, such as the cellulose genes of cyst and root-knot nematodes, no significant similarity to any *C. elegans* gene can be found.

Table 10.3 Mapped nematode resistance loci/genes

Crop	Species of origin	Locus	Nematode	Genetic location
Tomato	*L. peruvianum*	*Mi*	*M. incognita, M. javanica, M. arenaria*	Chromosome 6
Tomato	*L. peruvianum*	*Mi3*	*M. incognita, M. javanica*	Chromosome 12
Tomato	*L. pimpinellifolium*	*Hero*	*G. rostochiensis*	Chromosome 4
Potato	*S. tuberosum* spp. *andigena*	*H1*	*G. rostochiensis* pathotypes *Ro1* and *Ro4*	Chromosome 5
Potato	*S. spegazzinii*	*Gro1*	*G. rostochiensis, pathotypes Ro1* and *Ro5*	Chromosome 7
Potato	*S. spegazzinii*	*Gpa*	*G. pallida, pathotypes Pa2* and *Pa3*	Chromosome 5
Potato	*S. vernei*	*GroV1*	*G. rostochiensis, pathotype Ro1*	Chromosome 5
Potato	*S. bulbocastanum*	$R_{Mc\text{-}1}$	*M. chitwoodi*	Chromosome 11
Sugar beet	*B. patellaris*	$Hs1^{pat\text{-}1}$	*H. schachtii*	Chromosome 1
Soybean	*Glycine max*	Rhg_4	*H. glycines, race 3*	Linkage group A
Wheat	*Triticum aestivum*	*Cre*	*H. avenae*	Long arm of chromosome 2B
Wheat	*T. tauschii*	*Cre3*	*H. avenae*	Long arm of chromosome 2D

Table 10.4 Parasitism genes of major phytonematodes

Plant-parasitic nematodes	Parasitism genes
Heterodera glycines	*Hg-eng-1* and *ror-1,2,3*
Globodera rostochiensis	*Gr-eng-1*
Meloidogyne javanica	*Mj-cm-1*
Meloidogyne incognita	*Mi-cbp-1* and *mi-msp1*

Plant parasitism is believed to have evolved at least three times independently. The genes that were evolved from nematode ancestors of contemporary species are one likely possible mechanism for the origin of nematode parasitism genes and the other mechanism may be horizontal gene transfer (Table 10.5). It was reported that those genes expressed in the esophageal gland cells of plant-parasitic nematodes show strongest similarities to the bacterial genes which strengthened the existing hypothesis that parasitism genes in plant nematodes may have been acquired, at least in part, by horizontal gene transfer from bacteria and other microorganisms that inhabit the same parasitic environment. The genes *Mj-cm-1*and *Mi-cbp-1* show strongest similarities to the genes of bacteria. The complementation of a bacterial mutant with *Mj-cm-1* was also used to provide functional analysis of the gene. Most of the parasitism genes are found to be highly similar to bacterial sequences, thereby suggesting that these

Table 10.5 Cyst and root-knot nematode parasitism genes with predicted functions

Nematode secretions	Cyst nematode	Root-knot nematode
I. Cell wall-degrading enzymes		
1. β-1,4-endoglucanase	+	+
2. Pectate lyase	+	+
3. Polygalacturonase	−	+
4. Expansin	+	+
5. Xylanase	−	+
6. Cellulose-binding domain	+	+
II. Calreticulin	−	+
III. Chorismate mutase	+	+
IV. Ran BPM	+	−
V. Ubiquitin extension	+	−
VI. CLAVATA3 or other peptides	+	+
VII. Phytohormones	−	+

parasitism genes could have been acquired from bacteria through horizontal gene transfer. For example, the nematode endo-1,4-β-glucanases from the Tylenchomorpha, which belong to glycosyl hydrolase family (GHF5), show less similarity to plant endoglucanases but show resemblance to the bacteria.

The genes encoding the cellulase enzymes of both nematode and bacteria may have evolved from an ancient cellulase of a common ancestor of both the bacteria and nematodes. The endoglu-

canases from nematode show the highest similarity with the bacterial one, which also points to a horizontal gene transfer from bacteria to an ancestor of the cyst nematode. However, it is not possible and advisable to provide the conclusive evidence for a horizontal gene transfer from one organism to another organism germ line. There are examples of putative cases of horizontal gene transfer from eukaryote to prokaryote, from prokaryote to prokaryote, and from prokaryote to eukaryote. On the other hand, the presence of bacterial symbionts in nematode ancestors, such as the bacterium *Wolbachia* symbiont found in filarial nematodes, may also represent a source for transfer of genetic material from bacteria to nematodes (Ngangbam and Devi 2012).

Cellulase genes and several other genes cloned from nematode esophageal gland cells have striking similarities to microbial genes, suggesting that some nematode parasitism genes may have been acquired by ancient horizontal gene transfer. Horizontal gene transfer is a central process in shaping nematode genomes. High incidence of gene acquisition can happen through horizontal gene transfer. In nematodes, genes are gained and lost frequently. A major challenge in plant nematology is to identify the avirulence gene products of parasitic nematodes. Genes are also required for various major activities including egg hatching (*hch*), dauer formation (*daf*), and sex determination (*xol, her, and fem*).

10.14 Nuclear Localized Parasitism Proteins

A significant number of the proteins encoded by parasitism genes expressed in plant nematode esophageal gland cells (Gao et al. 2003; Huang et al. 2003) contain both a predicted secretion signal peptide and a motif encoding a putative nuclear localization signal (NLS). These data present the tempting hypothesis that the secreted products of some nematode parasitism genes become localized to the host cell nucleus. This is not without precedent since the antigens of secreted products from the animal-parasitic nematode *Trichinella spiralis* have been immunolocalized to the nucleus of host muscle cells. Expression of GFP- and GUS-tagged SCN parasitism proteins in plant cells has demonstrated that some of the predicted NLS domains do indeed function to import products into the plant cell nucleus. DNA-binding domains are also predicted in some of the NLS-containing proteins predicted to be secreted by plant nematodes, suggesting an extraordinary potential for regulatory control within the nucleus of host feeding cells if confirmed.

10.14.1 RanBPM

Ran-binding protein microtubule-organizing center (RanBPM) appears to function as a scaffolding protein in several signal transduction pathways. RanBPM is a crucial component of multiprotein complexes that regulate the cellular function by modulating and/or assembling with a wide range of proteins in different intracellular regions and thereby mediate diverse cellular functions. This suggests a role for RanBPM as a scaffolding protein. A homologue of a Ran-binding protein to microtubule (*RanBPM*) gene, initially known as IC5, was identified and characterized in second-stage juveniles (J2s) of *Globodera pallida* (Blanchard et al. 2005). The full-length cDNA (937 bp) was obtained by 5′ and 3′ rapid amplification of cDNA ends (RACE), and specific primers were designed to amplify the genomic sequences of 2,396 bp containing six introns. The ORF (798 bp) encodes a putative 265-amino acid sequence with a predicted SPRY domain and a signal peptide of 23 amino acids on the N-terminal part of the protein. In situ hybridization experiments showed that the transcript is located in the dorsal gland of the J2s, suggesting that the encoded protein has an extracellular function and can be involved in the late stages of parasitism such as feeding site establishment. This gene was specifically overexpressed in the juveniles before and during parasitism, but not in adult developmental stages. As this gene is presumed to be involved in plant–nematode interaction, particularly in the development and maintenance of the feeding structure that allows

the nematode to achieve parasitic development, homologous genes were sought in other cyst nematode species. One of them was cloned and sequenced in the closely related species *Globodera mexicana*.

Expressed genes encoding secretory proteins with high similarity to proteins that bind to the small G-protein Ran, so-called RanBPMs (Ran-binding protein in the microtubule-organizing center), were identified in the dorsal esophageal gland cell of cyst nematodes (Blanchard et al. 2005).

10.14.2 Ubiquitination/Proteasome

The proteasome is a protein-destroying apparatus involved in many essential cellular functions, such as the regulation of cell cycle, cell differentiation, signal transduction pathways, antigen processing for appropriate immune responses, stress signaling, inflammatory responses, and apoptosis. It is capable of degrading a variety of cellular proteins in a rapid and timely fashion, and most substrate proteins are modified by ubiquitin before their degradation by the proteasome. The proteasome is a large protein complex consisting of a proteolytic core called the 20S particle and ancillary factors that regulate its activity in various ways.

The most common form is the 26S proteasome containing one 20S core particle and two 19S regulatory particles that enable the proteasome to degrade ubiquitinated proteins by an ATPdependent mechanism. Another form is the immunoproteasome containing two 11S regulatory particles, PA28 alpha and PA28 beta, which are induced by interferon gamma under the conditions of intensified immune response. Other regulatory particles include PA28 gamma and PA200. Although PA28 gamma also belongs to a family of activators of the 20S proteasome, it is localized within the nucleus and forms a homoheptamer. PA28 gamma has been implicated in the regulation of cell cycle progression and apoptosis. PA200 has been identified as a large nuclear protein that stimulates proteasomal hydrolysis of peptides. Several parasitism genes expressed in cyst nematode esophageal gland cells encode secreted isotypes of cytoplasmic proteins involved in the ubiquitination pathway, namely, ubiquitin itself, along with proteins (i.e., RING-Zn-Finger-like and Skp1-like proteins) similar to those found in the host E3 ubiquitin protein ligase complex (Gao et al. 2003).

10.14.3 The 14-3-3 Protein Family

14-3-3 proteins constitute a family of eukaryotic proteins that are key regulators of a large number of processes ranging from mitosis to apoptosis. 14-3-3s function as dimers and bind to particular motifs in their target proteins. To date, 14-3-3s have been implicated in the regulation or stabilization of more than 35 different proteins. This number is probably only a fraction of the number of proteins that 14-3-3s bind to, as reports of new target proteins have become more frequent. An examination of 14-3-3 entries in the public databases reveals 153 isoforms, including alleloforms, reported in 48 different species. Two isoforms of a protein identified as a member of the 14-3-3 family were isolated from stylet secretions induced in vitro from second-stage juveniles of root-knot nematode (Jaubert et al. 2002). The expressed genes encoding each 14-3-3 were cloned, and in situ hybridization analysis indicated that one isoform (14-3-3a) was expressed in genital primordia and the other isoform (14-3-3b) was expressed within the dorsal esophageal gland of root-knot nematode juveniles.

To develop a better understanding of invertebrate anhydrobiosis, Kshamata Goyal et al. (2005) characterized dehydration-inducible genes and their proteins in anhydrobiotic nematodes and bdelloid rotifers. Initial work with the fungivorous nematode *Aphelenchus avenae* led to the identification of two genes, both of which were markedly induced on slow drying (90–98 % relative humidity, 24 h) and also by osmotic stress, but not by heat or cold or oxidative stresses. The first of these genes encodes a novel protein, anhydrin; it is a small, basic polypeptide, with no counterparts in sequence databases, which is predicted to be natively unstructured and highly hydrophilic. The second is a member of the group 3 LEA

protein family; this and other families of LEA proteins are widely described in plants, where they are most commonly associated with the acquisition of desiccation tolerance in maturing seeds. Like anhydrin, the nematode LEA protein, Aav-LEA-1, is highly hydrophilic, and a recombinant form has been shown to be unstructured in solution. In vitro functional studies suggested that Aav-LEA-1 was able to stabilize other proteins against desiccation-induced aggregation, which is in keeping with a role of LEA proteins in anhydrobiosis. In vivo, however, Aav-LEA-1 was apparently processed into smaller forms during desiccation. A processing activity was found in protein extracts of dehydrated, but not hydrated, nematodes; these shorter polypeptides are also active anti-aggregants, and it was hypothesized that processing LEA protein serves to increase the number of active molecules available to the dehydrating animal. Other LEA-like proteins were identified in nematodes, and it seems likely, therefore, that they play a major role in the molecular anhydrobiology of invertebrates, as they are thought to do in plants.

10.14.4 Surface Defense

Chemical composition, origin, and biological role of the surface coat of plant-parasitic nematodes are described by Spiegel and McClure (1995) and compared with those of animal-parasitic and free-living nematodes. The surface coat of the plant-parasitic nematodes is 5–30 nm thick and is characterized by a net negative charge. It consists, at least in part, of glycoproteins and proteins with various molecular weights, depending upon the nematode species. The lability of its components and the binding of human red blood cells to the surface of many tylenchid plant-parasitic nematodes, as well as the binding of several neoglycoproteins to the root-knot nematode *Meloidogyne*, suggest the presence of carbohydrate recognition domains for host plants and parasitic or predatory soil microorganisms (e.g., *Pasteuria penetrans* and *Dactylaria* spp.). These features may also assist in nematode adaptations to soil environments and to plant hosts with defense mechanisms that depend on reactions to nematode surfaces. Surface coat proteins can be species and race specific, a characteristic with promising diagnostic potential.

The hypodermis is a syncytial cell layer directly beneath the (nonliving) nematode cuticle that forms the new cuticle during molts and secretes a number of molecules for deposition on the cuticle surface of the nematode body. Several genes expressed within the hypodermis encode proteins deposited on the cuticle surface that are in direct contact with host cells during nematode invasion of plant tissues. Among the dynamic mixture of proteins at the cuticle surface are proteins with potential roles in mitigating host defense response. Peroxidase genes are expressed in the potato cyst nematode hypodermis (Jones et al. 2002), and the peroxidase proteins accumulate on the nematode body surface presumably to detoxify reactive oxygen species generated by the defense response of the host.

10.14.5 Stealth Signals

Several molecules from nematodes with observable effects on plant cells have been reported, but the nature and origins of these molecules from nematodes are unclear. The isolation of secreted cytokinins from root-knot nematode juveniles in the absence of plant hosts (De Meutter et al. 2003) confirmed earlier evidence suggesting that nematodes produce cytokinin endogenously (Dimalla and Van Staden 1977). It was suggested that cytokinins of nematode origin may be an excreted waste product of nucleic acid degradation. This hypothesis relates to the localized hyperplasia that is a hallmark of gall formation, cell cycle regulation in feeding sites, and other cytokinin effects stimulated in roots by root-knot nematodes (Lohar et al. 2004). The incubation of roots of the legume *Lotus japonicus* in the presence of viable juveniles of root-knot nematode prior to infection stimulated identical cytoskeletal activity in root hairs as observed by treatment with rhizobial Nod factors (Weerasinghe et al. 2005). The “NemF”

(Nem factor) from root-knot nematodes also provided a response identical to Nod factor in Nod-receptor mutants, and a similar response to NemF was observed in root hairs of tomato. The identity of NemF and its origins in root-knot nematode have not been reported (Davis et al. 2008).

10.14.6 Bioactive Peptides

Perhaps the most interesting group of parasitism genes are those that encode signaling peptides. Secretions collected and fractionated from hatched juveniles of the potato cyst nematode contained a peptide or peptides of less than 3 kDa that induced mitogenic activity in tobacco leaf protoplasts and human peripheral blood mononuclear cells (Goverse et al. 1999).

Below is a sequence/list of cyst and root-knot nematode parasitism genes with predicted functions:

- Peptide signaling – *H. glycines;* contained C-terminal domain similar to plant CLAVATA3/ESR-related (CLE) peptides
- Altered cellular metabolism – chorismate mutase *(CM)*
- Cell cycle augmentation – *RanBPMs*
- Nuclear localization *(NLS)*
- Cytokinins and protein degradation – ubiquitin proteasome pathway

Parasitism genes expressed in the esophageal gland cells of root-knot nematodes encode proteins that are secreted into host root cells to transform the recipient cells into enlarged multinucleate feeding cells called giant cells (Huang et al. 2006). Expression of a root-knot nematode parasitism gene which encodes a novel 13-amino acid secretory peptide in plant tissues stimulated root growth. Two scarecrow-like transcription factors of the GRAS protein family were identified as the putative targets for this bioactive nematode peptide in yeast two-hybrid analyses and confirmed by in vitro and in vivo co-immunoprecipitations. This discovery is the first demonstration of a direct interaction of a nematode-secreted parasitism peptide with a plant-regulatory protein, which may represent an early signaling event in the root-knot nematode–host interaction.

Meloidogyne hapla, a diploid root-knot nematode with a compact (54 Mbp) sequenced genome, has been established as a tractable model to study the genetic and biochemical basis for plant parasitism (Bird et al. 2012). The current annotation freeze (HapPep4) predicts 14,207 proteins, many of which have been confirmed by LC-MS^E. Comparative genomics, particularly with the sympatric species *M. chitwoodi* and the migratory species *Pratylenchus coffeae*, proved to be a powerful approach to deduce the evolution and mechanisms of parasitic ability. A current target of a functional analyses was genes encoding small proteins that exhibit sequence similarity to members of two classes of plant peptide hormones, viz., RAR (root architecture regulator) and CLE (clavata-like elements). In *M. hapla*, RAR mimics were encoded by a 12-member gene family, and 8 genes encode CLE peptide mimics. Ectopic exposure of roots to RAR peptides was sufficient to elicit galling even in the absence of rhizobacteria or RKN. Further genome analysis of these loci in *M. hapla* supported the model that these genes were acquired from an ancestral dicot via horizontal gene transfer. Bioassays were developed to study the biology of these and other ligands involved in parasitism and established methods for efficient isotopic labeling of nematode proteins to empower an MS-based approach to map plant and nematode proteins.

Plant-parasitic cyst nematodes are known to secrete proteins that mimic the function of plant CLE signaling peptides to promote a successful infection. However, the mechanistic details of this process have yet to be elucidated. Wang Wang Xiaohong (2011) identified and functionally characterized CLE-like genes from several cyst nematode species including the two species of potato cyst nematodes and demonstrated that nematode CLEs can be trafficked by the function of the variable domain to the place outside plant cells where they can act as plant CLE mimics by interacting with membrane-bound plant CLE receptors. A transient expression system was used to demonstrate the processing of nematode CLE in plants. The majority of the earlier research was focused on the identification of the

processed and bioactive forms of nematode CLE peptides that function in plant cells and the initial investigation of plant receptors that perceive nematode CLE signals. By overexpression of a nematode CLE protein in potato roots coupled with proteomic analyses, they determined that the bioactive forms of nematode CLEs share striking structural similarity to mature plant CLE peptides. These results provided the direct evidence that nematode CLEs, once being delivered into host root cells, can be recognized by host cellular machinery to become mimics of plant CLE signals. Using genetic and biochemical approaches, it was demonstrated that plant receptors of CLV2 and CRN were required for nematode CLE-mediated parasitism. They identified that BAM1 and BAM2 were additional host receptors that can bind to processed nematode CLE peptides. These results suggested that multiple host receptors might be involved in perceiving nematode CLE peptides to facilitate nematode parasitism. Mimicry of host plant signals was an extraordinary adaptation by a plant parasite to have evolved the ability to reprogram host plant cells for its own benefit.

10.14.7 Chorismate Mutase

Chorismate (or prephenate) is a precursor for a variety of compounds including cellular aromatic amino acids, phytohormone indole-3-acetic acid (IAA), plant defense-related salicylic acid, and a range of other secondary metabolites. These chorismate-derived compounds (CDCs) therefore play important roles in plant growth and development, in defense, and in interactions with other organisms. The activity of chorismate mutase is a key regulatory mechanism that determines the cellular balance of the aromatic amino acids phenylalanine, tyrosine, and tryptophan (Romero et al. 1995). Chorismate mutase (CM) catalyzes the pericyclic Claisen-like rearrangement of chorismate to prephenate in the last step of the shikimate pathway, which is a primary metabolic route found in plants and microorganisms (Huang et al. 2005).

Chorismate mutase is well characterized in microbes and plants and not described from any animals outside of phytonematodes. The first animal CM gene (Mj-cm-1) was cloned from the root-knot nematode *Meloidogyne javanica* and found to be expressed in the nematode esophageal gland cells (Lambert et al. 1999). Chorismate mutases are potentially involved in early development of the feeding sites induced by plant-parasitic nematodes, but how the nematode CMs alter the development of host plant cells is still not clear. Recently, CMs have been identified in soybean and potato cyst nematodes. The presence of CM homologues in different obligate, sedentary endoparasitic nematode species that induce elaborate feeding sites in roots suggests that they have key roles in plant–nematode interactions.

Some plant endoparasitic nematodes are biotrophic and induce remarkable changes in their hosts in order to ensure a continuous supply of food. Proteins secreted from esophageal gland cells have been implicated in this pathogenic process (Jones et al. 2003). A potentially secreted chorismate mutase has been isolated from *Globodera pallida*. The gene encoding this protein is expressed in the subventral esophageal gland cells of the nematode, and the mRNA derived from this gene is only present in the early parasitic stages. Sequence analysis of this gene showed that, like other genes involved in the host–parasite interaction of plant-parasitic nematodes, it is likely to have been acquired by horizontal gene transfer from bacteria. The presence of a signal peptide in the deduced amino acid sequence of the *G. pallida* chorismate mutase and its expression in the subventral esophageal gland cells suggested that it is secreted from the nematode, pointing to a role for the protein in the host–parasite interaction. The shikimate pathway, of which chorismate mutase is normally a part, is not found in animals but is present in plants and bacteria. In plants it gives rise to a variety of compounds which are important in amino acid synthesis and defense signaling pathways, as well as auxins, which have been implicated in the early development of nematode feeding sites.

Parasitism genes encoding secretory proteins expressed in the esophageal glands of phytoparasitic nematodes play critical roles in nematode invasion of host plants, establishment of feeding sites, and suppression of host defenses (Huang et al. 2005). Two chorismate mutase genes potentially having a role in one or more of these processes were identified from a *Meloidogyne incognita* esophageal gland cell subtractive cDNA library. These *M. incognita* enzymes (designated as Mi-cm-1 and Mi-cm-2), with amino-terminal signal peptides, were significantly similar to chorismate mutases in *M. javanica* and bacteria. The complementation of an *Escherichia coli* CM-deficient mutant by the expression of Mi-cm-1 or Mi-cm-2 confirmed their CM activity. In situ mRNA hybridization showed that the transcripts of Mi-cm-1 and Mi-cm-2 accumulated specifically in the two subventral esophageal gland cells of *M. incognita*. RT-PCR analysis confirmed that their transcript abundances were high in the early parasitic juvenile stages and low (Mi-cm-1) or undetectable (Mi-cm-2) in later parasitic stages of the nematode. Southern blot analysis revealed that these CM genes were members of a small multigene family in *Meloidogyne* species. The widespread presence of CMs in the specialized sedentary endoparasitic nematode species suggests that this multifunctional enzyme may be a key factor in modulating plant parasitism.

10.15 Genetic Analysis of Parasitism

The use of forward genetic strategies to add to our understanding of nematode parasitism and virulence has been complicated due to the obligate nature of phytonematodes, parthenogenetic mode of reproduction, and lack of tools for genetic mapping. A genetic linkage map for *H. glycines* has been constructed and its utility was demonstrated by mapping the *HG-Cm-1* gene. Of the four most damaging *Meloidogyne* spp., *M. hapla* has been found to reproduce by facultative meiotic parthenogenesis, so that both selfed and outcrossed progeny can be generated for classical genetic studies; hence it has been adopted as a model system. In addition to the *M. hapla* genome sequencing project, genetic mapping is also under construction and F2 mapping populations have been generated.

These new tools help researchers to identify the genes controlling various traits – nematode virulence and host range to understand pathogenesis. Molecular approaches to explore the altered gene expression in nematode feeding site (NFS) include differential screening and subtraction of cDNAs, promoter b-glucuronidase (GUS) fusions, mRNA in situ hybridization, reverse transcription-polymerase chain reaction (RT-PCR), Mabs, Pabs, ESTs, and microarray.

10.15.1 Molecular Analysis of Genes

These include cDNA RT-PCR, cDNA-AFLP, RNA fingerprinting, expressed sequence tag (EST) analysis, LD-PCR (long-distance PCR), PSORT II, and BLASTP analysis. Nematode parasitism genes are active in any or all parts of the parasitic cycle including preparasitic and parasitic life stages. Parasitism genes are expressed in their esophageal gland cells and secreted through their stylet into host tissue to control the complex process of parasitism. Sixty different proteins are secreted by the stylet during parasitism. Plant genes induced during a compatible plant–nematode interaction sequence include complex changes in plant gene expression. Pectate lyase genes are expressed in the early nematode developmental stages and their pectate lyase activities would help to degrade the pectin matrix and assist nematode migration between the cells in host roots.

10.15.1.1 *Hg-eng-1 and Gr-eng-1*

These genes have been isolated from *Heterodera glycines* and *Globodera rostochiensis.* These are produced in subventral glands. They play a role in the cell wall dissolution (cellulase) (Re Dong 1997). Two β-1,4-endoglucanases (EGases), Hg-eng-1 and Hg-eng-2, were cloned from the soybean cyst nematode, *Heterodera glycines,* and their expression was shown in the subventral esophageal glands of hatched second-stage juveniles (de Boer et al. 1999). They examined the

expression of these EGases in the subventral glands of all postembryonic life stages of *H. glycines* by in situ hybridization and immunolocalization. The first detectable accumulation of EGase mRNAs occurred in the subventral glands of unhatched J2. EGase transcripts remained detectable in J2 after hatching and during subsequent root invasion. However, in late parasitic J2 and third-stage juveniles (J3), the percentage of individuals that showed EGase transcripts decreased. In female fourth-stage juveniles and adult females, EGase transcripts were no longer detected in the subventral glands. EGase hybridization signal reappeared in unhatched males coiled within the J3 cuticle, and transcripts were also present in the subventral glands of migratory adult males. Immunofluorescence labeling showed that EGase translation products are most abundantly present in the subventral glands of preparasitic J2, migratory parasitic J2, and adult males. The presence of EGases predominantly in the migratory stages suggests that the enzymes are used by the nematodes to soften the walls of root cells during penetration and intracellular migration.

10.15.1.2 *Mj-cm-1 and Mi-cbp-1*

These genes are isolated from *Meloidogyne javanica* and *Meloidogyne incognita*. These are produced in subventral esophageal gland cells. The function of these genes is to convert chorismate mutase into aromatic amino acid and tyrosine. Those genes expressed in the esophageal gland cells of plant-parasitic nematodes show strongest similarities to the bacterial genes which strengthened the existing hypothesis that parasitism genes in plant nematodes may have been acquired, at least in part, by horizontal gene transfer from bacteria and other microorganisms that inhabit the same parasitic environment (Blaxter et al. 1998). The genes Mj-cm-1 and Mi-cbp-1 show strongest similarities to the genes of bacteria. The complementation of a bacterial mutant with Mj-cm-1 was also used to provide functional analysis of the gene. Most of the parasitism genes are found to be highly similar to bacterial sequences, thereby suggesting that these parasitism genes could have been acquired from bacteria through horizontal gene transfer.

In brief, the cloning and characterization of genes that promote nematode parasitism of plants is in its early stages, but already, some promising research directions and unexpected results have been realized. Genetic analyses will be merged with physical maps of plant-parasitic nematode genomes to isolate nematode (a) virulence genes, but it is unclear if these genes will represent a subset of modified parasitism genes or if they will have functions unrelated to plant parasitism. Direct molecular analyses of genes expressed in the nematode esophageal gland cells whose products are secreted into plant tissue during parasitism are proving to be a fruitful area of investigation.

10.16 Nematode Genomics

Combined with the extensive genetic and genomic analyses of *C. elegans,* the increase in genomic analyses of parasitic nematodes in recent years promises to provide an unprecedented understanding of nematode biology and pathogenesis. SPS of random clones from cDNA libraries to generate ESTs (expressed sequence tags) from different nematode life stages become a powerful tool for identifying genes important in nematode–host interactions, which contributed more than 400,000 publicly available parasitic nematode expressed sequences to databases enabling in identification of nematode-specific gene families.

To elucidate nematode genome organization, phytoparasitic nematode genome sequencing projects for both root-knot nematodes (*M. incognita* and *M. hapla*) and cyst (*H. glycines*) nematodes are currently underway and upon completion will provide genome-wide catalogues of nematode PGCs for both functional and comparative analyses. By utilizing this gene structure, organization and function can be compared across different genomes to facilitate evolutionary analyses within the phylum Nematoda.

A surprising result is that the nematode cellulase genes and several other genes cloned from nematode esophageal gland cells have striking similarities to microbial genes, suggesting that some nematode parasitism genes may have been acquired by ancient horizontal gene

transfer. The application of genomics to the study of nematode parasitism genes will help to address questions and allow the isolation of additional nematode parasitism genes to progress. The development of efficient assays for functional analysis of isolated parasitism genes will be of paramount importance to understanding the evolution and complexity of plant parasitism by nematodes.

Expanding genomic data on plant pathogens open new perspectives for the development of specific and environment friendly pest management strategies based on the inhibition of parasitism genes that are essential for the success of infection (Arguel et al. 2012). Identifying such genes relies on accurate reverse genetics tools and the screening of pathogen knockdown phenotypes. Root-knot nematodes are major cosmopolitan crop pests that feed on a wide range of host plants. Small interfering RNAs (siRNAs) would provide a powerful tool for reverse genetics of nematode parasitism genes provided that they could (1) target genes expressed in inner tissues of infective nematodes and (2) target genes expressed during parasitism. It was shown that siRNAs can access inner tissues of the infective juveniles during soaking and accumulate in the esophagus, amphidial pouches, and related neurons of the nematode. Evidence was provided that siRNAs could trigger knockdown of the parasitism gene *Mi-CRT*, a calreticulin gene expressed in the esophageal glands of *Meloidogyne incognita*. *Mi-CRT* knockdown in infective juveniles affected nematode virulence. However, *Mi-CRT* knockdown was not persistent after plant infection, indicating that siRNA-mediated RNAi is best suited for functional analysis of genes involved in preparasitic stages or in the early steps of infection.

10.17 RNA Interference in Phytonematodes

RNA interference (RNAi, also called RNA-mediated interference) is a mechanism for RNA-guided regulation of gene expression in which double-stranded ribonucleic acid inhibits the expression of genes with complementary nucleotide sequences (Mehmet Karaka 2008). Conserved in most eukaryotic organisms, the RNAi pathway is thought to have evolved as a form of innate immunity against viruses and also plays a major role in regulating development and genome maintenance. RNAi has recently been demonstrated in phytonematodes. It is a potentially powerful investigative tool for the genome-wide identification of gene function that should help improve our understanding of plant-parasitic nematodes. RNAi helps to identify gene and, hence, protein targets for nematode management strategies.

There is accumulating evidence for the efficacy of RNAi in plant-parasitic nematodes. A range of genes have been targeted for silencing in cyst and root-knot nematode species and both the phenotypic and the molecular effects (Table 10.6). Nevertheless, the molecular detail of the RNAi process in plant parasitic nematodes has yet to be elucidated. Infective stages of plant-parasitic nematodes are sufficiently small to make their microinjection with dsRNA a major technical challenge. In addition, they do not normally ingest fluid until they have infected a host plant. However, RNAi effects have been achieved using octopamine to stimulate oral ingestion by preparasitic second-stage juveniles of cyst nematodes *H. glycines* and *G. pallida* (Urwin et al. 2002) and root-knot nematode *M. incognita* (Bakhetia et al. 2005). Resorcinol and serotonin also induce dsRNA uptake by second-stage juvenile of *M. incognita* and may be more effective than octopamine for this nematode.

Alterations to the original method, including the addition of spermidine to the soaking buffer and an extended incubation time, were reported to increase the efficiency of RNAi for *G. rostochiensis*. The genes targeted by RNAi to date are expressed in a range of different tissues and cell types. The ingested dsRNA can silence genes in the intestine and also in the female reproductive system, sperm, and both subventral and dorsal esophageal glands (Huang et al. 2006). Uptake of dsRNA from the gut is a proven route to systemic RNAi in *C. elegans*. The systemic nature of RNAi in plant-parasitic nematodes following ingestion of dsRNA sug-

Table 10.6 Plant-parasitic nematode genes targeted by RNAi

Nematode species	Gene function	RNAi effect	Site of gene expression
Meloidogyne incognita	Cysteine proteinase	Delayed development	Intestine
		Decreased number of established nematodes	
	Dual oxidase	Decreased number of established nematodes	Presumed role in extracellular matrix
		Decreased fecundity	
	Splicing factor	Reduced galling	Unknown
		Reduced number of females	
	Integrase	Reduced galling	Unknown
		Reduced number of females	
	Secreted peptide 16D 10	Reduced galling	Subventral pharyngeal glands
		Decreased number of established nematodes	
Heterodera glycines	Cysteine proteinase	Increased male:female ratio	Intestine
	C-type lectin	Decreased number of established nematodes	Hypodermis
	Major sperm protein	Reduction in mRNA	Sperm
		No phenotypic effect at 14 dpi	
	Aminopeptidase	Decreased number of established nematodes	Female reproductive system
		Increased male:female ratio	
	Beta I,4-endoglucanase	Decreased number of established nematodes	Subventral pharyngeal glands
	Pectate lyase	Increased male:female ratio	Subventral pharyngeal glands
	Chorismate mutase	Increased male:female ratio	Subventral pharyngeal glands
	Secreted peptide SYV 46	Decreased number of established nematodes	Dorsal esophageal gland
Globodera pallida	Cysteine proteinase	Increased male:female ratio	Intestine
	FMRFamide-like peptides	Mobility inhibition	Nervous system
M. artiellia	Chitin synthetase	Delayed egg hatch	Eggs
G. rostochiensis	Beta I,4-endoglucanase	Decreased number of established nematodes	Subventral pharyngeal glands
	Secreted amphid protein	Decreased ability to locate and penetrate roots	Amphids

gests they share similar uptake and dispersal pathways. Alternative routes to dsRNA uptake may exist for plant-parasitic nematodes. RNAi of a chitin synthase gene expressed in the eggs of root-knot nematode *Meloidogyne artiellia* was achieved by soaking intact eggs contained within their gelatinous matrix in a solution containing dsRNA. The enzyme plays a role in the synthesis of the chitinous layer in the eggshell. Depletion of its transcript by RNAi led to a reduction in stainable chitin in eggshells and a delay in hatching of juveniles from treated eggs. The results implied that the eggs of this nematode and possibly others are permeable to dsRNA.

10.18 Evolution of Parasitism

Parasitism is an important life history strategy in many metazoan taxa. This is particularly true of the phylum Nematoda, in which parasitism has evolved independently at least nine times. The apparent ease with which parasitism has evolved

among nematodes may, in part, be due to a feature of nematode development acting as a preadaptation for the transition from a free-living to a parasitic life history. One candidate preadaptive feature for evolution in terrestrial nematodes is the dauer larva, a developmentally arrested morph formed in response to environmental signals (Stasiuk et al. 2012). For this parasitic clade, and perhaps more widely in the phylum, the evolution of parasitism co-opted the dauer switch of a free-living ancestor. This lends direct support to the hypothesis that the switch to developmental arrest in the dauer larva acted as a preadaptation for the evolution of parasitism and suggests that the sensory transduction machinery downstream of the cue may have been similarly co-opted and modified.

Despite extraordinary diversity of free-living species, a comparatively small fraction of nematodes are parasites of plants (Baldwin et al. 2004). These parasites represent at least three disparate clades in the nematode tree of life, as inferred from rRNA sequences. Plant parasites share functional similarities regarding feeding, but many similarities in feeding structures result from convergent evolution and have fundamentally different developmental origins. Although Tylenchida rRNA phylogenies are not fully resolved, they strongly support convergent evolution of sedentary endoparasitism and plant nurse cells in cyst and root-knot nematodes. This result has critical implications for using model systems and genomics to identify and characterize parasitism genes for representatives of this clade. Phylogenetic studies reveal that plant parasites have rich and complex evolutionary histories that involve multiple transitions to plant parasitism and the possible use of genes obtained by horizontal transfer from prokaryotes. Developing a fuller understanding of plant parasitism will require integrating more comprehensive and resolved phylogenies with appropriate choices of model organisms and comparative evolutionary methods.

It is unlikely that a species switches from a fully free-living to a parasitic lifestyle in one step, and it is generally accepted that prior to the transition to parasitism, preadaptations must exist. Preadaptations are features that evolved for different reasons but facilitated the step toward parasitism (Dieterich and Sommer 2009). Indeed, many free-living nematodes can be found in association with other organisms without being parasitic. These associations range from short-term, rather unspecific, phoretic interactions to long-term associations that can be highly species specific. For example, dauer juveniles of different species of the genus *Pristionchus* associate in a species-specific manner with scarab beetles (Weller et al. 2010). The worms seem not to harm the beetle, but instead wait until the beetle dies to resume development on the rich microbial fauna that emerges on the carcass. There is empirical evidence that at least for some parasitic nematodes, the infective stages, which are crucial for entering the host, are homologous to the dauer juveniles in free-living nematodes (Wang et al. 2009). Hence, dauer juveniles and phoretic and necromenic interactions are likely candidates for the preadaptations that facilitated the evolution of parasitism by allowing a stepwise formation of tight and specific interactions. The closely related species with very different ecologies and very distantly related species with similar ecologies make nematodes an interesting system with which to investigate how genomes are shaped by the environment and by evolutionary descent.

10.19 Horizontal Gene Transfer (HGT) in Nematodes

Horizontal gene transfer (HGT), the transmission of a gene from one species to another by means other than direct vertical descent from a common ancestor, has been recognized as an important phenomenon in the evolutionary biology of prokaryotes (Danchin 2011). In eukaryotes, in contrast, the importance of HGT has long been overlooked, and its evolutionary significance has been considered to be mostly negligible. However, a series of genome analyses has now shown that HGTs not only do probably occur at a higher frequency than originally thought in eukaryotes, but recent examples have also shown that they have been subject

to natural selection, thus suggesting a significant role in the evolutionary history of the receiver species. Surprisingly, these examples are not from protists in which integration and fixation of foreign genes intuitively appear relatively straightforward, because there is no clear distinction between the germ line and the somatic genome. Instead, these examples are from nematodes, multicellular animals that do have distinct cells and tissues and do possess a separate germ line. Hence, the mechanisms of gene transfer appear in this case much more complicated.

One of the most unexpected findings from whole genome sequencing projects in nematodes is the widespread occurrence of HGT, which is the transmission of genes between organisms in a form other than vertical inheritance. Although HGT is frequent in prokaryotes, it was thought to be rare among eukaryotes with sexual reproduction (Andersson 2005). Recent genome and EST sequencing projects, however, provide strong evidence for HGT from bacteria, fungi, amoebozoa, or endosymbionts into various nematode genomes. Best characterized are examples of HGT in parasitic *Meloidogyne*, *Heterodera, Globodera*, and *Pratylenchus* groups, the fungivorous *Bursaphelenchus*, necromenic *Pristionchus* species, and the filarial parasite *B. malayi* (Dieterich and Sommer 2009).

Horizontal gene transfer implies the nonsexual exchange of genetic material between species, in some cases even across kingdoms. Although common among Bacteria and Archaea, HGTs from pro- to eukaryotes and between eukaryotes were thought to be extremely rare. Recent studies on intracellular bacteria and their hosts seriously question this view. Recipient organisms could benefit from HGT as new gene packages could allow them to broaden or change their diet, colonize new habitats, or survive conditions that previously would have been lethal (Mitreva et al. 2009). About a decade ago, plant-parasitic nematodes were shown to produce and secrete cellulases. Prior to this, animals were thought to fully depend on microbial symbionts for the breakdown of plant cell walls. It was hypothesized that the ability of nematodes to parasitize plants was acquired by HGT from soil bacteria to (ancestral) bacterivorous nematodes. Since the identification of the first nematode cellulases, many more plant cell wall-degrading enzymes (CWDE) have been identified in a range of plant-parasitic nematode species.

HGT requires close physical contact between donor and recipient, and this could be achieved in, for example, a symbiont–host or a trophic relationship. The former type of relationship was indeed shown to potentially result in the transfer of genetic material (e.g., *Brugia malayi* and *Wolbachia*). However, currently known endosymbionts of nematodes may not be the source of CWDEs. Remarkably, all cellulases discovered so far within the order Tylenchida belong to a single glycoside hydrolase family (GHF5). A range of soil bacteria harbors GHF5 cellulases, but of course, nothing can be said about the gene content of soil bacteria at the time HGT took place, if at all.

The characterization of cellulases/other CWDEs and their genomic organization in more basal (facultative) plant-parasitic Tylenchida are needed to find out if CWDEs were indeed acquired via HGT from bacteria. A more complete picture about the evolution of CWDEs among plant-parasitic Tylenchida needs a detailed characterization of two – so far – fully unexplored basal suborders, Tylenchina and Criconematina. The natural acquisition of novel genes from other organisms by horizontal or lateral gene transfer is well established for microorganisms. There is now growing evidence that horizontal gene transfer also plays important roles in the evolution of eukaryotes (Mayer et al. 2011). Genome sequencing and EST projects of plant and animal associated nematodes such as *Brugia*, *Meloidogyne*, *Bursaphelenchus*, and *Pristionchus* indicate horizontal gene transfer as a key adaptation toward parasitism and pathogenicity. However, little is known about the functional activity and evolutionary longevity of genes acquired by horizontal gene transfer and the mechanisms favoring such processes.

Werner Mayer et al. (2011) attempted to transfer cellulase genes to the free-living and beetle-associated nematode, *Pristionchus pacificus*, for

which detailed phylogenetic knowledge was available, to address predictions by evolutionary theory for successful gene transfer. They used transcriptomics in seven *Pristionchus* species and three other related diplogastrid nematodes with a well-defined phylogenetic framework to study the evolution of ancestral cellulase genes acquired by horizontal gene transfer. Intraspecific, inter-specific, and intergenic analyses were performed by comparing the transcriptomes of these ten species and tested for cellulase activity in each species. Species with cellulase genes in their transcriptome always exhibited cellulase activity indicating functional integration into the host's genome and biology. The phylogenetic profile of cellulase genes was congruent with the species phylogeny demonstrating gene longevity. Cellulase genes showed notable turnover with elevated birth and death rates. Comparison by sequencing of three selected cellulase genes in 24 natural isolates of *Pristionchus pacificus* suggested that these high evolutionary dynamics are associated with copy number variations and positive selection.

Not surprisingly, nematodes have evolved to occupy diverse ecological niches as they are the most abundant and speciose metazoans and account for up to 80 % of the kingdom's members (Boucher and Lambshead 1994). Like the well-studied *C. elegans*, most are free living and graze on microbes or detritus, and, as such, have no obvious direct impact on humans. Others, however, are adapted as parasites and are responsible for such widespread problems as human disease, debilitation of livestock, and crop damage. Plant-parasitic forms are responsible for an estimated $100 billion in annual crop damage worldwide. The most damaging family (the *Heteroderidae*) includes the root-knot (*Meloidogyne* spp.) and the cyst (*Globodera* and *Heterodera* spp.) nematodes. Root-knot nematodes penetrate plant hosts and migrate between the cells in roots, where they induce the formation of large multinucleate cells called "giant cells." Galls form around the giant cells and the roots become distorted, often leading to compromised root function and retardation of plant growth. The origin of plant parasitism within the phylum Nematoda is intriguing. The ability to parasitize plants has originated independently at least three times during nematode evolution, and as more molecular data has emerged, it has become clear that multiple instances of horizontal gene transfer (HGT) from bacteria and fungi have played a crucial role in the nematode's adaptation to this new lifestyle (Jones and Danchin 2011). The first reported HGT cases in phytonematodes were genes encoding plant cell wall-degrading enzymes. Other putative examples of HGT were subsequently described, including genes that may be involved in the modulation of the plant's defense system, the establishment of a nematode feeding site, and the synthesis or processing of nutrients. Although, in many cases, it is difficult to pinpoint the donor organism, candidate donors are usually soil dwelling and are either plant-pathogenic or plant-associated microorganisms, hence occupying the same ecological niche as the nematodes. The exact mechanisms of transfer are unknown, although close contacts with donor microorganisms, such as symbiotic or trophic interactions, are a possibility. The widespread occurrence of horizontally transferred genes in evolutionarily independent plant-parasitic nematode lineages suggests that HGT may be a prerequisite for successful plant parasitism in nematodes.

Phylogenetic reconstruction of nematode cell wall-degrading enzymes strongly indicates the independent acquisition from distinct microbial donors (Danchin et al. 2010). For example, the characterized cellulases from plant-parasitic Tylenchida are from glycoside hydrolase family 5 (GHF5). A GHF5 gene cassette consisting of the catalytic domain and the carbohydrate-binding module 2 (CBM2) was acquired as an intronless ancestral gene from putative bacterial donors. In contrast, the pine wood nematode *Bursaphelenchus xylophilus*, which is part of the same clade as the Tylenchida, has independently acquired a different family of cellulases (GHF45) from fungi. Similar findings have been made for other families of cell wall-degrading enzymes of plant-parasitic nematodes by systematic investigations of the evolutionary history of the corresponding genes. These studies also suggested massive gene duplications after the ancestral

acquisition by HGT, a finding that has several evolutionary implications.

Horizontal gene transfer occurs frequently in prokaryotes, but seems to be rare in eukaryotes. For example, ~1 % of the gene repertoire in *Meloidogyne* probably originated by horizontal transfer (Scholl et al. 2003) compared to 1–5 % of single-copy genes and at least 22 % of gene duplicates in Y-proteobacteria *Meloidogyne hapla*, a plant-parasitic nematode, seems to have gained at least a dozen genes by horizontal gene transfer from bacteria that occupy similar niches in the soil and roots. Those genes gained are useful for the nematode's parasitic lifestyle, such as cellulases for digesting plant material, and signaling molecules that induce morphological changes in the plant, facilitating invasion. A distantly related plant parasite, *Bursaphelenchus xylophilus*, seems to have independently acquired a cellulase gene from a fungus. Perhaps horizontal transfer can spur the transition to parasitism. Several groups of parasitic nematodes, including *Brugia malayi*, live in symbiosis with specific bacteria carried by the nematodes. Some of these are extracellular symbionts, but others are intracellular, such as *Wolbachia* living in *B. malayi* and other filarial nematodes. The capture of the *Wolbachia* gene set seems to have been adaptive for filarial nematodes, since killing *Wolbachia* with antibiotics reduces the growth and fecundity of the nematodes.

Free-living nematodes may also have pinched genes from organisms that live nearby. Many nematodes use other animals, often arthropods and mollusks, as transport hosts. For example, *C. remanei* lives in close association with mollusks and isopods. Indeed, *C. elegans* has four genes, including an alcohol dehydrogenase, that have stronger sequence matches to fungi than to other animals. These *C. elegans* genes group with fungal genes in phylogenetic trees. Similar phylogenetic analyses will allow us to scan the eight new genomes for stolen genes.

It is not clear which genetic differences between the plant-parasitic and nonparasitic forms may be responsible for conferring parasitic ability. On the basis of phylogenetic analysis, it appears that plant parasitism arose independently at least three times over the course of nematode evolution. Consequently, one cannot be assured that any gene or set of genes that aid in the parasitic lifestyle in one nematode species will also exist in another. Conceptually, several mechanisms affecting evolution to parasitism can be envisioned. These include adaptation of preexisting genes to encode new functions, changes in genes regulating metabolic or developmental pathways, gene duplication, gene loss, and the acquisition of genes from other species, HGT. HGT has become a widely accepted mechanism of rapid evolution and diversification in prokaryotic populations (Ochman et al. 2001). Recent genome analyses of primitive eukaryotes, such as the sea squirt (*Ciona intestinalis*) and single-celled parasitic diplomonads, implicate HGT events in early eukaryotic evolution. In contrast, the extent of horizontal transfer involving higher eukaryotes has been controversial, with many cases of hypothesized horizontally transferred genes having been refuted by later studies.

On the basis of biochemical and immunological criteria, genes have been identified in *Globodera rostochiensis* and *Heterodera glycines* that allow these nematodes to endogenously produce enzymes that can degrade cellulose and pectin, the two major components of plant cell walls. A possible ancient bacterial origin of these genes has been theorized (Popeijus et al. 2000). A bacterial origin for a number of root-knot nematode genes also has been proposed, although their possible role in parasitism is less clear. Some, such as a gene encoding chorismate mutase, were likewise identified on the basis of biochemical properties, whereas others, including a polygalacturonase gene, were identified from expressed sequence tag (EST) data sets, the latter from our data using a keyword search. Veronico et al. (2001) isolated a presumed polyglutamate synthetase gene with bacterial homology by sequencing neighboring regions of the *M. artiellia* chitin synthetase locus. They were to determine whether other root-knot nematode genes might have been acquired by horizontal gene transfer, particularly as such genes might potentially be related to parasitism. Although bacteria-like *Meloidogyne*

genes that are not present in *C. elegans* and *Drosophila* comprise a preliminary pool of candidates, multiple gene loss may be responsible for the presence/absence pattern revealed by the filter. To test this more thoroughly, the authors established a screen to compare the now small pool of preliminary candidates with all other sequences in the public databases. The most parsimonious explanation to be drawn from candidates with no significant matches to any metazoan genes is that they arose by horizontal gene transfer from a non-metazoan pool, as opposed to multiple independent gene losses in the metazoan lineages. Candidates thus identified were subsequently validated through phylogenetic analysis of relationships between the most similar matches from our screening processes.

Elizabeth H. Scholl et al. (2003) carried out high-throughput genomic screening and found it as an effective way to identify horizontal gene transfer candidates. Transferred genes that have undergone amelioration of nucleotide composition and codon bias have been identified using this approach. Analysis of these horizontally transferred gene candidates suggests a link between horizontally transferred genes in *Meloidogyne* and parasitism.

10.20 Identifying "Parasitism Genes"

Parasitism of plants and animals has evolved independently at least nine times in the history of the nematodes (Dorris et al. 1999). Four of the nematodes whose genomes are being sequenced are parasites: *Haemonchus contortus*, *Meloidogyne hapla*, *Brugia malayi,* and *Trichinella spiralis*. The adoption of parasitism in nematodes probably required adaptation of genes present in their free-living ancestors (Blaxter 2003). For example, the modification of nutrient-acquisition genes found in *C. elegans*, such as digestive enzymes or secreted hydrolases, is likely to have been important for the evolution of parasitism. The ability of parasitic nematodes to survive immunological attack, some living in an infected individual for years, has long been a puzzle. The cuticle is the main site of interaction between a nematode and its environment, and many nematode genes so far implicated in evading host defenses are secreted or cuticle proteins.

Identifying parasitism genes encoding proteins secreted from a plant-parasitic nematode esophageal gland cells and injected through its stylet into plant tissue is the key to understanding the molecular basis of nematode parasitism of plants (Hussey et al. 2011). Parasitism genes have been cloned by directly microaspirating the cytoplasm from the esophageal gland cells of different parasitic stages of cyst or root-knot nematodes to provide mRNA to create a gland cell-specific cDNA library by long-distance reverse transcriptase-polymerase chain reaction. cDNA clones are sequenced and deduced protein sequences with a signal peptide for secretion are identified for high-throughput in situ hybridization to confirm gland-specific expression.

The main soluble surface glycoprotein of filarial nematodes, a secreted glutathione peroxidase (GPX-1), is hypothesized to have a role in immune evasion (Zvelebil et al. 1993). In viral, bacterial, and protozoan parasites, genes involved in host immune evasion or recognition are often under positive selection and so show patterns of rapid amino acid substitution. Indeed, *B. malayi* GPX-1 shows signs of positive selection. By scanning for *Haemonchus contortus* genes that have diverged sharply in sequence from their *Pristionchus* and *Caenorhabditis* orthologs and that bear secretory signals, it may be possible to identify *H. contortus* genes that have adapted for a parasitic lifestyle. Some genes essential for parasitism in worms may be novel genes. One possible source is gene duplication, which allows one duplicate to keep the original role and the other duplicate to take on a parasitic role. For example, the *alt* gene family of filarial nematodes, which has been implicated in establishing infection, has a single *C. elegans* ortholog. On the other hand, other novel genes adapted for parasitism may have been assembled de novo or have been gained by horizontal gene transfer. Plant-parasitic nematodes seem to have

acquired “parasitism genes” from bacteria in their environment.

Some “parasitism genes” may by identifiable by examining the expression pattern of their *C. elegans* orthologs. In *Haemonchus contortus* and *Brugia malayi*, the infective stage of the life cycle is the third larval developmental stage (Blaxter 2003). In *C. elegans* the larval developmental stage is an alternative developmental pathway adopted when food is scarce, called the dauer larva. Thus, identifying the orthologs of *C. elegans* genes expressed in the dauer larva (Wang and Kim 2003) may be a route to pinpointing *Brugia* and *Haemonchus* genes involved in infection. The ability of nematodes to live on plant hosts involves multiple parasitism genes. The most pronounced morphological adaptations of nematodes for plant parasitism include a hollow, protrusible stylet (feeding spear) connected to three enlarged esophageal gland cells that express products that are secreted into plant tissues through the stylet. Reverse genetic and expressed sequence tag (EST) approaches are being used to discover the parasitism genes expressed in nematode esophageal gland cells. Some genes cloned from root-knot (*Meloidogyne* spp.) and cyst (*Heterodera* and *Globodera* spp.) nematodes have homologues reported in genomic analyses of *Caenorhabditis elegans* and animal-parasitic nematodes. To date, however, the candidate parasitism genes endogenous to the esophageal glands of plant nematodes, such as the ß-1,4-endoglucanases, have their greatest similarity to microbial genes, prompting speculation that genes for plant parasitism by nematodes may have been acquired by horizontal gene transfer.

Nematode feeding sites are constructed from plant cells, modified by the nematode to feed itself. Powerful new techniques permit to begin to elucidate the molecular mechanisms that produce the ultrastructural features in nematode feeding cells (Gheysen and Fenoll 2002). Many plant genes that are expressed in feeding sites produced by different nematodes have been identified in several plant species. Nematode-responsive plant genes can now be grouped in categories related to plant developmental pathways, and their roles in the making of a feeding site can be illuminated. The black box of how nematodes bring about such elaborate cell differentiation in the plant is also starting to open. Although the information is far from complete, the groundwork is set so that the functions of the plant and nematode genes in feeding site development can begin to be assessed.

Secretory proteins encoded by genes expressed in the esophageal gland cells of plant-parasitic nematodes have key roles in nematode parasitism of plants (Gao et al. 2001a). Two venom allergen-like protein cDNAs (designated hg-vap-1 and hg-vap-2) were isolated from *Heterodera glycines* gland cell cDNA libraries. Both cDNAs hybridized to genomic DNA of *H. glycines* in Southern blots. The hg-vap-1 cDNA contained an open reading frame encoding 215 amino acids with the first 25 amino acids being a putative secretion signal. The hg-vap-2 cDNA contained an open reading frame encoding 212 amino acids with the first 19 amino acids being a putative secretion signal. Genes of hg-vap-1 and hg-vap-2 contained four introns, which ranged in size from 44 to 574 bp, and five exons ranging in size from 43 to 279 bp. In situ hybridization analyses showed that mRNAs of both vap genes accumulated specifically in the subventral gland cells of *H. glycines* during parasitism. In this study, the gland cell-specific expression and presence of predicted secretion signal peptides in both VAPs suggested that these proteins are secreted from the nematode and may play a role in the infection of host plants by this parasite.

10.21 Mechanisms Involved

With many of the resistance genes, mechanisms have been described in genetic basis of host–pathogen interactions, which include gene–gene relationship, localized necrosis (hypersensitive response), the degradation of feeding structures in the host (a sign of poor nutrition for nematode), changes in gene expression, and molecular responses.

10.21.1 Gene–Gene Relationship

Plants have a repertoire of resistance genes that protect them from many pathogens, including fungi, bacteria, nematodes, and viruses (Williamson 1999). In many cases, pathogen recognition by the host is mediated by single resistance genes (*R genes*) in the host and single gene in the pathogen called avirulence (*Avr*) genes. This relationship proposes that hypersensitive reaction occurs when the product of plant resistance gene (*R*) interacts with the product of pathogen virulence or avirulence gene (*Avr*). Disease resistance requires a dominant resistance (R) gene in the plant and a corresponding avirulence (Avr) gene in the pathogen. R genes are presumed to either enable plants to detect Avr gene-specified pathogen molecules or initiate signal transduction to activate defenses or possess the capacity to evolve new R gene specificities rapidly. The following steps are involved in plant pathogens:

- Pathogen entry into plant cell or connection with infected cells.
- Release of protein and other molecules.
- Binding of R gene products to certain molecules from pathogens (Avr gene products).
- Binding activates R gene product and triggers protective hypersensitivity response (HR).
- When R and Avr gene products do not match, no HR occurs and plant becomes susceptible to disease.

Recently, R genes have been cloned from several plant species. Most encode proteins that carry a structural motif with a repeating pattern of 20–30 amino acids called a leucine-rich repeat (LRR). A few have been cloned and a number of additional genes are likely to be cloned in the near future.

Mi-1: The *Mi* gene of tomato confers effective resistance against several root-knot nematode species. This gene was introduced into cultivated tomato, *Lycopersicon esculentum*, from the wild species *L. peruvianum* by embryo rescue of the interspecific cross. The Mi gene was isolated by positional cloning and its identity confirmed by complementation of function. Transgenic plants with *Mi* were also found to be resistant to the potato aphid, *Macrosiphum euphorbiae*, indicating that this gene confers resistance to the aphid as well as to root-knot nematodes.

Gpa2: Recently Gpa2, a gene that confers resistance against some isolates of the potato cyst nematode, *Globodera pallida*, was cloned by a positional cloning strategy. This gene is a member of the NBS-LRR gene family and contains a possible LZ near its amino terminus.

GroI: A gene mediating resistance against the potato cyst nematode, *Globodera rostochiensis.*

Hs1$^{pro\text{-}1}$: The first nematode resistance gene to be cloned was *Hs1$^{pro\text{-}1}$*, a gene from a wild relative of sugar beet that confers resistance against *Heterodera schachtii*.

Cre3: A gene mediating resistance against cereal cyst nematode (CCN). Using the sequences of NBS-LRR gene as a probe, homologues were detected in other regions of the wheat genome, some of which correspond to other CCN R gene loci. In addition, loci with homology to R genes have been found to be associated with CCN and aphid resistance in barley.

The recognition of pathogen by host initiates a cascade of defense responses, often including a hypersensitive response consisting of localized cell necrosis at the infection site. Feeding site becomes surrounded by necrosing tissues and eventually collapses. For example, *Mi*-mediated resistance is characterized by a localized necrosis of host cells near the invading nematode (Zacheo et al. 1993). Additional molecular changes occur rapidly after the infection of resistant plants. For instance, the activity levels of the enzymes phenylalanine ammonia lyase and anionic peroxidase are induced early in the resistance response in tomato to many pathogens including nematodes. With *Hi*-mediated resistance of potato to *G. rostochiensis*, the feeding site begins to develop and the nematode becomes sedentary. However, the developing feeding site becomes surrounded by necrotic tissues and collapses. The few nematodes that do develop on *H1* potato plants are mostly male, a sign of poor nutrition for the nematode.

10.21.2 Changes in Gene Expression

Changes in gene expression are suggestive of a stress or defense response, which have been observed after infection with either cyst or root-knot nematodes. For example, changes in gene expression in potato leaves after root infection by the cyst nematode *G. rostochiensis* include the induction of pathogenesis-related proteins; in tomato roots infected with root-knot nematodes, genes with homology to several known plant defense genes (including peroxidase, chitinase, lipoxygenase, and proteinase inhibitors) are induced locally within 12 h of inoculation; the induction of the family of genes encoding glycoproteins that form a major component of plant cell walls and are induced in plant defense responses is significantly increased in *M. javanica-induced* galls at 1 week after infection as well as in tomato root tips by 12 h after infection.

Molecular responses include wounding or stress caused by nematode infection as well as perturbations directed toward the initiation and maintenance of feeding sites. The complex morphological and physiological changes that occur during the establishment of feeding sites are reflected by altered gene expression in the host. Because many of the genes identified in the response are members of gene families with complex regulation, their regulation is difficult to interpret. Not surprisingly, phytohormone levels are also abnormal in root-knot nematode-infected roots, providing an additional level of complexity in understanding plant responses to nematode infection.

In total, intraspecific genetic variation in host range and response to specific resistance genes is high, especially for the sexually reproducing plant-parasitic nematode species. This heterogeneity has made it difficult to breed for nematode resistance in crops. There may be multiple genetic mechanisms by which a nematode can acquire the ability to circumvent resistance. Loss of a nematode gene product could result in failure of the plant to recognize the nematode. Hence, efforts to clone several nematode resistance genes are currently in progress. To fill the gap, a variety of strategies to engineer synthetic resistance are being developed. Strategies that combine one or more natural resistance genes with synthetic resistance may be the most effective.

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11 Nematological Techniques

It is difficult to detect a nematode damage merely on the basis of visible symptoms. Without a proper diagnosis, suitable nematode management schedule cannot be designed. Identifying a nematode problem comprises various steps, viz., drawing of suitable samples of soil and plant parts from the infested field, processing the samples to extract nematodes, separating and identifying specific parasitic species of the phytonematode based on morphological criteria, preparation of mounts, staining plant tissues to observe endoparasitic nematodes, etc. Some of the major techniques commonly adapted in plant nematology are furnished hereunder.

11.1 Sampling for Phytonematodes

It is essential to manage the nematode population in the field if it is above the economic threshold level (ETL). ETL is the population level at which nematodes cause economic damage to the crop. Therefore, nematode analysis is necessary before planting the crop to estimate possible damage and to help decide the suitable management options. The collection of soil samples and plants are the first step in the diagnosis of crop disorders caused by nematodes that attack root systems. An accurate diagnosis depends on proper collection and processing of samples. Improper collection and handling of samples may lead to the dismissal of nematodes as part of the problem; hence, any management strategy developed to alleviate the problem will be deficient. Information such as crop and cultivar, previous cropping history, history of other known or suspected problems, irrigation or rain fed, and previous applications of soil amendments (organic or any pesticides) are needed along with samples to assist in the diagnosis of the problem. The exact location of the samples is important. This information will permit comparisons with other problems reported previously from the region or indicate if the samples represent the first report of a nematode species from the area (Hafez and Pudasaini 2012).

Nematode sampling has become increasingly important in modern agriculture as the concepts of integrated pest management (IPM) and integrated crop production are developed and utilized. Scientists concerned with nematode populations have improved methods for their assay; however, data from the best extraction methods are of limited value if the sample is not representative of the area. Effective diagnostic sampling may involve rating plant roots (e.g., galls caused by *Meloidogyne* spp.), bioassays, or visually assessing aboveground growth for effects of foliar pathogens in addition to collecting soil and root samples for nematode counts. Since adoption of nematode management practices is need based, growers are advised to treat the soil only if they have identified the specific nematode that causes damage to that particular crop.

Nematode sampling is the basis for determining the occurrence and distribution of many plant-parasitic nematodes. Quarantine or phytosanitary

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regulations of many countries, or political subunits, require that planting materials be produced on land certified free from nematodes. Soil sampling for certification of widely distributed planting materials requires extreme precision for detection of quarantined pests. Although the objective of detection seems simple, a negative result does not necessarily prove absence of the pest, but only indicates that a nematode population is below the detection level. The fact that initial numbers of nematodes can be related to the yield of annual crops has enabled nematologists to develop functional advisory programs, even though relationships between nematode numbers and crop damage may be modified by environment. Because of the importance of reliable detection, most sampling for advisory purposes is conducted when population densities are near their maximum levels, often at the end of the growing season after harvest. Sampling at the time of planting, however, theoretically will give a better estimate of the initial nematode problem where population levels are high enough for detection. Follow-up sampling may be necessary with perennials because low, nondetectable populations sometimes increase over time to damaging levels.

Root and soil samples containing roots can be taken at any time as long as the soil is not frozen. During the active growing season, however, nematodes live and feed inside or along roots particularly during hot dry seasons. If nematodes are suspected of contributing to the decline of a particular area of a young crop during the growing season, collect entire root systems with surrounding soil separately from plants with symptoms and plants without symptoms. If the decline is noticed in a fruit tree orchard, vineyard, or other perennial crop, carefully dig and sample from the feeder root zone approximately 10–20 g fresh weight of roots from the infected plants and submit for analysis. Do not sample the roots from dead plants because the nematodes will have already died or moved away from dead roots into the soil. Place samples in a plastic bag out of direct sunlight and in a cool place during transportation to the diagnostic lab.

11.1.1 Suitable Time to Collect Soil Samples

The best time to sample soil for nematode population assessment is in the spring after the soil has warmed up or during the fall, soon after harvest. It is not advisable to take nematode samples when fields are very wet. Fields with a history of nematode problems may be sampled routinely to determine if the nematode population is approaching or has exceeded an economic threshold. Soil populations of most phytonematodes tend to be highest in September and October after crops have senesced and died. This is the best time of the year to sample for nematodes. Sampling in the early fall allows growers time to make decisions on whether to fumigate during the fall or spring or what crop should be planted the following spring. It also allows time to implement an integrated management strategy prior to growing a susceptible crop in that field. Sampling in the spring prior to planting a crop may also be reliable.

11.1.2 Where to Look for Sample?

Where to sample soil for nematode assessment depends on the purpose for taking the soil sample, the type of crop in the field, and the type of nematodes being sampled. The rhizosphere zone of a plant is the right place to collect soil and root samples. If the purpose of sampling soil for nematodes is to diagnose a problem during the growing season in a row crop, take 8–10 soil cores from areas where plants are unhealthy or near plants along the margin of a severely affected area. Sample another 8–10 soil cores separately from areas of healthy growing plants for comparison (Fig. 11.1). When sampling soil from row crops during the growing season, or from trees or perennial crops, it is very important to get the feeder roots of the crop in the soil sample, since this is where many nematodes live.

For individual fruit trees or ornamental shrubs suspected of being infested with nematodes, it is best to take soil samples from just below the drip

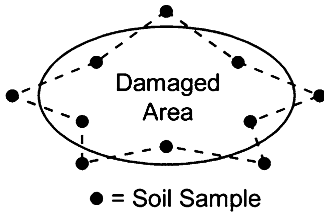


Fig. 11.1 Sampling pattern for damaged area or infected patch in a crop

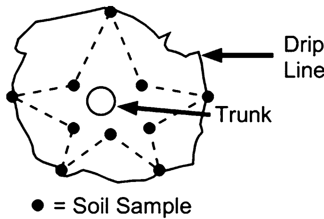


Fig. 11.2 Sampling pattern for individual tree or shrub

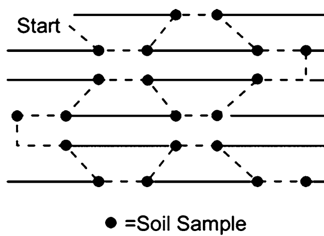


Fig. 11.3 Soil sampling pattern for row crops

line and in the area between the outer branch tips and the tree trunk (Fig. 11.2). If the purpose of sampling a field is to determine whether the nematode population has reached an economic threshold in a row crop, take soil cores within the row of actively growing plants to obtain samples that contain feeder roots (Fig. 11.3). When sampling from fallow fields, in the autumn after the crop has senesced or in the spring prior to planting, it is best to walk in a Z, W, or M pattern across the field (Fig. 11.4). The soil sample should represent not more than 2.5 ha.

11.1.3 How to Collect Soil Sample?

Nematodes are rarely distributed evenly throughout a field, and nematode populations fluctuate throughout the growing season. Soil should be

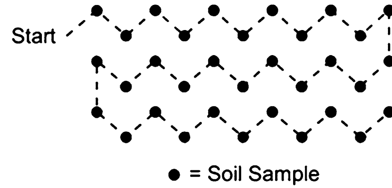


Fig. 11.4 Sampling pattern in the autumn after the crop has senesced or in the spring prior to planting. This pattern can also be used for fallow fields and crops that are not planted in rows

Table 11.1 Number of soil core samples/area required to estimate nematode populations

Area	Number of soil cores/sample
<500 m^2	8–10
500 m^2–0.5 ha	25–35
0.5–2.5 ha	50–60

sampled approximately 20 cm (8 in.) deep using a 2.5-cm (1-in.)-diameter soil core probe (Fig. 11.4). Alternatively, soil can be sampled with a narrow-bladed shovel or trowel; however, this method is less reliable than using a soil core probe. Extremely wet, dry, hot, or cool seasons can influence the population levels particularly in the top 2.5–5 cm (1–2 in.) of soil. Discard the top 2.5–5 cm (1–2 in.) of soil where nematodes would not usually live due to extreme environmental conditions. Collect soil cores in a clean bucket, mix the soil thoroughly but gently, and place in a labeled plastic bag or container. Never allow soil samples to heat up or dry out. Place soil samples in a cooler with ice until they can be stored in a fridge or analyzed for nematode populations.

11.1.4 How to Decide the Number of Soil Cores?

The number of soil core samples required to estimate nematode soil population levels depends on the size of the area under investigation (Table 11.1). The sample submitted to the laboratory should not represent more than 2.5 ha. Enough soil to give a good representation of the soil population is all that is necessary. The chart below is a guide of how many cores are necessary to

make up a representative sample. If soil type changes within the field, take separate samples from each soil type. Send the soil samples to a pest diagnostic clinic or laboratory that is qualified to isolate, identify, and enumerate nematodes.

While collecting soil samples, it is also best to collect root samples to aid in the diagnosis. When doing so, one should dig up the plant so as to obtain as many of the fine feeder roots as possible. If the plant is pulled from the ground, most of these feeder roots will be lost. If sampling a perennial crop, it is also important to collect feeder roots specifically from the current year's growth rather than larger and older roots. It will be difficult to make an accurate diagnosis from a sample that only contains large roots. Of course, some nematode parasites are rarely found in the soil or roots but are found primarily in the bulbs, corms, stems, or foliage. In such cases, care must be taken to collect the appropriate symptomatic tissues. Again, samples should not be taken from long dead plants, as the parasites may be difficult to detect in such samples. It is best to collect samples from a number of live plants that are exhibiting a range of symptoms. Extra effort in sampling plant tissues and soil is required to obtain accurate results. The accuracy in determining relative numbers and developmental stages of nematodes may be greatly affected by sample handling or extraction. Design and management for sampling may need to be modified for each specific type of study.

11.1.5 Sampling Tools

There are different tools to collect soil, such as soil probes, trowels, hoes, narrow-bladed spades, or shovels. However, they are collected most efficiently with sampling tools designed for the procedure, such as a standard Oakfield soil probe with a diameter of 1 in. If using a shovel or spade, it is best to collect only a narrow column of soil from each shovelful of soils to avoid excessive sample volumes. The multiple subsamples should be thoroughly mixed together in a large bag or bucket and a final sample of 1–2 L of soil placed in appropriately labeled plastic bags.

11.1.6 Care of Samples After Collection

The biological vitality of the sample should be preserved after the collection of plant and soil samples as the extraction and identification procedure needs live nematodes to achieve the best accuracy. Samples should be delivered or shipped to the diagnostic laboratory without delay. Samples should be protected from extremes of temperature, i.e., freezing (less than 32 °F) or temperatures above 95 °F. Thus, they should be packed in insulated containers and kept in a cool environment. Refrigeration (storage at 40 °F) is not required if the sample is being processed within a day or two but is helpful if the samples will be stored for a longer time period. It is usually not necessary to pack samples in ice for shipment, but shipping over a weekend or holiday period should be avoided. This will reduce the possibility of the samples being left unprotected on a loading dock or in a warehouse for several days. A good rule to follow is to treat the samples like perishable food that one wishes to consume in 3–4 days.

11.1.7 Considerations to Design Sampling Procedures

Various aspects are to be considered while designing sampling procedures, viz., influence of nematode distribution patterns on the results, the capacity of the nematode species to move or be moved by man or other carriers, presence of majority of nematodes in most annual cropping systems in the upper layer of soil (to 15 in.), influence of biology, feeding habits and environmental interactions of the nematode species involved, and the effect of crop rotation and cultural practices.

11.1.8 Field Mapping

The distribution of nematodes is seldom uniform or constant and changes may occur rapidly. Most of the time nematode distribution is patchy. For these reasons, the field to be sampled should

be mapped into subdivisions. Any observable variation in previous crop growth, soil texture, moisture and draining patterns, or cropping history will constitute a subdivision. An effective sampling map may then be constructed.

11.1.9 Sampling in Different Crops

11.1.9.1 Established Perennials

Several points are to be borne in mind while sampling in perennials, which include the following: collect separate samples for each plant species, collect from the feeder root zone of plants showing decline, and avoid sampling directly around dead plants; perennial crops will typically fluctuate during the year with the timing of the fluctuations varying by crop and location; it is important to always sample at the same time each year (because of population fluctuation); and if one wants to compare populations from year to year, it is important to leave some areas untreated for comparative sampling if treatments are applied to try to reduce nematode numbers. If this is not done and samples are taken several months after a treatment, populations may be either lower or higher than before treatment just because of normal population fluctuations.

11.1.9.2 Annual Crops

In this category, nematodes typically are at a low level during planting and increase toward harvest as long as roots are healthy enough to support nematode feeding. Following harvest, nematode numbers typically decrease until another susceptible crop is planted or weeds become available that will support reproduction. Under weed-free fallow conditions, for instance, root-knot nematode populations may drop as much as 85–90 % in a year's time, while sugar beet cyst nematode will only decrease by 20–60 % depending on the location. *This principle is illustrated in an example of population decrease of Heterodera schachtii following harvest of a susceptible crop until the planting of another susceptible crop (cabbage) during which time populations increase dramatically and then decrease once again during successive nonhost crops. Nematode populations are usually easier to detect at harvest when they are highest than several months following harvest. For example, after several months in a fallow or nonhost crop, populations of root-knot nematode may not be detectable through sampling but may still be high enough to cause significant damage when a host is planted.*

11.1.9.3 Ornamental Plantings

Population fluctuations on ornamental crops including turf is almost similar to other perennial crops. Those on ornamental plantings will likely vary depending on whether the planting is an annual or a perennial. Samples should be collected when soil is moist and one pint of soil should be collected for each sample. When sampling fields in row crops, samples should be collected to represent the top 8 in. of soil. When possible, sample directly in the root zone. For pastures, lawns, and other areas, take samples of only the top 5 in. of soil. For shrubs, each sample should be composed of soil taken from three or more places in the area. Even when the sample is to be collected from one shrub, take soil from three or more places around the plant. Collect the sample to represent the top 6 in. of soil.

11.1.9.4 Field Mapping

In general, the distribution of nematodes is seldom uniform or constant and changes may occur rapidly. Most of the time nematode distribution is patchy and not uniform. Hence, the field to be sampled should be mapped into subdivisions. Any observable variation in previous crop growth, soil texture, moisture and draining patterns, or cropping history will constitute a subdivision. An effective sampling map may then be constructed.

11.1.9.5 Sampling Nursery Stock

Strategies for individual nursery situations involve subjective on-site judgment. The greater the potential loss, the greater is the sampling intensity that can be justified. For routine monitoring of container grown plants to maintain plant health and product quality, extraction of nematodes from single pots may not adequately reflect the situation in a whole block. Removing

single cores from a series of pots and bulking them into a composite sample provide a sample representing more plants. A convenient sampling rate of 1- and 5-gal containers is one core per 100 containers. If the plant is particularly susceptible or if nematode problems are suspected, the block of containers should be divided into groups of 2,000, with each group represented by a single sample of 20 cores. For a more routine sampling, cores from the whole block may be composited into a bucket and mixed thoroughly and a 1-quarter sample removed to represent the block.

The proportionally larger sample taken from smaller containers by this approach will help detect earlier stages of nematode infestation on young plants. Core sampling is excessively destructive in containers smaller than 1 gal, but the same criteria can be applied by destructively sampling one container per hundred as representative of a single core. The number of cores and sampling pattern for nursery plants in raised beds depends on the value of the plants and potential magnitude of the problem. Representing each bed by one sampling of several cores provides information on the occurrence of nematode problems in individual beds and allows individual bed treatments. If a nematode problem is unlikely, a single sample may represent several beds, which may result in a need for subsequent sampling to identify distribution of a population.

11.1.9.6 Effects of Cropping History

Differences in cropping history of a field that has now uniformly planted crop susceptible to root-knot nematodes may lead to the irregularity of root-knot damage within that field. If a portion of that field had an established vineyard earlier, on which root-knot nematodes increased to high levels and the remainder of the field has a grassy pasture free of root-knot nematode, the pattern of plant damage due to root-knot nematode infection will correspond to the previous vineyard area where nematode population density is much higher.

11.1.9.7 Soil Sampling Strategies

There are mainly three sampling strategies, viz., scouting, diagnostic, and predictive.

Scouting Sampling: When sampling for scouting purposes, soil should be collected from areas that are likely to be first infested with the nematode, called "high risk" areas. These areas include spots of the field where equipment enter along fence lines where windblown soil accumulates, in low spots of the field where surface water accumulates, or in areas of the field where unthrifty soybean growth had been observed in the past. However, if collection of soil samples for the purpose of scouting for soybean cyst nematode is not feasible, soil samples collected for soil fertility analysis also can be assayed for the nematode.

Diagnostic Sampling: Diagnostic soil sampling is performed when the soybean crop is in the field and the plants are showing obvious aboveground symptoms. Two separate samples have to be collected, one from the infected spot and another from a nearby spot which does not appear to be affected. Soil should be collected from near plants showing the most dramatic symptoms as well as near some that are not as severely affected.

Predictive Sampling: Predictive soil sampling is performed to gain information on the severity of a known soybean cyst nematode infestation for use in making management decisions for the upcoming growing season. It is done after the crops have been harvested, or in early spring prior to planting. If sampling for predictive purposes, collect the soil in a systematic, zigzag pattern within the area. Limit the area sampled to no more than 15–20 acres; if a larger field is to be sampled, divide the field into 15–20-acre parts and collect separate samples from each part. Define the part of land to be sampled based on agronomic parameters such as soil type, pH, drainage, elevation, or prior cropping history. The fewer the number of acres represented in each sample, the more accurate and representative the results will be.

Nematode Sampling Data Sheet: This Sheet Should Always Accompany the Sample							
1.	Collector: ____________________						
2.	Sample number: ____________						
3.	Date of collection: ________						
4.	Locality/village: ____________ Taluk: ____________						
	District: ____________ State: ____________						
5.	Precise location: ________________						
	Grower's name and address: ____________________						
6.	Host: ________ Variety: ________ Crop stage/age: ______						
7.	Condition of crop (healthy/diseased): ____________						
8.	Previous crop/intercrop: ________ Soil type: ____________						
9.	Rain fed: ______ Irrigated: ________ Water stable: ______						
10.	Symptoms observed:						
	i.	Above ground/part:					
		Stunting [ ]	Yellowing [ ]	Necrosis [ ]	Wilting [ ]	Slow decline [ ]	
	ii	Below ground/root system:					
		Galls [ ]	Cysts [ ]	Decay [ ]	Lesions [ ]	Rotting [ ]	
11.	Severity/disease index: ____________						
12.	Others:						
	i.	Distribution of affected plants:					
		General [ ]	Localized areas [ ]		Scattered plants [ ]		
	ii.	Percent of planting affected: ____________					
	iii.	Acre age (size of the field):					
		<½ ac [ ]	½ ac [ ]	1 ac [ ]	5 ac [ ]	10 ac [ ]	>10 ac [ ]
	iv.	Stand of the crop:					
		Very good [ ]	Good [ ]	Fair [ ]	Poor [ ]		
	v.	Comments: ________________________________ ______					
	vi.	Remarks, if any: ____________					

11.2 Techniques of Nematode Extraction

Once the samples, either soil or plant part, are collected, they need to be processed in the laboratory for extracting nematodes for further studies. Extraction is a fundamental and the most essential technique in nematological research (Ravichandra 2010). Plant-parasitic nematodes can be extracted from soil and plant parts by using different techniques. Some techniques are more effective than others for particular types of nematodes or for special kinds of plant materials. Some techniques are better adapted than others to specific purposes such as nursery stock inspection or quarantine enforcement. Others require expensive equipment or are too laborious to be practical except in extraction research work. Plant-parasitic nematodes can be extracted from both soil and plant parts. The technique depends upon the type of nematodes or kind of plant materials. Processing of samples consists of separating the nematodes from soil or plant materials in order to count them. Some techniques of processing samples are adapted to specific purposes. Nematode extraction techniques can be categorized into major groups like extraction from soil and plant material and cyst extraction.

Extraction of Phytonematodes from Soil: For smaller sample size, the soil may be directly placed in the funnel and processed by the mist extraction of Baermann's funnel technique. Larger ones may be processed first by the combined screening-funnel technique or by the gravity-screening technique followed by the mist extraction.

Extraction of Cyst Nematodes from Soil: The centrifugal floatation technique or the special water floatation technique is appropriate.

Extraction of Phytonematodes from Roots: The roots are to be cut into small bits, processed by mist extraction, blending, or Baermann's funnel technique or by gently washing and subjecting to jar or plastic bag incubation. The mist chamber is more effective for the extraction of both endoparasitic and ectoparasitic nematodes from both roots and soil samples. Root samples from nursery stock are processed by jar incubation to recover migratory endoparasites only. Direct examination can be done if the roots show galling or other clear evidence of nematode damage.

Extraction of Nematodes from Other Plant Parts: Other plant parts may include tubers, bulbs, stems, leaves, crowns, etc. Baermann's funnel and mist extractions are the most effective techniques.

Extraction of Sluggish Nematodes: Sluggish nematodes like *Hemicycliophora* and *Criconemoides* are difficult to extract by funnel method but can be extracted using centrifugal floatation technique.

11.2.1 Techniques of Nematode Extraction from Soil

These techniques are adapted to extract phytonematodes from soil, particularly from rhizosphere by using various techniques. Plant-parasitic nematodes can be extracted from soil surrounding the roots on which they feed, in several ways. The following are some of the most commonly followed ones:

1. Petri plate technique
2. Baermann's funnel technique
3. Cobb's sieving/gravitation technique
4. Combined Cobb's and Baermann's funnel technique
5. Mist chamber technique/Mistifier
6. Elutriation technique
7. Centrifugation technique
8. Fenwick can technique

11.2.1.1 Petri Plate Technique

This is a very fundamental technique to extract nematodes from a small quantity of soil sample.

Procedure Take a Petri plate filled with water; above that place a wire gauge and over that place tissue paper; water level per plate should be in touch with the wire mesh; place the soil sample above tissue paper. Leave this for 24–48 h; take out the Petri plate and observe for nematodes present in it under a stereo binocular dissecting microscope.

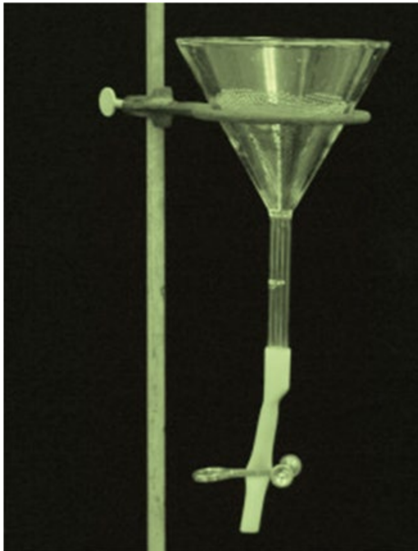


Plate 11.1 Baermann's funnel apparatus

This is a very simple, basic, less expensive, and quick technique. It can be used to extract nematodes from both soil and plant tissues. However, it is not very precise because the Petri plate cannot hold large amounts of water. It can hold only a small quantity of soil sample. Since there is not much space in the Petri plate, there may be suffocation.

11.2.1.2 Baermann's Funnel Technique

This technique is an excellent system of separating nematodes from soil and also plant parts including roots and condensing them for examination. This technique utilizes a long-necked funnel that has been named after its inventor, "Baermann" (Plate 11.1).

Procedure Attach a 10-cm length of rubber tubing to the funnel stem and clamp the tubing; mount the funnel on the ring stand; fill the funnel two-thirds full with water; place the wire-mesh basket on top of the funnel and use it to support tissue; mix the soil sample and remove plant debris, stones, inert matter, etc.; spread the soil subsample (50–200 cm^3) evenly on tissue; fold the edges of the tissue paper without allowing them to face downward, extending outside the wire mesh. This is to avoid trickling of water drops from the edges which might carry nematodes. Fill the funnel completely with water so that the water level is about 5 mm above the wire mesh; do not let the water and soil lose contact during the extraction period; add water as needed; maintain a temperature at 22–25 °C so that it is conducive to nematode movement; nematodes move through tissue and settle in the funnel; only active stages are recovered; after 24–48 h, recover the extracted nematodes by releasing 20 ml of water from the stem of the funnel into a counting dish/beaker.

In this technique, most nematodes are recovered after 24–48 h. However, nematode yield may vary depending on several factors including the size of the sample, temperature, time of storage and extraction, and nematode species.

This technique is simple and easy to set up, and the equipment is inexpensive. Nematodes from a small quantity of soil sample can be effectively recovered. However, lack of aeration in the water may reduce nematode movement, hindering recovery. Only active nematodes can be recovered; sluggish/inactive forms cannot be extracted. Recovery of active nematodes from large samples is poor. The funnel capacity is small; hence it may be too small to be a representative. Frequent care needs to be taken to check whether the wire gauze is in touch with the water surface.

11.2.1.3 Cobb's Sieving and Gravity Method (Decanting and Sieving Method)

This is the most basic technique that consists of mixing soil (the volume varies) with a large volume of water (normally three- to five-folds), allowing a brief time for heavy particles to settle, and then pouring the mixture through one or more sieves of a mesh size expected to retain large debris or nematodes. The sizes of screens used vary depending on the type of nematodes expected to be recovered and soil characteristics. Some begin with a course sieve of 10–20 mesh/in. which will catch large debris but allows nematodes to pass through. The solution is then passed through sieves of 60–500 mesh/in. to catch nematodes. Nematodes and soil particles caught on the sieves are "backwashed" into containers.

If not too murky, this solution can then be viewed under a microscope or subjected to an additional technique to further purify the sample. A set of three or four sieves is used here. Although the size of the sieves may vary slightly, big-, medium-, and small pore-size sieves are employed.

The following is a list that gives the different sieve sizes (British Standard Sieve Series) (Southey 1986).

Sl. No.	Mesh/in.	Pore aperture (μm)
1	4	4,000 (=4 mm)
2	6	2,800 (=2.8 mm)
3	8	2,000 (=2 mm)
4	12	1,400 (=1.4 mm)
5	16	1,000 (=1 mm)
6	20	840
7	22	710
8	30	500
9	44	355
10	60	250
11	72	210
12	85	180
13	100	150
14	120	125
15	150	105
16	170	90
17	200	75
18	240	63
19	300	53
20	350	45

Most adults of large dorylaimids are caught on a 250 μm-aperture sieve, adults of average-size nematodes on a 90 μm-aperture, and many larvae and small adults on a 63 μm-aperture sieve. A 45 μm-aperture sieve is necessary to recover small larvae. Only a proportion of the nematodes are caught when a suspension is poured once through even the finest sieve (65 % of nematodes), 500 μm long or 25 % of those 250 μm long when the suspension is poured once through a 50 μm-aperture sieve. It is therefore advisable to use a bank of sieves to pour the suspension three or four times through the finest sieve in use, collecting the residue off the sieve each tine. The diameter of the sieve, the quantity of water used, and the amount of debris collected on the sieve will affect the number of nematodes retained.

Plate 11.2 Set of sieves

Procedure Mix soil sample and pass through coarse sieve to remove debris, inert matter, roots, etc.; take a 200-cm^3 subsample of soil, pack lightly into beaker for uniformity; place soil in one of the buckets or pans; mix water in the ratio of 1:3 (soil:water); sieving and decanting process (various combinations of the following) (Plate 11.2): mix soil and water by stirring with hand or paddle; allow to stand until water almost stops swirling; pour all but heavy sediment through a 20-mesh sieve into the second bucket; discard residue in the first bucket; discard material caught on sieve (mostly it contains inert matter, saprophytic nematode forms, etc.); stir material in the second bucket; allow to stand until water almost stops swirling; pour all but heavy sediment through a 200-mesh sieve into the first bucket; discard residue in the second bucket; backwash material caught on the 200-mesh sieve (which includes large nematodes) into a 250-ml beaker; stir material in the first bucket; allow to stand until water almost stops swirling; pour all but heavy sediment through a 325-mesh sieve into the second bucket; discard residue in the first bucket; backwash material caught on the 325-mesh sieve (which includes small- to midsized nematodes and silty material) into a 250-ml beaker; sample in the 250-ml beaker will probably be too dirty with fine soil particles for direct viewing; sample may be placed on Baermann's funnel or subjected to sucrose centrifugation; the combined procedure allows extraction of nematodes from larger volumes of soil.

Soil particles may block the aperture of fine sieves before all the suspension had passed through. Blocking can be avoided by pouring the suspension carefully onto sieves inclined at about 30° above the horizontal and by ensuring (by mean of small spacers) that airtight joints forming air locks do not occur between sieves in a bank. Should a blockage occur, tapping the frame or gently stroking the underside of the gauze with the fingers may clear it; alternatively, the sieve may be partly immersed in water and gently shaken until the mesh clear.

The technique is not dependent on nematode movement; hence, sluggish nematodes can also be recovered. It allows recovery of most nematodes from large soil samples. Nematodes are available for direct examination in less than half an hour. Nematodes can be differenced based on their size. However, the technique requires expensive sieves and experienced workers. The suspension may not be very clear. Difficulty may be encountered in observing nematodes because of fine soil particles. It cannot be used for nematode extraction from plant tissues. Careful handling is required as mesh is delicate.

11.2.1.4 Combined Cobb's and Baermann's Funnel Technique

This technique avoids the disadvantages of both techniques. Both active and sluggish nematodes can be extracted by this combined technique.

Procedure Nematodes may be separated from soil particles after sieving process by the use of sieving and gravitation technique; put the sieved soil in the tissue paper on top of the wire mesh in the funnel; fill the funnel with water up to the rim; nematodes will pass through settling at the bottom of the funnel; collect 5–10 ml after 24–48 h.

This method allows recovery of most nematodes from large soil samples. The resultant sample contains less silt and debris compared to Cobb's sieving method, and it is easier to examine under a dissecting microscope. However, sluggish nematodes recovered during the sieving part of the technique may fail to pass through the tissue in the funnel, although recovery of mobile nematodes is good. Processing takes longer and requires considerable equipment.

11.2.1.5 Mist Chamber Technique/Mistifier

This technique is a modification of Baermann's funnel technique and can be used for both soil and plant samples. A continuous fine mist of water is sprayed over soil samples. Active nematodes emerge which can be recovered from the water which collects below. Nematodes recovered by this method are often more active than those extracted by some of the above methods because oxygenation is better and sap and toxic decomposition products are washed away (Seinhorst 1950).

Procedure Before operating, make sure the equipment is functioning properly. The nozzle should deliver a fine, fairly uniform mist at the proper temperature (70–75 °F) and be on the correct time cycle (1.5 min on, 8.5 min off). When the nozzle valve shuts off, do not allow water from the nozzles to drip into the funnels; place one funnel for each sample into the funnel rack. Cover the inside of the stainless steel wire basket with a double thickness of tissue. Avoid puncturing or tearing the tissue. Carefully place the material for extraction into the tissue-lined basket, then set the basket in the funnel; samples may include moist soil (1/4 cup), small root bits (1/4–1/2 in.), bulb, corm, sucker, or tuber tissues cut into bits; label the culture tubes with sample number and date. Insert the stem of each funnel into the corresponding labeled culture tube; insert the loaded funnel rack into the mist chamber; incubate samples under the mist for 3–5 days (2 days for foliar and bud samples); the water should gradually fill the culture tubes and overflow slowly enough so that the nematodes remain at the bottom of the tubes; carefully remove each culture tube from its funnel without disturbing the contents; place the labeled tubes in a test tube rack; with a tube attached to an aspirator or with a large pipette, draw off the water from each tube to within 1 in. of the bottom; this must be done very carefully to avoid stirring up of nematodes which have settled to

the bottom; the inside and outside of the aspirator tube or the pipette must be rinsed between samples or a fresh pipette used for each sample to avoid contamination; the nematode suspension remaining in each culture tube is now ready for examination or prepared for shipment to the laboratory.

By this technique, more nematodes can be recovered from a given quantity of soil sample or plant material than by Baermann's funnel. It prevents the loss of nematodes which occurs in the jar incubation technique when rinsed water is poured through the sieves. There is no accumulation of toxic materials during the incubation period. However, this technique involves relatively expensive specialized equipment.

11.2.1.6 Sieving and Sucrose-Centrifugation Technique

This technique is also useful for the nematode extraction both from the soil and plant parts. Some difficulty may be encountered with fine clay soil. Only a small quantity of the sample can be used. It is also useful to isolate cysts and juveniles of *Heterodera* and *Globodera* species (Barker 1985).

Procedure Prepare sucrose solution; add deionized water to 454 g sugar to bring total volume to 1 L; stir until sugar is completely dissolved; mix soil sample and pass through coarse sieve to remove inert matter, roots, etc.; collect a 100-cm^3 subsample of soil. Pack lightly into beaker for uniformity; remove inert and organic material; mix soil subsample in 500 ml water by pouring between beakers ten times; rinse residues in the second beaker into beaker with sample; swirl beaker with sample; allow to stand for 15 s (for settling of sand); pour supernatant through 20/500-mesh stacked sieves. Tap gently the side of 500-mesh sieve to facilitate drainage.

Larger particles will remain in the beaker; organic debris is caught on the 20-mesh sieve; nematodes and silt are retained on the 500-mesh sieve. Using the coarse-spray water bottle, gently wash nematodes into one sector of the 500-mesh sieve. Using the fine spray water bottle, wash sample into a centrifuge tube.

Add water to centrifuge tubes to equalize volumes; place tubes in centrifuge in balanced pairs; spin at 1,700 rpm (810 g) for 5 min without using the brake; allow to settle for 5 min; aspirate the supernatant to approximately 1 cm above the pellet; fill tubes with sucrose solution at room temperature; stir with a spatula to break up the pellet (must be completely dispersed). Spin the sample and bring the centrifuge up to 1,000 rpm (280 g) in 30 s and then apply brake.

Nematodes and clay are suspended in sucrose supernatant; silt and larger particles are in the pellet. Pour supernatant through a 635-mesh sieve. Rinse gently with water and transfer to labeled vials using the fine spray water bottle.

11.2.1.7 Elutriation Techniques

In this technique, a measured flow of water in an upward direction will support nematodes in a given range of specific gravities but will allow heavier soil debris to pass downward so that nematodes can be collected in a relatively clean state. There are two major types of elutriation system which differ mainly with respect to the equipment and the processing methods.

Advantages: Nematodes are separated according to their size.

Disadvantages:

(a) Time-consuming and cumbersome. It normally takes about 35–40 min per sample.
(b) May be expensive in case of any breakages.
(c) Major glass units, if broken, are difficult to repair.

The Oostenbrink Elutriation Technique

Procedure Fill Oostenbrink elutriation the apparatus with clear water till the outlet of the funnel (up to level 1) by a constant water stream of 1,000 ml/min through a perforated pipe from the bottom of the can (Oostenbrink 1960); thoroughly mix the soil. Place the moist soil sample (100–500 ml) in the 1-mm-pore-size top sieve; wash the sample into the can via the funnel by means of a nozzle delivering about 700 ml/min until two-thirds of the column is filled up (up to level 2); turn off the top nozzle. Reduce the constant water stream from the

bottom to 600 ml/min until the water reaches level 3; pour the suspension into 4 sieves of 325 or 400 mesh sizes, 30 cm diameter, placed on top of one another; the catch is immediately washed into a 250-ml beaker; nematode suspension is poured onto a wire gauge sieve containing two layers of tissue paper; the wire gauge with tissue paper is placed in a funnel holding sufficient water to remain in contact with the bottom of the wire gauge. Leave the funnel overnight. The final suspension containing the nematodes is ready for analyzing at the bottom of the funnel system.

Seinhorst's Elutriation Technique

Procedure Stir the soil sample (500 cm^3) in 750 ml water and strain through a coarse sieve into a 2-l Erlenmeyer flask (A) provided with a funnel cap (C) and a rubber plug (O) (Seinhorst 1962); close the flask with the cap and place in such a way that only the outlet protrudes into the funnel C1 + C2, which is connected with a second funnel of similar shape but smaller in size (D1 + D2); C1 has an overflow pipe at the upper end which discharges into a small funnel; C2 and D2 have outlet pipes provided with rubber sleeves and stopcocks; the end of funnel D2 discharges into funnel E1; the outlet pipe (I) is fitted with a clamp; a feed pipe (N) is connected to the upper end of funnel E1; E1 discharges into E2 which is a straight tube; the lower end of E2 is closed with a plug provided with a device (M). A cable connects E1 and E2 and a free plug is used to close the passage between them if E2 has to be emptied; the floatation apparatus is filled with tap water up to the overflow pipe. The Erlenmeyer flask is opened and the sample is poured through a funnel using a length of wire. By regulating the counterflow of water, the nematodes and small-sized soil particles are maintained in a suspended state or settle slowly, while the heavier particles of soil settle relatively more rapidly than in container E2; regulate the flow of water so that the rate of rise in D2 is 975 or 380 cm/h in C2. The reserve tank (P) ensures the constant flow of water through an outlet pipe located above the overflow pipe (F) and connected by a hose with syringe (G); the small-sized nematodes with a settling rate below 380 cm/h remain in the funnel C1 + C2, whereas particles above 50 μ size settle to the bottom; in the funnel D1 + D2 the nematodes with a settling rate of 380–975 cm/h are separated from particles over 100 μ in size. The large nematodes (above 2 mm) settle within 7–9 min in E1 and the largest soil particles are collected in E2; particles of more than 50 μ size will settle within 20 min in sandy soil type, 30 min in loamy soil type, from the flask and may be removed; 10–15 min later, the clamp may be opened and the contents of C1 + C2 are poured into vessel 1. Later, D1 + D2 are emptied into vessel 2 and E1 into vessel 3; empty E2 and the whole apparatus is washed; contents of vessel 2 is sieved through seven 100 μm-aperture sieves of 10 cm diameter, washing the residues into vessel 1; sieve the contents of vessel 1 through seven 50 μm-aperture sieves of 20 cm diameter, collecting the residues in smallest quantity of water; the contents of vessel 3 are poured through 250 μm-aperture sieves; wash all the residues and concentrate their suspension by pouring through 1 50 μm-aperture sieve; wash the residues into a beaker small amount of water.

Using this technique, nematodes are separated according to their size. However, it is time-consuming and cumbersome and normally takes about 35–40 min per sample. It is expensive in case of any breakages and major glass units, if broken, are difficult to repair.

11.2.1.8 Sugar Floatation Technique

Preparation of Sugar Flocculant Solution Add 908 g (2 lb) sugar to 1 L warm water and stir for 20 min or until dissolved. To this, add 25 ml of 0.1 % flocculant solution (1 g Super Floc 16 added slowly to 1 L of warm water and stirred).

Procedure Pour sample to pan A, add an equal amount of water to the sample, and mix soil and water, breaking up soil clods; pour surface liquid in pan A through the 20-mesh sieve, held over pan B until the soil begins to flow onto the sieve. Still holding the 20-mesh sieve over pan B, wash the soil through the sieve with the fogger

nozzle; discard soil retained on the 20-mesh sieve and in pan A. Pour the material in pan B through 100-mesh sieve, held over pan A until soil begins to flow onto the sieve. Still holding the 100-mesh sieve over pan A, wash the soil through sieve with the fogger nozzle; backwash the material retained on the 100-mesh sieve into a 1,000-ml beaker. Pour the material in pan A through the 200-mesh sieve, held over pan B until soil begins to flow onto the sieve. Still holding the 200-mesh sieve over pan B, wash the material through the sieve with the fogger nozzle; backwash the material retained on the 200-mesh sieve into a 1,000-ml beaker. Pour it through the 500-mesh sieve, held over the sink, until soil begins to flow onto the sieve. Backwash the material retained on the 500-mesh sieve into a 1,000-ml beaker. Add water to the1,000-ml beaker containing the sieved material, raising the total volume of liquid and residue to 300 or 400 ml; add an equal volume of sugar flocculant solution to the contents of the 1,000-ml beaker; with the high-speed stirrer, stir for 1 min at high speed. Allow the suspension to settle for 1 min; wash sugar off the residue on the 500-mesh sieve with the fogger nozzle. Backwash the residue on the 500-mesh sieve into a 250-ml beaker; keep the final volume in the beaker to an absolute minimum (50 ml maximum). Label the beaker and examine the sample.

Nematodes are recovered quickly, making timely identification possible. But this technique needs some relatively expensive equipment. Sugar, if left on the sample too long, may distort nematodes, making identification difficult.

11.2.1.9 Floatation-Modified Fenwick Can Technique

This technique is useful for extracting cysts from dry soil although centrifugation with 50 % sucrose (by weight) and other methods are also popular (Fenwick 1940).

Procedure Mix the soil thoroughly; fill the modified Fenwick can with water; place the sample (100 cm^3) in the top sieve (20.5 cm diameter, 18- or 24-mesh sieves); wash the sample into the apparatus via the funnel (Plate 11.3). The coarse material is retained on the top sieve, heavy soil particles such as sand sink to the bottom of the apparatus, and the floating cysts are carried off over the overflow collar; cysts, root debris, and other particles are collected on a 20-cm-diameter sieve (60–80 mesh).

Plate 11.3 Fenwick can apparatus

Particles of 175 μm or smaller pass with water through the sieve; after washing, dry the debris at room temperature. Transfer the somewhat dried debris retained on the sieve to a 250-ml capacity flask; pour technical acetone or a mixture of three parts acetone and one part carbon tetrachloride into a volumetric flask up to the neck of the flask. Shake the flask and fill it completely (use an exhaust hood); one minute later, decant the floating cysts and debris through a filter paper (18.5 cm diameter) in a glass funnel into a volumetric or Erlenmeyer flask while rotating the original flask; the acetone passes through the filter; place the filter in a Petri dish and view it through a stereoscopic microscope (50× magnification) with overhead light; pick up the cysts with a camel hairbrush and transfer them to a small watch glass containing moist filter paper. Identify the cysts under the dissection microscope using an overhead light; transfer the cysts of desired species with a camel hairbrush into a small drop of water in the glass tube of the homogenizer. Place the piston in the tube and

carefully rotate it by hand; pour the eggs and juveniles that were released from the cysts into a bottle; fill the bottle with water up to 100 ml; mix the suspension carefully using compressed air; pipette out two 100-ml aliquots and place in dishes for counting.

11.2.2 Techniques to Extract Cyst Nematodes

These techniques are necessary to extract cysts of cyst nematodes (*Heterodera* and *Globodera* spp.) from soil. Efficient assays for cyst nematodes (*Globodera* and *Heterodera* species) must include numbers of cysts, eggs, and juveniles. The use of a reliable technique for determination of numbers of eggs in cysts, in egg masses if present, is essential for the evaluation of efficacy of nematicides and other management practices on *Heterodera* or *Globodera* species. A glass house homogenizer is enough for this purpose. The sodium hypochlorite (NaOCl) technique is also a good method in dissolving cysts and releasing eggs. The NaOCl concentration should be two to three times more for egg masses of root-knot nematodes. The following are the most commonly used cyst extraction techniques.

11.2.2.1 Centrifugation Technique with Heavy Sugar

This technique is useful for isolation of cysts and juveniles of *Heterodera* and *Globodera* species. Some difficulty may be encountered with fine clay soils. Sugar flotation and centrifugation utilize a concentrated sugar solution to float nematodes away from soil particles. Typically, these procedures are used following a sieving-type procedure (e.g., elutriation–sugar centrifugation). The concentration of the sugar solution varies from and can be adjusted to facilitate recovery of different-sized nematodes. The length and speed of the centrifugation also vary. A typical procedure consists of elutriation, followed by centrifugation of the material retained on the screen, after which the pellet is suspended in a sugar solution, recentrifuged during which nematodes float and soil particles sink. The supernatant is poured through a sieve and retained nematodes are "backwashed" and saved for identification. This procedure greatly increases the recovery of ring nematode relative to Baermann funnels and works well for smaller nematodes such as lesion and juveniles of root-knot and cyst nematode. The recovery of larger nematodes such as dagger and needle is typically lower with this technique than Baermann funnels lined with cheesecloth. Increasing the sugar concentration or adjusting the length and speed of centrifugation can increase the recovery of larger nematodes, but there may be a trade-off with respect to nematode survival or identification because of the increased osmosis.

Procedure Wash 100-cm^3 soil through a 25-mesh sieve and collect it in a beaker (use 1-l water); mix the suspension thoroughly and allow it to settle for 5 s; pour the supernatant through a 100-mesh screen; add a 400-mesh sieve for juveniles; wash any residue from the screen into a centrifuge tube or tubes with 1.8 M sucrose solution; centrifuge at 420 g for 2.5 min. Collect the supernatant on a 100-mesh screen; add a 400-mesh sieve for juveniles; rinse thoroughly; wash the sample into a beaker, using about 20 ml of water; crush the cysts with a homogenizer or dissolve them with NaOCl as described earlier and count the eggs and juveniles.

11.2.2.2 Semiautomatic Elutriator Technique

This technique includes an elutriator similar to Oostenbrink along with a sample splitter and sieve shaker. It may be used in combination with Baermann's trays or centrifuge (Oostenbrink 1960).

Procedure Add 500-cm^3 non-mixed soil to the elutriator, with air and water flowing at desired rates; run the elutriator for 3–4 min, catching roots on the 35-mesh sieve over a sample splitter and free nematodes on the 400-mesh sieve on the motorized shaker; rinse the sieves; cysts of *Heterodera* and *Globodera* may be collected on the 60-mesh sieve under 10- or

20-mesh sieves; the eggs from the cysts may be extracted by the NaOCl method or with a glass tissue grinder.

11.2.2.3 Sodium Hypochlorite (NaOCl) Extraction Technique

This technique is very commonly used to extract cysts of *Heterodera* and *Globodera* and also for dissolving egg masses of *Meloidogyne* species. Care should be taken to minimize the exposure to the NaOCl. Even with normal precautions, only about 20 % of the eggs extracted with NaOCl produce infective juveniles (Barker 1985).

Procedure Collect and cut 6–10-week-old infected roots into 1–2-cm segments; shake root segments in 200 ml of a 0.5–1.0 % a.i. NaOCl solution for 8–10 min (for cyst extraction) and 1–4 min (for egg extraction); pass NaOCl solution through a 200-mesh (75 μm) sieve, nested over a 500-mesh sieve to collect freed eggs/cysts; quickly place the 500-mesh sieve with eggs under a stream of cold water to remove residual NaOCl (rinse for several minutes); rinse remaining roots with water to remove additional eggs/cysts and then collect them by sieving; for maximum precision in experiments, the eggs should be placed on 500-mesh nylon hatching sieves in 1–2-cm-deep chlorine-free water; tap water allowed to sit in the laboratory for 2–4 days before use is practically chlorine-free due to evaporation; the hatching juveniles are then collected and used as inoculum.

11.2.3 Technique to Enhance Extraction of Nematodes from Clayey Soils

The extraction of nematodes from clay soil is difficult using floatation sieving techniques of soil water suspensions. Aggregated soil particles trap nematodes and prevent them from passing through the top coarse sieves during decanting. In addition, the soil aggregates clog the sieves which slow the decanting operation. Clay particles are difficult to clean from centrifuge tubes that are used in the centrifugal floatation extraction process. Hence, this technique helps to extract nematodes from clay soil samples by replacing exchangeable calcium with sodium on soil particles to induce deflocculation/dispersal of the clay soil. In this technique, there is no need for soaking and agitating the clay soil during extraction (Wehunt 1973).

Procedure Collect the soil sample, pass twice though a sieve with 1.3-mm openings to mix and remove large clods and other debris; divide the soil into 150-ml subsamples and impose the following treatments: a soaking in 150 ml water for 2, 12, and 24 h; a shaking in 150 ml water at three reciprocations/sec on a wrist action shaker for 2, 12, and 24 h; a soaking in 20 ml Electrasol solution (a detergent containing sodium metasilicate, sodium carbonate, and sodium tripolyphosphate) (454 g in 3 L water) plus 150 ml water for 5 min, 30 min, and 8 h; a soaking in 150 ml water for 24 h plus Electrasol solution for 8 h; and a shaking in 150 ml water for 24 h plus Electrasol solution for 8 h.

11.2.4 Techniques of Extraction of Nematodes from Plant Tissues

Plant material containing nematodes should be kept cool and moist and examined at the earliest. Whole plants are stored free from soil. Shoots often decompose more quickly than roots and hence should be kept in separate bags if to be stored for more than a day or two. Polyethylene bags are better containers for samples. Many nematode species will survive for several days, sometimes weeks, in samples stored at 5 °C, but it is safer to store samples from the tropic at about 10 °C.

11.2.4.1 Direct Examination

This is the most conclusive and direct technique to diagnose a nematode infecting plant material is the microscopic examination (Hooper 1970). Small amounts of plant tissue can usually be examined directly for nematodes under a stereomicroscope at magnifications from

15 to 50×, using transmitted and/or incident light. For root-knot nematode and cyst nematode this is the best method since swollen females can be directly observed. Careful examination of roots/plant material can reveal the presence of nematodes, i.e., galls or swellings on roots, tubers, or suckers/rhizomes; white, yellow, or brown pinhead-size bodies adhering to the roots; swollen or malformed leaf, stem, or other tissue; and root lesions or unusual root proliferation. If nematodes are located, they can be dissected out from the plant tissue.

11.2.4.2 Dissecting Technique

Procedure The infected plant tissue is washed and thoroughly cut into small bits with a scissor, and a few of them are placed in a Petri plate or Syracuse dish containing water; the pieces are sliced/dissected/shred with dissecting needles under stereo binocular dissecting microscope; if nematodes are not traced out in the tissues, keep aside with proper labeling and reexamine after an hour for nematodes; if nematodes are traced out, pick them up with a fine needle or a bamboo splinter; transfer them into a Syracuse dish for identification.

11.2.4.3 Baermann's Funnel Technique

This technique is an excellent system of separating nematodes in plant material and also soil and condensing them for examination.

Procedure Attach a 10-cm length of rubber tubing to the funnel stem and clamp tubing; mount the funnel on the ring stand; fill the funnel two-thirds full with water; place a wire-mesh basket on top of the funnel and use it to support tissue; mix soil sample and pass through coarse sieve to remove rocks, roots, etc.; spread soil subsample (50 cm^3 soil) evenly on tissue; fold in edges of tissue; complete filling funnel with water so that water level is about 5 mm above the wire mesh; do not let the water and soil lose contact during extraction period – add water as needed; maintain temperature at 22–25 °C so that it is conducive to nematode movement.

Plate 11.4 Waring blender

Nematodes move through tissue and settle in the funnel; only active stages are recovered. After 48 h, recover extracted nematodes by releasing 20 ml of water from the stem of the funnel into a counting dish.

11.2.4.4 Waring Blender Technique or Maceration Technique

This technique is quicker, popular, and more efficient to extract migratory and sedentary endoparasitic nematodes from various plant parts like roots, foliage, and stems. A similar technique may be used for recovery and estimation of stem and leaf endoparasites and for detecting *Anguina* larvae in grass-seed samples. This is commonly used for extracting *Meloidogyne* eggs in roots to be used for inoculation.

Procedure This is a quick and useful method of examining roots for the presence of endoparasitic nematodes including *Pratylenchus, Helicotylenchus, Hirschmanniella,* and Radopholus; wash the roots gently to remove soil particles; cut 10 g of roots into short pieces (1–2 cm) with a scissor and place 50 ml water in the blending jar (Plate 11.4); run the motor for 10–30 s intermittently for three times; pour the mixture through the coarse sieve into a plastic pail; wash the macerated tissue with water and discard the material on the sieve; stir the mixture until the residue is all in suspension; pour gently through the

200-mesh sieve into the pail; stir the mixture in the pail and gently through a 325-mesh sieve; wash gently to transfer residue into the 100-ml beaker or set up in a Baermann funnel (it is optional, to be followed to get a clear suspension, free of soil particles) for 48 h prior to observation and counting under the dissecting microscope.

11.2.4.5 Root Incubation Technique

Most plant-parasitic nematodes are basically aquatic in nature. When roots are stored moist or immersed in water, migratory endoparasites tend to leave them. This method extracts potential root endoparasites such as *Pratylenchus* and *Radopholus* and also the immature stages and males of sedentary parasites.

Procedure Wash the roots gently to remove soil particles; place roots while still wet in a glass jar and add a small amount of water and close the jar lid loosely; leave the sample 24–48 h at 27 °C; add 50–100 ml water, shake gently, and invert the jar several times to wash off nematodes; pour water through the coarse sieve mesh size 100 on top of a very fine sieve mesh size 325; collect residues from very fine sieve in a beaker; place suspension in Syracuse watch glass or counting dish and count the nematodes with the use of a dissecting microscope.

11.2.4.6 Mist Chamber Technique/Mistifier

The details and the procedure of this technique has been mentioned under Sect. 11.2.1.5.

11.2.4.7 Maceration–Sedimentation Technique

This technique is effective in extracting plant-parasitic nematodes like coconut red ring nematode, *Rhadinaphelenchus cocophilus*, from coconut palm tissue. These nematodes are extremely thin and active and remain suspended in water for several hours.

Procedure The material is chopped and well macerated and the suspension transferred to a 2-l conical flask, which is filled with water; allow to stand for 30 min; shake the flask and invert with its neck in a vessel of water and sedimented for 30 min; the sediment which has passed from the flask to the lower vessel is discarded; the flask contents are poured four times through a sieve of 63 μm aperture or less, washing off the residue each time; collect it in a beaker.

11.2.5 Techniques for the Extraction of Eggs from Cysts

The procedure comprises three stages: extracting the cysts from the soil, crushing the cysts to extract the eggs, and microscopic observation of the suspension of eggs for counting (Southey 1970).

Extraction of Cysts from Soil Cysts are recovered from soil through a combination of wet sieving and decanting. The technique is a modification of the Cobb (1918) sifting and gravity technique.

Procedure Combine a well-mixed 100-cm^3 soil sample (approx. 1/2 cup) in a bucket with two (2) quarts of water; break clumps, if any, with fingers and mix the soil suspension well for 15 s; pour the soil suspension through an 8-in.-diameter #20 (850 mm pore) sieve into another bucket; briefly rinse the debris caught on the 20-mesh sieve; pour the soil suspension in the second bucket through a #60 (250 mm pore) sieve; backwash the debris caught on the 60-mesh screen into a pan; repour the suspension through the 60-mesh screen; hold the screen at an angle to concentrate the cysts and debris; backwash into a pan using a minimal (<250 ml) amount of water. Pour the cysts and debris into a 250-ml beaker. Discard the heavier material that quickly settles to the bottom of the buckets/pans during the above sieving process.

11.2.5.1 Extraction of Eggs from the Cysts

The above technique will result in a suspension of cysts, along with organic debris and sediments similar in size to the cysts. The cysts in this

suspension could be counted using a simple dissecting microscope. Egg content of cysts is highly variable and will not yield reliable counts of the population in the sample.

Procedure Allow cysts/debris to settle for 30 min in the 250-ml beakers. Pour off excess water, resuspend sediments, and transfer to 50-ml beakers; allow cysts to settle in the 50-ml beakers; pour off excess water (30 ml) and transfer the cyst/debris suspension to a 55-ml Wheaton Potter–Elvehjem tissue grinder; grind at 7,500 RPM for 10 s; rinse pestle into grinding tube; after grinding, pour the suspension in the tube through an 8-in.-diameter #200 (75 mm pore) sieve over a stainless steel #500 (25 mm pore) sieve; rinse the tube several times with tap water, each time pouring the contents through the sieves; discard sediments caught on the #200 sieve. Carefully wash sediments and eggs caught on the #500 sieve into a clean beaker with as little water as possible.

11.2.5.2 Technique to Estimate the Egg Content of Cysts in *Heterodera* spp.

Several techniques are available to open the cysts to release the eggs. A cyst is cut open individually with needles of an oculist's scalpel. This routinely followed method is tedious and time-consuming and is of importance only in assessing the proportion of cysts with contents or "full cysts," in a population. A more rapid and convenient technique has been given below (Reid 1955).

Procedure Squash cysts on a channeled aluminum slide, 7.5×2.5 cm (3×1 in.); the channel is 15 mm (0.6 in.) wide and 0.05 mm (0.002 in.) deep; draw off the water in which the cysts have been soaking with a Pasteur pipette; provided that the end of the pipette is straight and is pressed against the bottom of the staining block, no cysts will be sucked up; transfer this water to the marked boiling tube or graduated cylinder to which the rest of the cyst contents will later also be transferred; draw the cysts to the edge of the staining block with a dissecting needle, the end of which has been bent round in a loop; transfer the batch of cysts with this needle to the slide; place them in a thin line down the center of the channel and with the pipette draw off most of the water, leaving a thin film around the cysts; roll a glass rod backward and forward about three times over the slide, applying gentle pressure; the raised edges of the slide prevent damage to the eggs; if the aluminum slide is not available, cysts can, with care, be squashed between two glass slides; wash the squashed sample from the slide and the glass rod, with a liter of water, into the marked container; agitate the suspension with a electric stirrer for about 30 s to separate the eggs. Make up to a known and convenient volume, viz., 25 or 50 ml.

11.3 Precautions to Be Taken While Extracting Phytonematodes

Certain hazards prevail for some of the techniques of nematode extractions. Some precautions to be considered and safety measures to be followed are listed below (Barker et al. 1978).

- When using the NaOCl extraction procedure for eggs of *Meloidogyne* spp. and other nematodes that produce external egg masses, work should be done in a fume hood to avoid inhaling the vapors.
- Soil samples should be collected within 14 days after application of highly toxic nematicides like organic carbamates or phosphates.
- When soil assays are carried out within 2–4 weeks after applying chemicals at any residual concentration, appropriate precautions (rubber or plastic gloves) should be taken in handling and mixing soil.
- When selecting techniques for extracting different nematode species, the biology and population dynamics of the nematodes should be considered to choose the most suitable method for each sampling time.
- Root samples are best suited for endoparasitic nematodes like and semi-endoparasites in evaluating chemical soil treatments, because a significant portion of these nematode populations may exist in the roots.

- Root sampling may not be necessary shortly after a chemical treatment, because the fractions of the populations in the soil reflect the relative efficacy of the test material.
- Many sedentary ectoparasites can be extracted only by floatation techniques.
- For soil samples collected within 1–3 weeks after chemical soil treatments, techniques that yield only motile nematodes are the best.
- Vital stains like Phloxine B and new Blue R may be used with procedures like the centrifugal floatation method, which yields dead and live worms.
- Many non-fumigant nematicides act over a period of 6 weeks or more, causing nematode starvation and slow disappearance from the soil.
- Many factors may affect the efficiency of specific extraction techniques.
- Certain problems/difficulties like losing nematodes through sieve openings occur with numerous techniques.
- Some procedures and major potential difficulties are listed below (Barker et al. 1978).

11.4 Techniques of Handling, Killing, Fixing, Staining, and Mounting of Phytonematodes

Handling of nematodes includes several steps: picking nematodes and transferring them from one solution to another, observing under a stereo binocular dissecting microscope with a range of magnifications (10–100×), and counting the number of nematodes present in a population.

11.4.1 Examination of Nematode Suspensions

Procedure Place all or part of the suspension in an open counting dish and examine under microscope; Petri dish or flat bottomed Syracuse watch glasses or dishes are used for counting and a grid is etched or scratched with a marking diamond, on the inside of the base to act as a guide when searching; Doncaster's circular, rotating, perspex dish which has concentric channels is very convenient; the space between the grid lines should be a little less than the field width of the microscope at the magnification used to be sure of searching over the whole area of the dish; a sample being searched for large nematodes would be examined at about 15× magnification in a dish with guide lines about 1 cm apart, whereas an extract containing average-sized nematodes would be examined at about 50× in a dish with lines about 3 mm apart; a hand tally counter or a bank of counters is required for counting nematodes.

11.4.2 Transferring Nematodes

There is a need to transfer individual nematodes from one dish or vial to another. It can be done by two ways, viz., the traditional method relies on a picking device such as a handle equipped with a fine needle, eyelash, nylon fiber, platinum wire, etc.; it usually requires a good deal of exercise before you will get the hang of it. The other type is pipetting, which is usually much easier but requires that the nematodes are more or less suspended in liquid.

11.4.3 Technique of Picking Nematodes

The objective of the technique are to pick nematodes from a suspension. Practicing of picking nematodes is a basic and essential to all those who want to work with nematodes.

Procedure Picking devices are sharpened to a fine point under a stereomicroscope and mounted later in a needle holder or stuck on to the end of a mounted steel needle or a perspex rod; a hair or a fine wire loop secured to the end of a mounted needle or mounted at the end of a glass pipette may also be used; for smaller nematodes, an eyebrow hair stuck onto the end of the mounted needle is very useful. It has the following advantages: it has very little damage to the nematodes, is highly

durable, and does not split. It is also useful for handling nematodes in glycerol or lactophenol; a quill toothpick with a spatulate end is suitable for handling short and plump specimens like insect-parasitizing nematodes.

Handpicking This is carried out with any one of the above mentioned picking devices; picking worms out of a suspension is a common step in almost all techniques. Selecting a good picking device is a very important basic step, e.g., a fine and rigid insect needle tapped against the table to bend its tip to a minute hook or a handpicked hair from the brushy mustache. Mount one of these on a handle and use as a nematode pick; choose a particular nematode to pick in a suspension taken in a container like Petri dish, cavity dish, and Syracuse dish; lift the nematode from the bottom with a pick and slowly get it up to the surface of the suspension by placing the pick at the middle of the body and also changing the fine adjustment knob of the microscope so that while lifting the nematode does not go out of the focus; once the pick along with the nematode on it (mostly coiled around the pick due to the viscosity and surface tension of the liquid) reaches the surface of the solution, suddenly lift the pick out of the surface of the solution and place it in the drop of water/any liquid on a glass slide; check the slide for the presence of the nematode.

11.4.4 Technique of Anesthetizing Nematodes

Live and stationary nematodes are more suitable than dead ones or fixed or processed nematode specimens in order to observe certain structures (Hoper 1970). It is, therefore, desirable to prepare temporary mounts of anesthetized nematodes in the following manner.

Procedure Mount nematodes in a 0.5–1.0 % solution of propylene phenoxetol in tap water (a weak solution of dichlorodiethyl ether can also be used); at the lower strength nematode juveniles can be immobilized within 30 min which can survive for about 2 h.; add two drops of the ether to 50 ml of water in a small stoppered bottle; shake well and allow it to clear; ether dissolves to make a solution which will immobilize nematodes mounted in it; nematodes will recover when placed in fresh water, or they may be killed and fixed for subsequent processing and mounting.

11.4.5 Technique of Sterilization and Inoculation of Nematodes

Procedure Transfer a mono-population of the nematodes with a handling needle in a small drop of water in a glass tube; fill the tube with a mixture of 0.02 % ethoxy-ethyl-mercury chloride (Aretan) and 0.1 % di-hydro-streptomycin sulfate; shake the closed tube for about 1 min; allow the nematodes to settle; remove carefully the supernatant with a standard pipette until a small droplet of the nematode suspension remains in the tube; transfer this nematode suspension over a cotton wool filter which is placed on top of a glass tube drawn to a fine point and closed at the tip, which is filled with Aretan–streptomycin sulfate mixture; the nematode will pass the filter; allow the nematodes to settle; after about 60 min pour the nematodes in a silicone watch glass by opening the tip of the glass tube; remove the excess water with a micropipette; sterilize a chosen area of the foam bag with 70 % ethanol; make a slit and transfer the sterilized nematodes with a needle in an air space of the agar bag; seal the slit; inject sterile water through a hypodermic needle since about 2 weeks after planting, the seedlings have used up the water in the agar bag; sterilize the puncture area with 70 % ethanol before injecting; seal the hole made by the hypodermic needle with adhesive tape.

11.4.6 Techniques of Killing and Fixing Nematodes

Nematode killing is an essential part of most studies since live nematodes get distorted and spoiled when they are treated with cold fixatives.

There are several ways to kill nematodes. Nematodes must be killed instantaneously, either by using hot fixative or by heat-killing them prior to adding fixative. Both procedures result in "heat relaxation," relying on a knock-out heat shock to instantly relax the musculature. Another method is cooling the nematodes prior to adding hot fixative on melting ice, but this is not fail-safe. It may sometimes be advisable to starve the nematodes for a few days prior to killing and fixing, because well-fed specimens can contain so many intestinal granules that other organs remain obscured even after transfer to glycerin.

However, one of the best ways to kill live nematodes that are collected in a small volume of water (e.g., from an extraction tray or an agar plate) is to transfer them to a glass vial and plunge this in a 70–90 °C water bath. Stir the vial for 20–30 s and check under the stereomicroscope that they are all motionless and stretched out. Make sure they are not boiled – this messes up the cellular structure. After heat-killing, it is usually best to fix with hot fixative because this will be more chemically active.

11.4.6.1 Seinhorst's Technique of Killing

Procedure Collect specimens in a small drop of water in a glass staining block or similar deep concave vessel (Seinhorst 1966); heat formalin acetic acid fixative 4:1 (FAA) to 100 °C and an excess 3–4 ml is added to the nematodes; the fixative can be heated in a special dropper, whose orifice can be cleaned by an internal rod; sufficient fixative is picked up with the dropper from a stock bottle, and the dropper is later stood in boiling water for 3–4 min; the fixative can be heated in a small tube stood in boiling water and then poured on to the nematodes; if a pipette is used, it should have a wide aperture to allow a quick discharge and be stood in, and heated with, the fixative; this technique fixes glands and gonads and also nuclei are clearly visible; in place of acetic acid in killing propionic acid may also be used.

11.4.6.2 Flame Killing

Procedure Transfer a few nematodes to a drop of water on a plain or cavity slide which is heated over a small flame for 4–6 s until the nematodes suddenly assume straight in some genera and curved or spiral in a few; remove the slide as soon as nematodes appear in this state since overheating will spoil the nematodes. On the other hand if they are not properly heated, they begin to twitch; transfer them immediately to fixative or fix on the slide by adding double-strength fixative.

11.4.7 Technique for Killing and Preserving Nematodes in Soil Samples Before Extraction

This technique involves killing and preserving phytonematodes present in soil samples using chemicals and microwave energy, prior to routine extraction (Barker et al. 1972). The most uniform and reliable results can be obtained by adding chemical preservatives directly to the soil. In addition to providing identifiable specimens when extracted by sugar floatation techniques, the addition of 2 % NaN_3, 5 % formalin, or FAA + picric acid results in increased recoveries of nematodes as compared to nontreated controls. The increased recovery may be due to individual dead specimens having an increased buoyancy when placed in sugar solution. Sometimes adding chemicals like formalin directly to the soil may cause slight distortion of the nematode, but still they may be identified easily. These chemicals affect the morphological characters of the nematodes less than exposure to methyl bromide. The use of hot chemicals reduces distortion of the nematodes.

Procedure

Soil Preparation and Assay Procedures: Premix soil in either a concrete mixer or a sample splitter; collect 50 cm^3 soil sample for treatment.

Direct Addition of Chemical Preservatives: Add fixatives and other toxic or preservative chemicals to the soil directly, viz., 2 % NaN_2, 5 % formalin, and FAA + picric acid (15–20 ppm

concentration); place the samples in closed containers, stored at room temperature; extraction of nematodes can be made weekly or monthly intervals after treatment; most nematodes get killed by these chemicals.

Microwave Energy: A microwave oven model that emits 2,450 MHz (6.30w) has to be used; at a specific exposure duration, killing of nematodes is greatly influenced by the volume of soil and type of container; all juveniles of *M. incognita* can be killed by 3 min exposure of 150 cm^3 of soil, 2 cm deep in sealed 700-cm^3 cellophane bags, approximately 25 cm from the emitter; soil moisture often influences the effects of microwave treatments and rates of nematode killing. Nematode killing was observed at soil moisture levels ranging from 4.5 % (oven dry weight basis) to saturated soil; a 5-min exposure to microwave energy can kill all stages of soybean cyst nematode (*Heterodera glycines*). This nematode is resistant to microwave energy compared to root-knot nematode. This is mainly due to the basic structure of the cyst. They are resistant to ultrasonics and gamma radiation also; pre-treatment saturation of the soil with water can greatly increase the killing effect of microwave energy, but incubation at 15 °C can slightly enhance the effectiveness.

11.4.8 Fixatives and Preservatives Used for Nematode Fixing

The main objective of this technique is to prepare different fixatives and preservatives for fixing and preserving plant-parasitic nematodes in order to study the various structures of nematode body (Hooper 1970). A wide range of fixatives are used for preserving nematodes.

FA 4:1 (FA 4:10): Formalin (40 % formaldehyde), 10 ml; glacial acetic acid, 1 or 10 ml; and distilled water, up to 100 ml

Specimens are not usually distorted in these fixatives but they may become brown and the posterior half of tylenchid stylets transparent after more than a few days, especially in FA 4:10. Several observations on the swelling of the cuticle of nematodes like *Trichodorus pachydermis* in FA 4:10 have been reported.

TAF (Triethanolamine Fixative): Formalin (40 % formaldehyde), 7 ml; triethylamine, 2 ml; and distilled water, 91 ml. After fixation, nematodes appear lifelike. The solution remains stable for a long time and the TAF neutralizes any free formic acid, and being hygroscopic, it prevents specimens from drying even if the fixative evaporates.

Formalin–Glacial Fixative (3 % Formaldehyde Solution + 2 % Glycerol): Formalin (40 % formaldehyde), 8 ml; glycerol, 2 ml; and distilled water, 90 ml. Directly from this fixative, nematodes can be brought to glycerol by slow evaporation. Another advantage here is that the specimens will not dry if vials are not properly sealed, but remain finally in glycerol.

Ditlevsen's Fixative or FAA: 95 % ethanol, 20 ml; formalin (40 % formaldehyde), 6 ml; glacial acetic acid, 1 ml, and distilled water, 40 ml. In this fixative, shrinkage of the specimens may be observed since it has ethanol. This fixative is useful while preparing plain like structures as incisures and annulations.

Formalin Fixative: Dilute solutions of 2–4 % formaldehyde (5–10 % formalin) are used for fixing dead nematodes although in this fixative, nematodes tend to appear dark and granular. By adding a little quantity of calcium carbonate granulation may be prevented as it neutralizes free formic acid. By adding hot formalin, muscles in the killed nematodes can be clearly seen. However, 4 % formaldehyde may also be used for fixing nematodes killed by formalin propionic acid 4:1 (FP 4:1).

11.4.9 Formalin–Glycerin Technique (FG Fixation) for Fixing Nematodes

Procedure Prepare double-strength formalin–glycerin (FG) fixative containing 8 % formalin and 2 % glycerin in distilled water; transfer live nematodes to a small glass vial and allow them to settle to the bottom; draw off surplus water until they are left in about 2 ml water; kill the nematodes

by stirring the vial for 20–30 s in a 70–90 °C water bath, check if they are all dead and stretched, and then add an equal volume of 65–70 °C fixative; stir and then leave the vial alone for a day to allow the fixative to penetrate and act on all tissues; take a clean jam jar with airtight-sealable cap; prepare a "weck pot" by adding ethanol to the jam jar until there is a layer of about 5–10 mm on the bottom; place a small platform (a small inverted Petri dish or cavity block) on the bottom of the jam jar so that its top surface is raised above the ethanol; take the vial with FG-fixed worms – if this has a narrow opening, transfer the worms to a cavity block; draw off as much fixative as possible without losing nematodes and then fill the vial or block with 5 % glycerin–95 % ethanol solution (to the brim if in a cavity block, to about 5 mm high if in a vial); place the block or vial on the platform inside the jam jar and seal this; leave the "weck pot" overnight in an incubator at 35–40 °C.

This will allow all water in the suspension with the nematodes to be replaced with ethanol; the next day, take the vial or block out of the weck pot and leave it open in the 35–40 °C incubator for 2–3 h, to evaporate about half of the ethanol (if necessary, cover partly to prevent complete evaporation); refill with 5 % glycerin–95 % ethanol solution, leave for another 2–3 h, and refill one last time before leaving the vial or block overnight in the incubator at 35–40 °C. After 24 h, the nematodes will be impregnated in pure glycerin and ready for mounting in slides, or for stocking without fear of desiccation; the entire FG/ethanol procedure takes only 3 days and usually results in well-fixed worms that will not decay for decades; transferring through ethanol dissolves cuticular lipids, however, and may result in a finely wrinkled cuticle that will show up as such under the scanning electron microscope. To avoid this, the following slightly slower technique may be followed.

11.4.10 Techniques of Staining Nematodes

For clarity in observation and detailed investigations of the internal structures of the nematode body, staining phytonematodes is an essential technique (Ravichandra 2010). A wide range of stains have been used by several nematologists. Some are specific in their function and some are nonspecific. Important stains include carmine propionic acid, acetic orcein, propionic orcein, gold chloride, silver nitrate, vital stains, new Blue R, chrysoidin, aqueous Phloxine B, alcoholic eosin-Y, acridine orange, pronase, collagenase, polychrome blue, methyl red pH indicator dyes, redox indicator, 1-naphthol-2-sodium sulfonate-iodine, acid fuchsin–lactophenol, acid fuchsin, Johansen's quadruple stain, Sass safranin, fast green, triarch quadruple stain, etc.

Acetic Orcein: This is useful mainly to study the reproductive system in nematodes. The composition of this stain is glacial acetic acid, 1 part; absolute ethanol, 6 parts; and chloroform, 3 parts.

Procedure Kill nematodes by gentle heat and fix in Carnoy for 10–20 min; stain the nematodes in 1 % acetic orcein for 1 h; mount in 45 % acetic acid.

Gold Chloride: This is more suitable to stain gonads, esophageal glands, and nerve ring.

Procedure Wash the fixed nematodes in distilled water; place in an aqueous solution of 0.1 % gold chloride and 0.1 % mercuric chloride until they are light yellow; after washing the nematodes for 19 min in distilled water, place them in 1 % formic acid; expose them to the light of a photoflood lamp which causes breakdown of the gold chloride to colloidal gold which stains organs bright red. Nematodes can be processed to glycerol and mounted permanently.

Silver Nitrate: This technique is used on live nematodes and to stain the peripheral nervous system of nematodes.

Procedure Place the live nematodes in a hypertonic solution of 10 % sodium nitrate for 5 min, which lessens their size by exosmosis; place in 0.5 % silver nitrate for 15 s and finally in distilled water; endosmosis occurs in the hypotonic silver nitrate which gets flushed into the tissues; this treatment kills the nematodes, but after illumination the peripheral nervous system becomes apparent

due to the deposition of colloidal silver; it remains visible in nematodes subsequently processed to glycerol.

Vital Staining: Since cuticle of nematodes is almost impermeable and few nematodes ingest liquid in vitro, it is difficult to use vital stains on nematodes. Methyl red redox indicator, 1-naphthol-2-sodium sulfonate-indole, and neutral red pH indicator dyes have been shown to be ingested by rhabditids with their food in vitro. The dyes are absorbed in different concentrations by various regions on the intestinal wall.

11.4.11 Technique of Mounting Nematodes

The objective of this technique is to mount processed plant-parasitic nematodes either temporarily or permanently for future studies. In nematodes that are fixed, internal structures like gonads may be obscured by the granular appearance of the intestine. Nematodes may be cleared by processing to lactophenol or glycerols which are also suitable mountants (Hooper 1990). Phenol is supposed to be a dangerous poison so lactophenol is a better option which should be used with caution. Lactogylcerin also can be used which is quicker than glycerol in preparation of mounts in lactophenol/lactogylcerol, which if well sealed may last for several years. Aqueous solution of dimethyl formaldehyde resin 70 % (w/v), 30 % solution of Bovine Ox Plasma fraction V, immersion oil, saturated aqueous picric acid, araldite, etc., have also been used by several nematologists. If a stain is used, some features are more readily observed than in glycerol.

Lactophenol: Phenol (liquid), 500 ml; lactic acid, 500 ml; glycerol, 1,000 ml; and distilled water, 500 ml. A stain, usually cotton blue, is used in water before mixing the ingredients.

Glycerol: Ethanol 96 %, 20 parts; glycerol, 1 part; and distilled water, 79 parts

Rapid Technique to Glycerol: Fixed nematodes are cleared in lactophenol (with cotton blue added if required) and then processed through a series of solutions, all kept at 55 °C; the solutions are kept in a series of numbered dropping bottles fitted into holes of wooden box. Their composition is as follows (in parts) (Franklin and Goody 1949):

Solution	1	2	3	4	5
Glycerol	55	70	82	90	100
Lactic acid	15	10	05	2.5	0
Phenol (crystals)	10	05	05	2.5	0
Formalin (40 % formaldehyde)	05	05	03	2.5	0

From lactophenol transfer nematodes in a series of solutions, allowing a minimum of 10 min in each in oven; after the last solution, i.e., glycerol, mount immediately in pure anhydrous glycerol; any tendency for cotton blue stain to come out during processing can be counteracted by using glycerol containing 0.0025 % cotton blue as the last solution and for final mounting.

11.4.12 Mounting Nematodes in Temporary Slides

11.4.12.1 Technique of Mounting Dead Nematodes

By preparing temporary slides of killed nematodes, many observations can be made on refractive structures like spear, head skeleton, lumen of esophagus, excretory pore, and spicules.

Procedure Place a few nematodes in a drop of water on the glass slide; place three pieces (1 mm) of glass fiber around; check that nematodes are at the center of the drop and not floating; put the cover slip; seal the edges with nail polish or wax or recently extinguished candle; a mixture of eight parts paraffin wax to three parts petroleum jelly is better than ordinary wax for sealing cover slips (Doncaster 1962).

11.4.12.2 Technique of Mounting Live Nematodes

If the detailed morphology of live nematodes to be studied, a temporary slide with live nematodes can be prepared on a thin layer of agar.

Procedure Administer one or two drops of hot 4–5 % agar on a glass slide; flatten this agar with another glass slide provided with spacer strips of thick plastic tape; carefully remove the top slide when the agar has set; add a drop of water on the agar, transfer the nematode to it, and put a cover slip on top; the pressure between the cover slip and the hard agar will slow down the nematode sufficiently making it observable with oil immersion magnification; if nematode has to be immobilized, smearing some vaseline on the rims of the cover slip may be done; place it on top of the agar and nematode, and carefully press down the rims of the cover slip until the nematode is trapped but not squashed; a complete vaseline seal will also prevent desiccation.

11.4.13 Technique of Mounting Nematodes in Permanent Slides

Preparing permanent slides is easy once nematodes have been fixed and transferred to glycerin. Various types of slides and mounting aids exist, but glass slides using paraffin as seal and separator is more common. The most luxurious and safe mount for microscopic nematodes is a "Cobb slide" which consists of an aluminum carrier supporting two cover slips between which the nematodes are sandwiched and sealed. Unlike glass slides, Cobb slides will not break when dropped and they can be turned over and observed from both sides at high power. Several techniques are available to mount nematodes in permanent slides.

11.4.13.1 Glycerin Technique

Procedure

Preparing a Glass Slide: Fill a Petri dish with paraffin granules, melt them at about 60 °C, and allow the paraffin to set into a solid layer (Daykin and Hussey 1985); take a 10-cm-long cross-cut metal tube with smooth, thin rim and slightly smaller diameter than the cover slips (e.g., a 16-mm-diameter tube for 18-mm-diameter cover slips); heat one end in a flame; when the other end of the tube is beginning to get hot in your hand, push the heated end down vertically in the paraffin so that it gets covered by melting paraffin; press this end down vertically on the middle of a glass slide; lift the tube, and a complete 3–4-mm-thick ring of setting paraffin should be left behind; transfer a small drop of pure glycerin to the center of this wax ring, with a thin glass rod, leaving a spot of 4–5 mm on the slide; repeat this for as many slides required to prepare; getting the proportions of wax and glycerin right is important because too little paraffin and too much glycerin will result in an incomplete seal and too much wax and too little glycerin will result in nematodes being covered or trapped by paraffin.

Transferring Nematodes: Pick out the specimens with a needle and transfer them to the glycerin drop in the center of a wax-ringed glass slide; up to ten of them per slide can be mounted; more may result in nematode overlapping or ending up in paraffin; after transferring the required number to a slide, put it under the stereomicroscope; push all nematodes to the bottom of the glycerin drop with the pick, making sure none overlap with one another.

Sealing and Shuffling: Drop a cover slip over the wax ring and glycerin drop; put the slide on a moderately hot plate or a mesh or metal plate above a small flame; make sure one end of the slide sticks out over the rim of the plate; allow the paraffin to melt around the glycerin drop and allow all air to escape from under the cover slip; then put the slide back under the stereomicroscope and check that no nematodes are overlapping; if so, gently push the cover slip in the required direction to dislodge one of the overlapping nematodes; if the paraffin has set by now, return the slide to the hot plate; re-heating can also be done and gently push the cover slip sideways to turn nematodes over; once set, the paraffin will act both as a seal and a separating layer between the cover slip and the glass slide, and the slide will contain just a small circular central area with glycerin and nematodes; if some nematodes are covered by smudges of paraffin under the cover slip, and/or the paraffin is too thick to observe specimens

with high power objectives, put the slide back on the hot plate and allow the wax to heat and spread out further so that it forms a thinner layer; if specimens are to be picked for transferring to another slide or for use in scanning electron microscope or cross sections, gently raise the cover slip open with a scalpel or thin needle while keeping track of nematodes under the stereomicroscope.

11.4.13.2 Seinhorst's Technique

Procedure Kill nematodes by pouring in an equal volume of hot (80 °C) 1.0 acetic acid to create 0.5 %. Alternatively, kill in hot FA 4:1 fixative; fix for 48 h in FA 4:1 fixative; rinse specimens in distilled water; place in Seinhorst I solution (ethanol–glycerin mix) in watch glass; add 1–2 drops of saturated aqueous picric acid to provide a yellow stain and prevent clearing of stylet; place this open watch glass in a larger container (desiccator) surrounded by 95 % ethanol and incubate at 35–40 °C for 12 h; add Seinhorst II solution (ethanol–glycerin mix) and store in a partly closed container (covered Petri dish) at 40 °C; transfer to pure glycerin.

FA 4:1: 10 parts formalin (40 % formaldehyde), 1 part glacial acetic acid, and 89 parts distilled water

Seinhorst I Solution: 20 parts 95 % ethanol, 1 part glycerin, and 79 parts water

Seinhorst II Solution: 95 parts 95 % ethanol and 5 parts glycerine

11.4.14 Major Steps for Preparation of Nematode Mounts

There are three basic steps in preparation of nematode mounts, viz., killing and fixing nematodes, processing nematodes to glycerin, and mounting nematodes.

1. *Killing and Fixing Nematodes*: Collect live nematode specimens in distilled or deionized water in a small beaker or watch glass; concentrate the nematodes in a minimal volume of water and add equal volume of hot (90 °C) fixative solution, buffered formalin, to it. Nematodes may be killed with heat before adding fixative although adding hot fixative directly is also effective. Buffered formalin provides very good fixation; leave the specimens in the fixative for 1–2 days. Nematodes may be stored in buffered formalin indefinitely, it does not clear characters
2. *Buffered Formalin Solution*: Formalin (ca 40 % formaldehyde), 10.0 ml; water, 90.0 ml; sodium acid phosphate, 0.4 g; and anhydrous disodium phosphate, 0.65 g
3. *Processing Specimens to Glycerin*: Prepare Seinhorst I and II solutions (procedure as mentioned above) and keep them at room temperature. Place fixed nematodes in a dish; draw off excessive fixative and concentrate the nematodes in a small volume; add ca 6–8 ml of Seinhorst solution I to the nematode suspension; place the dish in a larger closed glass container (desiccator) with 95 % ethanol at the bottom and place in oven at 35–40 °C for at least 12 h. This removes most of the water in the dish; remove dishes from oven and draw off excess Seinhorst solution I from the dish, using a pipette under a dissecting microscope to avoid loss of specimens; add Seinhorst solution II to the dish; place it in a partially covered Petri dish and return to oven at 40 °C; several hours (at least 3 h) later, draw off excess solution from the dish; keep the dishes in oven until all the alcohol has evaporated (at least 3 h) and nematodes are in pure glycerin.

11.4.15 Mounting Nematodes

11.4.15.1 Temporary Mounts

Procedure Place a small drop of the fixative in the center of a clean glass slide; using a "nematode pick" under a dissecting microscope, pick up the desired specimens and place them in the fixative on the center of the slide; place the slide under the dissecting microscope; arrange the nematodes in the center of the slide and bottom of the drop; place glass wool (about 5 mm in length) or glass

micro beads in a triangular position near the edge of the drop; place a cover glass (18 mm wide) gently over the drop using a forceps or supporting it with a needle; draw off excess fixative carefully using filter paper; apply zut, glyceel, or nail polish with a small brush to the edge of the cover glass, to seal it; after the sealant has dried, the slides can be observed under microscope.

11.4.15.2 Semipermanent Mounts in Lactophenol

Procedure Collect the nematodes and kill them as explained before; transfer them to a dish containing fixative; cover with cover slip and leave it overnight; transfer the nematodes into a drop of warm, vaporizing lactophenol and allow to cool.

Lactophenol Liquid phenol, 94 ml; lactic acid, 83 ml; glycerine, 160 ml; and distilled water, 100 ml

Heat the lactophenol (or lactophenol with a little 0.1 % cotton blue in it) on a cavity slide with a depression above a small flame on a brass heating table; mount the nematodes in a small drop of lactophenol on a final glass slide; apply a cover slip and ring with glyceel.

11.4.15.3 Permanent Mounts

Procedure Fix a clean cover glass (25 mm wide) in the center of a Cobb aluminum slide by supporting with appropriate-sized white cardboard pieces; place a small drop of anhydrous glycerin in the center of the cover glass in the aluminum slide; pick up nematodes from the fixative and place them in the drop of glycerin; arrange the nematodes in the center of the slide and place glass wool; carefully place a cover glass (18 mm wide) over the drop and seal the edges of the cover glass; after the sealant has dried, a second coat of sealant may be added. Allow to dry, label the slides on the white cardboard, and examine under a compound microscope; excess glycerin on the slide is difficult to remove and can cause smudges, which interferes with the sealing process; store the slides in a flat position to avoid settling of nematodes toward the edge of the cover glass; use of aluminum slides enables viewing of the nematodes from both sides of the slide.

11.4.16 Technique to Prepare Permanent Mounts of Nematodes Within Plant Tissues Stained with Cotton Blue or Acid Fuchsin

The major objective of this technique is to prepare permanent mounts of nematodes within plant tissue. The selected portions of the tissues containing well-stained nematodes have to be cut out and further processed by following techniques (Hooper 1970).

Procedure

Glycerol Mounts: Transfer 5–10 % glycerol in water; set aside in a desiccator until the glycerol is concentrated; mount in pure glycerol; seal the cover slip with zut/glyceel.

Canada Balsam Mounts: Pass the plant tissue through graded ethanol series to absolute; clear in clove oil; mount in Canada balsam.

"Euparal" Mounts: Pass the plant tissue through 30, 50–70 % ethanol; pass through isobutanol; mount in "Euparal."

11.4.17 Technique of Remounting

Sometimes, mounts prepared long back may get spoiled due to some reasons. Such damaged or partially damaged specimens can be remounted using this technique (Hooper 1970).

Procedure Peel-off the sealing ring as much as possible; add excess mounting fluid around the edge of the cover slip; carefully lift the cover slip with a fine needle. While doing so, avoid it from slipping laterally by holding another needle vertically against the far edge; place the cover slip upside down on a clean slide and add excess mounting fluid and to the old slide; if nematodes are stuck to the glass, dislodge them by gently stroking with a mounted eyebrow hair or place them in an oven at 50 °C for 3–4 h in excess

mounting fluid; deteriorated nematodes will improve if put into hot cotton blue lactophenol and then reprocessed to glycerol.

11.4.18 Techniques of Preservation and Staining of Nematodes in Plant Tissues

These techniques are very important to investigate host–parasitic relationships involving endoparasitic nematodes, which requires techniques that facilitate observation of nematodes inside the plant tissues. Preserving and staining of nematodes in plant tissues will be useful and essential in many studies, particularly, in order to properly understand host–parasitic relationships. The studies on the penetration and development of endoparasitic nematodes within intact plant tissues are of importance in nematological research.

The most suitable technique of preserving plant tissue containing nematodes depends on the ultimate treatment of the plant tissue.

11.4.18.1 Lactophenol/FAA/Technique

Procedure Immerse the cleanly washed nematode-infected plant material (roots or whole plants) in cold 5–6 % formaldehyde solution or FAA, in appropriate airtight plastic or glass jars (Hooper 1970); display the jars or keep for museum purposes; better nematode specimens will often be obtained if the infested tissue is immersed in hot fixative (70–80 °C); if the infested tissue is immersed in lactophenol, tissues are softened and nematodes are easily dissected from them.

11.4.19 Technique for Retaining Green Color of the Plant Material

Procedure Dissolve 14 g copper sulfate in 2.5 L tap water; slowly bubble sulfur dioxide through the solution until cloudiness has cleared; fresh plants are introduced and are stored or displayed in the same solution; the color of the plants fades at first but retains after some time; if the container is not airtight, some more quantity of sulfur dioxide may be added.

11.4.20 Technique to Preserve Juveniles of *Meloidogyne* spp.

Procedure Incubate *M. graminicola* juveniles in 10 % ethylene glycol at 37 °C for 15 min (Bridge 1985); incubate later in cold 40 % ethylene glycol for 30 or 45 min before freezing in liquid nitrogen at −196 °C; the treatment reduces number of active nematodes, but those that recovered after thawing infected rice roots produce large populations of females and eggs after 40 days.

11.4.21 Technique to Preserve Infected Roots

Procedure The roots are carefully washed, air dried, and fully labeled; wrap the roots in paper bags or sheets; in this condition they may be kept well for prolonged periods; the roots may be soaked for 30 min in warm water and later may be cut into bits of 1–1.5 cm for examination; in case of cyst infected cereal roots by *Heterodera avenae*, seedling roots collected 3–4 weeks after beginning of growth clearly show white females and those examined at 6–8 weeks show brownish cysts and males.

11.4.22 Technique for Killing and Preserving Nematodes in Soil Samples Before Extraction

The objective here is to kill and preserve plant-parasitic nematodes present in soil samples using chemicals and microwave energy, prior to routine extraction (Barker et al. 1972). The most uniform and reliable results can be obtained by adding chemical preservatives directly to the soil. In addition to providing identifiable specimens when extracted by sugar floatation techniques, the addition of 2 % NaN_3, 5 % formalin, or FAA + picric

acid results in increased recoveries of nematodes as compared to nontreated controls. The increased recovery may be due to individual dead specimens having an increased buoyancy when placed in sugar solution. Sometimes adding chemicals like formalin directly to the soil may cause slight distortion of the nematode, but still they may be identified easily. These chemicals affect the morphological characters of the nematodes less than exposure to methyl bromide. The use of hot chemicals reduces distortion of the nematodes.

Procedure

Soil Preparation and Assay: Premix soil in either a concrete mixer or a sample splitter; collect 50 cm^3 soil sample for treatment.

Direct Addition of Chemical Preservatives: Add fixatives and other toxic or preservative chemicals to the soil directly, viz., 2 % NaN_2, 5 % formalin, and FAA + picric acid (15–20 ppm concentration); place the samples in closed containers, stored at room temperature; extraction of nematodes can be made weekly or monthly intervals after treatment; most nematodes get killed by these chemicals. If needed, potato dextrose agar, water agar, and nutrient agar dilution plates (1:10 to 1:1,000) of soils from each treatment/chemical can be made at monthly intervals to determine the relative rates of fungal and bacterial killing. In order to kill all soil-inhabiting fungi and bacteria in addition to nematodes, it is important to increase the concentration to 2 %.

Microwave Energy: A microwave oven model that emits 2,450 MHz (6.30w) has to be used. At a specific exposure duration, killing of nematodes is greatly influenced by the volume of soil and type of container. All juveniles of *M. incognita* can be killed by 3 min exposure of 150 cm^3 of soil, 2 cm deep in sealed 700-cm^3 cellophane bags, approximately 25 cm from the emitter. Soil moisture often influences the effects of microwave treatments and rates of nematode killing. Nematode killing was observed at soil moisture levels ranging from 4.5 % (oven dry weight basis) to saturated soil. A 5-min exposure to microwave energy can kill all stages of soybean cyst nematode (*Heterodera glycines*). This nematode is resistant to microwave energy compared to root-knot nematode. This is mainly due to the basic structure of the cyst. They are resistant to ultrasonics and gamma radiation also. Pretreatment saturation of the soil with water can greatly increase the killing effect of microwave energy, but incubation at 15 °C can slightly enhance the effectiveness.

11.4.23 Staining Techniques

In order to observe endoparasitic nematodes present inside the tissue, it is important to clear and stain the nematode-infected plant material. A wide range of staining techniques have been reported depending upon the different types of plant tissues (Daykin and Hussey 1985). It includes the most common technique, the acid fuchsin–lactophenol technique that was developed by Mc Beth et al. (1941). In general, for staining dense, bulky material is not suitable, unless sliced thinly, because it will not transmit sufficient light even when cleared. Cotton blue or acid fuchsin, in lactophenol, methylene blue is useful for routine assessment of nematodes in fresh or preserved plant tissue.

11.4.23.1 Sodium Hypochlorite–Acid Fuchsin Technique

For clearing and staining nematode-infected root tissues, this technique is most suitable which has the following advantages over other techniques (Byrd et al. 1983). It eliminates exposure of personnel to toxic compounds like phenol, which are utilized in other techniques. Since plant tissue is cleared with NaOCl prior to staining with acid fuchsin, it does not become heavily stained. Hence, the time required for destaining is reduced; frequent destaining is not required. This is also a very good technique to stain endoparasitic nematodes in cotton and soybean roots, in particular.

Procedure Place the washed infected plant tissue (e.g., roots) in a 150-ml beaker. Cut the plant tissue into small bits and stain. Add 50 ml of tap water to clear the tissue along with an appropriate quantity of chlorine bleach (5.25 %

NaOCl). The quantity depends on the age of the plant material as indicated below.

(a) Young aged: 10 ml 5.25 % NaOCl
(b) Medium aged: 20 ml 5.25 % NaOCl
(c) Older or more ligneous: 30 ml 5.25 % NaOCl

Soak the roots in the NaOCl solution for 4 min and agitate occasionally; rinse roots for 45 s in running tap water and soak in tap water for 15 min to remove any residual NaOCl which may affect staining with acid fuchsin; drain the water and transfer the roots into a beaker with 30–50 ml of tap water; add one ml of stock acid fuchsin stain solution to the water (stock solution is prepared by dissolving 3.5 g acid fuchsin in 250 ml acetic acid and 730 ml distilled water); boil the solution for about 30 s on a hot plate or in a microwave oven; cool the solution to room temperature, drain it from the roots, and rinse the roots in running water; place the roots in 20–30 ml of glycerin acidified with a few drops of 5 N HCl and heat to boiling for destaining; roots may be stored in acidified glycerin with little change in contrast between nematodes and root tissue.

11.4.23.2 McBryde Technique

This technique does not require heating and destaining. It is comparatively a rapid technique and can be easily regulated (Southards 1965).

Procedure Fix and stain infected plant tissue/roots for 6–24 h in a solution of equal parts of 95 % ethanol and glacial acetic acid, containing 0.0175 mg/ml acid fuchsin (17.5 mg/l); destain root tissue by soaking in a saturated solution of chloral hydrate (4.5 g/ml of water) for 12–24 h; rinse roots with stained nematodes in tap water and store in acidified glycerine. The nematode color, if it fades with time, the roots may be stained again by this technique after being rinsed in tap water.

11.4.23.3 Lactophenol Technique

This one is the very commonly adopted technique to stain nematodes in plant tissues. Nematodes in the infected roots of either young plants or small roots of older plants can be well stained by this technique (Mc Beth et al. 1941). This technique may not suit well for roots with a high fat content like most perennials as their roots will retain more stain. The destaining step cannot be easily regulated and may take many days. By keeping stained roots in clear lactophenol and autoclaved for 10 min at 15 lb pressure, the procedure can be accelerated.

Procedure Prepare lactophenol (by mixing liquid phenol, 500 ml; lactic acid, 500 ml; glycerine, 100 ml; distilled water, 500 ml); prepare stain solution by adding 5 ml of a 1 % stock solution of acid fuchsin or cotton blue per 100 ml of lactophenol. The concentration of stain may require variation according to the age of the tissue; stain is added to a beaker and brought to a boil on a hot plate; immerse infected roots in boiling water; destain in clear lactophenol solution until maximum contrast between the nematodes and root tissue is observed; heat the solution in a water bath (100 °C) or an oven (70 °C) for about 90–120 min in lieu of direct boiling of the stain; destaining time may vary from a few hours to several days.

11.4.23.4 Sodium Hypochlorite–Acetic Acid Technique

This technique is most suited for fresh roots or roots fixed in any of the common fixatives (formalin, alcohol, FAA, etc.). It is important that the roots be either stained or fixed as soon as possible after collection since nematodes may migrate from the roots (Byrd et al. 1983).

Procedure Wash soil from roots with tap water; place roots in a 250-ml conical flask with approximately 70 ml of 1.5 % sodium hypochlorite (or half strength household bleach) and bleach for 5 min with occasional stirring; rinse roots with water and soak for 15 min in 1 % acetic acid. This acid rinse step is critical for consistent staining, especially of fixed roots; drain off acid solution and place roots in 30 ml distilled water to which 1 ml stain has been added; heat over a low flame until boiling; boil gently for 30 s and then allow to cool for 30 min at room temperature; remove excess stain by rinsing with water; place roots

in 20 ml acidified glycerol and heat to boiling; remove from heat immediately as boiling commences and cool quickly by standing flask in shallow water; pour roots in glycerol into a Petri dish; gently tease apart and mount on a microscope slide; nematodes are stained red.

11.4.23.5 Cold Staining Technique

This technique provides better differentiation between nematodes and plant tissues.

Procedure Wash plant material and quickly dry it on a filter paper; immerse it in boiling lactophenol for 2 min (without stain); remove and allow it to cool in the air and immerse it in cold lactophenol containing 0.001 % cotton blue. By using this technique, *Meloidogyne* stains well in about 10 days; *Scutellonema* in yam tubers takes 1–2 days. The contrast remains as long as the material is left in the stain solution.

11.4.23.6 Technique to Stain Nematodes in Leaf Litter

This is a technique to determine the number of nematodes left in forest litter (viz., oak) (Minderman 1956). This technique helps in staining nematodes in litter, after removing most of them by modified Baermann's technique.

Procedure Bleach the dead leaves for 24 h in the mixture (NH_4OH, 2 parts; H_2O_2, 5 parts; H_2O, 11 parts); wash the litter in water and pour on hot (65 °C) tinted lactophenol; leave for 5 min and pour off the stain; wash out the excess with clear lactophenol until little dye remains in the washing fluid; mount in lactophenol.

11.4.23.7 Flemming's Technique

Staining infected plant material in Flemming's solution helps in proper detection of nematodes. The osmic acid of Flemming's solution has been reported to blacken fats within nematodes, due to its reduction, causing the nematodes to show clearly against the comparatively unstained plant tissues (Godfrey 1929).

Procedure Immerse the nematode-infected plant material in Flemming's solution in a suitable container, for 4–6 h; nematodes are stained in black, while tissue remains unstained. Observe the nematodes under a stereo binocular dissecting microscope.

11.4.23.8 Technique to Stain Green Shoot Material

This is a modified technique of Flemming's solution technique that avoids the darkening of the tissues due to rapid reduction of osmic acid (Godfrey 1935).

Procedure Green shoots/leaves are treated with hot 80 % acetone before soaking them in Flemming's solution; pour sufficient 80 % acetone into a small conical flask; place it in a large beaker of water heated over a small flame (the boiling point of 80 % acetone is about 63 °C); drop into the boiling acetone small pieces of shoot or leaf material containing nematodes; allow to boil for a few minutes; leave the material in the slowly cooling acetone for 3–4 h or until the green color is removed; pour off the acetone and wash in 2–3 changes of water; stain as described for roots.

11.4.23.9 Techniques to Stain Root Material

Flemming's Solution Technique

Procedure Wash the roots free from soil and plunge them into hot water (70–80 °C) for 2–3 min to kill nematodes present within; transfer the roots to strong Flemming's solution for 5 min; control the depth of staining by examining under the stereomicroscope; a closed container should be used to protect the eyes from osmic acid vapor; wash the stained roots in running water for a few hours to overnight; pass them through graded ethanol solutions to absolute ethanol; clear in clove oil and mount in Canada balsam.

Modified Aceto-osmium Technique

This technique is helpful to stain endoparasitic nematodes present in more opaque roots.

Procedure Fix and stain washed roots for 2 h at 52 °C in the solution (prepare the solution by

mixing acetic acid 10 %, 10 parts; aqueous osmic acid 2 %, 2 parts; distilled water, 16 parts); wash in running water for 1 h; bleach in 10–30 % hydrogen peroxide at 30 °C till the darkened tissues lighten perceptibly; wash several times in water; pass through a graded series of ethanol solutions half an hour in each at 52 °C: 30, 50, 70, 95, and 100 %; for nematodes within roots, start at 70 %, but for ectoparasitic ones start at 30 %; clear in methyl salicylate at 52 °C for half an hour or in clove oil; mount in Canada balsam.

11.4.23.10 Technique to Stain Fresh/ Preserved Shoot Material

This technique is used to stain fresh or preserved shoot material to observe the stained (bright red) endoparasitic nematodes and their stages against a green background of the shoot (Hooper 1970).

Procedure Wash the material, if fresh, to remove dirt and plunge it into hot water (70–80 °C) for 2–3 min to kill nematodes; preserved material if used should be brought to water and passed through 30, 50, and 70 % ethanol, giving one or two changes in the last; transfer the material to a specimen tube; fill the tube with a saturated solution of scarlet R in 70 % ethanol with 2 % acetone added; cork the tube and leave overnight or for several days; overstaining should not occur; wash the stained material in 70 % ethanol and then transfer it to isobutanol which displaces ethanol; control the steps under a stereomicroscope; mount in "Euparal"; nematodes are stained bright red against a green background of the shoot material.

11.4.23.11 Techniques for Counting Nematodes in Root Tissue

This is a useful technique to count the stained endoparasitic nematodes, either directly from intact root tissue or following root maceration technique (Daykin and Hussey 1985).

Direct Counting

Procedure Distribute the roots in a small amount of glycerin on a Petri dish cover (glass or plastic); press against the cover with the Petri dish bottom; mark a grid on the Petri dish that aids in counting the nematodes under a stereoscopic microscope at 40× magnification.

Root Maceration Technique

Objective of this technique is to free nematodes from the roots by maceration technique and to remove a subsample for counting as considerable time is required for direct counting nematodes inside large root systems.

Procedure Macerate the roots in a Waring blender or laboratory mixer as explained earlier; separate the nematodes from the root tissue by sieving; take care to ensure that nematodes are not ruptured or distained during maceration.

11.5 Histopathological and Histochemical Techniques (Microtome Techniques/ Microtomy)

A microtome is a mechanical instrument that is used to cut biological specimens including nematodes into transparent thin sections for microscopic examination. It is used for cutting histological sections from tissue block. A microtome consists of steel, glass, or diamond blades depending upon the specimen being sliced and the desired thickness of the sections being cut. Steel blades are used to prepare sections of animal or plant tissues for light microscopy histology. Glass knives are used to slice sections for light and to slice very thin sections for electron microscopy. Industrial-grade diamond knives are used to slice hard materials like bone, teeth, and plant matter for both light microscopy and electron microscopy. Gem-quality diamond knives are used for slicing thin sections for electron microscopy (Zacheo 1987). The microtome mechanically holds a definite thickness of tissue between the undersurface of a transparent plate and the advancing edge of a thin, flat razor blade. Multiple slices can be made with a minimum trauma and with considerable reproducibility.

11.5.1 Types of Sections

11.5.1.1 Traditional Histological Sections

In this technique, tissues are hardened by replacing water with paraffin. The tissue is cut using the microtome at the thickness varying from 2 to 25 µm. The tissue is mounted on a microscope slide, stained with appropriate aqueous dyes after prior removal of the paraffin, and examined using a light microscope.

11.5.1.2 Cryosection

This technique involves hardening of water-rich tissues by freezing. They are cut in the frozen state with a freezing microtome or microtome. Cryostat sections are stained and examined with a light microscope. This technique is much faster than traditional histology (5 min vs. 16 h). This technique can be used in conjunction with medical procedures to achieve a quick diagnosis. Cryosections are also used in immunohistochemistry since freezing tissue stops degradation of tissue faster than using a fixative and does not alter or mask its chemical composition as much.

11.5.1.3 Electron Microscopic Sections

After embedding tissues in epoxy resin, a microtome equipped with a glass or gem-grade diamond knife is used to cut very thin sections (typically 60–100 nm). Sections are stained with an aqueous solution of an appropriate heavy metal salt and examined with a transmission electron microscope (TEM). This instrument is often called an ultramicrotome. This is also used with its glass knife or an industrial-grade diamond knife to cut survey sections prior to thin sectioning. These survey sections are generally 0.5–1.0 µm in thickness. They are mounted on a glass slide, which are stained to locate areas of interest under a light microscope prior to thin sectioning for the TEM. Thin sectioning for TEM is done with a gem-quality diamond knife.

11.5.1.4 Botanical Microtomy Sections

Sledge microtome is required for hard materials like wood, bone, and leather. These microtomes have heavier blades and cannot cut as thin as a regular microtome.

11.5.2 Spectroscopic Sections

Infrared spectroscope, in particular, requires thin polymer sections as the infrared beam penetrates the samples. Samples are cut into a thickness of 20–100 µm.

11.5.2.1 Laser Microtomy Sections

Sample is cut with a femtosecond laser instead of a mechanical knife. This technique is contact-free and does not require sample preparation techniques. It has the ability to slice almost every tissue in its native state. Thicknesses of 10–100 µm are feasible depending on the material in consideration.

11.5.3 Microtechniques in Phytonematology

Microtechniques allow a complete analysis of the tissue which includes samples of both healthy and infected root tissue and collection of the infected tissues at different stages of nematode development (Ravichandra 2010). Study and comparison of reactions of tissues to nematodes and treatments is possible. A matrix of either paraffin or plastic provides easy sectioning and handling of the tissues. The paraffin-embedded tissue sectioning needs sophisticated equipment which provides a rapid examination of a large quantity of tissue. It is useful to enable greater cytological details with the help of thinner sections by using plastic-embedded tissue, and to prepare the infected plant material for histopathological and histochemical investigations, the following procedure has to be followed (Daykin and Hussey 1985).

11.5.3.1 Selection and Preparation of the Plant Tissue

Always include tissues of both healthy and nematode-infected plant material; it is advisable to have infected tissue at various stages of nema-

Table 11.2 Tertiary butyl alcohol dehydration schedule

			Quantity (ml) needed for solution			
Step	% alcohol	Time	Distilled water	95 % ethanol	100 % ethanol	100 % TBA
1	50	2 h or more	50	40	0	10
2	70	Overnight	30	50	0	20
3	85	1–2 h	15	50	0	35
4	95	1–2 h	0	45	0	55
5	100	1–3 h	0	0	25	75
6	100	1–3 h	0	0	0	100
7	100	1–3 h	0	0	0	100
8	100	Overnight	0	0	0	100

Note: It is important to keep the tertiary butyl alcohol changes in a warm place as the chemical solidifies at 25.5 °C

tode development; collect the roots carefully and wash gently and thoroughly under running tap water to remove all soil particles because presence of a small soil particle may come in the way of the sharpness of the microtome knife while sectioning; cut the tissue into small pieces; place them into fixative immediately; give longitudinal cuts to the roots, small roots into 1 cm and larger roots and stems into more than 1 cm diameter.

Table 11.3 Isopropyl alcohol dehydration schedule

			Quantity (ml) needed for solution	
Step	% alcohol	Time	Distilled water	100 % IPA
1	70	1 day–1 week	30	70
2	90	1 day–1 week	10	90
3	100	1 day–1 week	0	100
4	100	1 day–1 week	0	100

11.5.3.2 Fixation

The objective is to kill and harden the tissue and also to preserve the cellular structures. In fixation, the tissue is submerged in a volume of fixative at least ten times greater than that of the volume of the tissue to ensure that the fixative does not become overly diluted by water from the tissue. If pieces do not sink rapidly after they are put into the fixative, the container of fixative maybe placed under a mild vacuum to draw air out of the tissue more quickly. Tissue must remain in the fixative for a minimum of 24 h to several days depending on its thickness. Material may also be stored indefinitely in the fixative. One of the best and most common fixatives is formalin-aceto-alcohol (FAA): 90 ml of 50 % ethanol, 5 ml of glacial acetic acid, and 5 ml of 37 % formaldehyde. Another fixative which is also good is formalin-propiono-propanol (FPP): 90 ml of 50 % isopropyl alcohol, 5 ml of propionic acid, and 5 ml of 37 % formaldehyde. The proportions of reagents given for FAA and FPP are satisfactory for most tissues, but the concentrations of acids and formaldehyde may be varied for certain types of material if poor results are obtained with the standard concentrations.

11.5.3.3 Dehydration

The purpose of dehydration is to remove water from the tissue. Water must be removed gradually if plasmolysis is to be avoided. Hence, dehydration is accomplished by moving the tissue stepwise through increasingly higher concentrations of alcohols. When FAA is used as the fixative, the tertiary butyl alcohol (TBA) dehydration schedule should be followed (Table 11.2). If the material has been fixed in FPP, the isopropyl alcohol (IPA) dehydration schedule should be followed (Table 11.3).

When solutions in the dehydration schedule are changed, the liquid is drained from the container holding the tissue and then the tissue is covered immediately with the next solution. Care must be taken that the solution is never allowed to desiccate. The time the material is allowed to remain in the various dehydrating solutions depends on its thickness. Fine roots

may need the minimum length of time whereas thick woody material will require the maximum length of time. Material allowed to remain too long in the higher concentrations of alcohols may become brittle.

11.5.3.4 Infiltration

In this step, alcohols in the tissue are replaced by paraffin so that the tissue is saturated with a pure solution of paraffin. When the TBA dehydration schedule has been followed, the 100 % TBA solution is first replaced with a 1:1 mixture of 100 % TBA and paraffin oil. The tissue is allowed to remain in this solution for 1 h or more, depending on its thickness. Shortly before the next step, another container is 3/4 filled with melted paraffin and the paraffin is allowed to solidify slightly. The tissue in the TBA–paraffin oil mixture is then placed on top of the solidified paraffin and is covered with a layer of the TBA–paraffin oil solution. This container is placed uncovered in an oven that is set slightly above the melting point of the paraffin. The tissue sinks to the bottom of the container as the paraffin melts. After 1–3 h, the TBA–paraffin oil–paraffin mixture is poured off and is placed with pure melted paraffin. The uncovered container is placed back in the oven for about 3 h. This step should be repeated at least once more. The melted paraffin is replaced by a specialized type of melted paraffin, like paraplast or tissueprep, which is made especially for histopathological use. Tissue should remain in the oven in this paraffin overnight. After one more exchange with fresh melted paraplast or tissueprep for about 1 h, the tissue is ready for embedding. The infiltration procedure to be followed when the IPA dehydration schedule has been used is much simpler but requires a greater length of time.

To start with, part of the 100 % IPA in the last dehydration step is poured off so that the tissue in the bottom of the container remains covered; the container is then filled with chips of paraplast or tissueprep and is placed uncovered in an oven set at 59–60 °C; once the chips have melted, the paraffin–IPA mixture is poured off and replaced with pure melted paraplast or tissueprep; this paraffin should be exchanged for freshly melted paraplast or tissueprep at least twice more at 3–4-day intervals; small, fine roots should be ready to embed 1 week after beginning the infiltration procedure, while thicker tissues may take 1–2 weeks longer.

11.5.3.5 Embedding

The tissue is positioned in cooling paraffin so that it can be sectioned after hardening. Molds for embedding may either be constructed in the lab out of folded paper, or metal base colds and embedding rings designed especially for histology may be purchased. Molds should first be coated with a thin layer of glycerin. The tissue sample is then poured or carefully lifted into the mold with heated forceps and additional melted paraffin is added to fill the mold. This step may be done on a hot plate set at 60 °C. However, an embedding table, consisting of a rectangular metal plate which has a heat source at one end and which becomes progressively cooler toward the other end, gives increased control of the embedding procedure. The filled mold is next moved to a cooler surface either on the laboratory bench or on the embedding table. As soon as the paraffin begins to solidify on the bottom of the mold, the tissue is rapidly oriented in the desired fashion with a heated dissecting needle. Once the paraffin begins to solidify over the top of the mold, the mold is plunged into ice water and left there until the paraffin is completely solidified. After hardening, the paraffin is removed from the mold and may be cut into smaller blocks which can either be mounted on wooden blocks with melted paraffin or inserted directly into the microtome. Samples of tissue may be stored in these blocks indefinitely if kept in a cool place.

11.5.3.6 Sectioning

Paraffin block sectioning is carried out on a rotary microtome equipped with a knife or disposable razor blade. A knife must be used when the tissue is tough or woody. However, for other types of tissue, razor blades will cut sections equal in quality to the knife-cut sections. Because razor blades can be frequently replaced with fresh blades, they have the advantage of not requiring sharpening and therefore are useful when working with root tissue, which often carries soil particles that can rapidly dull a cutting edge. Excess paraffin

surrounding the tissue should be trimmed away before sectioning, leaving at least 1 mm around the tissue. Care should be taken so that opposite edges of the trimmed block face are parallel. The block is then cooled in ice water for at least 5 min, inserted into the microtome clamp, and one edge of its face aligned parallel to the knife edge. Sections 8–12 μm in thickness are usually cut for histological studies. Tissue that is especially tough or woody will section more easily if the trimmed block is first soaked overnight in the refrigerator in a softening solution consisting of 90 ml of 1 % sodium lauryl sulfate and 10 ml of glycerin. The excess paraffin on the face of the block must first be trimmed away exposing the tissue so that the softening solution can penetrate. As the sections are cut, the edge of each section should adhere to the previous section to form a ribbon.

A sharp knife edge and proper knife angle are most important in obtaining a ribbon; the back of the knife edge should also be checked frequently and cleaned if necessary as paraffin buildup there will adversely affect ribbon formation; as the ribbon increases in length, it should be held away from the microtome with a dissecting needle or brush; it is then removed from the knife edge with a second needle and transferred, shiny side down, to a clean, flat surface; the ribbon should never be touched with hands; the ribbons can either be mounted immediately or stored in a cool, dust-free place for several weeks, if necessary.

11.5.3.7 Ribbon Mounting

Ribbon mounting adheres sections to glass microscope slides so that they can be stained. Ribbons must first be cut into shorter lengths so that they can be fit onto the slides. Slides may be labeled with a diamond pencil or, if the glass on one end is frosted, with a lead pencil. The surface of the slides is then coated with a small amount of Haupt's adhesive.

Preparation Dissolve the gelatin in the water at 30 °C; add the phenol and glycerin and filter the solution; before the adhesive dries on the slides, the slides are flooded with a 2–3 % formalin solution, which should be made fresh each day; the flooded slides are then placed on a warming tray held at 35–40 °C, and segments of the ribbon are floated on the slides; as the slides warm up, the ribbon will flatten out and the liquid will evaporate after several hours, when the slides are completely dry; remove and dry them indefinitely.

11.5.3.8 Staining

The process of staining removes the paraffin from the sections and increases the contrast in the tissues. The staining procedures that have been most used for nematode-infected root material are Johansen's quadruple stain (Table 11.4), Sass safranin and fast green stain (Table 11.5), and triarch quadruple stain (13) (Table 11.6). Solutions made from dry, powdered stains should always be filtered before they are first used. If only a few slides are being stained, the alcohols and staining solutions may be kept in Coplin jars and the

Table 11.4 Johansen's quadruple stain

Step	Solution	Time
1	Xylene	5 min
2	Xylene–absolute ethanol (1:1)	5 min
3	95 % ethanol	5 min
4	70 % ethanol	5 min
5	Safranin O solution[a]	6–24 h
6	Rinse in tap water	
7	1 % aqueous methyl violet 2B	10–15 min
8	Rinse in tap water	
9	95 % ethanol-methyl cellosolve-tertiary butyl alcohol (1:1:1)	15 s
10	Fast green FCF solution[b]	10–15 min
11	95 % ethanol-tertiary butyl alcohol (1) plus 0.5 % glacial acetic acid	15 s
12	Orange G solution[c]	3 min
13	Clove oil–methyl cellosolve–95 % ethanol (1:1:1)	15 s
14	Clove oil–absolute ethanol–xylene (1:1:1)	15 s
15	Xylene	5 min
16	Xylene	5 min or longer

[a]The Safranin O solution is prepared by dissolving 4 g Safranin O in 200 ml methyl cellosolve. When the safranin is dissolved, add 100 ml 95 % ethanol and 100 ml distilled water. Finally, add 4 g sodium acetate and 8 ml formalin

[b]The fast green FCF solution is prepared by adding 0.25 g fast green FCF to 50 ml of a solution composed of methyl cellosolve and clove oil (1:1). After the fast green has dissolved, 150 ml 95 % ethanol, 150 ml tertiary butyl alcohol, and 3.5 ml glacial acetic acid are added

[c]The orange G solution is prepared by dissolving 1 g orange G in 200 ml methyl cellosolve and then adding 100 ml 95 % ethanol

Table 11.5 Sass safranin and fast green stain

Step	Solution	Time
1	Xylene	5 min
2	Absolute ethanol	5 min
3	95 % ethanol	5 min
4	70 % ethanol	5 min
5	50 % ethanol	5 min
6	30 % ethanol	5 min
7	1 % aqueous Safranin O	1–12 h
8	Rinse in tap water	
9	30 % ethanol	3 min
10	50 % ethanol	3 min
11	70 % ethanol	3 min
12	95 % ethanol	3 min
13	0.1 % fast green FCF in 95 % ethanol	5–30 s
14	Absolute ethanol	15 s
15	Absolute ethanol	3 min
16	Xylene–absolute ethanol (1:1)	5 min
17	Xylene	5 min
18	Xylene	5 min or longer

Table 11.6 Triarch quadruple stain

Step	Solution	Time
1	Xylene	5 min
2	Xylene	5 min
3	Xylene–absolute ethanol (1:1)	5 min
4	95 % ethanol	5 min
5	70 % ethanol	5 min
6	1 % Safranin O in 50 % ethanol	5–15 min
7	Rinse in distilled water	
8	1 % aqueous crystal violet	1–2 min
9	Rinse in distilled water	
10	Absolute ethanol	30 s
11	Absolute ethanol	30 s
12	Orange G[a]–fast green[b] (135 ml–15 ml)	3 min
13	Orange G–fast green (145 ml–5 ml)	2 min
14	Orange G–fast green (148 ml–2 ml)	2 min
15	Orange G	2 min
16	Absolute ethanol	1 min
17	Xylene	5 min
18	Xylene	5 min or longer

[a]Orange G is prepared by dissolving 0.4 g orange G in 100 ml clove oil
[b]Fast green is prepared by dissolving 1 g fast green FCF in 100 ml absolute ethanol

slides moved individually after each time period. However, larger containers and racks that hold 25 or 50 slides are much more convenient when staining larger quantities. Stains and alcohols that are not being used over long periods should be stored in tightly capped bottles. In these three staining schedules (Tables 11.4, 11.5, and 11.6), containers holding water rinses should be emptied and refilled with fresh water after each group of slides moves through. Rinses containing alcohols should be changed when the liquid becomes heavily stained. Staining solutions and xylene require replacement less often. After completion of the staining procedure, cover slips are mounted with a few drops of either balsam or permount.

Slides are first removed from the xylene, which is always the final step in a staining procedure and laid on a flat, absorbent surface; the mounting medium is then applied to the surface of the slide before the xylene evaporates, and a cover slip is lowered gradually over the slide; a minimum of mounting medium should be used, as any excess will run out over the surface of the cover slip; finished slides should be left flat to dry for at least 24 h at room temperature. However, the medium will harden better if the slides are held on a 60 °C, warming tray overnight.

The safranin in the previously discussed stains turns lignified or cutinized cell walls red, whereas the fast green generally turns cellulose walls greenish. Starch grains stain purple by methyl violet and crystal violet. Nematodes in tissue vary from brownish to red in color. Triarch quadruple stain gives the best contrast consistently and takes the least amount of time. Also, a wide variety of tissue types may be stained without altering the staining time. However, since it requires a large amount of clove oil, it is the most expensive stain to prepare. Staining times in the Johansen's quadruple stain and Sass safranin and fast green stain may need to be adjusted for the best contrast between cell types to be obtained.

11.5.3.9 Plastic Embedding of Plant Tissue for Light Microscopy

The previous sections presented information on preparation of histological sections by the traditional embedding method in which paraffin is used. Plastic-embedded material, however,

provides superior preservation of cell structure. Plastic-embedded material will not entirely replace paraffin-embedded material, which still can be used for initial examination of infected tissue. However, critical tissue evaluation is enhanced with plastic-embedded material due to the added resolution obtainable with thin sections. The cytological detail seen in 3-μm-thick sections of plastic-embedded material is superior to that which can be obtained in paraffin-embedded sections. Several procedures for plastic embedding are available. The most commonly followed procedure is given below (Feder and O'Brien 1968).

Procedure Fix small pieces of tissue in 4 % glutaraldehyde in 0.1 M phosphate buffer at pH 7.2; rinse twice in buffer and dehydrate in a graded, acetone series; infiltration is done by embedding in soft Spurr's resin; Sections 3 μm thick are cut from a carefully trimmed block on a Sorvall MT-2 ultramicrotome on a glass knife and floated on distilled water on a gelatin-coated slide at 60'C; sections are stained with toluidine blue and permanently mounted with polymount resin; excellent results have been obtained with plastic-embedded tissue that is infected with nematodes.

11.6 Techniques to Evaluate Botanicals Against Phytonematodes

11.6.1 Technique to Screen Plants for Their Efficacy Against Nematodes

Procedure Prepare leaf extract by grinding 2 g of fresh leaves of a botanical plant (Table 11.7) in 5 ml of distilled water using pestle and mortar (Bhatti 1988); filter the water extract through a four-ply muslin cloth to remove the plant debris; centrifuge for 5 min at 4,000 rpm; filter through Whatman's filter paper No.1; preserve the extract in plastic bottles in refrigerator for 24 h and use it as stock solution for further evaluation against nematodes.

Table 11.7 List of some plants possessing nematicidal properties

Sl. No.	Common name of the plant	Botanical name of the plant
1	Neem	*Azadirachta indica*
2	Castor	*Ricinus communis*
3	Datura	*Datura stramonium*
4	Pudina	*Mentha piperita*
5	Subabul	*Leucaena leucocephala*
6	Bhang	*Cannabis stramonium*
7	Aak	*Calotropis procera*
8	Bathua	*Chenopodium album*
9	Safeda	*Eucalyptus naundina*
10	Paper flower	*Bougainvillea spectabilis*
11	Kandal	*Cirsium arvense*

11.6.2 Effect on Nematode Mortality

Procedure Pour 10 ml of larval suspension into each of 5-cm-diameter Petri dishes; add measured volumes of stock solution to these dishes to make the resultant dilutions of 1:5, 1:10, 1:20, 1:40, and 1:80; water alone can be taken as control; replicate each treatment three times; place the Petri dishes with leaf extracts and larval suspension in BOD incubator at 28 ± 1 °C for *Tylenchulus semipenetrans*, *Heterodera cajani*, and *Meloidogyne javanica* at 18 ± 1 °C for *H. avenae* and *Anguina tritici*; after 48 h, record the observations on larval mortality by counting living and dead second-stage juveniles under the stereo binocular microscope; calculate the percent mortality, and data obtained is subjected to angular transformation and statistically analyzed by applying complete randomized design.

11.6.3 Effect on Egg Hatching

Procedure Fit Petri dishes of 5 cm diameter with molded pieces of wire gauges and upon each of them place two-ply filter paper; prepare two dilutions from stock solution of

plant extract (viz., 1:5 and 1:20); pour 10 ml from each dilution into each Petri dish containing 4–5 cysts of *H. avenae, H. cajani*, and 5 egg masses of *M. javanica* separately; replicate each treatment thrice; water can be used as control; place the Petri plates containing cysts of *H. avenae* in BOD incubator at 18 ± 1 °C; place the Petri plates containing egg masses of *M. javanica* and cysts of *H. cajani* at 28 ± 1 °C; count the number of larvae emerging from cysts or egg masses at 3, 6, and 9 days interval; subject the data to square root n or square root $n+1$ transformation and analyze the data by applying complete randomized design.

11.6.4 Techniques to Study the Effects of Few Physicochemical Factors on the Nematicidal Property of Botanicals

11.6.4.1 Effect of Temperature on the Efficacy of Leaf Extracts

Procedure Expose freshly prepared leaf extracts (stock solutions) to varied temperatures, viz., 40, 50, and 60 °C, and boiling temperature for 5 min each; prepare two dilutions, viz., 1:5 and 1:20, from the stock solutions by adding the required volume of distilled water; replicate each dilution three times; use water alone as control; place the Petri dishes containing leaf extracts and larval suspension in incubator at 27 ± 1 °C for *M. javanica*, *T. semipenetrans*, or *H. cajani* and 18 ± 1 °C for *A. tritici*; record the data on mortality after 48 h by observing under the stereo binocular microscope.

11.6.4.2 Effect of Longevity/Persistence on the Efficacy of Leaf Extracts

Procedure Fill the beakers (100 ml) with sterilized river sand; add leaf extracts in dilutions of 1:5 and 1:20 into these beakers to drench the river sand on the same day; add the 100 larvae/beaker at 0, 1, 2, 4, and 6 weeks after drenching the river sand; replicate each dilution three times; in control, drench the river sand with sterile water; keep the river sand moist during the period of experiment; place the beakers under shade; wash the river sand of beakers carefully with water, 72 h after liberation of larvae; pass through a series of sieves (10 and 300 mesh size); subject the suspension to Baermann's funnel extraction technique; count those larvae which migrate down as alive and calculate the percent recovery in each extract.

11.6.4.3 Effect of Ageing on the Efficacy of Leaf Extract

Procedure Prepare the stock solutions of the fresh plant leaf extracts and keep in corked plastic bottles for 5, 10, 15, and 45 days at room temperature; prepare two dilutions, viz., 1:5 and 1:20, from this stock solution; test the efficacy of these at 27 ± 1 °C for 48 h against *M. javanica*, *T. semipenetrans,* or *H. cajani* and at 18 ± 1 °C against *A. tritici* for 48 h; replicate each dilution three times; use water alone as control; record the data on mortality by observing under the stereo binocular microscope.

11.6.4.4 Effect of pH on the Efficacy of Leaf Extracts

Procedure Maintain different pH range of the extract from 4 to 10 (i.e., 4, 8, and 10) by adding 1 N HCl or 6 N NaOH; note down the actual pH of the extract; place these extracts at room temperature for 1 h for final check of the pH; prepare two dilutions (1:5 and 1:20) from the stock solution by adding the needed volume of distilled water; replicate each dilution three times; use water alone as control; place the Petri plates containing leaf extracts and larval suspension of *M. javanica*, *T. semipenetrans*, or *H. cajani* at 27 ± 1 °C and *A. tritici* at 18 ± 1 °C for 48 h in incubators; record the data on mortality by observing under the stereo binocular microscope after 48 h.

11.6.5 Preparation of the Plant Leaf Extract

Procedure Wash fresh leaves of the plants in ordinary water two or three times; sterilize these leaves with 0.1 % $HgCl_2$ solution for 30 s; wash them thoroughly with sterilized water two to three times and ground with a sterilized pestle and mortar to prepare paste; prepare the extract by adding 2 g of the paste in 5 ml of sterilized distilled water; filter this through a sterilized muslin cloth till the whole liquid separates from green leaves or leaf debris; centrifuge the extract that contains more quantity of chlorophyll at 4,000 rpm for 5 min; filter the supernatant through double Whatman's filter paper No.1; prepare the extract under sterilized conditions and store in sterilized flasks in a refrigerator; for bacteria, filter the supernatant after centrifugation through microfilters and store at low temperature.

11.6.6 Efficacy of Chopped and Finely Ground Plant Leaves Mixed in Soil Under Green House Conditions

Procedure Chop and finely grind the leaves of plants at 10, 20, 40, and 80 g leaves/kg soil; mix them with sterilized soil in 15 cm pots; after a week, sow the seeds of the required crop (pigeon pea against *H. cajani*, wheat/castor/aak/ bougainvillea against *H. avenae*); when the plants are 1 week old, inoculate 1,000 larvae; replicate each treatment five times; record the data on plant growth parameters and final nematode population in roots and soil after 4 months in pigeon pea and 5 months in wheat.

11.6.7 Field Evaluation of Nematicidal Plants Against Phytonematodes

Procedure Select the fields having uniform infestations of *H. avenae* and *M. javanica* to study the efficacy of chopped leaves of castor and aak at 20 and 40 g/kg soil in tomato and wheat, respectively; the desired plot size for wheat is 1 m×1 m and for tomato 2 m×1 m; calculate the quantity of chopped leaves to be added for each plot based on the weight of soil/plot (weight of 6′ furrow soil of 1 ha is estimated to be 2×10^6 kg); estimate the initial population for each nematode before mixing of chopped leaves for each plot separately; mix the calculated amount of each plant leaves thoroughly in each plot; arrange the treatments in randomized block design; replicate the treatments four times; water the pots individually; 15 days after adding the chopped leaves (after decomposition), take up the sowing of wheat and transplant tomato seedlings; plots receiving no plant leaves serve as control; for comparison, carbofuran 3G is applied at 2 kg a.i./ ha before sowing/transplanting; record the data on final yield/plot and final nematode population both in roots and soil.

11.6.8 Technique to Isolate and Characterize the Active Principles of Nematicidal Plants

11.6.8.1 Plant Material

Dry the samples of plant materials under shade; the material may consist aerial parts of bhurt (*Xanthium stramonium), Chenopodium album, C. murale, Calotropis procera, Ricinus communis, Nerium oleander, Datura stramonium, Ipomea carnea,* palmarosa (*C. martinii* var. *motia*), lemon grass (*C. flexuosus)*, and citronella java (*C. minternianus*).

11.6.8.2 Preparation of Crude Extract

Dip the fresh, dried, and crushed parts of the plants in methanol for 48 h at room temperature and filter; remove the solvent at reduced pressure that gives extract which is stored in refrigerator for further analysis.

11.6.8.3 Isolation of Essential Oils

Obtain essential oils of bhurt, palmarosa, lemon grass, and citronella java by hydro-distillation of fresh aerial parts of the plants in a laboratory hydrodistillation unit.

11.6.8.4 Chromatographic Resolution

Resolve the crude extracts/oils by repeated column chromatography/preparative TLC over silica gel using a series of solvent system in increasing order of polarity.

11.6.9 Identification of Constituents

Routine checking of the purity of the compounds is done on silica gel G TLC plates. Record the melting points in Gensons electrical melting point apparatus; identify the compounds mainly by spectral studies; record IR (max. cm^{-1}) spectra on Perkin-Elmer infrared 157 spectrophotometer; run ^1H NMR spectra (in units) on Varian EM-360 instrument using TMS as internal reference and mass spectra on Jeol-JMS-D300 mass spectrometer.

11.6.9.1 GLC of Lipid Fraction

Prepare the fatty acid methyl esters from the lipid by transesterification in methanol using sodium methoxide as catalyst; separate by GLC using Aimil Nucon Gas Chromatograph series column (1/8 in. O.D.X feet) packed with 15 % polydiethylene glycol succinate on Chromosorb W, under usual operating conditions; identify the compounds by comparison of their retention times with those of authentic samples recorded under similar operating conditions.

11.6.9.2 Preparation of Acetates

The compounds amenable to acetylation are converted into acetoxy derivatives using acetic anhydride pyridine at room temperature.

11.6.9.3 Monoterpenes and Related Derivatives

Citral (XIV), geraniol (XV), methanol (XXIa), cyclohexanone (xxiv), and cyclohexanol (xxva) required in this estimation are available commercially (Aldrich Chemical Company Ltd.); prepare the N-phenyl-carbamates (xxb, xxib, and xxvb) of xxa, xxia, and xxva, respectively, by their reaction with phenyl isocyanate in refluxing benzene; prepare the esters xxc-xxa, xxib-xxid, and xxvc-xxvd by the esterification of xxa, xxib, and xxva with corresponding acid anhydride or acid chloride, 3-chloro-p-menthane (xxii), and P-3-menthane (xxiii) by treatment of menthol (xxia) with phosphorus pentachloride and p-toluene-sulfonic acid, respectively; check for the purity of all these compounds by thin-layer chromatography over silica gel G.

11.6.9.4 Bis-(3-Substituted Amino/Aryloxy-2-Hydroxy or Phenyl-Carbamoyloxy-Propoxy) Benzenes

Prepare the compounds 1,4-bis-(3-substituted amino/aryloxy-2-hydroxy propoxy) benzene xxvii-xxix/xxx as per the standard procedure starting from the alkylation of quinol with spichlonohydrin followed by nucleophilic ring opening of the epoxide xxvii with various secondary amines or phenoxide ions; refluxing of the amines xxviii-xxix and quinol xxvi with phenyl isocyanate gives the corresponding carbamoyloxy derivatives xxxi–xxxii and xxxiii.

Phenolics: Select phenols with various substituents having electron-withdrawing or electron-donating tendency, which are commercially available.

Ethyl (Aryloxy) Acetates: Prepare these by the standard procedures, by stirring the corresponding phenol with ethyl bromoacetate in the presence of potassium carbonate at room temperature.

Azides: The esters on refluxing with hydrazine hydrate in ethyl alcohol give the corresponding azide.

11.7 Techniques of Remote Sensing

Remote sensing is a technique of measurement of acquisition of information on some property of an object or phenomenon by a recording/measurement device that is not in physical contact with the object or phenomenon under study. Remote sensing measurements make use of the visible, infrared, and microwave sensors with specific spatial and radiometric character-

istics in the acquisition of required data. The data thus acquired is stored in the form of photographs, images, or digital tapes depending on the sensor used and the mode of acquisition. The data is interpreted either manually, machine assisted, or totally automated, and the information thus obtained is used for purposes of inventory, survey, monitoring, planning, and management. Remote sensing is especially useful where speed, repetitive observation, and a synoptic view are needed. It provides an important new dimension in the detection and quantification of damage to plants, assessment of the distribution of the principal host plants or habitats of pests, and surveillance of environment factors favorable for the development, spreading, and outbreak of nematode pests.

11.7.1 Remote Sensing Techniques Used in Plant Protection

Various techniques of remote sensing applicable in plant protection against pests including plant-parasitic nematodes are as follows (Nageswara Rao et al. 1991). They include photography and videography from ground and aircraft, satellite-borne photography, multispectral scanning, thermal imaging, ground-based and airborne radar, and acoustic sounding.

The range of wavelengths employed extends from the ultraviolet end of the visible spectrum to the radars.

11.7.2 Aerial Photography

Although aerial photography cannot give as great a synoptic view as satellite imagery, it provides an accurate delineation and recording of affected area of vegetation damage (Blakeman 1990). Photographic response has been used by many workers for the detection of plant diseases, including nematode diseases. It is possible to pick up primary infection of crop plants by pathogens 2–3 days before the aboveground symptoms are visible on the ground surveys. Different levels of crop diseases can be identified using small-scale color infrared (CIR) photography (1:60,000). Changes in the appearance of the foliage and other aboveground plant parts due to the infestation by nematodes may be detected by aerial photography. The CIR infrared photography has been the most widely used technique in detecting plant stress because it portrays the combination of differential response from visible as well as near infrared spectrum (Heller 1978). Identification of host plants of pests with aerial CIR photography offers a means to plan large area pest management and eradication programs. This technique may be used to investigate the distribution of host plants of plant-parasitic nematodes. It can also be used to map areas of infestation by a specific nematode. It may be used in annual surveys to monitor the postharvest plant removal to prevent outbreaks. CIR photography can serve as a unique tool for monitoring the effectiveness of plant growth regulators/chemicals applied to manage various pests. CIR photography (1:8,000 scale) has been used to show that crop rotation from cotton to grain sorghum can significantly minimize the infestation level of the reniform nematode (*Rotylenchulus reniformis*) in cotton fields.

11.7.3 Aerial Videography

Videography has been used in various remote sensing applications for estimating freeze damage; discriminating plant species, weeds from crop plants, and infested plants from the healthy ones; etc. In a video camera, the image of remote objects is cast onto a photoconductive surface rather onto a film and forms a charge distribution that duplicates the optical image. This distribution is read by a scanning electron beam and is converted into electrical signals, which are stored on a video tape recorder.

Major advantages of videotaping include the ability to display imagery while it is actually being acquired, high sensitivity to light, suitability for digitization and analysis by computer, and low price and reusable nature of video recording tape. However, a disadvantage

Table 11.8 Satellite sensors

Type/name	Sensor type	Spectral regions (μm)	Spatial resolution (M)
IRS-1A Operational	LISS I and II	0.45–0.52	36.5 and 73
		0.52–0.59	
		0.62–0.68	
		0.70–0.86	
IRS-1B	LISS I and II	0.45–0.52	36.5 and 73
		0.52–0.59	
		0.62–0.68	
		0.70–0.86	
IRS-1C/1D	LISS I and II	0.52–0.59	23
		0.62–0.68	23
		0.77–0.86	23
		1.56–1.76	69
	Panchromatic WIFS	0.50–0.75	10
		0.62–0.68	180
		0.77–0.86	180

of commercial video systems is their low resolution (Manzer and Cooper 1982).

11.7.4 Multispectral Sensing (MSS)

This can form images over a much wider range of electromagnetic wavelength than photographic techniques. Instead of generating an instantaneous image of the whole scene, MSS uses sensors with very narrow fields of view to scan an image systematically; an image is built up as the scan progresses. Satellite sensors presently operational and those going to be available in the near future for use in pest management and plant protection are listed in the following table (Table 11.8).

Satellite-based MSS has been concentrated in two principal areas of plant protection, i.e., the detection of vegetation changes due to diseases/pests and the measurement of meteorological data. The identification of crop stress and disease from satellite data is a tough task. Timeliness of data acquisition and repetitive coverage are even more important as symptoms are often transient and closely linked to crop growth stage. Satellite sensors can cover and provide useful information on crop stress and disease when the symptoms are extensive (Epstein 1975). Satellite MSS has been used to assess disease severity on wheat. Satellite remote sensing is an ideal tool to investigate the environmental factors controlling the pest and disease development, particularly in monitoring rainfall and air temperature. Meteosat data can be used to monitor rainstorms that may affect pathogen development and for rapidly locating potential outbreaks. Several methods/programs like SPOT and Landsat TM and Advanced Very High Resolution Radiometer (AVHRR) of NOAA meteorological satellite that records vegetation index maps and rainfall estimates have been developed in detecting and studying disease aspects.

11.7.5 Radar

Radar has been extensively used as a tool in the study of long-distance migration and flight behavior of insect/disease inducing organisms. Information on its usage with respect to nematode diseases is but meager. However, in future, progress in this regard is expected, especially, aboveground feeding nematodes. These radars use a wavelength of 3–10 cm. Millimetric radar is used in case of small insect pests. Migration of a pest can be deduced from the orientation and directive properties of the radar antenna and target range from the time elapsing between transmission of the illuminating pulse and the reception of the

echo. Typical maximum detection ranges would be 1.5–2.8 km for individual pest and up to several tens of kilometers for dense swarms (flight behavior of locusts, grasshoppers, and moths of various species of *Helicoverpa*, *Spodoptera*, etc.). Radar (C-band) has been demonstrated to be an effective tool to detect blight in corn (Ulaby and Moore 1973). Microwave backscatter from crops is strongly dependent on the size of the scattering elements within a crop, crop geometry, row direction, canopy height, and the dielectrical properties of the canopies. A time series data of canopy scattering properties at different microwave frequencies (Ku, X, C, L bands) and polarizations (HH, VV, and HV) over the season could provide information about crop vigor and stress.

11.7.6 Sodar

The working of sodar is similar to that of pulse radar, except that high-frequency sound waves are transmitted rather than electromagnetic waves. Low-power, short-range sounding device has been used to count pests approaching pheromone traps (Hendricks 1980). Acoustic sounds can be used to monitor the atmospheric structure and vertical temperature profiles which favor the flight of many insect pests.

11.8 Technique of Soil Solarization

Soil solarization is a nonchemical method for managing phytonematodes and other soil microorganisms apart from weed seeds, bacteria, and fungi. Soil solarization is a unique approach to handle nematode problems. It is a potential alternative to chemical fumigation. As a nonchemical management method, it fulfills organic or low toxicity control (Ravichandra 2010). The technique is a simple and easy way to clean nursery/garden soil to grow nematode-free plants. Early summer is a great time to use solarization to clean up nematode "hot spots." Soil solarization is usually only considered as a remedy for nematode management in nursery and in the home garden. The basic procedure is to cover the soil with a clear plastic and trap the sun's heat to raise soil temperatures high enough to kill nematodes present in the soil. No plant material should be present in the area to be solarized. The extremely high soil temperatures will kill any plants left in the solarized area, and plant remains/debris will interfere with even heating.

Nursery, vegetable garden plots, and annual flower beds are the best areas for control. Soil solarization is a simple, safe, and effective alternative to the toxic, costly soil nematicides and the lengthy crop rotations now needed to manage soil nematodes. The technique utilizes the sun's heat to zap nematodes by trapping solar energy in the soil with a polythene layer. There are several procedures that must be followed closely to insure the soil temperature is raised to the level where nematode control is obtained (Killebrew 1999). Radiant heat from the sun is the lethal agent involved in soil solarization. A clear polyethylene mulch or tarp is used to trap solar heat in the soil. Over a period of several weeks to a few months, soil temperatures become high enough to kill nematodes to a depth of nearly 8 in. Nematodes may not be fully eradicated from the treated area, but their numbers in the plow layer (top 6–8 in.) will be greatly reduced, allowing successful production of a crop. In sandy or sandy loam soils, nematodes may survive at depths below the lethal temperature zone. As a result, some damage may be seen on deep-rooted crops, but those with shallow root systems should escape serious injury.

11.8.1 Points to Consider

The soil should be moist and well tilled before tarp installation. Solarization should be practiced during late spring months, or ideally at the hottest time of the year when the incidence of sunlight is greatest. Normally, 4–6 weeks is an adequate period of solarization. Six weeks is the preferred period if the technique is used in the late spring. Selection of a plastic or polyethylene cover is probably the most important step in the process. The cover must be clear and no more than 2 mm thick. It should be strong and durable enough to allow for stretching. Placement of the cover is also important.

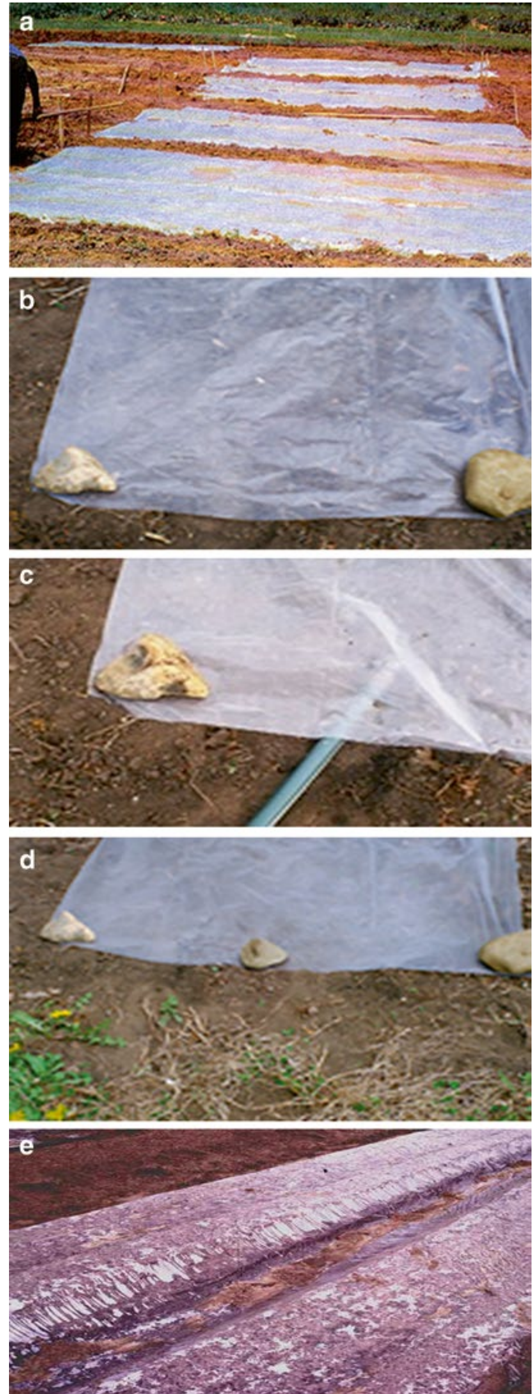
It should be stretched tight and in direct contact with the soil surface. Care must be taken to bury the edges of the tarp in the soil to a depth of at least 6 in. so as not to allow the wind to lift the tarp during the solarization period. The tarp has to remain in place for a 4- to 6-week period. When removing the tarp, avoid contamination of the treated area with untreated soil. This is also true at planting and best results are obtained when beds are prepared for planting before solarization and immediately planted after removing the tarp. Solarization also controls other soilborne organisms such as fungi, insects, and certain weeds.

Procedure

Soil Preparation: The soil to be solarized must be worked up to seedbed condition, that is, cultivated until it's loose and friable with no large clods or other debris on the soil surface (Plate 11.5a–e); a rotary hoe or rototiller will eliminate clods or other debris that create air pockets that reduce heating of the soil and keep the tarp from fitting tightly over the soil surface; a clean, flat surface will also prevent the accidental puncturing of the thin plastic mulch by debris.

Soil Moisture: Make sure moisture levels are adequate for working the soil before laying the plastic tarp; if the soil is dry, water the areas to be solarized before laying the tarp, because most soil pests are more sensitive to high temperatures in wet soil than in dry soil; when possible, lay a soaker hose or drip irrigation lines under the tarp to maintain moisture levels during soil solarization; tarped raised beds may also be watered by flood-irrigating the adjacent furrows.

Trenching: Dig a trench approximately 6–8 in. deep and wide around the perimeter of the plot. The trench will be used to bury the edges of the plastic to be placed over the plot.



Plates 11.5 (**a**)–(**e**) Steps in soil solarization (Courtesy: http://Okeechobee.ifas.ufl.edu)

11.8.2 Plastic Tarp

Use a clear, UV-stabilized plastic (polyethylene or polyvinyl chloride) tarp or sheeting 0.5–4 mils thick. The tarp material must be flexible enough to stretch across the soil surface. Using two layers of thin plastic sheeting separated by a thin insulating layer of air increases soil temperatures

and the overall effectiveness of a solarization treatment. The edges of the sheets must be buried to a depth of 5 or 6 in. in the soil to prevent blowing or tearing of the tarp by the wind. White or black plastic usually does not transmit enough solar radiation to raise soil temperatures to lethal levels for many soil pests. Thinner sheets (0.5–1 mil) are less costly, but they tear or puncture more easily.

Thicker plastic sheets (2 or more mils) should be used where damage from high winds or similar problems is likely. Patch holes or tears immediately with duct tape to prevent heat loss. Plastic mulches may be laid by hand or machine in a continuous sheet using glue or heat as a sealant and in strips over flat or raised beds. Continuous sheets are the best method for nematode control because the entire area is disinfested. Plastic strips, 2–3 ft wide, are often more convenient and economic for many bed-grown vegetable crops. Crops may be seeded or planted directly into slits or holes in UV-stabilized strip mulches after soil solarization is completed. For effective solarization, the edges of tarps laid over raised beds must be buried in the adjoining furrows. Expect some increase in pest and weed problems along the edge of the stripped mulches. Do not cultivate solarized areas, because healthy weed seed will be brought to the soil surface.

11.8.2.1 Timing

Long, hot, sunny days are needed to reach the soil temperatures required to kill nematodes. The longer the soil is heated, the better and deeper the control will be. During hot summers, a tarping period of 4–6 weeks is most favorable to control nematodes (although a slight variation is commonly seen in different regions), while a 2-week tarping period may not be very effective.

11.8.3 Other Benefits

Managing damaging nematode, soil fungi, bacteria, and pests with soil solarization can increase the growth and yield beyond expected levels. Some increases in yield may result from the control of usually minor and often unnoticed diseases. Populations of beneficial, growth-promoting, and pathogen-antagonistic bacteria and fungi quickly recolonize solarized soil, adding a biological control component to soil solarization. Plant-pathogenic fungi weakened by high soil temperatures are more susceptible to these antagonists. *Rhizobium* bacteria are also sensitive to high soil temperatures, but reduced nodulation of the roots of legumes such as peas or beans in solarized soils should be temporary. Improved soil tilth and the increased availability of essential plant nutrients such as nitrogen, calcium, and magnesium may also account for increases in plant growth following soil solarization. Soil solarization will tie up land for a period of 1–3 months. Commercial growers and gardeners must plan to pull areas out of production sometime during the 6-month period when solarization is possible. The benefits of higher crop yields with quality will reduce nematicidal use, particularly for gardeners, may far outweigh the cost and inconvenience associated with soil solarization.

11.8.4 Factors Influencing the Success

Soil characteristics such as color, structure, and depth have an effect on the effectiveness of soil solarization. Dark colored soil tends to absorb more solar radiation in comparison to light colored soils. Loose, friable soils enable heat to be transmitted throughout the soil and allow heat to go deeper. Control is much better in the top few inches of the soil and decreases with soil depth. The level of control achieved depends on a combination of the duration of the solarization process, the high temperatures reached, and the susceptibility of the weed species to be controlled. Nematodes that live in the top 2–3 in. of soil are easier to manage with solarization.

11.9 Technique of Soil Fumigation

The optional growing conditions provided for repeated cropping in intensive, irrigated horticulture favors the growth and multiplication of plant-parasitic nematodes. If not checked,

nematode pests build up to such levels that disease outbreaks progressively lower crop quality and yield, until a point is reached where crop plants are destroyed. Soil fumigation is the only answer to the problem, both as a cure for severely diseased soils and as a means of preventing soils from becoming diseased. Soil treatment with fumigant gases can reduce soil infections to safe levels and reduce the carry-over of nematodes to successive crops (Ravichandra 2010). Among various fumigants, methyl bromide is the most commonly used that gives the most efficient and economic management of plant-parasitic nematodes. It is the most effective fumigant that kills nematodes in soil and also kills nematodes within their cysts and galls and in plant residues which have not yet decomposed, viz., cyst nematodes, root knot, root lesion, pin, stylet, dagger, stem nematodes, etc. This technique is also an effective way to manage soil nematode pests present in seedbeds, nurseries, and other small plots.

11.9.1 Methyl Bromide

It is a liquid fumigant, it boils and becomes a gas at 4 °C, but this temperature is too low for soil treatment. Soil may be treated when its temperature at 15–20 cm is 8 °C, the optimal temperature, however, being about 25 °C. Methyl bromide sinks into soil at first, because it is heavy (about three and a half times heavier than air), but because it is very volatile, it is applied under plastic tarpaulins that serve to prevent its escape. It is sold in cans as a liquid under pressure. When the can is opened, all the methyl bromide flows out rapidly as a liquid or an odorless gas. Because it has not odor, it would be dangerous if not mixed with 2 % chloropicrin (trichloronitromethane) which has an odor and is highly irritating to the eyes. Methyl bromide is also obtainable mixed with larger percentages of chloropicrin and other nematicides.

Procedure Correct soil preparation is the key to successful nematode management by methyl bromide; nematodes are exposed and activated in the process of correctly preparing soils for fumigation; soils correctly prepared for methyl bromide treatment must be free of crop trash; cultivated to porous seedbed tilth to the maximum depth possible, without a plow sole; neither too wet nor too dry; about 2/3 of filed capacity is ideal; level.

11.9.2 Soil Preparation Schedule

For soil to be thoroughly ready for methyl bromide treatment, the below given order of operations is to be followed.

Clear the soil of plant trash; cultivate the soil to 40 cm depth at least. The deeper the cultivation, the better the penetration of the gas; disc at right angles to the prior operation. In heavy and medium soils, use a roller to break up any clods and obtain an even surface. For the third and last cultivation, a harrow should be attached. It will help pulverize clods and remove plant trash and will give a level surface finish. An excellent implement, especially in hothouses (green houses/poly houses), is the rotary hoe or the spade plow. It penetrates the soil to a great depth, turning and pulverizing clods without forming a plow sole, and gives a good, level surface finish. Irrigate to saturation to an 80-cm depth at least, not less than 2 weeks prior to treatment. Irrigate lightly, as required, to maintain constant moisture status (about 2/3 FC), until and especially at treatment time.

11.9.2.1 Manures

Soil should be manured before the first cultivation, but the dressing must not exceed 200 m^3/ha. If a dressing is required or if the manure is lumpy, it must be applied at least 3 months before fumigation so that it can decompose and mix with the soil. Large lumps of manure on or in the soil are to be avoided.

11.9.2.2 Chemical Fertilizers

High levels of available nitrogen are released by soil fumigation. To prevent possible excessive vegetative growth, the application of N fertilizers as a post-planting top dressing is advisable instead of a preplanting basic dressing.

Table 11.9 Dosage of methyl bromide based on crops

Crops	Dosage (in kg/ha)	Aeration (in days, by soil type and temp.)	Remarks
1. *Nurseries*: vegetables and flowers	350–500	7–14	Do not fumigate heavy soils to be used for celery nurseries
2. *Vegetables*: cucurbits, tomato, eggplant, pepper	350–500	7–14	For Beit Alpha- type cumbers, soil leaching is required
Strawberry (nursery and field)	350–500	14	–
3. *Flowers*: annual and perineal cut flowers	350–500	14–21	Even light soils must be leached before planting carnations
4. Bulbs and corms (on light soils only)	350	14	–
5. Citrus replanting	500	14	–
6. Deciduous replanting	750	14	–

11.9.3 Fumigant Application

Irrespective of the type of method chosen to apply the fumigant to soil, use of plastic tarpaulins is common, to confine this volatile gas in the soil for a 48–96 h exposure/treatment period. This variation in length of exposure is determined by the soil temperature at 15–20 cm at the time of treatment. The longer periods are required for lower temperatures. Polyethylene (polythene) tarps are recommended, either thin (0.03–0.05 mm) for one-time use or thick (0.1–0.15 mm) for reuse. Polyvinyl chloride (PVC) tarps are also used. Black tarps, made of recycled plastics, can reach a temperature of 60 °C on hot sunny days, but their use is to be avoided. Methyl bromide tends to move down slopes. On gently sloping sites, apply more gas at the upper, than at the lower end. On steep slopes, treat on the contour. To ensure that run-off water from irrigation or rain will not wash infected soil into the fumigated area, always fumigate a few meters beyond the ends of the planting rows and open a trench around the fumigated area. Crops may be planted 3–21 days after the tarps are removed. The length of this aeration period depends on the crops as shown in Table 11.9.

For light soils and/or high temperatures, the shorter aeration period is sufficient; for medium and heavy soils and all soil types at low temperatures, the longer aeration period is required; the long aeration is also desirable for direct seeded crops; if rain is expected during the aeration period, do not remove the plastic sheets but allow for aeration while protecting the soil from direct rain.

For crops sensitive to bromide residues (e.g., onion, carnation, citrus seedbeds, cotton, pepper, celery), a leaching irrigation after an initial 2–3 days aeration of 200–400 mm is essential. These crops may be planted as soon as soils have drained to suitable moisture levels. As for all nematicides, carefully read the label before starting work. Methyl bromide can be applied by various methods like in cylinders either by cold gas manual method or hot gas manual method, in cans, as capsules, through drip irrigation system, through fumigation of hothouse soils, or by machine. In most places, fumigation of hothouse soils is a commonly followed method.

11.9.4 Methyl Bromide Fumigation of Polyhouse Soils

In polyhouses and glass and plastic hothouses, physical barriers like walls and supporting posts, affect work methods and order. Uncovered

plastic houses may be regarded as being on a par with the open as far as precautions are concerned, but the generally limited ventilation of covered plastic and glass hothouses makes working to safety rules imperative. The dosage of the fumigant is normally fixed based on the crops and stage of the crop (Table 11.9). Work out the glasshouse doors as much as possible. Seal, above and below soil level, all drains and other openings that connect hothouses adjacent to the one being fumigated, so that the gas will not escape into them. Open all doors and windows to the full. Start fumigating at the bay furthest from the entry and complete it at the bay closest to it. The one-time, overall treatment should be the rule for hothouse fumigation. It has the great advantage of the gas being delivered from outside the structure. When preparing the soil, pay special attention to the usually more compacted soil close to the hothouse door. To seal the soil, trench soil away from the walls to form a channel along the wall base and a soil mound parallel to it. Brush all soil from the walls into the channel. Hold the tarp smooth against the wall, so that its margin hangs in the channel rather like a bed sheet, with the soil being pushed from the mound beneath the tarp rather like the mattress holds a bed sheet margin firmly and smoothly against a wall, the mattress (here the soil) being sealed in by the sheets under flap, in the process. The sealing around posts can be problematic. It is sometimes worth slitting the margin of the tarp and firmly taping the overlaps to the post. No soil particle must be left above soil level on wall surfaces, post bases, etc., because it could cause recontamination after fumigation. If tarps between bays are not to be glued together, allow for a 35-cm overlap at each margin.

At the end of methyl bromide delivery, close all side ventilators and hothouse doors from the outside and hang up the required warning notices on doors and in conspicuous positions around the hothouse. Roof ventilation must be left open. Unauthorized personnel (and pet animals) must be forbidden from closely approaching or entering the hothouse during and for 48 h after fumigation or until it is shown by halide detector that it is free of gas. A gas mask with a methyl bromide filter or one supplying compressed air must be worn if there is a need to enter the hothouse during treatment or after when tarp edges must be partly raised for initial aeration (in most places, the use of a compressed air breathing apparatus is compulsory). Open wide all doors and side ventilators at the end of the 48–96-h treatment time. Lift tarp edges here and there and leave the hothouse immediately. After a 2-h period, remove the lay flat tubes and tarps, and if the tarps are to be reused, aerate them in the pen before storing. Ventilate the hothouse either for 4 days or until it is shown to be free of gas by a halide detector, before allowing operations to start up again. Ensure that gas mask filters are used according to manufacturer's instructions. Begin each day with a new filter. Clearly mark those filters which are lapsed and never reuse filters. If the gas concentration is high, change the filter more often than specified.

11.9.5 Safety Precautions

11.9.5.1 Handling the Containers

Cans must be opened with special can openers; instructions for using the special openers must be rigidly followed. Empty cans should be crushed and buried away from habitations. Cylinders have a dip pipe reaching to the concave bottom; gas (and added nitrogen) pressure will make the liquid methyl bromide rise through the tube when the valve is opened and the cylinder will empty itself. Cylinders must never be handled roughly, dropped, bumped, or dragged and must never be unloaded by rope sling, hooks, tongs, etc. The heavier cylinders must be firmly cradled and secured for transport on a suitable hand or fork truck. The valve-protection cap should be removed only when methyl bromide is to be delivered from the cylinder. This cap must be opened by an adjustable or other smooth spanner only. Instruction for delivering the gas must be rigidly followed. Empty cylinders must be returned to the manufacturer with closed valves and valve-protection and cylinder caps in place.

11.9.5.2 Storing the Containers

Cans and cylinders must be stored under lock and key, away from excessive heat, and upright and

tightly capped in well ventilated, dry, cool storage areas secured from children, unauthorized persons, and animals. Cylinders should be examined periodically for leaks by means of a halide detector lamp.

11.9.5.3 While Handling Methyl Bromide

Avoid inhaling gaseous fumes and splashing liquid fumigant on the skin, eyes, etc. Clothing, shoes, bandages, rings, etc., must be removed immediately if splashed. Since splashes will cause burns, they must be washed immediately from skin or eyes with copious amounts of water. Gloves must not be worn. They impede the rapid evaporation of the fumigant from the bare hands and, by prolonging contact, will only worsen skin injury. Air contaminated shoes and clothes fully before wearing them again. Do not eat, drink, or smoke during fumigation operations and shower and change into clean clothes and shoes at the end of the operation.

11.9.5.4 Hothouse Fumigation

The general provisions noted above also hold for hothouse fumigations. There are, however, some extremely important additional safety measures required, relating to the proper use and care of approved safety equipment, which, if strictly observed, will make hothouse fumigation a safe, effective operation. The use by all operators of approved, full-face, air-purifying respirators with methyl bromide canister filters and/or compressed air breathing apparatus. The use of suitable methyl bromide detection equipment (halide leak detector, gas detection tubes, etc.) to establish that safe entry levels (i.e., atmospheric gas concentrations of less than 5 ppm) have been reached inside the hothouse. The rule that anyone not wearing an approved full-face respirator or compressed air breathing apparatus shall under no circumstances enter the hothouse throughout the fumigation operation (i.e., from the start of gas delivery until the hothouse is declared safe for entry). The rule that work must be performed by qualified, responsible personnel and that it must be done in teams (or at least in pairs), so that anyone entering the hothouse for operational reasons shall, although wearing breathing equipment, be under observation by an operator outside the hothouse. A full-face respirator must be used strictly according to the manufacturer's instructions. Always check airtight fit before use, by placing face piece over the face, kinking the hose, and trying to breathe. An airtight mask will be sucked against the face and no air will leak into it from the edges. Prevent misting of the eyepiece by using an impregnated cloth to clean it.

During gas delivery under the polyethylene tarpaulins, the methyl bromide vapor canister is to be used for a maximum of 1 h. A new vapor canister is to be fitted routinely at the start of each day's operations. This vapor canister is suited to a safe upper working limit of 20 ppm – if it becomes necessary to come in contact with higher concentrations (e.g., when a lay flat or manifold must be reattached or a tear must be repaired), wear a compressed air breathing apparatus. Never reuse partially used canisters, even if not time-expired, and always keep them apart from new ones. Crushing the inlet of used canisters will insure that they are not used again. When using compressed air breathing apparatus, move to an area free of gas immediately if the warning whistle indicates the level of air in the cylinder is low. When wearing a respirator, a person with punctured eardrums may draw fumigant vapors in through his ears as a result of creating a slight negative pressure during inhalation. Any fumigant drawn this way will be exhaled into the inside of the respirator face piece and a poisonous concentration may build up inside the respirator. It is usually possible for persons with this defect to obtain complete protection by using cotton earplugs covered with oil.

11.10 Microplot Techniques

Microplots refer to the small field plots with barriers to retain the nematodes, which are useful to differentiate damage caused by single specie/genus of nematode and related population dynamics (Barker et al. 1985). They are specifically designed to overcome many of the difficulties associated with field plots. Microplots that give more control of the experimental variable than do

typical field experiments have been used by many scientists. Using microplots, types of barriers that may be constructed, methods of installations with various nematodes can be determined.

Microplot experiments yield useful quantitative data for most crops. They are useful to develop estimates of the effects of several nematode species on growth and yield potential of a given crop; to determine the relative importance for various physical and climatic parameters like soil type, temperature, and soil moisture; to study the differential responses of several cultivars to specific species; to characterize the population dynamics of several nematodes; to determine reproductive factors, equilibrium densities, and rates of decline of a single nematode species; to characterize nematode–host interactions; to identify and differentiate the roles of some soil microflora and microfauna associated with various disease complexes; to develop and evaluate nematode management tactics; to determine the differential efficacy of several nematicides on single and multiple nematode species; to manage nematodes in the study of biocontrol agents; and to determine the effect of rotation regimes and other management practices on nematode populations.

11.10.1 Types of Microplots

Most microplots used for nematological research vary in size from 25×25 cm to 1 m^2 soil surface area (Plates 11.6 and 11.7). Since available soil moisture becomes limited quickly, the smaller plots may pose problems for moderate- to large-sized plots, which can be minimized when automated irrigation facilities are adopted. Perennial plants may need a plot size of 5×5 m, while plots of approximately one-half m^2 are enough for most annual plants and also for small perennial plants.

11.10.2 Preparation and Infestation of Microplots

Preparation of microplots for infestation by the specific numbers and types of nematodes needs considerable effort and expense. Treat the soil with a fumigant like methyl bromide at a rate of 50–150 g/m^2 to destroy nematodes and other soil pests. Till the soil of each plot to a depth of 20–30 cm and 3 or more holes at least 40 cm deep should be made in each plot to facilitate penetration by the fumigant. Cover the entire plot area with plastic prior to treatment with the fumigant. It is advised to avoid introducing nematodes or plants into the plots for 1–2 months after treatment. Tillage of the soil two to three times after removal of the plastic cover will enhance the dissipation of the toxic gas. Nematode inocula to be used to infest microplots to the desired levels may be collected from greenhouse or monoxenically grown culture (Townshend and Potter 1980). Before the harvesting of nematode inocula, allow

Plate 11.6 Microplots with different treatments

Plate 11.7 Microplots-General view

plants to grow for 10–12 weeks. Standardized, chopped, infected roots or NaOCl-extracted eggs may be utilized to infest the plots to desired levels. It is better to add mycorrhizal fungi like *Glomus* spp. and other beneficial organisms like *Rhizobium* spp. for legumes into each plot at the time of nematode infestation. Add these organisms at the same rates to all plots including the control plots.

If infected root material is used to establish the required level of nematode level, a suitable quantity of healthy root material should be added into the control plots and lower density plots to give an equal amount of root material for reach plot. Several factors influence the quality of data obtained from microplots like preparation, precise infestation, and thorough mixing of the inoculum into each plot. Due to minimal competition, an abnormal result may be encountered sometimes like an annual crop yield two- to three-fold more than that which occurs with normally spaced plant populations that can be overcome with continuous normal spacing of plants within and between the microplots.

11.10.3 Major Limitations of Microplots

As with greenhouse and phytotron experiments, results obtained in microplots should be validated in parallel full-scale tests. Data thus obtained may be applicable to limited geographical and climatic regions. Contamination with multiple species is a major problem in microplots, particularly with nematodes like *Paratrichodorus* spp., which are difficult to eradicate with fumigants. Special efforts are needed to eradicate nematodes on deep-rooted plants prior to initiating experiments. There is a need for much hand labor and frequent use of expensive nematicide treatments. Data interpretation and inferences normally cannot be extrapolated directly to the field situation as in the studies involving the relationship of initial nematode densities to growth and yield of the plant.

11.11 Techniques of Evaluating Nematicides

To know the nematicidal activity of a wide range of agricultural chemicals, evaluation techniques are necessary. They are helpful to understand the influence of systemic nematicides on nematodes by continued and discontinued exposure and to know the persistence of systemic nematicides in the soil under various conditions. Experiments with nematicides provide convincing evidence that yields can be increased by nematode control. If use of nematicides is economically feasible, experiments

provide useful information on methods, results, and profits. When the use of nematicides is not profitable, demonstration of yield increases provides a strong incentive to find other nematode control methods. Experiments demonstrating yield increase in farm fields are evidence that nematologists are working on a subject which will be of benefit to farmers and will increase food supplies. Standardized nematicide experiments by cooperators of international projects may provide comparison of data from different parts of the world (Taylor and Sasser 1978).

11.11.1 Procedure for Measuring the Root-Knot Infestation by Using the "Indicator Plants"

Collect soil samples at 5-m intervals. Each sample should be taken from an area about 1 m^2 and should be a composite of four samples of about 500 cm^3 each. Samples taken from the soil layers about 10–20 cm deep are best. Number the samples and make a drawing showing the location of each. Place the composite samples in pots (tin cans/plastic bags) and plant two or three tomato seedlings grown in nematode-free soil or ten tomato seeds in each pot. Fertilize lightly and water every day. If seeds are planted, remove all but two or three seedlings from each pot. Allow to grow for 4 or 5 weeks and examine the roots. These are indicator plants to be used for estimating infestation of the soil. Remove the tomato plants from the pots and wash off the soil. Examine the roots for *Meloidogyne* knots. Rate each plant by using the scale (0 = no galls, 1 = 1–10 galls, 2 = 11–20 galls, 3 = 21–50 galls, 4 = 51–100 galls, 5 = 101 or more galls).

The results will give an indication of the nematode infestation of the plots. Make a diagram of the plots, locating the samples and indicating gall rating. Fields can also be selected at the end of a crop season by examination of living roots remaining in the soil. If the crop has a moderate infection and roots are not excessively decayed, there will probably be a good infestation for experimental purposes the next season.

11.11.2 General Methods

11.11.2.1 Preparation of Plots

Plow the soil and prepare it for planting (Taylor and Sasser 1978). Lay out plots. For example, if sixteen plots are needed, plots may be in four blocks of four plots each. Plots may be square or rectangular according to the shape of the land. Mark each plot with a stake showing the treatments. These stakes should be 4 or 5 cm wide and 50–60 cm long. They should be strong enough to last for the duration of the experiment.

11.11.2.2 Treatments

The four treatments are as follows:

1. Control, no nematicide
2. One-half the amount recommended by the manufacturer of the nematicide
3. The amount recommended by the manufacturer
4. Two times the amount recommended

Each treatment is applied to four plots randomized so that no two plots receiving the same treatment are adjacent.

11.11.2.3 Preparation for the Treatment

Calculate the number of grams of granular nematicide or the number of milliliters of liquid needed for each plot (T3), that is, the amount recommended by the manufacturer of the nematicide. The information is obtained from the label of the container or from the manufacturer's circulars. The amount may be specified as application rate per acre or per hectare. The following table (Table 11.10) gives amounts per square meter and amounts per 100 m of row for various application rates (Taylor and Sasser 1978).

Table 11.10 Calculating application rates for nematicides

Application rates for liquid nematicides				Application rates for granular nematicides			
US gal/acre	L/ha	ml/m^2	ml/100 m of row 30 cm wide	lb/acre	kg/ha	g/m^2	g/100 m of row 30 cm wide
1.0	9.353	0.94	28.06	1.0	1.121	0.11	3.36
2.0	18.706	1.87	56.10	2.0	2.242	0.22	6.73
3.0	28.059	2.81	84.18	3.0	3.363	0.34	10.09
4.0	37.412	3.74	112.24	4.0	4.484	0.45	13.45
5.0	46.765	4.68	140.30	5.0	5.605	0.56	16.81
6.0	56.118	5.61	168.35	6.0	6.726	0.67	20.17
7.0	65.471	6.55	196.41	7.0	7.847	0.78	23.54
8.0	74.824	7.48	224.47	8.0	8.968	0.90	26.90
9.0	84.177	8.42	252.53	9.0	10.089	1.01	30.27
10.0	93.530	9.35	280.59	10.0	11.210	1.12	33.63

Notes
1 US gal = 3785.3 ml = 0.832 imperial (British) gal
1 m = 3.281 ft = 1.094 yd = 39.37 in.
1 ha = 10,000 m^2 = 2.471 acres = 11,959.64 yd^2
1 acre = 43,560 ft^2 = 4,840 yd^2 = 4,046.9 m^2
1 US gal/acre = 9.353 L/ha
1 kg = 2.205 lb
1 lb/acre = 1.121 kg/ha

Check the calculations for T3 carefully. Then calculate one-half the amount for T3 plots and two times the amount for T4. Assemble all materials and equipment required and make a trial application on a piece of ground not to be used for the experiment. If this goes well, proceed with the application. Make a diagram of the plots and the field. This will help in locating the plots even if the stakes are lost and also in finding their location again in the future.

11.11.2.4 Planting and Care of the Plots

After the waiting period recommended by the manufacturer of the nematicide, plant the plots, following the best procedure being followed in the region. It is suggested that the experimental plots be weeded and cultivated. Plots should be visited at frequent intervals to be sure that nothing is going wrong, like infestation by insect/disease or accidental interference.

11.11.3 Publicity to the Farmers

Before harvest when plots show large differences, it is better to invite farmers to visit the plots, explain them about the procedure followed there, and advise them how to improve the yields by controlling nematodes. If possible, prepare and distribute the leaflets/handouts/literature on the importance of the particular nematode problem of that area with all details including the symptoms, spread, life cycle, survival, and management with more stress on the integrated management including the major insect pests, diseases, weeds, and nutrient deficiency, with good quality photographs. Arrange radio talks and television programs on the specific problem in detail rather than a general talk/show on nematode problems of several crops. Field days/farmers' meetings may be arranged during the completion of the experiment/demonstration in collaboration with the line department officials of agriculture, horticulture, sericulture, etc., and

also scientists of the nearby research stations/ institutes. The program may be given a wider publicity through local newspapers to reach large number of farmers.

11.11.4 Collection of Data

During the growing season, make notes of any differences in growth, death of plants, or wilting. If possible, take numerical data, plant height, percentages of dead or wilted, etc. Four to six weeks after planting, dig 5–10 plants from each plots and rate for galling as with the indicator plants. At harvest time, bags, crates, or other containers will be required to hold the produce, preferably one for each plot. Scales to obtain weights are needed. Data include yield of each plot, and if the produce is usually graded, the weight of each grade to be taken. After harvest, dig up the plant roots and rate them for root-knot galls, using the same method as for indicator plants.

Calculations: Calculate weights and value of produce from each plot and averages of the four plots of each treatment. This data can be analyzed by standard methods of analysis of variance.

Reporting: Prepare a report of the experiment in good form for publication and send to the appropriate journal.

General: Photographs of difference in growth in plots are useful. These may be color slides for use at scientific meetings and also black and white for publication, if needed. It may be possible to photograph the harvest in a way which will show differences between treatments and control. Be sure that administrators and supervisors have a full report of results.

11.11.5 Types of Specific Techniques

Three major types are common.

1. The penetration inhibition test (PI TEST)
2. The therapeutic test (T TEST)
3. The modified gall index test (GI TEST) (Bunt 1975)

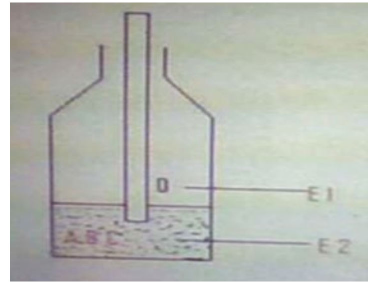


Fig. 11.5 Penetration inhibition test

11.11.5.1 The Penetration Inhibition Test

This test rates the property of all known nematicides to inhibit invasion by repellence, narcosis, or antifeeding.

Procedure Pipette a nematode suspension (*Ditylenchus dipsaci*) of 1 ml comprising about 400 specimens (B) into a 10-ml glass vial containing 5 ml of dry silver sand (A) (Fig. 11.5). Another 1 ml with the substance to be tested in the desired concentration (C) is also pipetted into vial. The sand is then just saturated. After 24 h incubation at room temperature, a 4-cm stem section of field bean, *Vicia faba* L., is placed a few mm into the moist sand (D). After another 24 h, the infective fourth larvae invade particularly the lowest 1 cm of the stem, which is washed free from adhering sand and cut off. After cutting the stem section once longitudinally, the pieces are placed in a counting tray in 5 ml water for 24 h to extract the number of penetrated but still viable nematodes (E_1), which are examined and counted after extraction. Untreated stem pieces in water instead of chemical solution usually have about 100 nematodes. The silver sand can also be analyzed for surviving *D. dipsaci* by placing the sand sample on a nematode extraction filter in a counting tray (E_2). Before field bean stem sections were found suitable as bait for *D. dipsaci*, pieces of potato tubers and carrots and also stem sections of potato and other plants should be tested.

11.11.5.2 The Therapeutic Test

This test is used to investigate nematicides which might affect nematodes already in

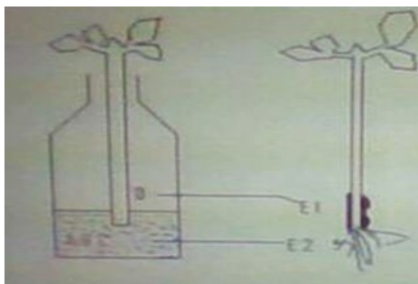


Fig. 11.6 Therapeutic test

infested plants. It is useful to study the effect of delayed treatment when nematicides have to be converted by the plant into nematicidal active derivatives; such nematicides may be missed in the PI test.

Procedure The test follows the procedure of the PI test. A 1-ml suspension with 400 *D. dipsaci* (B) and 1 ml solution of the test chemical (C) are pipetted into a vial containing 5 ml of dry sand (A), just saturating the sand (Fig. 11.6). After incubation at room temperature for 24 h, a tomato cutting is placed a few mm in the moist sand (D). At 15–20 °C it takes 6–8 days for *D. dipsaci* to cause swellings and discolor the tomato stem just below to a few cm above the soil (E_1). The silver sand is analyzed for surviving nematodes by placing the sand sample on a nematode extraction filter in a counting tray (E_2). An effective nematicide prevents nematode attack and no symptoms are seen. One week after placing the tomato cutting, the attack may be evaluated with the naked eye. Two to four replicates suffice for reliable results over a range of concentrations of a candidate nematicide. Thus, some hundreds of compounds can be tested by one person per week. The influence of the chemicals upon egg laying and numbers of larvae produced may also be determined. If further observation is needed, fertilizer is added and the plants are allowed to grow for 2–3 weeks. The first new larvae have then appeared if an inoculum of L_4 stage larvae is used. If such a longer growth period is desired, vials of a greater content, e.g.,100 ml instead of 10 ml, can be used to keep the sand moist. The cuttings can be inoculated in a small vial and the rooted cuttings infested with *D. dipsaci* transplanted to a larger container later on. For a complete therapeutic test, the cuttings can be allowed to root in the sand and *D. dipsaci* allowed invading the stems for 3–4 days before the chemical is introduced. A therapeutic systemic will prevent multiplication or kill the nematodes in the plant tissue. If more than 200 *D. dipsaci* specimens are used as inoculum, the first symptoms are visible after 3–4 days, but they do not become severe if effective systemics are added.

The test may also be used to study adsorption rate of a nematicide on organic matter and on other soil components. Inoculated, rooted cuttings transplanted to various soils are treated with a range of concentrations of the test chemical; the influence of adsorption can be estimated from the test plants by evaluation of symptoms, egg laying, number of larvae and adults, and sex ratio of the adult nematodes. The best time for evaluation depends on the information wanted. The techniques for nematode extraction are the same as for the PI test.

11.11.5.3 The Modified Gall Index Test

This technique is used to study the nematicide effect against *Meloidogyne* spp. in pots/greenhouse.

Procedure The gall index or GI test resembles the T test, but *M. incognita* is used instead of *D. dipsaci* and root galling is recorded instead of stem swellings (Fig. 11.7). A 1-ml suspension with about 500 *M. incognita* larvae (B), obtained by placing egg masses upon a nematode extraction filter, and a 1 ml solution of the chemical to be tested (C) are pipetted into a vial containing 5 ml silver sand (A). After incubation at 20 °C for 24 h a tomato seedling, from which the roots are taken off, is placed a few mm into the moist sand (D). Some days later new roots are formed and active *M. incognita* larvae in the sand will penetrate and initiate galling of the roots. After 7 days the roots are washed free from sand and are rated for galling under the dissecting microscope. With the naked

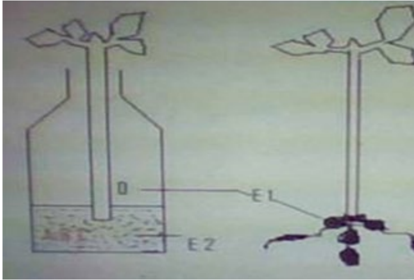


Fig. 11.7 Modified gall index test

eye the degree of galling can be estimated after about 10 instead of 7 days. The results may be given as estimates, but counted num ber of galls on the root is more exact (E_1). Surviving larvae can be extracted by placing the sand on a nematode extraction filter for 24 h (E_2).

The modifications compared to the original gall index test are as follows: tomato seedlings without roots are used, the bioassay takes place in vials of 10 ml comprising 5 ml pure sand, less chemical is required, the whole test is shortened from 6 weeks to 10 days, and less labor and greenhouse space is needed.

11.11.6 Points to Remember

The PI and T test appear to be adequate for screening large numbers of chemicals to detect nematicidal activity independent of the mode of action. Each method has advantages and disadvantages, but both tests cover a wider field of nematicidal effects and are easier to handle than methods in current use. In special cases, the GI test may be preferable, e.g., for tropical regions and also if microscopes are not available for evaluation; the last reason also holds for the T test. The advantages of all these tests is that they are bioassays; the test plants or plant parts are also used to indicate phytotoxicity of candidate nematicides, namely, by blackening of stem parts in the PI test and by various other symptoms in the GI and *T* tests. A phytotoxic systemic may damage the top leaves of the young seedlings and phytotoxic fumigants more often damage roots or lower parts of the stem.

In a large-scale primary screening program, it is possible with each of the three techniques to test about 250 substances per person per week.

11.11.7 Preliminary Screening of Nematicides

11.11.7.1 Water Screening

Add 1 or 2 ml of nematode suspension containing approximately 50 juveniles into a vial. Add equal amount of stock solution of nematicide to give half the concentration prepared. Keep four replications of each treatment and check. Examine under stereo binocular microscope after fixed time.

11.11.7.2 Sand Screening

Add 1 or 2 ml of nematode suspension containing approximately 50 juveniles into a vial. Fill 2/3 of vial with thoroughly washed sand. Add 1/10th of the volume of the vial the prepared concentrations of the nematicide. Shake to mix sand, nematodes, and the chemical. Fill the vial with dry sand. Keep screw for 12 h. Wash the contents of the vial into a beaker and decant. Examine under a stereo binocular microscope.

11.11.7.3 Test Systems

Several test systems are available for the evaluation of nematicidal effects of fungal extracts or the ability of fungi to act as biocontrol organisms. Important ones are as follows.

11.11.7.4 The Microtiter Plate Assay

It is a fast assay for testing the nematicidal activity of crude extract or pure compound. Large numbers of samples can be handled while using relatively small sample amounts.

11.11.7.5 Water Agar Assay

In the water agar assay, the antagonistic properties of fungi for a potential use in biocontrol can be evaluated. These fungi can be divided into endoparasitic species which grow only within their host and precious species which grow in the soil and form special mycelial structures

functioning as nematode traps. Nematode trapping is restricted to mobile host stages whereas parasitism can be performed on mobile or immobile stages. A migration assay with lettuce seedlings in a Sephadex resin can be used for detecting toxic effects of fungal extracts or isolated compounds and their interference with the migration of larvae to the root tips. Phytotoxic effects of the compounds may also be seen. The ability of a compound to interfere with the invasion of second-stage larvae into the root tips and the gall formation can be evaluated in a test system for protective activity on agar plates. This assay also gives information about the stability of the compound and the duration of the effectiveness of the compound under favorable nematode growing conditions.

11.11.8 Techniques to Test the Efficacy of Nematicides

These techniques may be used for various purposes, viz., to evaluate the effects of chemical/nematicide treatments on hatching and emergence of juveniles and to obtain juveniles for in vitro evaluation of chemicals and for inoculations to evaluate chemical soil treatments (Steele 1978).

11.11.8.1 Procedure to Evaluate Nematicidal Effects on Hatching and Emergence of Juveniles from Cysts

Separate newly hatched cysts from sugar beet roots and soil by washing, floating, and decanting suspended debris into screens. Select the newly formed cysts with eggs and juveniles, manually separate from washed debris, and store until required. Transfer 2–5 ml of test solutions to the portion cups using a precalibrated pipette. Transfer groups of 20 nematode cysts with a fine bristle brush to a small wedge-shaped piece of filter paper before going to the collection cups (sieves). This prevents dilution of the test solution during transfer of the cysts. Incubate the hatching vessels and their contents at 24 °C during the entire test period. Treat the cysts with the nematicidal solutions for 1–7 days. Transfer to tap water, which is changed daily, for 4 days to remove the test materials. Place the cysts in a hatching agent for 2–4 weeks. Inoculate the host plant (e.g., sugar beet) with the cysts to evaluate nematode viability.

11.11.8.2 Procedure for Hatching Juveniles

Add washed and screened root debris containing cysts to a large screen (about 10 cm in diameter), which is placed in a funnel containing a hatching solution. Adjust the level of the solution so that the debris is wet but not completely covered. Insert the stem of the funnel through the top of the refrigerator cabinet. Maintain the cabinet interior around 8 °C and that of the solution bathing the cysts at 24 °C using an electronic thermoregulator equipped with a thermistor and heating tape. If ambient temperatures are above 24 °C, supplementary heating of the solution may not be needed.

11.11.8.3 Procedure for Evaluating Chemical Effects on Hatched Second-Stage Juveniles

Treat newly hatched second-stage juveniles for 24 h at 24 °C with aqueous solution of 1, 5, 10, 25, 50, and 100 μg/ml of test chemical. Estimate the effects of chemical treatments on mobility by placing the juveniles on tissue paper supported by collection cups (sieves) that are in turn placed in the chemical solution. Juveniles remaining on the tissue paper are assumed to be either immobilized or incapable of purposeful movement (disoriented). After the initial treatment, wash the juveniles with several liters of tap water. The nematodes are easily concentrated in a small volume of water using a Buchner-type funnel with fritted disc. Inoculate the roots of the host plant (sugar beet) grown in a steam-sterilized sand–soil mixture with treated and nontreated juveniles. At 18 and 30–35 days post inoculation, harvest the plants. Examine the roots and soil for adult nematodes. Specify the numbers of juveniles added per plant or unit weight of soil. Not less than 2,000 juveniles or eggs and juveniles from 20

selected viable cysts should be added per plant. If cysts are used, estimate the numbers of eggs and juveniles by counting them or by hatching the eggs in a solution containing a hatching agent.

11.11.8.4 Procedure to Assess the Efficacy of Nematicides

Evaluate the nematicides' efficacy one or more times during the growth period of the host plant. Obtain at least one count before production of the second nematode generation. Take up the counts on nematodes extracted from soil or plant tissues and may include any or all stages. To get the counts of adult males, harvest plants 18 days after inoculation and place roots on funnels in a moist chamber for 5–10 days. For counts of adult females, harvest the plants 30–35 days after inoculation.

11.11.8.5 Techniques to Evaluate Nematicides for Systemic Eradication of Root-Knot Nematodes

The objectives may include the evaluation of a nematicide for systemic eradication properties against root-knot nematodes (*Meloidogyne* spp.) present inside the root tissue prior to chemical treatment. This technique helps in evaluation of nematicides and their potential for post-planting use (Thirugnanam 1978).

Inoculum Procedure (Modified Baermann's Technique)

Wash the root-knot infected host (e.g., tomato) roots containing egg masses free of soil in tap water. Cut into small pieces and macerate in a blender for about 30 s. Pour the macerate evenly onto a screen (15–25 mesh) that is already layered with wet strength tissue and supported by two Petri dishes at one end in an inclined shallow tray. Add water to the tray and maintain at a level sufficient to keep the tissue layer moist during the incubation periods. During this period, the juveniles hatch on the screen and migrate toward water. Aeration of water keeps them alive. After 5–7 days of incubation, the aqueous larval suspension is stirred well to get uniform distribution of nematodes for microscopic counting. Dilute the aliquot further with water to facilitate counting.

Inoculation Procedure Wash 3–4-week-old seedlings grown (4–6 leaf stage) in steam-sterilized soil in running water to free the roots from soil particles. Place the root system of each seedling in a Petri dish. Pipette out 2 ml of aqueous nematode suspension containing about 500 second-stage juveniles directly onto the roots. Sprinkle the steam-sterilized soil over the entire rot system. Maintain this soil moist throughout the inoculation period. After 24 h, remove the seedlings and wash their roots thoroughly in running water prior to chemical treatment.

Nematicidal Treatment

Soil Incorporation: Treat the steam-sterilized soil with test compounds to obtain desired dosages. Apply the chemicals by thorough incorporation, drench, or surface treatment or by fumigation in the case of volatile compounds. Plant the seedlings exposed to nematode infection for 24 h in treated soils. Appropriate checks/standard chemical treatments/replications are to be maintained.

Foliar Application: Hold the seedlings horizontally and spray the foliage to run off with test compounds. Cover the root system, during spraying, to prevent direct contact with the chemical. When leaves are dry, pot the plants in steam-sterilized soil. Appropriate checks/standard chemical treatments/replications are to be maintained.

11.11.8.6 Determination of Eradicant Activity

Extraction of Eggs

After 4–6 weeks of treatment, remove the plants carefully from the soil. Wash the root system in water. Determine the number of eggs on each plant by extraction with 0.5 % NaOCl for 10 min and microscope counting. Express the eradicant activity of the test compound as the percent control of egg production using the following formula.

$$\text{Percent control of egg production} = \frac{\text{Number of eggs in check plant} - \text{Number of eggs in treated plant} \times 100}{\text{Number of eggs in check plant}}$$

11.11.8.7 Technique to Test the Phytotoxicity of a Nematicide

After having disinfested the soil with a nematicide, particularly in case of volatile chemicals, the grower may need to start planting or sowing as soon as possible, however, running the risk of phytotoxicity. The "watercress germination" test is a useful method to establish whether traces of the nematicides are still present in the soil.

Procedure Mix the soil thoroughly. Take a sample of 300 ml and pour this in the jars. Treat the soil with one of the nematicides. Close the jars with the lid or with plastic film. Ten days after treatment a small pad or moist cotton wool with watercress on it is suspended in the jar. The germination is indicative of the disappearance of the nematicide.

11.11.8.8 Points to Be Considered During Field Evaluation of Nematicides

Various test materials and environmental and cultural conditions influence the results of evaluation of nematicides under field conditions. The information that should be recorded during field testing of experimental nematicides for an effective evaluation is given hereunder (Johnson 1978).

Test Materials

Compare all the test materials with an untreated control and with a known standard, normally one of the materials currently recommended. The knowledge on the biological activity and chemical and physical properties of the test material is necessary. Review pertinent literature or technical reports before designing field trials.

Formulation

Record the following: formulation type, viz., emulsifiable concentrate, wettable powder, flowable, water soluble, and granular (mesh size); the names and percentage of every ingredient in the formulation; lot number on the package label; and dates sent and received. If a nematicide is diluted before application, specify the amount of diluent used by common and chemical names.

Rate or Rates of Application

Mention clearly and precisely the rates as formulation and active ingredient in one or more of the following terms: the quantity per unit of area if treated overall (broadcast), the quantity per linear distance and row spacing if row treated, and width of band and row spacing if band treated.

Number and Timing of Applications

Record the dates including the month, day, and year of preplant or postplant applications or both. Proper timing of application is very important. Specify the time of application in terms of crop planting date, emergence date, growth stage, preharvest interval, and intervals between applications. Specify the levels of the target nematode population and incidence. Record the plant size, stage of growth, or number of days since emergence for postplant applications or a combination of these.

Method of Application

Specify the method of application including specialized equipment. Specify the appropriate terms like spraying, injecting, soaking, spacing, rinsing, and flooding. Specify details in case of soil application like band width, row spacing, chisel spacing, depth of application, and time interval between application and incorporation. If applied on the surface, mention the method and depth of incorporation, if any. Row application details should include furrow row, band over row, or side dressed (preplanting, at planting, postplant, post emergence). For side-dressed applications, mention the placement in relation to seed or plant. Treatment may be broadcast, strip, row, site, root dip, or foliar spray and may be injected into the soil or applied to the soil surface as a drench, spray, granule, or solution in irrigation

water. Incorporate the granules into the top few centimeters of soil, or the active ingredient may be washed from the granules by irrigation or rainfall. Take care to prevent recontamination of treated areas by cultivation of other means, whereby soil from nontreated areas is blended with soil from treated areas. Application methods in irrigation water should specify overhead sprinkler, row or furrow, and basin.

Environmental and Cultural Conditions

Record the information on pretreatment, at-treatment, and posttreatment environmental and cultural factors that might affect the efficacy of the nematicides. Sometimes, inconsistent/erratic results of incomplete experiments may occur due to some effects of these factors as relative humidity, wind, rainfall, and air temperature during the test period. Consider and explain/correlate the relationship among environmental and cultural factors to host, nematode, and nematicides.

Soil factors should include the identity of target and nontarget nematodes and their relative density before, during, and after testing; temperature; soil types including textural variations with depth; pH; field capacity; nutrient levels; organic matter percentage; presence or absence of crop trash/refuse; percentage of soil moisture; an estimate of drainage; amounts and frequency of rainfall or irrigation or both and type of irrigation (flood, sprinkle, row, basin etc.); other information that may affect the application and performance of the nematicide being tested; the occurrence and quantity of other organisms that affect crop growth and nematode populations; the application of fertilizers, lime, or other soil amendments like herbicides, fungicides, bactericides, or insecticides; and the cropping history and pesticide usage.

11.12 Crop Loss Assessment Due to Nematodes

Yield losses in crops due to phytonematodes are influenced by several criteria including the pathogenicity of the species of nematode involved, the nematode population density at planting, and the susceptibility and tolerance of the host and by a range of environmental factors. Because of this, available models only estimate yield losses as proportions of the nematode-free yield. Estimating threshold levels further involves various economic calculations. Consequently, predicting yield losses and calculating economic thresholds for most nematode/crop problems is not yet possible. What is needed is more field-based information on the relationship between nematode population densities and crop performance, and various approaches to obtaining such data are described. Measuring the population density, especially of *Meloidogyne* species, is a major problem (Trudgill 1992).

Major objective of crop damage assessment and prediction is to form a basis for nematode management decisions. A fundamental principle of nematode management is that disruptive management techniques should only be used if and where necessary (Ferris 1981). The decision basis may be economic but constrained by environmental considerations; it requires knowledge of the relationship between the organisms present and the expected crop loss. Environmental monitoring may be involved to determine the occurrence of conditions dictating the emergence or arrival of nematodes in the field, rather than by direct analysis and quantification of population levels. Management decisions based on knowledge of the relationships involved may be made at the plant, field, farm, crop, regional, national, international, or global levels. There should be some measure of the confidence which can be placed in the predictive capabilities of models used at each level of management decision. Users of crop loss models include growers for crop management decisions, nematode management advisors for developing and justifying appropriate management systems, and extension and advisory services. Another objective for crop damage assessments is to allow quantification of damage caused by phytonematodes at various levels of structural organization, so that research objectives and priorities can be planned, funded, and administered. The information is also used by horticultural economists and resource planners for nematicide development and benefit

assessment and by national and international policy-makers in determining the allocation and availability of food and fiber throughout the world.

Reliable sampling of nematode communities in crop loss assessment provides basic characterization of the population dynamics and damage potential of given species in a particular host–cultivar environment (Barker et al. 1985). Loss assessments for annual plants may be based on samples taken at time of planting, mid-season (where nematicide or resistant-cultivar treatments are used), and at harvest. Population levels at, or shortly after, harvest are most useful in projecting nematode hazards for a subsequent crop, especially where survival rates are known. Root symptoms, root-gall, or root-necrosis indices, taken at mid-season or at harvest, also can be correlated with yield losses caused by *Meloidogyne* spp. and associated fungi. A composite sample of 20–50 soil cores (2.5 cm diameter × 20 cm deep) collected in a stratified or systematic pattern over a 1- to 2-ha area provides nematode population estimates within about 30–50 % of the mean. The major problems encountered in relating these estimates to potential hazards and eventual crop losses are related to the striking variation in crop–nematode responses as influenced by environment, cultivar, and general crop management practices. Relative precision in crop loss assessment should be improved as more research on crop–nematode responses and sampling methodology is completed.

Disease assessment is the process of determining disease intensity in a population of plants using an accepted method. Disease incidence is the proportion of plants infected in a population, commonly expressed as a percentage, while disease severity is the proportion of plant tissue infected. The FAO has used disease intensity to mean either disease incidence or disease severity (Chiarappa 1981). Nematode-induced crop losses are a feature of both primitive and modern agricultural systems involved in food and fiber production. Because crop losses continue to be encountered while agriculture generally has become more input and energy intensive, much research has been directed toward identifying the causes of loss, quantifying losses, and forecasting losses for disease management.

11.12.1 Strategies for Loss Assessment

Using various approaches, crop loss data may be obtained dependent on the resolution of the data required for decision making and the definition of loss used. Crop loss actually refers to the difference between attainable yield and actual yield, according to the FAO (Chiarappa 1981). Loss is the reduction in quantity and/or quality of the economic yield of a crop. Some workers suggest that it is necessary to obtain loss data in quantities like kg/ha, while others feel that knowing the magnitude of loss (e.g., 1 = none, 5 = very severe) may be acceptable for setting policy on crop protection.

The major approaches employed to collect regional crop loss data are mentioned below (Teng 1981).

1. *Expert Testimony*: In this approach, knowledgeable scientists are asked to make a "statement of authority" on the extent of loss based on their experience with the crop and diseases in an area.
2. *Enquiries*: Estimates are solicited from a broad range of people concerned with the production of a crop in an area and a consensus is developed on the extent of loss. This approach resembles the "delphi" procedure used in research management.
3. *Literature Reviews*: Evaluation of published work not specifically designed for loss assessments for its value in giving estimates, for example, multiplication chemical and cultivar evaluation trials.
4. *Remote Sensing*: Satellite imagery is used in estimating crop area, crop yield, and crop loss. This is best suited for diseases that result in total plant loss, as in nematode caused diseases.
5. *Experiment/Survey Approach*: Yield and crop loss models are developed through field experiments and the models coupled with survey data to provide regional loss estimates. This is supposed to be the most objective method of deriving crop loss data for decision making at all levels (James and Teng 1979).

There is no single perfect approach for collecting regional crop loss data that has been generally accepted. While other approaches with the exception of remote sensing are mostly indirect that can be used to improve the reliability of direct approaches like experiment and remote sensing, the experiment/survey approach is the most direct and empirical approach, which requires several strategies, viz., quantification of nematode/disease, collection of data to measure the disease–loss relationship, modeling of the disease–loss relationship, and development and use of regional crop loss databases.

11.12.2 Quantification of Nematode/Disease

Nematodes have generally been quantified in terms of their numbers per unit of soil or plant part. Number of nematodes may be related to the intensity of plant symptoms, which in turn is a visual indication of the stress imposed on the plant, resulting in measurable loss. With nematodes, preplant nematode density is the most common descriptor used in quantitative relationships of loss. Field disease assessment is done normally, by using disease keys, standard area diagrams, remote sensing, and population counts.

Disease keys and standard area diagrams rely on the determination of severity in comparison with a predefined key or series of diagrams depicting different degrees of severity. The severity assesses for a plant part like a leaf includes the infected area as well as any accompanying chlorosis or necrosis. Remote sensing has been successfully used for assessment of losses due to nematode pests that cause total plant loss but has been only marginally successful with pests that affect only plant parts (James and Teng 1979). Population counts are widely used in quantifying diseases caused by nematodes. A problem in loss assessment is the determination of a representative mean value of the nematode/disease in a cropping unit using the designated method of assessment. Sampling for diseases populations is a relatively under-researched area in comparison with insect sampling. The distribution of a nematode/disease in any spatial unit may be mathematically described as a frequency distribution with estimated parameters, for example, normal or negative binomial.

Preliminary indications of the type of distribution are obtained by examining the mean: variance ration of the sample mean of disease intensity. Knowledge of the type of distribution in a field enables sampling protocol to be designed to obtain a representative mean in an economic manner (Lin et al. 1979). Nematode populations commonly occur as clusters, suggesting that the pattern of taking samples from a field is important (Barker and Olthof 1976). Various microprocessor techniques have been designed for use in fields. Portable, low-cost data acquisition system for measuring canopy reflectances, which may be used for determining the mean effect of a pest on a crop in terms of reduced crop vigor; laboratory-based video image analysis unit to measure the area of infected leaf tissue and proportion of infection; testing the taping of images of diseased leaves in the field with portable video cameras and analyzing the images in the laboratory; image analysis is routinely used for measuring the root area of plants and it is conceivable that there will be developments allowing its use for measuring nematode number in a sample (Lindow and Webb 1983).

11.12.3 Collection of Data to Measure the Disease–Loss Relationship

It represents the experimental phase of a disease–loss program, in which field data are collected either from fields with natural epidemics or from experimental treatment plots with different disease intensities. Several major methods have been briefed hereunder.

11.12.3.1 The Single Tiller/Plant Method

This method is used for collecting data to model the disease–loss relationship in fields in any cropping area, with varying disease in one season, and it includes the steps like tagging of hundreds

of tillers/shoots with care being taken to select tillers reflecting a wide range of severities, including zero and maximum disease; visit the fields using a predefined survey procedure, and in each field, disease intensity is assessed and tillers are harvested; each tiller becomes a single datum point for regression analysis; this method is a derivation of the paired-plant method in which pairs of healthy and diseased plants are tagged and observation is made on them through the growing season; natural epidemics are used in this method and there is economy of labor, space, and time (James and Teng 1979); it has mathematical limitations and models developed have only been able to explain a small proportion of the variation in yield due to disease; interplant differences in yield are a major source of variation in single tiller/plant studies. It may be improved by using measurements of plant parts not affected by disease, but related to potential yield, to correct for differences in observed yield and are able to reduce some of the variation.

11.12.3.2 The Synoptic Method

Initial procedures are to determine which of the parameters measured are appropriate in the yield-loss analysis. An advantage of the approach is that it requires and promotes consideration of all the factors likely to influence crop yield or crop loss (Ferris 1981). Predictive regression models are based on those variables deemed relevant by the initial analyses. As mentioned earlier, a real danger in the study of crop damage from a disciplinary standpoint is that factors outside of the discipline are not considered. This results in inflated estimates of crop losses by additive consideration of losses from individual pests. Consequently, interaction and discounting effects of damage overlap are ignored.

The synoptic approach represents a classical mutivanate statistical version of the systems analysis approach to an agroecosystem. All significant components of the system are considered. The complexity of the system is reduced through principal components analysis and determination of those parameters which provide the greatest explanation of the observations. In a systems analysis approach, the parameters and state variables considered important in determination of the output variables to be monitored would be selected intuitively. Generally, they would be in greater number and in greater detail than necessary and would be reduced by trial simulations of the system. Sensitivity analysis determines which state and input levels affect the system when they change. The multivariate statistical approach (Stynes et al. 1979) would be a rational and logical way of choosing the components for an agroecosystem model for a systems analysis and simulation approach to the assessment of crop damage.

11.12.3.3 Field Plot Techniques

Plots arranged using an experimental design like a randomized complete block are common in crop loss studies. The plots are either paired treatment or multiple treatment, where treatments are desired levels of disease or pathogen population. In crop loss assessment, the aim of treatments is to ensure that epidemics with different characteristics are generated using methods that may not necessarily be economic (James 1974). In the paired plot approach, healthy (protected) and infected (unprotected or inoculated) plots are situated near each other to constitute a replicate and the pairs repeated over many locations. With multiple treatment experiments, treatment extremes range from healthy (no disease) to maximum disease, with intervening levels of disease as the other treatments. Levels of disease have been generated on cultivars with different susceptibilities but comparable potential yield, by variation in planting date, by use of chemicals, and by use of differential inoculation.

Nematode population can be varied by growing hosts or nonhosts prior to experimentation, by inoculation, or by mixing infested with non-infested soil (Seinhorst 1981). Plot size is a major consideration in this technique. Use of micro-plots is a common practice by many nematologists. In practice, there is a trade-off between reducing inherent yield variation by increased plot size and increasing the variation due to soil factors when plot size is increased. In general, small plot size, as opposed to large plot size, results in higher variation between plots and

requires a larger number of replicates for the same difference between two treatments to be detected. The relationship between yield loss and disease at different growth stages has been conceptualized as a three-dimension response surface, where at each growth stage, disease–loss may be represented by curves. Experiments to model nematode population–loss relationships commonly focus on the initial nematode population. The precision of nematode models could be greatly improved if data is also collected on population levels during the cropping season.

11.12.4 Mathematical Models for Crop Damage Assessment

Models predicting final nematode densities from initial preplant nematode densities are generally based on modifications of the Verhulst logistic model (Seinhorst 1970). Models proposed for describing the relationship between numbers of nematodes and plant growth have been developed by Seinhorst (1965). They are elegant in concept, recognizing the decreasing influence per nematode as population densities increase. In general, the models pertain to annual crops and predict proportional yield loss in relation to preplant nematode populations. All these models are well described (Ferris 1981). One rationale for developing mathematical models is the simplification of the real world. Attention is focused on the components of the system that are critical as predictors of its output and, further, on the environmental parameters which affect the interactions among the components. Therefore, the model acts as a conceptual framework within which literature can be searched and current information assessed. It provides a summary of the state of knowledge of the system, and it allows the organization of available data and the determination of research needs by focusing on information gaps. A series of steps are prescribed in the development of the mathematical model. The regimentation involved in the procedure forces simplification and understanding of the system.

Step 1: The limits of the universe to be considered in the model are defined. It may be a horticultural field and the nematode communities limiting the production. Such a model would be extremely complex and involve a series of interacting subsystems. A simpler model would be a single plant and the phytonematode community parasitizing its root system. A danger of this simplification is that the resultant model may be misrepresentative in isolating one set of stresses on the growth of the plant and considering them apart from other interacting stresses in determining crop loss. Another simpler and less realistic situation is to consider the effect of parasitism of one nematode species on plant growth.

Step 2: The components of the system are defined. In the simplest case described, the components might consist of the nematode population system, the photosynthetic system of the plant, and the harvestable yield of the crop; even simpler components might be the numbers of nematodes and the amount of harvestable yield.

Step 3: The relationship between these components should be quantified. With nematodes on annual crops it is well documented that predictable relationships can be developed between harvestable yield and preplant nematode densities.

Step 4: The effect of environmental parameters on the defined relationship should be determined. This allows use of the model under a range of environmental conditions.

Step 5: Validation of the model is necessary before any quantification of crop loss due to nematodes is possible. One result of validation might be a realization that the original definition of the system was too narrow and that interaction with other organisms and other environmental conditions should be considered.

11.12.5 Types of Models

The empirical disease–loss models available in literature may be classified into various types, viz., single-point models, multiple-point models, integral models, response-surface models, nonlinear models, synoptic models, critical-point models, and simulation models.

11.12.5.1 Single-Point Models

These models relate to disease intensity at a specific time in the life of a crop, either a critical growth stage or a predetermined number of days into the growing season. Several models have been explained in case of fungal diseases and not much for nematode diseases, although similar concept holds good for nematode also. For example, in wheat stem rust, loss may be estimated from the percent stem rust severity at the ¾-berry stage (X) using this model (Romig and Calpouroz 1970).

$$\%\text{Loss} = -25.53 + 27.17 \ln X$$

In this example, the ¾-berry stage is identified as a time when the crop is most sensitive to rust. Fitting a single-point model to a data set does not imply that no other growth stages respond to diseases but rather that a particular stage only shows good statistical correlation. It is necessary to incorporate some physiological knowledge into regression models to ensure that the models are biologically meaningful. Another form of single-point model is that for estimating losses in potato due to late blight from the number of blight-free days (X), using the model as proposed by Teng and Gaunt (1985).

$$\text{Yield, t / ha} = 234.0 - 1.706\,X$$

Single-point models are the most commonly used disease–loss models mainly because they require relatively less data to develop. However, their application appears restricted to short-duration, late epidemics with stable infection rates. This model type assumes that disease dynamics before and after the single-point in fields resembles that encountered in the original experiments.

11.12.5.2 Multiple-Point Models

These models relate yield loss to several disease assessments during the life of a crop. The disease descriptors used in models are either disease increments during a defined period or disease intensities at identified growth stages. For example, use of model for estimating wheat yield loss due to leaf rust from three growth stages, X_2 (% rust/tiller at boot stage), X_5(% rust on flag leaf at early berry stage) and X_7 (% rust on flag leaf at early dough stage), using the model (Burleigh et al. 1972).

$$\%\text{Loss} = 5.3788 + 5.5260X_2 - 0.3308X_5 + 0.5019X_7$$

This type involves repeated measurement of the disease progress to predict losses. Such models have not been explored thoroughly in nematology (Ferris 1981). They may have potential for use with perennial crops but are unlikely to be useful with annual crops. In perennial crops, yield during a current year is frequently influenced by the amount of stored products accumulated during a previous year, which would be affected by previous levels of nematode stress. Consideration of nematode populations at a point in time in successive years might allow prediction of yield losses. The cost involved in sampling to determine population development at various points during the growing season would probably become limiting in the use of these models in annual crops. Further, the lack of available management techniques during the growing season would negate their usefulness. The workers who developed this model observed that though they could determine several single-point models from the same data, the multiple-point model explained the most variation in yield loss due to rust. Multiple-point models are particularly suited for epidemics that are long in duration, have unstable infection rates, and affect more than one yield component.

11.12.5.3 Integral Models

These models relate loss to a disease descriptor derived from summing disease intensities over a specified period of crop growth (Vander Plank 1963). Vander Plank proposed the area under the disease progress curve (AUDPC) as a method for analysis of wheat stem rust data. The model for estimating loss in cowpea due to leaf spot caused by *Cercospora* is an example (Schneider et al. 1976).

$$\%\text{Loss} = 0.43\,\text{AUDPC} - 14.95$$

AUDPC, in general, cannot distinguish between late or early epidemics since two progress curves with very different onset times and

infection rates could give the same area under the curve. AUDPC models can be applied for short-duration, late epidemics. The predictive ability of AUDPC models can be improved by assigning weighting factors to the disease assessments made at different growth stages which are used to calculate the AUDPC (Hills et al. 1980).

11.12.5.4 Critical-Point Models

The classical models of Seinhorst relating expected plant growth to preplant nematode population levels fall into this category. These involve measurement of a nematode population at one point in time, usually before planting, and they predict losses based on knowledge of some damage function. Critical-point models are frequently used in plant pathology. They allow prediction with a minimum of measurement and monitoring. The development of critical-point models has certain advantages for nematodes over other pest groups. Nematodes are relatively slow-reproducing organisms compared to fungi or bacteria. They do not have winged stages which may result in crop invasion at unpredictable times. For annual crops, the critical population density is that which is present at the time of planting. This allows the development of predictive relationships between preplant population densities and crop growth (Ferris 1981). The existence of such relationships is fortunate, since most management alternatives, including varietal selection and soil fumigation, are preplant decisions. Since the nematode population is present at the time of planting, it is not necessary to consider the phenological state of the crop at the time of pest invasion, as might be the case with a foliar pathogen or an insect pest.

The basic damage function model of Seinhorst (1965) [$y=m+(1-m)zW$-]I has a strong foundation in biological theory. Critical-point relationships have been used in determination of economic threshold levels of nematode populations. The nature of both the empirical and theoretical critical-point models is governed by three parameters: the tolerance level, the slope of the lines, and the minimum yield. These parameters are influenced by environmental and physiographic conditions. There is a need for researching the relationship between environmental conditions and the magnitude of the determinant parameters to allow interpolation and generalization of the critical-point models to a range of conditions. Critical-point models are usually developed for single nematode species, often in microplots. Another approach to generalizing critical-point models for multispecific communities is to make use of available information on nematode feeding, habits, biology, pathogenicity, and ecology. A "critical species" model is developed for crop damage relative to a nematode parasite of significant importance, and the pathogenicity of other nematode species is weighted relative to the critical species.

11.12.5.5 Simulation Models

Simulation models mimic the biology of the interaction of nematode, plant, and environment at their prevailing levels at any point in time. Rather than predicting crop damage based on a population density at a single point in time or at a series of points in time, such models are real-time explanatory models that are descriptive of both pest and plant biology. They deal with the actual conditions influencing the interaction rather than assuming average seasonal conditions affecting the relationship. Simulation models, when initially implemented, require frequent biological monitoring for confirmation. As confidence is gained in the model, monitoring can become less frequent (Ferris 1981). Simulation models deal with relative rates of growth of the nematode pest and plant populations. In poikilothermic systems, the metabolic rate is proportional to the temperature to which the system is exposed. Between definable upper and lower limits, the cumulative development is proportional to the cumulative heat to which the system is exposed. In damage assessment modeling, it is useful to consider the plant as a supply/demand system. The energy supply of the plant is a function of the rate of photosynthesis and the amount of photosynthetic surface at any point in time. The energy fixed is transformed into various plant parts according to a genetically determined sequence of growth priorities. More data are necessary to construct and use simulation models than are needed for critical- and multiple-point models. Plant and pest biology must be known to develop the

explanatory framework of the system. Much required information on growth and development of the organism and plant relative to environmental conditions can be extracted from the literature once this framework is established.

A major problem in real-time simulation with available weather data is that ambient conditions of temperature and moisture may not be the microclimate perceived by the plant or the pest. The microenvironment for a pest shaded under a plant leaf is very different from ambient conditions (Ferris 1981). Similarly, conditions for a nematode 6 in. below the soil surface differ from ambient atmospheric conditions. Soil texture, irrigation status, phenological growth stage, and plant shading effects are all important. There is a real need for on-site measurements of microclimate and for models which predict microclimate from ambient conditions relative to growth status of the plant and physical environmental parameters. A critical portion of simulation models is the coupling between subsystems to allow determination of crop damage relative to varying pest densities attacking the plant over time and to varying rates of plant growth. Both plant and pest subsystems are dynamic, and the state and nature of the interaction varies constantly. The modeling rationale is that the rate of photosynthesis is proportional to the efficiency of the root system, which is related to the proportion of the root system not damaged by vascular disruption. Simulation models are useful for research and analysis of crop damage through the partitioning of energy flow. They are also useful for prediction in the pest management decision process. A simulation model may be the most logical way of abstracting and conceptualizing the system in an analysis of the crop damage due to nematodes and other biological and environmental stresses in perennial crops. The numbers of computations involved in real-time simulation models require the use of a computer. The advent of high-speed, low-cost computers has made the use of simulation models a more practical reality. The flexibility of computer and simulation languages allows the modeler certain advantages not available in classical, analytical approaches, such as critical-point models.

11.12.5.6 Other Models

Teng and Gaunt (1981) conceptualized the relationship among disease, stage of the crop growth, and loss as a three-dimension response surface, which has been generalized as % Loss = f (disease, crop stage), thereby enabling loss estimation if the disease intensity and growth stage are known. This response-surface model may also be considered as integrated series of single-point models and various models have been developed fitting this concept. Response models require substantially more data to develop than the other models discussed previously and have led to research on alternative ways of experimentation to collect data, as explained in the previous section. Most disease–loss models assume a linear relationship, while it is generally recognized that biological relationships may be nonlinear (Madden et al. 1981). More than one model can commonly be found to fit a set of experimental data on any disease–loss system.

Although it is generally advisable to collect more data than is required for modeling because of the lack of prior knowledge on the form of the model, with some diseases and crops, however, enough is known of yield physiology to enable postulation of potential relationships (Teng and Gaunt 1981). This approach helps in guiding the design of experiments and pinpoint growth stages where it may be useful to have more treatments. The intended use of a model is another consideration in determining the form of the model, whether it is single point or multiple point. Several single-point models may be required in surveys, where fields may be visited only once. A multiple-point or integral model may be required to forecast potential yield loss that can account for fluctuating rates of disease progress in response to factors like nematicide application.

11.12.6 Modeling of the Disease–Loss Relationship

A mathematical model is a concise way of representing any system. In crop loss experiments, the usefulness of the data generated would be limited if the data is not reduced into a simple form.

Because of the many forms of the disease–loss relationship, there is no universal mathematical model to fit all these forms. The forms of relationships range from linear to sigmoid, and there are nine possible shapes of the disease–loss curve, according to Teng and Gaunt (1985). The mathematical description of the relationship depends on the disease descriptor (independent variable) used, like disease severity at one growth stage or area under the disease progress curve. In case of plant-parasitic nematodes, the log of nematode density is commonly used as the independent variable (Barker and Olthof 1976). The majority of mathematical models describing the disease–loss relationship have been derived using least squared regression techniques, although, recently, simulation modeling has been also tried (Ferris 1978). With regression models, several statistical criteria can be used to evaluate each model: F, r, s, and t (James and Teng 1979). Assumptions in the data collected for modeling need to be recognized and tested. For example, regression assumes that the variables show a normal distribution, yet this assumption is often violated in taking samples of disease data.

Among specific needs are the development of crop loss assessment methodology, the development of models and survey systems for collating the crop loss information and measurements, and estimates of confidence intervals and reliability which can be placed on the data developed.

11.12.7 Model Validation and Prediction

A problem with validating any model used to predict plant growth relative to a nematode population is that crop yield in a field without nematode stress will vary, even in the same region, due to microclimate differences and grower expertise (Ferris 1981). Seinhorst (1965) approached this problem using a relative yield measurement with yield loss represented as proportional decrease from the maximum yield for that field. A further generalization is the concept of minimum yield below which no further reduction was seen even at high nematode densities. Measurements of actual crop yield relative to nematode communities assessed prior to planting and weighted relative to edaphic and physiographic conditions can be used for validations of the conceptual models. This validation may result in a perception that the simplistic approaches suggested are inadequate and reveal a need for further consideration of the interaction among the determinant factors. A convenient tool for handling the problem of multiple-species nematode communities, differential host ranges, and varying environmental and edaphic requirements is to use an interactive scenario on a minicomputer. Information would be input for the actual conditions of a field for which the prediction is being made. Hence, the damage prediction is customized according to the nematode community, environmental conditions, crop type, and grower expertise. Management decisions are based upon the best current information of nematode biology, ecology, and expected damage (Ferris 1980).

Howard Ferris (1981) opined that the implementation of quantitative approaches involves real problems. The cost-effectiveness and reliability of nematode population assessment are poor. Since any use of predictive models in a management mode is based on population assessment, reliability becomes critical. There is a tremendous need for development of basic damage functions for key nematode species and crops as a basis for predicting yield losses from preplant densities. Simulation models require systems approaches to nematode biological problems and the availability of suitable plant models with which pest models can be interfaced.

Parts of farmers' fields are intensively sampled throughout the season (Stynes 1975). Variables measured include disease, nematodes, insects, soil, and water properties. Models are developed which explains a significant proportion of the yield variation caused by several factors. This method allows crop loss profiles to be developed, showing the contribution of each constraint in reducing attainable yield to actual yield. A limitation both for this and the single tiller method is that the range of disease severities for each disease may not be wide enough in any season, leading to an underestimation of its importance as a yield constraint.

11.12.8 Parameter Measurement and Estimation

Predictions of yield loss based on the assessment of nematode populations are limited by the reliability of the soil samples representing the field population. Problems in reliably estimating nematode population densities are well documented. They relate to variation in the macro- and micro-distribution of nematodes as a function of their edaphic and environmental requirements and of their biological requirements in terms of feeding habits and reproduction pattern. Phytonematodes are primarily distributed according to the root system of their host, but their distribution and density varies with soil texture, previous cropping history, soil moisture-holding capacity, drainage and cultural patterns, and plant spacing (Ferris 1981). The vertical distribution of nematodes varies with food availability, root distribution pattern, and cultural and tillage procedures. Nematodes aggregate around specific feeding sites, such as root tips, and around areas of egg deposition, particularly in those species which are sedentary and deposit eggs in masses. The most reliable method of determining potential nematode stress on a crop would be to extract and count the nematodes from all of the soil in a field. A more practical reduction is to represent the field by a series of soil samples.

It is often desirable to assess or predict crop losses for areas larger than a single field. In such cases it is necessary to have data on nematode densities and distribution on a regional basis. In areas where nematode advisory programs have been conducted by public institutions for many years, a wealth of quantitative information is available on nematodes. Such information includes sampling sites, cropping histories, soil texture, and nematode population densities. If yield-loss prediction models were available, such data would allow assessment of losses, either predicted or historical, on a regional basis, according to the actual distribution of the nematode parasites of each crop. It is a practical impossibility to measure the response of each variety of a crop to each of its nematode parasites under every set of environmental conditions. Levels of horizontal resistance and tolerance will, however, dictate the expected quantitative response of individual varieties to the same nematode density. An approach to the problem is to determine the damage function relative to a range of nematode population densities for one common variety of the crop and to rank all other useful varieties in terms of their relative intolerance by greenhouse and field measurements of growth under nematode stress (Ferris 1980). The intolerance can be expressed on a zero to one basis relative to the least tolerant variety and used in weighting the damage function for specific cultivars.

It may be possible to evaluate the environmental suitability of the physiographic region and edaphic conditions based on literature currently available on nematode biology and ecology. The use of textural preference indices (Ferris and Duncan 1980) involves transforming the effect of the soil texture on nematode multiplication to a zero to one scale, in which one represents the maximum multiplication detected in a set of experimental data. Soil texture is arbitrarily quantified and a tripartite model fitted to the data with an optimal range of soil texture and a region of declining favorability on either side. For any nematode species, any portion of this tripartite model may be inoperative. Nematode counts from soil samples can be weighted for use in predictive models. Similar information can be gathered on temperature requirements of nematodes and used to express a regional preference or physiographic preference index for each nematode species. Depending on the location of the nematode community and the crop to be grown, it may be possible to weight the populations in that community according to their physiographic and textural preference indices, pathogenicity relative to a major pathogen of the region, and the relative intolerance of the host variety to be grown. This allows development of a weighted nematode stress total which is on the same relative basis as the population series for which the nematode damage function was determined (Ferris 1980). It allows prediction of potential yield losses for the proposed crop which can be used for management purposes and which are customized to the physiographic region, nature of the nematode community, the edaphic conditions, and crop variety.

11.12.9 Development and Use of Regional Crop Loss Databases

Regional crop loss databases have been developed for many countries through the use of sample surveys and the indirect methods previously dealt. This endeavor represents the next phase in most crop loss programs (James 1974). A regional database is considered to be a set of crop loss data collected over several definable spatial units like counties. Although the method of procuring the crop loss data may be different, it is common that such databases provide information on the prevalence/distribution, incidence and severity of diseases, and/or the related losses in percentage or tonnage (Teng and Gaunt 1981). Sound statistical sampling methods are to be used to determine field averages and to select representative fields. Field selection that represents a region has been based on simple random sampling, stratified sampling, or multistage sampling.

Stratification based on county or similar lines, crop reporting districts, or state boundaries appears to be most common in the literature, although stratification according to soil types, agroecological zones, or production systems has more interpretative value to scientists. The literature on ad hoc disease surveys is plentiful, and although not allowing historical trend analysis, these surveys have been useful in identifying diseases for further research and extension. With development in computer technology, isoloss lines have been plotted for Western Europe showing the mean annual loss in cereals due to each key pest. A combination of expert opinion, mail surveys, and literature reviews has been used to provide the data. Risk maps have also been plotted to show parts of the region where a certain disease has historically caused significant loss. A national pest survey database has also been developed in several countries for use in expert certification. Such and the Cooperative National Plant Pest Survey and Detection programs rely on computers and telecommunications for exchange of weekly pest data among states and central computer and will be capable of providing real-time pest and crop data during the growing season.

Individual state systems for computerized, online pest management are also in place. In Europe, the Dutch "EPIPRE" system of national pest management has been extended to other countries (Zadoks 1983). This system projects increases in pest populations and the potential economic loss, thereby providing a basis for control. In several countries like the USA, research has been directed at linking regional crop loss databases to decision making at the farm level. The "RUSTMAN" model for timing fungicide sprays on sweet corn makes recommendations based on rust severity in the field, estimated field loss, and rust severity in surrounding fields (Teng and Montgomery 1982). With increasing miniaturization of electronic equipment, matched by increasing computer power, it has been possible to deploy highly sophisticated models of nematode-loss systems in farmers' fields for nematode monitoring and management.

11.12.10 Nematode Damage Models

The nematode, the host, and the environment are the three interacting variables influencing the extent of yield loss in infested soils. An understanding of the mechanisms and principles involved in these interacting relationships is basic to being able to predict yield reductions from estimates of preplanting nematode population densities (Trudgill 1992). When modeling the damage caused to plants by root-feeding nematodes, several basic principles are to be considered like damage is proportional to the nematode population density; the degree of damage is influenced by environmental factors; and the yield harvested is determined by the amount of light intercepted by the crop, by how efficiently the intercepted light is converted into dry matter, and finally by how that dry matter is partitioned into non-harvested and harvested yield. For some crops significant variations in moisture content will also affect final yield; the above principles are more complex in practice.

Damage may be proportional to the nematode population density, but there are several qualifications of this statement, viz., the relationship is usually curvilinear, increasing numbers of nematodes having proportionally diminishing effects; there is some evidence that at low densities the host plant can repair the damage and that growth may even be slightly stimulated; Seinhorst (1965) termed the population density (Pi) at which damage first became apparent as the tolerance limit (*T*); equally, at very high values of Pi, increasing numbers of nematodes may not further reduce dry matter productivity. This minimum yield is termed "*m*." There are various reasons for the occurrence of "*m*"; there may be some growth before attack starts or after it finishes, and a significant biomass may be planted (e.g., potato tubers). However, "*m*" applies to total dry matter and because of effects on partitioning, the harvest value of *m* may be greater or less than that for total dry matter; the third parameter in the Seinhorst equation is *z*, a constant slightly less than one. The equation is as follows:

$$Y = m + (1 - m)z^{(\mathrm{Pi}-T)}$$

For $\mathrm{Pi} > T$

$y = 1$ where Pi is less than / equal to T

where y is the yield.

An important qualification is that *y* is expressed as a proportion of the nematode-free yield. Hence, according to Seinhorst, the greater the yield potential, the greater the loss in tons per hectare for any value of Pi. The Seinhorst equation is usually plotted with Pi on a logarithmic scale, producing a sigmoidal curve. In practice *T* is usually small and the Pi value at which *m* is reached is so large that it is only the central part of the curve that is of practical use. This approximated to a straight line (Oostenbrink 1966). The equation for such a line is $y = y\,(\max) - \text{slope constant} \times \log \mathrm{Pi}$.

The slope of the regression varies for several reasons. These include differences in pathogenicity (capacity to cause damage) between species, e.g., *Meloidogyne* spp. may be inherently more damaging than *Tylenchus* but there is no measure of their relative pathogenicities. Different plant species and varieties within species differ in their tolerance (capacity to withstand nematode damage). Also, there are large environmental influences on the damage suffered and particularly how that damage is translated into effects on final yield. An important consideration, often overlooked, is the basis of measuring Pi. Usually it is given as numbers per gram of soil. A more appropriate measure is per unit volume of soil as this allows for bulk density differences. Numbers per gram of root is probably the most appropriate, but is difficult to measure because it is always changing. This latter aspect becomes important when trying to relate results from experiments where root densities are very different, e.g., pot and field trials.

A further problem is encountered when considering damage by nematodes that have two or more generations in the lifetime of a crop. Usually the Pi is measured at planting, but on a good host population of, for example, *Meloidogyne* spp., they can increase from below the value of *T* to a level in mid-season where they cause significant damage. Even so, it is a race between increasing Pi and increasing plant size that brings with it increasing tolerance (in Seinhorst terms, increasing *m*). In such situations suitability as a host (susceptibility) and tolerance can have a marked effect on the degree of damage. To conclude, both the Seinhorst and Oostenbrink equations are, without the addition of a substantial amount of additional information, purely descriptive and cannot be used to predict actual yield losses.

11.12.11 Approaches of Estimating Yield Losses

Pot studies can be used to determine some of the basic information on yield-loss relationships, but because of environmental differences and interactions, field studies are also needed. There are two major approaches, viz., to use nematicides at relatively uniformly infested sites and to work at sites with a range of population densities but which are uniform in other respects (Trudgill and Phillips

2006). A combination of both approaches is often a happy compromise. The former gives practical information on the effectiveness and potential value of a particular treatment but tells little about the nature of the relationship. It also suffers from the criticism that nematicides have a range of side effects. The latter has the benefit of producing information on the relationship between Pi and yield, but it requires experimental errors to be minimized. Because Pi estimates have large errors, accuracy is improved by reducing plot size and by taking and processing multiple samples from each plot.

However, plot size must be large enough to obtain a realistic yield and adequate guard plants are essential. Another option is to establish many small plots in large but otherwise uniform fields. These can be at random, in a grid pattern or along known trends in Pi. The plots can be split and a nematicide applied to one-half. For each plot the Pi and yield are determined. The results will produce a scatter of points, hopefully with yield decreasing as Pi increases. Much of the scatter is due to errors in estimating Pi and yield, and it can be minimized by taking the average of all the results within each error band. Such an approach requires a wide range of initial populations, a uniform field, a large number of plots (100 or more), and the plots to be part of an otherwise uniform crop.

Chemical management of *Tylenchulus semipenetrans* consistently increased yield of grapefruit on sour orange rootstock in Texas (Timmer and Davis 1982). In this study, data from chemical control tests conducted from 1973 to 1980 were analyzed to determine the relationship between nematode counts and grapefruit yield and fruit size. The correlation between yield and nematode counts was negative ($r = -0.47$) and highly significant ($P < 0.01$). The data best fit the exponential decay curve: $y = 160.3x\,(-0.0000429)$ where y = yield in kg/tree and x = nematodes/100 cm of soil. The correlation between fruit size and nematode counts was not significant because yield and fruit size were inversely related. Yield loss in an average untreated orchard was estimated to be 12.4 tons/ha. Economic loss to citrus nematode in Texas grapefruit, assuming no treatment and an average on-tree price of S60/ton, was estimated to be S13.2 million annually.

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12 Nematodes of Quarantine Importance

Phytonematodes have ever been an unseen and often underestimated menace in horticultural crops across the globe. Besides causing damage due to their own feeding, nematodes also incite or aggravate fungal and bacterial diseases and serve as vectors of many plant viruses. The maxim "prevention is better than cure" is particularly true with nematodes. Once established in a given area, the nematodes are almost impossible to eliminate. Substantial crop losses from phytonematodes could be much greater if species currently causing localized damage became more widespread.

"Quarantine" refers to regulatory actions aimed at preventing or retarding the introduction, establishment, and spread of dangerous pests in crop protection (Maas 1987). In quarantine and certification programs, intensive sampling may be needed to determine if lots or shipments of plants, pots, cuttings, or other units are free of plant-parasitic nematodes and other plant pests. Because it is seldom feasible, or even possible, to examine and test the entire lots for such harmful pests, these determinations must nearly always be made on the basis of samples drawn from the lots.

A "quarantine nematode" refers to a nematode species that can cause a pest of potential economic importance to the area, which can be one specific country or a whole continent, endangered thereby and not yet present there or present but not widely distributed and being officially controlled, as given by the International Plant Protection Convention (IPPC). Opportunities for the introduction of new species of nematodes have been numerous, but its introduction does not necessarily mean its establishment as a pest. Often a complicated set of conditions and events must be present before an organism can survive in a new environment. Frequent introductions, particularly of large numbers of the pest organism, increase that organism's chance of becoming established in a new area. Once a nematode is introduced and established, it may be years before numbers sufficient to cause severe crop injury are produced. Ordinary plant quarantine regulatory action is not well adapted to the detection of plant-parasitic nematodes.

The potential phytosanitary importance of all named phytonematode species is determined by evaluating available information on species characteristics, association with economically important crop hosts, and ability to act as vectors of viruses or form disease complexes with other pathogens. Most named species of phytonematodes are poorly known, recorded from a single location only, not associated with economically important crops, and not known to be associated with other plant disease organisms.

Phytonematodes during quarantine inspections may also act as a bioindicator for consignments that do not meet the phytosanitary requirements of plants being grown in sterile environments and could be carrying other plant pathogens and microorganisms (Hockland and Anderson 2012). Quarantine measures can also prevent spread of nontarget species which are potentially invasive (Schrader and Unger 2003). Quarantine and other phytosanitary measures are particularly important for PPN because other management methods such

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as chemical control or crop resistance can be far more costly and difficult to implement without other adverse effects (Nicol et al. 2011).

First step in implementing quarantine measures is to determine which species should be regulated under international trade rules. The international plant pest convention defines a quarantine pest as "a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled." Organisms which meet this definition can be regulated. Countries determine their lists of regulated pests according to guidelines set by the International Plant Protection Convention (IPPC) and International Standards for Phytosanitary Measures (ISPM). Countries may also establish lists of regulated non-quarantine pests. The IPPC defines these as follows: "A non-quarantine pest whose presence in plants for planting affects the intended use of those plants with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party." All lists of regulated pests are dynamic, with species added or removed from lists as phytosanitary risks change or as new species emerge as pests.

Many phytonematodes have low impact in their native range, but much greater impact when introduced to new areas, for example, *Bursaphelenchus xylophilus* (pine wood nematode) in Japan, China, Portugal, and most recently in Spain (Robertson et al. 2011) and the potato cyst nematodes in Europe, the USA, Canada, and Australia (Sun et al. 2007). Quarantine measures against known damaging nematodes are effective in preventing their spread, thus effectively and economically preventing crop losses. The soil habitat that elaborates survival mechanisms of nematodes protects them and enables their quick establishment in a new locality. The low environmental resistance in a new locality also allows their quick growth.

Species other than those on official lists of quarantine pests may also pose a biosecurity risk. Only a small proportion of nematode species have been described (Blaxter 2011; Hodda 2011). New species are being described regularly, and species new to science have been intercepted during phytosanitary inspections (e.g., *Radopholus bridgei* and *Meloidogyne thailandica*, both described from material intercepted in quarantine (Handoo et al. 2005)).

Table 12.1 Nematodes of quarantine importance specific to crop and country

Nematode species	Crop	Country
Globodera rostochiensis	Potato	India
Ditylenchus destructor		
Meloidogyne spp.		
Pratylenchus spp.		
Radopholus similis	Banana	India
Helicotylenchus multicinctus		
Rhadinaphelenchus (Bursaphelenchus) cocophilus	Palms	Japan, East Asian Nations
Heterodera schachtii	Sugar beet	European countries
Heterodera goettingiana	Vegetables	Europe
H. humuli	Hops	Europe
H. cacti	Cacti	Europe
Aphelenchoides fragariae	Narcissus	Europe
Pratylenchus vulnus	Temperate fruits	Europe and the USA

Table 12.2 A general list of quarantine nematodes

Nematode species
Meloidogyne chitwoodi, Meloidogyne fallax
Globodera rostochiensis, Globodera pallida, Heterodera glycines
Hirschmanniella spp. (except *H. gracilis* and *H. loofi*), *Nacobbus aberrans, Radopholus similis*
Ditylenchus dipsaci, Ditylenchus destructor
Aphelenchoides besseyi, Bursaphelenchus xylophilus
Longidorus diadecturus, Xiphinema americanum, Xiphinema bricolens, Xiphinema californicum, Xiphinema rivesi
Heterodera schachtii, Cactodera cacti, Radopholus citrophilus
Aphelenchoides fragariae, A. ritzemabosi, Heterodera humuli, Heterodera goettingiana
Rhadinaphelenchus cocophilus, Aphelenchoides arachidis

A list of major phytonematodes of quarantine importance in horticultural crops has been furnished in Tables 12.1 and 12.2.

Yield loss caused by phytonematodes is often used to determine economic importance, but

there are limitations in its calculation. Yield-loss calculations from different studies and countries do not necessarily use the same methods, with some reporting damage as percentage yield loss and others reporting as tons per hectare or as percentage yield gained after the application of nematicides or as correlations of yield gains with declining nematode abundance. Indirect losses also generally remain unaccounted for in most yield-loss figures. Yield-loss estimates based on work done long ago and under different nematode management regimes are also likely to require updating. Impacts can also be difficult to estimate or severely underestimated because nematodes may injure plants in many different ways. One way is by direct feeding action, but this may take many different forms including direct damage, root galls, root stunting, or withdrawal of resources from other parts of the plant (Norton and Niblack 1991). Another aspect to consider when assessing phytosanitary status is intraspecific variation. This is often not considered because most phytosanitary regulation is on the taxonomic level of the species. However, species from the economically important genera *Belonolaimus, Ditylenchus, Globodera, Heterodera, Meloidogyne, Nacobbus, Radopholus, Rotylenchulus*, and *Tylenchulus* all have pathotypes or races with distinctive host responses and differences in host range.

12.1 Biosecurity Implications

Despite these limitations, making the best systematic predictions of impact and risk possible based on as much data as can be obtained is preferable to the alternative of empirical measurements of actual damage following real introductions of potentially damaging species. Where real introductions of exotic nematodes have occurred and been measured, the impacts have mostly been substantial. Of course, by this stage, eradication is seldom an option. More recently nematode species have been used as bioindicators during quarantine inspections (Hodda et al. 2008). In the UK, *Helicotylenchus dihystera* is often intercepted with planting materials supposedly grown in sterile conditions, indicating that phytosanitary standards were not met (Hockland and Anderson 2012). Other microscopic plant pathogens such as fungi, bacteria, and viruses may also use similar pathways to nematodes (Grousset et al. 2012); hence, targeting the generally larger nematodes during quarantine inspections has assisted in reducing the risks from other microscopic quarantine organisms generally. Phytosanitary risks are specific to countries or regions. Regulated organisms differ among countries depending on species distributions and regulatory or biosecurity policy in different countries and formal pest risk analysis processes.

Most quarantine action utilizes visual inspection supplemented by a hand lens or low-power microscope – to detect parasitic organisms themselves or to check symptoms of infection. Symptoms of nematode injury usually are not diagnostic, because they are largely plant reactions such as poor growth, chlorosis, and other symptoms equally indicative of root injury from many other causes. Specific symptoms also may vary with the reaction of different plants. Large obvious lesions are formed on the roots of certain plants attacked by root lesion nematodes, while such lesions are not found on the roots of many other kinds of plants attacked by the same nematode, although serious injury may be caused on both. Nematodes also may enter and be carried in roots of plants which are not preferred hosts. These nematodes later may prove to be of importance on other crops in the area or to subsequent crops on the same land. Nursery stock is a common means of disseminating plant-parasitic nematodes, either as parasites of nursery plants involved or as contaminants in or on these plants. Regulations established by the Department of Horticulture require that locations, where nursery stock is grown or sold, be inspected for phytonematodes.

Fortunately, the active dispersal of nematodes is rather limited, but this is more than compensated by the ease of passive dispersal with movement of infested soil, plant material, and water. Man has aided in the indiscriminate spread of nematode while carrying the plant materials and other items infested with nematodes for research, trade, charity, or other purposes. With

the recent trade liberalization, the bulk movement of commodities has become common, and consequently the risk of entry of pests and diseases has increased manifold. Several devastating phytonematodes have entered into new locations and established successfully on major horticultural crops, leading to losses.

12.2 Nematodes Under Quarantine Act

Nematodes under the following families have been included under quarantine act, viz., Anguinidae, Aphelenchoididae, Atylenchidae, Belonolaimidae, Caloosiidae, Criconematidae, Dolichodoridae, Ecphyadophoridae, Hemicycliophoridae, Heteroderidae, Hoplolaimidae, Longidoridae, Meloidogynidae, Neotylechidae, Parasitaphelenchidae, Paratylenchidae, Pratylenchidae, Psilenchidae, Rotylenchulidae, Sphaeronematidae, Telotylenchidae, Trichodoridae, Tylenchidae, Tylenchulidae, and Tylodoridae. The major phytonematodes include *Ditylenchus angustus, Globodera pallida, Globodera rostochiensis, Heterodera goettingiana, Heterodera schachtii, Heterodera zeae, Meloidogyne chitwoodi, Meloidogyne fallax, Nacobbus aberrans, Radopholus citrophilus, Radopholus similis*, and *Xiphinema index.* However, of all the phytonematodes, in most countries, quarantine measures have been strictly followed in potato cyst nematodes.

12.2.1 Potato Cyst Nematodes

Golden cyst nematode of potato (*Globodera rostochiensis*) and pale cyst nematode (*Globodera pallida*) are the major cyst nematodes infesting potato.

12.2.1.1 Host Range

Potatoes are by far the most important host crop. Tomatoes and aubergines are also attacked. Other *Solanum* spp. and their hybrids can also act as hosts. Both species of *Globodera* have several different pathotypes (Kort 1974). The pathotypes are characterized by their ability to multiply on certain tuberous *Solanum* clones and hybrids used in breeding. Five pathotypes are recognized within *G. rostochiensis* (Ro1-Ro5 international notation) and three in *G. pallida* (Pa1-Pa3) (Kort et al. 1977). Some of these pathotypes are recognized by their almost total inability to multiply on specific cultivars of potato (single-gene resistance); for example, the most commonly grown resistant potato cultivars (based on gene H1 derived from clones of *S. tuberosum* subsp. *andigena*) are resistant to pathotype Ro1 of *G. rostochiensis* only. Other pathotypes show different levels of ability to multiply on different cultivars; the testing of this form of resistance is discussed by Mugniéry et al. (1989). The internationally recognized system of classification of pathotypes refers mainly to the pathotypes present in Europe (and spread from there) and may not have relevance to South America (Kort et al. 1977). It is probable that pathotypes exist there which were never transferred from the Andean region (Canto-Saenz and Mayer de Scurrah 1978).

The golden nematode is one of the world's most damaging potato pests. First detected in the USA in 1941 in Nassau County on Long Island, New York, it was subsequently found in eight other New York counties. For over 60 years, an effective federal and state quarantine program has confined the pest to nine counties in New York. If golden nematode were to become more widely established in US potato, tomato, and eggplant production areas, annual crop losses could reach $4.8 billion. The golden nematode is primarily a pest of potatoes and is also referred to as a species of potato cyst nematode. In the larval stage, it bores into the roots of host plants and feeds on their juices. Because the aboveground damage is not visible during the early stages of infestation, the pest can remain undetected for years. The first sign of a golden nematode infestation is poor plant growth in one or more areas of a potato field. As an infestation builds, the damaged area increases and eventually the entire field displays poor plant growth. Large numbers of the nematodes cause wilting, stunted growth, poor root development, and early plant death. Golden nematodes can also reproduce on the roots of eggplant, tomatoes, and several solanaceous weeds.

The pale cyst nematode, *Globodera pallida*, is a major pest of potato crops in cool–temperate areas. It primarily affects plants within the potato family including tomatoes, eggplants, and some weeds. If left uncontrolled, pale cyst nematodes can cause up to 80 % yield loss in potato fields. The pale cyst nematode is widely distributed in many potato-growing regions throughout the world. In North America, besides the current find in Idaho, the nematode is also known to be present on the island of Newfoundland, Canada. Pale cyst nematode infestations may be associated with patches of poor growth in potato fields. At high nematode populations, affected potato plants may exhibit yellowing, wilting, or death of foliage – none of which has been observed in Idaho potato fields.

12.2.1.2 Geographical Distribution

The center of origin of the two species is in the Andes Mountains in South America from where they were introduced to Europe with potatoes, probably in the mid-nineteenth century. From there, they were spread with seed potatoes to other areas. The present distribution covers temperate zones down to sea level and in the tropics at higher altitudes. In these areas, distribution is linked with that of the potato crop.

12.2.2 Golden Cyst Nematode of Potato (*Globodera rostochiensis*)

EPPO Region: Albania, Algeria, Austria, Belarus, Belgium, Bulgaria, the Czech Republic, Cyprus, Denmark, Egypt, Estonia, the Faroe Islands, Finland, France, Germany, Greece (including Crete), Hungary (one locality only), Iceland, Ireland, Latvia, Lebanon, Libya, Lithuania, Luxembourg, Malta, Morocco, the Netherlands, Norway, Poland, Portugal (including Madeira; unconfirmed in Azores), Spain (including Canary Islands), Russia (Central Russia, Eastern Siberia, Far East, Northern Russia, Southern Russia, Western Siberia), Slovakia, Sweden, Switzerland, Tunisia, the UK (England, Channel Islands), Ukraine, and Yugoslavia (unconfirmed). Found in Israel on only two occasions in 1954 and 1965 in a small area in the Sharon region and was successfully eradicated.

Asia: Cyprus, India (Kerala, Tamil Nadu), Japan (Hokkaido), Lebanon, Pakistan, the Philippines, Sri Lanka, Tajikistan, and Russia (Eastern Siberia, Far East, Western Siberia)

Africa: Algeria, Egypt, Libya, Morocco (intercepted only), Sierra Leone, South Africa, and Tunisia

North America: Canada (Newfoundland, British Columbia Vancouver Island only), Mexico, and the USA (New York; eradicated in Delaware)

Central America and Caribbean: Costa Rica and Panama

South America: Throughout the high Andean region: Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Peru, and Venezuela. More southerly in range than *G. pallida*

Oceania: Australia (two outbreaks, one in Western Australia in 1986, the other in Victoria in 1991; both are subject to official eradication programs), New Zealand, and Norfolk Island

EU: Present

12.2.3 Pale/White Cyst Nematode of Potato (*Globodera pallida*)

EPPO region: Algeria, Austria, Belgium, Cyprus, the Faroe Islands, France, Germany, Greece (Crete only), Iceland, Ireland, Italy, Luxembourg, Malta, the Netherlands, Norway, Poland, Portugal (mainland), Russia (unconfirmed in European Russia), Slovakia, Spain (including Canary Islands), Sweden, Switzerland, Tunisia, the UK (England, Scotland, Channel Islands), and Yugoslavia

Asia: Cyprus, India (Himachal Pradesh, Kerala, Tamil Nadu), and Pakistan

Africa: Algeria, Tunisia, and South Africa

North America: Canada (Newfoundland)

Central America and Caribbean: Panama

South America: Throughout the high Andean region: Argentina, Bolivia, Chile, Colombia, Ecuador, Peru, and Venezuela. More northerly in range than *G. rostochiensis*

Oceania: New Zealand

EU: Present

Diagnosis: When suspected nematode introductions are found in diagnostic samples, their identity should be confirmed, voucher specimen should be deposited in an appropriate reference collection, and quarantine authorities should be notified. In addition to detecting newly introduced nematodes, diagnostic services may become involved in quarantine issues through their role in assessing samples of produce designated for overseas export market. As international trade increases, there will be an increasing demand from overseas quarantine authorities to ensure that agricultural products (e.g., tubers, rhizomes, rooted plants) are free of specific nematodes. If protocols for collecting and processing samples are set by the importing country, these must be followed by the diagnostic service. In the absence of specific protocols, the level of risk should be assessed and appropriate assessment procedures need to be developed.

Detection and Inspection Methods: Ordinary plant quarantine regulatory action is not well adapted for the detection of phytonematodes. Most quarantine action utilizes visual inspection supplemented by a hand lens or low-power microscope – to detect parasitic organisms themselves or to check symptoms of infection. Symptoms of nematode injury usually are not diagnostic, because they are largely plant reactions such as poor growth, chlorosis, and other symptoms equally indicative of root injury from many other causes. Specific symptoms also may vary with the reaction of different plants. Large obvious lesions are formed on the roots of certain plants attacked by root lesion nematodes, while such lesions are not found on the roots of many other kinds of plants attacked by the same nematode, although serious injury may be caused on both.

The symptoms described for *Globodera* spp. can have many other causes and cannot be taken as proof of presence of nematodes. For positive detection it is necessary to find cysts in soil samples or females or cysts on host roots. Mature females and cysts are just visible to the naked eye and can be seen as minute white or yellow globes on the root surface. Specific identification is just possible by observation of the female color at the appropriate stage of development, either a change from white to yellow in *G. rostochiensis* or prolonged white (slightly cream but no yellow phase) in *G. pallida*. Several methods are available for extracting juveniles or larvae from the soil (Southey 1986; OEPP/EPPO 1991), and thereafter specialist microscopical examination of juveniles, females, or cysts is necessary for precise identification. The species can also be distinguished by the morphological characters and measurements of second-stage juveniles and cysts. The use of biochemical techniques such as DNA probes is being investigated as morphological identification can be difficult.

Means of Movement and Dispersal: These nematodes have no natural means of dispersal and can only move the short distances traveled by juveniles attracted toward roots in the soil. They are spread into new areas as cysts on, in order of importance, seed potatoes, nursery stock, soil, flower bulbs, and potatoes for consumption or processing. The last named are only important if there is a risk of their being planted or if care is not taken with disposal of waste soil.

Phytosanitary Risk and Phytosanitary Measures: Both species of potato cyst nematode are A2 quarantine pests for EPPO (*OEPP/EPPO* 1978, 1981). They are also of quarantine significance for APPPC and NAPPO. In addition, *G. rostochiensis* is a quarantine pest for CPPC and IAPSC. The nematodes are already established in most or all areas in the EPPO region that are important for the cultivation of potatoes for consumption or the production of starch; therefore, regular attention to control is needed in such areas. Where domestic legislative measures are in force, import regulations are justified to ensure comparable standards for imported material. It is essential that areas of seed potato production be kept as free as possible from these nematodes. *G. pallida* is generally less common than *G. rostochiensis* in most of the EPPO region (with the exception of the southern part

of the UK) and is absent from some countries; it therefore merits greater attention from the phytosanitary standpoint. At some time in the future, it may be worthwhile to consider the individual pathotypes as being the quarantine organisms, rather than the two species themselves. It is obvious that some pathotypes are very widely distributed, and furthermore some are more important economically than others. Unfortunately, information is still limited on the precise distribution of the pathotypes.

Measures to prevent the introduction of the nematodes to areas where they are not already established include soil sampling surveys and regulations concerning movement of seed potatoes, nursery stock, flower bulbs, and soil. These apply nationally as well as internationally (CEC 1969). Consignments of potato tubers, rooted plants, and bulbs from countries where the nematodes occur may be examined to check amounts of adhering soil, if any, or to take samples of soil for laboratory examination. Additional safeguards during transit of consignments could be washing of tubers and flower bulbs to remove soil, although it should be noted that cysts can remain embedded in tubers, especially in the eyes. Alternatively, tubers may be dipped in dilute sodium hypochlorite solution (Wood and Foot 1977). The EPPO specific quarantine requirements (OEPP/EPPO 1990) for these nematodes require that the field in which seed potatoes or rooted plants being imported were grown was inspected by taking soil samples according to an EPPO-recommended method (OEPP/EPPO 1991) and found free from viable cysts of both species. The sampling must have been performed after harvest and after removal of the previous potato crop.

Best Management Schedule: Once established, these nematodes are nearly impossible to eliminate as cysts can lie dormant in the soil without a host crop for decades. A new generation of PCN is produced each time a host crop is grown. However, the following are the best management practices to help prevent infection and reduce the nematode population over time:

(a) *Minimize soil movement:* Avoid sharing farm machinery, equipment, tools, and containers; do not spread tare dirt or debris onto agricultural land or areas where it could be spread to other agricultural land. Tare dirt or debris is the loose soil knocked off either during potato processing operations or during storage filling/emptying operations; never use bags, containers, etc., more than once for potato transport unless they are free of soil. Be sure all commercial transport vehicles are free of soil.

(b) *Keep farm equipment and vehicles clean:* Ensure equipment is available to conduct proper cleaning and disinfection of farm machinery and vehicles; clean and disinfect all used equipment before using on a farm; clean and disinfect all machinery, vehicles, and other equipment before going between fields. This includes those of temporary help, custom applicators, and utility companies.

(c) *Healthy seed:* Plant certified seed potatoes produced on land determined not to be infested with nematode. Avoid continuous planting of potatoes in the same field(s). A long rotation cycle is critical for managing and preventing PCN. During interim years, plant nonhost crops. Contain water and soil during tuber washing to avoid contaminating farmland. Processing facilities should not return soil, water, cull tubers, and debris to agricultural land. This includes managing and limiting waste used as livestock feed. Segregate potatoes in storage – each field should be stored separately.

(d) *Best land practices:* Plant cover crops when fields are not in use to prevent wind and water from moving soil. Keep hedgerows, sod barriers, or sod strips between fields and along highways to provide a physical barrier to soil movement. Do not use common headlands, farm roads, and public roads as turning areas.

(e) *Long-term management:* Successful management or eradication of cyst nematodes

is complex and takes many years. It is important to develop appropriate long-term strategies to deal with the pest. These strategies will help protect the local, provincial, and national potato industries as well as other affected industries while being sensitive to the situation of positive growers. With appropriate management, it will be possible to grow most nonhost crops on affected fields, and it may, over several years, become possible to grow potatoes in an appropriate rotation.

(f) *Regulatory measures:* In the event of cyst nematode detection, immediate regulatory measures are to be taken to contain potential sources of spread. Surveys and investigations to trace the infestation back to an infested field and to trace the infestation forward are also conducted to prevent any further spread. No potatoes could be grown on land known to be infested through soil sampling; movement of farm machinery and construction equipment out of the regulated area was permitted only after steam cleaning and fumigation; no soil nor sod could be moved out of the restricted area; potato grading stations could be operated only under permit, and the tare soil had to be treated; potatoes grown on Long Island could not be used for seed; potatoes shipped for table stock must be washed. Potatoes must be packaged in nonreusable paper bags.

12.2.4 Stem and Bulb Nematode (*Ditylenchus dipsaci*)

D. dipsaci is known to attack over 450 different plant species, including many weeds. However, it occurs in more than ten biological "races," some of which have a limited host range. The races that breed on rye, oats, and onions seem to be polyphagous and can also infest several other crops, whereas those breeding on lucerne, *Trifolium pratense*, and strawberries are virtually specific for their named hosts and seem to have relatively few alternative host plants. The tulip race will also infest *Narcissus*, whereas another race commonly found in *Narcissus* does not breed on tulip. It is known that some of the races can interbreed and that their progeny has different host preferences (Eriksson 1974).

The principal hosts are faba beans, garlic, *Hyacinthus orientalis*, leeks, lucerne, maize, *Narcissus pseudonarcissus*, oats, onions, peas, *Phlox drummondii*, *P. paniculata*, potatoes, rye, strawberries, sugar beet, tobacco, *Trifolium pratense*, *T. repens*, and tulips. It has also been reported on carnations, celery, *Hydrangea*, lentils, rape, parsley, sunflowers, and wheat.

Geographical Distribution: D. dipsaci occurs locally in most temperate areas of the world (Mediterranean region, North and South America, northern and southern Africa, Asia, and Oceania), but it does not seem able to establish itself in tropical regions except at higher altitudes that have a temperate climate. In most countries regulatory measures (e.g., certification schemes) are applied to minimize further spread of *D. dipsaci*.

EPPO Region: Albania, Algeria, Austria, Belarus, Belgium, Bulgaria, Croatia, Cyprus (unconfirmed), the Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Latvia, Liechtenstein, Malta, Moldova, Morocco, the Netherlands, Norway, Poland, Portugal, Romania, Russia (European), Slovakia, Spain, Sweden, Switzerland, Syria, Turkey, Tunisia, the UK, Ukraine, and Yugoslavia

Asia: Armenia, Azerbaijan, China (Gansu, Hebei, Henan, Shandong), Cyprus (unconfirmed), India, Iran, Iraq, Israel, Japan (Honshu), Jordan, Kazakhstan, Korea Republic, Kyrgyzstan, Oman, Pakistan, Syria, Turkey, Uzbekistan, and Yemen

Africa: Algeria, Kenya, Morocco, Nigeria, Tunisia, Réunion, and South Africa

North America: Canada (Alberta, British Columbia, Ontario, Prince Edward Island), Mexico, and the USA (Alabama, Arizona, California, Florida, Hawaii, Michigan, New York, North Carolina, Utah, Virginia, Wyoming)

Central America and Caribbean: Costa Rica, the Dominican Republic, and Haiti

South America: Argentina, Bolivia, Brazil (Pernambuco, Parana, Rio Grande do Sul, Santa Catarina, São Paulo), Chile, Colombia, Ecuador, Paraguay, Peru, Uruguay, and Venezuela

Oceania: Australia (New South Wales, South Australia, Tasmania, Victoria, Western Australia), and New Zealand

EU: Present

Detection and Identification: Major symptoms, in general, include swellings and distortion of aerial plant parts and necrosis or rotting of stem bases, bulbs, tubers, and rhizomes.

On Allium spp. (*Onions, Garlic, Leeks, etc.*)*:* The penetration of onion leaves by *D. dipsaci* causes leaf deformation and leaf swellings or blister-like areas on the surface. The leaves grow in a disorderly fashion, often hang as if wilted and become chlorotic. Young plants can be killed by high infestations. The inner scales of the bulb are usually more severely attacked than the outer scales. As the season advances, the bulbs become soft and when cut open show browning of the scales in concentric circles. Conversely, *D. dipsaci* on garlic does not induce deformation or swellings, but causes leaf yellowing and death (Netscher and Sikora 1990).

On Lucerne: The crop declines in patches in the field, and damage is more serious in humid climates. The whole plant becomes desiccated and presents symptoms of stunting and swelling at the base of the stem with conspicuous shortened internodes. With heavy infestation, plants can be killed.

On Tobacco: Invasion by the nematode of the lower part of the stem causes stunting and deformation of the plant followed by "stem break."

On Faba Beans: D. dipsaci causes swelling and deformation of stem tissue or lesions which turn reddish-brown then black, depending on cultivar and environmental factors. Newly formed pods take on a dark brown appearance. The lesions envelop the stem and increase in length, often advancing to the edge of an internode. Leaf and petiole necrosis is also common under heavy infestations, but can be confused with symptoms induced by fungal leaf pathogens. Infected seeds are darker, distorted and smaller in size and may have speckle-like spots on the surface. Heavy infestations often kill the main shoots, stimulating secondary tiller formation. The more severe symptoms are usually induced by the "giant race" on faba beans (Sikora and Greco 1990).

Detection and Inspection Methods: D. dipsaci can be isolated from samples of suspected seed material (according to symptoms) by dissection in water at 20 times magnification. Nematodes leave the dissected tissue and swim actively in the water. Microscopic examination at 800 times magnification is necessary for correct identification of the nematode species.

Means of Movement and Dispersal: In international trade *D. dipsaci* is liable to be carried on dry seeds and planting material of host plants. In the field the fourth-stage, juvenile can withstand desiccation for many years, and although soil densities seem to decrease rapidly, the nematode can survive for years without a host plant. Nematode survival and damage are greater in heavy soils as compared to sandy soils. It can also survive on a number of weeds. Irrigation water and cultivation by contaminated farm tools and machinery are other sources of inoculum dissemination. Nematode-free (certified) seeds and planting material are most essential to prevent crop damage by *D. dipsaci*. Hot water treatments with different temperature–time combinations, depending on type and state of seed material, are major preventive measures.

Phytosanitary Risk and Phytosanitary Measures: At present, the distribution of the different races throughout the region is patchy, and some countries apply official control measures to limit spread. Other countries regard the pest as being a quality pest which can be effectively controlled by production and use of healthy planting material. It is certainly true that, without control, *D. dipsaci* may cause complete failure of host crops within the EPPO region. EPPO lists it as an A2 quarantine pest, and CPPC, IAPSC, and NAPPO also consider it to be of quarantine significance.

The implementation of certification schemes for the production of host plants of *D. dipsaci* can

provide planting material free from the pest. Imports of soil and plants for planting and seeds of host plants from countries where this nematode occurs should be restricted.

12.2.5 Potato Rot Nematode (*Ditylenchus destructor*)

Host Range: Potatoes are the main host of *D. destructor*, but the nematode can also occasionally be found on bulbous *Iris*, carrots, *Trifolium* spp., groundnuts, and garlic. Overall, some 70 crops and weeds and a similar number of fungus species have been recorded as hosts.

Geographical Distribution

EPPO Region: Albania, Austria, Belarus, Belgium, Bulgaria, the Czech Republic, Estonia, Finland (intercepted only), France, Germany, Greece, Hungary, Ireland, Latvia, Lithuania, Luxembourg, the Netherlands, Norway, Poland, Romania, Russia (European), Slovakia, Spain, Sweden, Switzerland, Turkey, and the UK

Asia: Azerbaijan, Bangladesh (unconfirmed), China (Hainan, Hebei, Jiangsu, Liaoning, Shandong), India (unconfirmed), India, Iran, Japan, Kazakhstan, Malaysia (unconfirmed), Saudi Arabia, Tajikistan, Turkey, and Uzbekistan

Africa: South Africa

North America: Canada, Mexico, and the USA (Arkansas, California, Hawaii, Idaho, Indiana, New Jersey, North Carolina, Oregon, South Carolina, Virginia, Washington, West Virginia, Wisconsin)

South America: Ecuador

Oceania: Australia (New South Wales, Victoria, South Australia, Western Australia, restricted distribution in Tasmania), and New Zealand (on hops only; Foot and Wood 1982)

EU: Present

Detection and Identification

Symptoms

On Potatoes: There are, in general, no obvious symptoms in the aerial parts of the plant, although heavily infested tubers give rise to weak plants which usually die. Early infections can be detected by peeling the tuber which can reveal small, off-white spots in the otherwise healthy flesh. These later enlarge, darken, are wooly in texture, and may be slightly hollow at the center.

On badly affected tubers, there are typically slightly sunken areas with cracked and wrinkled skin which is detached in places from the underlying flesh. The flesh has a dry and mealy appearance, varying in color from grayish to dark brown or black. This discoloration is largely due to secondary invasion of fungi, bacteria, and free-living nematodes (the latter are easily confused with *D. destructor*).

In contrast, the skin of potatoes infested with *D. dipsaci* is not usually cracked, and the rot darkens toward the inside of the tuber. The symptoms are more obvious in the foliage, which is shortened and malformed.

On Iris and Tulips: Infestations usually begin at the base and extend up to the fleshy scales, causing gray to black lesions; roots may be blackened and leaves poorly developed, with yellow tips.

On Groundnuts: Hulls of groundnuts show black discoloration which appears first along the longitudinal veins. The kernels are shrunken. The infected testae are brown to black and the embryo shows a brown discoloration (Jones and De Waele 1988).

Detection and Inspection Methods: Prior to planting, soil can be sampled using a standard extraction procedure for nematodes of this size (Hooper 1986). It is difficult to detect the presence of *D. destructor* from external tuber appearance alone. Sample tubers should be cut or peeled to look for the characteristic whitish pockets in which most of the nematodes are found. Microscopic examination of the nematode is necessary for correct identification of the species.

Means of Movement and Dispersal: The nematodes can move only short distances in the soil and have no natural means of long-range movement. The main means of dispersal is with infested potato tubers or other subterranean organs of host plants, e.g., bulbs and rhizomes (especially of *Iris*). Transport in infested soil is another important means of

spread. Irrigation water can also carry the nematodes.

Phytosanitary Risk: D. destructor was considered to be an EPPO A2 quarantine pest (OEPP/EPPO 1978) but was deleted from the quarantine list in 1984 because of its minor importance and very wide distribution throughout the EPPO region, in particular in those areas where it would be likely to cause crop damage. *D. destructor* is of quarantine significance for the APPPC and COSAVE. The requirement of the nematode for high relative humidity means it would be unlikely to become a problem in areas with warm, dry soils; it may therefore be of concern to potato production only in the northern parts of the EPPO region. However, its establishment as a groundnut pathogen in South Africa has shown its potential to adjust to different (and normally unfavorable) climatic conditions (De Waele and Wilke 1990).

Phytosanitary Measures: Fumigation under vacuum (650 mmHg) with hydrogen cyanide (initial dose, 4 g/m^3) for 1 h at above 10 °C gives good control of the nematode in bulbs, rhizomes, and tubers and especially asparagus roots and strawberry plants. Infestation in *Iris* bulbs can be controlled by immersion in water containing 0.5 % formaldehyde at 43.5 °C for 2–3 h, but some varieties may be injured during this treatment. In garlic bulbs, nematodes were controlled by drying at 34–36 °C for 12–17 days (Fujimura et al. 1989).

12.2.6 The Burrowing Nematode (*Radopholus similis*/*R. citrophilus*) on Citrus

12.2.6.1 Parasitism and Ecology of the Burrowing Nematode on Citrus

The burrowing nematode is a dimorphic species. Females have a well-developed stylet and are endoparasitic. Males have a poorly developed stylet and are not considered to be parasitic. Development and reproduction take place inside root tissues of plant hosts. Nematode feeding and migration in roots cause large cavities and necrosis in the cortical and vascular tissues. Nematode life stages require healthy root tissue for development and reproduction. They migrate out of roots that have been colonized and damaged as a result of nematode feeding activity and the subsequent decay caused by the invasion of bacteria, fungi, mites, and other organisms. The migratory habit of the nematodes in soil provides the opportunity for burrowing nematodes to search for healthy roots. Burrowing nematodes can survive in the absence of a host for about 6 months.

Burrowing Nematode Races: Studies have shown that there are two races with different host preferences (Inserra et al. 2005). One race, called the banana race, parasitizes banana, but not citrus. The other race, called the citrus race, parasitizes both citrus and banana. Both races have extended, overlapping host ranges that include ornamentals, agronomic crops, and weeds. There are no obvious and specific morphological differences between the two races. The citrus race was eventually elevated to the status of species, *Radopholus citrophilus* and had differences between the two races in oocyte maturation, sex pheromones, enzymes, and proteins. However, recent molecular and mating studies by Kaplan and Opperman (2000) show that *R. similis* and *R. citrophilus* are nonspecific and they have been returned to their previous status as races of *R. similis*.

Several biological factors need to be considered for regulatory management of different phytonematodes, including burrowing nematode (Table 12.3) (O'Bannon and Esser 1987).

Damage and Symptoms: The burrowing nematode severely damages the fibrous roots of citrus trees. The debilitated root system does not adequately support the aboveground portion of the tree which exhibits sparse foliage and weakened and dead branches. This condition results in severely reduced yields of as much as 80 % fruit loss compared to healthy trees, rendering infested orchards economically nonviable.

Soil factors play a major role in the expression of "spreading decline" symptoms, which occur mainly in deep well-drained sands (90–95 % sand) poor in organic matter (<1 %).

Table 12.3 Comparative biological factors to be considered for regulatory management of major phytonematodes

Factor	Burrowing nematode	Golden cyst nematode	Soybean cyst nematode
Soil depth occurrence	15 cm–5 m	7–30 cm	7–30 cm
Longevity, without food	6 months	8–10 years	4 years
Host range (plant species)	290	3	500
Parasitic habit	Endoparasitic	Semi-endoparasitic	Semi-endoparasitic
Natural protection	None	Cyst	Cyst
Climate	Tropical	Temperate	Temperate

This soil type makes up much of the subsoil in some locations known as the "ridge" and favors the burrowing nematode. The combination of low soil moisture conditions (5–7 %) in the subsoil and nematode damage results in "spreading decline" symptoms. Soils rich in organic matter, silt and clay components, and high moisture levels inhibit the increase of burrowing nematode populations and are not conducive to the expression of the disease (O'Bannon and Tomerlin 1971).

Quarantine Actions and Management of the Burrowing Nematode on Citrus: Long-term research has shown that the burrowing nematodes were spread in the "ridge" with infected citrus propagative material originating from infested nurseries. The nematode occurs mainly in areas where burrowing nematode-infected plants, such as citrus, banana, and many ornamentals, were introduced by human intervention. Nematodes may also occur on introduced nematode-infected aquatic plants, such as aquatic aroids. It is essential to implement appropriate phytosanitary actions to prevent the spread of the nematode in uncultivated areas and the adoption of effective management practices in citrus orchards.

(a) *Eradication:* A burrowing nematode eradication program, called "Push and Treat," was attempted in Florida by removing infected citrus trees from orchards and applying high doses of fumigant nematicides. This program was discontinued due to environmental concern and chemical contamination of the water table. However, these eradication attempts on more than 6,000 ha were effective in containing the spread of the nematode from infested orchards into noninfested ones.

(b) *Isolation:* Infested orchards can be separated from healthy orchards by areas, known as barriers. In the past, these barriers were kept weed and root-free by the application of large amounts of fumigant nematicides to chemically devitalize the citrus roots in the barrier and prevent nematode migration. Today they are maintained by mechanical plowing and root pruning. Maintenance of the mechanical barriers is costly.

(c) *Certification:* In several locations like Florida, the Citrus Nursery Certification Programme has been implemented to prevent the spread of citrus nematode pests in Florida citrus orchards (Rule Chapter 5B-44.003, Florida Administrative Code). The Citrus Nursery Certification Programme requires the following: Commercial citrus nurseries produce propagative material free of citrus nematode pests. Soil, peat, and rock material for use in citrus orchards and nurseries must be certified free of these nematodes by the Florida Department of Agriculture and Consumer Services.

The Citrus Nursery Certification Programme: The Citrus Nursery Certification Programme consists of three phases, viz., site approval, pit approval, and premovement certification of young citrus trees before they are moved from the nurseries and transplanted into orchards.

(a) *Site approval:* For economic reasons, commercial citrus nurseries are normally established directly on the ground, as in Florida. Pasture lands are often used for commercial citrus nursery sites. These potential nursery sites are sampled to check for the presence of nematode pests and certified if found free from regulated

Table 12.4 Enzymes/toxins released from nematode-infected plant tissues

Crop	Nematode	Enzymes/toxins released
Potato	*D. destructor*	Polygalacturonase (PG) pectinase
Onion	*D. dipsaci*	Endo-polygalacturonase (E-PG) alkaline phosphatase
Potato	*G. rostochiensis*	Amylase ß-galactosidase, ß-glucosidase
Tubers/cuttings	*Meloidogyne* spp.	Acid phosphatase, alkaline phosphatase, amylase, proteolytic cellulase, esterase, proteases, pectinase
Banana	*R. similis*	Cellulase, invertase
Sugar beet	*H. schactii*	Amylase, cellulase
Citrus	*T. semipenetrans*	Alkaline phosphatase, amylase

citrus nematode pests. Populations of *Tylenchulus* spp. occur in several pastures and uncultivated lands. At one time these populations were called “wild” strains of the citrus nematode and, because of their morphological similarity, were confused with the regulated citrus nematode.

(b) *Pit approval:* Land destined for citrus nurseries and certified nematode-free must be planted with nematode-free citrus rootstock seedlings, which are grown from seeds or tissue culture in soil mixes containing peat and sand free from regulated nematode pests that parasitize citrus. In Florida, peat and sand for use in citrus nurseries are mined from deposits located in natural fields free from the citrus nematode pests. Construction material, such as gravel, clay, and shell, to be used in citrus orchards for construction of roads or other purposes, must be extracted from sites free from nematode pests of citrus. These deposits of construction material are sampled as for pit and nursery sites.

(c) *Premovement:* Citrus rootstock seedlings are grown in certified media on elevated benches that prevent nematode contamination from the ground and transplanted into nematode-free growing areas. The Citrus Nursery Certification Programme requires that any soil or infill unloaded within 30 m of the borders of a citrus nursery or orchard must be certified free of any citrus nematode pest. This successful Citrus Nursery Certification Programme resulted in a rapid reduction in the number of burrowing nematode-infested citrus nurseries. Hence, the implementation of the eradication, isolation, or barrier and Citrus Nursery Certification Programmes initiated in the 1950s reduced the area of orchards infested with the burrowing nematode to less than 4,000 ha in 1984. Present distribution of burrowing nematode in citrus orchards is <1 % of the total area.

Nature of Damage: Since nematodes affect the storage/planting materials which are dormant, their tissues are physiologically different from actively growing plants. Nematodes employ mechanical energy and biochemical components, viz., enzymes and toxins (Table 12.4) to cause diseases. Nematodes degrade either the planting material (rotting or drying) or the growth and development of plant. Nematodes come in the way of respiration and photosynthesis. “True seeds” may be shriveled/distorted and become chaffy resulting in crop loss.

12.2.7 Procedure for Quarantine Inspection for Nematodes

The following is the practical procedure for quarantine inspection of plant material for nematode infestation/contamination:

- Maintaining of records relating to the type of plant material, country of origin, destination, date of receipt and date of disposal, phytosanitary certificate, etc.
- Visual examination of underground and aerial parts of plant material for symptoms of nematode injury, if any, for example, surface or deep necrosis of roots, root rot, root galls, lesions, excessive root branching, stubby root,

coarse root, curly root tips, dead or devitalized buds, crinkled or distorted stems and foliage, seed galls, leaf lesions or spots, leaf galls, and distorted or discolored seed. Each of these symptoms could give indications of infestation by nematodes which cause these specific symptoms.

- Use of appropriate technique for the recovery of nematodes from plant tissue and separation of plant debris, soil clods, free soil, and other extraneous inert matter mixed with true seed or adhering to the seed or plants. Examination of such soil and debris for incidence of nematodes, using appropriate techniques.
- Detailed examination and identification of the nematodes intercepted, under microscope, followed by risk analysis of the species intercepted.
- Decision on the disposal of the plant sample (clearance or rejection) in view of the regulations, risk involved, and the possibility of salvaging the material.
- Salvaging of infested material by fumigation; hot water treatment; nematicidal dip; manual cleaning, washing, and drying; etc., whenever appropriate methods of denematization are available. It should be mentioned here that the salvaging methods used in quarantine must ensure a hundred percent kill of the nematode without injury to the planting material. Since tested and standardized methods (for quarantine operations) are available only for a limited number of plants and planting material (and these too pertain to limited number of species of endoparasitic nematodes), the most ideal procedure for vegetative propagation is to grow the material in nematode-free soil, especially when the plant consignments are large and the suspected nematodes have not been recorded from the country.

12.2.8 Molecular Aids to Nematode Diagnosis

The traditional morphological methods of identification have formed the basis of nematode taxonomy for more than 100 years, and for the foreseeable future, they will continue to be important. However, rapid advances in biotechnology have provided taxonomists with a wide range of new tools, which may eventually revolutionize nematode identification. These new technologies are already widely used in areas such as medical and veterinary diagnosis, where they have been adopted because of their discriminatory power and their ease of use and interpretation. Protein electrophoresis and DNA-based technologies are major methods that may eventually be used for the routine identification of nematodes.

Morphological characters have long been used for nematode taxonomy and will continue to be the mainstay. It is an essential component of any higher level classification of nematodes, and in many cases morphology provides a quick and unambiguous diagnosis to species (Ravichandra 2008). Nevertheless, morphological taxonomy suffers from some limitations. Morphological taxonomy cannot be used for intrasubspecific groups such as races, pathotypes, and strains. Identification by other methods including host range testing is very time consuming and not full proof. Since the genotype of the nematode is examined directly in case of molecular markers, problems associated with phenotypic variation in taxonomic characters are avoided.

Furthermore, greater discrimination is possible because the entire genome is available for study, including 75–80 % of the genome, which is noncoding and contains many highly variable sequences. This DNA sequence variability provides a large pool of information that can provide useful diagnostic characters for the separation of taxa and intrasubspecific categories for the unraveling of evolutionary relationships between nematode groups. Diagnostic kits can be developed for the identification, which can be used for advisory services and intercepting the consignments for domestic as well as international quarantine purposes. Another advantage of molecular markers is that it is independent of the stage of the nematode to be identified and one or few nematodes are enough. The results are interchangeable among laboratories worldwide and reproducible. DNA analysis also helps to understand the genetic pool similarity of any nematode species in a given geographical area,

Table 12.5 Hot water treatment for denematizing planting material

Sl. No.	Nematode	Planting stocks	Time (min.)	Temp. (°C)
1.	*A. ritzemabosi*	Chrysanthemum stools	15	47.8
2.	*A. fragariae*	Easter lily bulbs	60	44.0
		Strawberry runners	10	46.0
3.	*Ditylenchus dipsaci*	Onion	120	43.5
		Narcissus bulbs	240	43.0
4.	*D. destructor*	Irish bulbs	180	43.0
		Potato	60	48.0
5.	*Meloidogyne* spp.	Potato tubers	120	46–47.54
		Sweet potato	65	46.7
		Grape rooted cuttings	10	50.0
		Strawberry cuttings	30	47.8
		Rose cuttings	5	52.8
		Ginger rhizomes	60	45.5
		Peach root stocks	10	55.0
		Tuberose tubers	5–10	50–51.0
		Begonia tubers	60	49.0
		Yam tubers	30	48.0
		Caladium tubers	60	45.0
		Cherry root stocks	5–10	51.0
6.	*Pratylenchus penetrans*	Strawberry	7.5	51.0
7.	*Radopholus similis*	Banana suckers	30	50.0
		Citrus rooted cuttings	20	55.0
8.	*Tylenchulus semipenetrans*	Citrus rooted cuttings	10	46.7
			25	45.0

and this information is highly useful in resistance breeding. There are various molecular markers available at present, which can be used in nematode identification depending on the availability of time, facilities, purpose, and quantity of nematodes.

12.2.9 Avoiding Infiltration of Nematodes/Restricting the Spread

- Set up a nematode diagnostic unit with basic apparatus for extraction, observation, and decontamination and a trained nematologist at each port of entry. Measures like hot water treatment of the planting stock can detect and kill nematodes (Table 12.5).
- Set up regional laboratories with equipments and two nematologists (one taxonomist and one applied nematologist).
- Establish a centralized database of indigenous and exotic nematode pests.
- Carry out pest risk analyses (PRA) for exotic and localized indigenous nematode pests.
- Introduce mandatory phytosanitary inspection of bulk imports of plant materials at the time of entry.
- Introduce post-entry periodic monitoring of sites where imported materials are sown/planted.
- Introduce mandatory destruction at entry-time and post-entry decontamination and, if necessary, destruction of infested material.

The use of treated planting stock is one of the best measures to avoid nematodes, which may be carried out in the following manners:

1. *Heat: steam* (potato cyst nematode, stem, and bulb nematode) and autoclave small quantities of soil.
2. *Hot water:* More common. Time and temperature factors are specific (Table 12.5).

3. *Irradiation: G. rostochiensis* and *D. myceliophagus*.
4. *Washing processes:* Proper and careful washing of material to remove adhering nematodes.
5. *Seed cleaning:* Mechanical seed cleaning to remove dormant structures in the seed lot.

12.2.10 Some Requirements and Solutions

These include basic information on occurrence and distribution of nematode species already present in the country. Categorization of nematodes depending on the information collected into groups based on their damage potential is important. Prohibited species, restricted species, unrestricted species, and import policy-bulk assignments are difficult to examine. Importing small quantities and multiplying of seed/planting material are more suitable. Post-entry quarantine facilities and post-entry isolation growing facilities are essential requirements at all quarantine agencies. Sometimes, mere lab examinations may not be sufficient to detect the nematodes. Operational requirements like socioeconomic factors of the country, lack of public awareness on the nematodes, and programs of educating the public through films, lectures, and pamphlets require immediate attention of the authorities. Training and communication on various aspects of nematodes is also an essential requirement.

12.2.11 Future Thrusts

Major future thrusts include detailed and precise information on the occurrence, distribution, pathogenicity, and host range of phytonematodes of quarantine significance, classification of nematode species into categories according to their damage potential to be incorporated into quarantine regulations under DIP Act, regulation of quantity of hazardous material by the authorities, post-entry quarantine facilities to be improved, attempts to create more public awareness, qualified nematologists to be appointed at quarantine centers, minimum usage of chemicals, safer and eco-friendly approaches like bio-agents, and the use of genetically modified planting material.

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13 Novel Methods of Nematode Management

A range of phytonematodes can injure various horticultural crops, among which one or more of them occur in most soils. Although some can cause significant losses when present in low numbers, most do not cause economically significant damage unless their numbers are unusually high or the plant is also subject to unusual levels of stress caused by other factors. Careful integration of most suitable management components will help keep most nematode pests below damaging levels and may simplify the decisions that must be made about selecting and applying nematicide when they are needed.

The major means of phytonematode management include regulatory, physical, cultural, biological, chemical, host resistance management and integrated nematode management (INM). Abundant published information is already available on these various measures of managing phytonematodes, and the major ways to manage nematodes attacking specific horticultural crop have already been discussed under the chapter "Nematode Diseases of Crop Plants." The latest and the novel methods of nematode management in horticultural crops are discussed in this chapter.

Development of nematode management tools with novel sites of action often relates to the physiological or biochemical variations among phytonematodes and their hosts. Approaches to novel management of nematodes may be acting either on nematode targets, at the nematode–plant interface, or in the plant response. Strategies aimed at nematode targets include disruption of nematode intestinal function through recombinant plant expression of protease inhibitors or *Bacillus thuringiensis* (BT) toxins, expression of double-stranded RNAs (dsRNAs) that cause silencing of essential nematode genes, disruption of sensory nervous system function, and generation of nematicidal metabolites (Mc Carter 2008). Methods directed at disruption of the nematode–plant interface include expression of proteins or dsRNAs that block the function of nematode parasitism gene products involved in migration through the plant vasculature or feeding site establishment, production of molecules repellent to the nematode, or conversion of the plant to a nonhost. Approaches acting through the plant response include expression of a cloned plant resistance gene triggering a hypersensitive response, expression of gene(s) deleterious to the feeding site with a feeding site-specific promoter, and conversion of the plant from sensitive to tolerant.

It is to be noted that nematodes and their hosts are provided with hormonal systems that differ in organization and chemical composition, which has lead to the details of nematode endocrine system studies (Chitwood 1987). The existence of specific endocrine tissues or developmental events under hormonal control in nematodes is a known fact. The next attempt is to isolate and identify the compounds from the nematode with hormonal function in other animals, especially the insects. Finally, because many hormones are steroids and because nematodes possess a dietary requirement for the metabolic precursors of steroid hormones, the disruption of nematode

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development by the compounds that interfere with the metabolic dietary sterols is mainly discussed.

Novel Concepts: The major and potential novel concepts in the nematode management include manipulation of genes/transgenic nematode management, nematode ecdysteroids, juvenile hormones, vertebrate steroid hormones, inhibition of steroid metabolism, biological activity, and mode of action of avermectins, endotoxins of *Bacillus thuringiensis,* 2,4-diacetylphloroglucinol (DAPG), phytoecdysteroids, and others.

13.1 Plant Nematode Resistance Genes

One of the greatest uses of recombinant DNA technology is the production of large quantities of scarce and novel proteins coded by the structural genes. The productional command of these structural genes is with the "promoter" sequences which act as transcriptional control elements. With increasing restrictions on chemical pesticides, the role of host resistance for nematode control has grown in importance. A number of genes that mediate nematode resistance have now been, or soon will be, cloned from a variety of plant species. Various approaches are being developed to provide resistance to a range of nematode species. Natural, R gene-based resistance is currently exploited in traditional breeding programs, and research is ongoing to characterize the molecular basis for the observed resistant phenotypes. A number of transgenic approaches hold promise, the best described being the expression of proteinase inhibitors to disrupt nematode digestion. The application of plant-delivered RNA interference (RNAi) to silence essential nematode genes has emerged as a potentially valuable resistance strategy (Fuller et al. 2008).

Plants are defined as resistant to nematodes when they support reduced levels of reproduction (Trudgill 1991). Nematode resistance genes are present in several crop species and are an important component of many breeding programs including those for tomato, potato, soybeans, and cereals. Several resistance genes have been mapped to chromosomal locations or linkage groups. A few have been cloned and a number of additional genes are likely to be cloned in the near future (Table 13.1).

One of the most exciting developments is the cloning and characterization of the first R gene directed against a nematode, *Heterodera schachtii* (Cai et al. 1997). This R gene, *Hs1 Pro-1*, was isolated from *Beta procumbens,* a wild species of beet. No commercially used beet varieties of *Beta vulgaris* carry a natural resistance gene. The designation of R genes in beet follows a convention which identifies the gene, wild beet parent, and chromosome carrying the gene. For example, the cloned gene, *Hs1*, derives

Table 13.1 Some cloned phytonematode resistance genes

Gene	Host plant	Nematode	Gene structure
Hs1 pro-1	*Beta procumbens* (wild relative of sugar beet *B. vulgaris*)	*Heterodera schachtii*	Amino terminus leucine-rich region
Gpa2	*Solanum tuberosum*	*Globodera pallida* – resistance to narrow range of pathotypes	LZ-NBS-LRR
Gro1-4	*S. tuberosum*	*G. rostochiensis,* pathotype Ro1 only	TIR-NBS-LRR
Hero A	*S. pimpinellifolium* (wild relative of cultivated tomato *S. lycopersicum*)	Resistance against broad range of *G. pallida* and *G. rostochiensis* pathotypes	CC-NBS-LRR
rhg1 and Rhg4	*Glycine max*	*H. glycines* – resistance against pathotypes 0	LRR, transmembrane and kinase domains
Mi-1.2	*S. peruvianum* (wild relative of cultivated tomato *S. lycopersicum*)	*M. incognita, M. arenaria, M. javanica*	CC-NBS-LRR

from chromosome 1 of *B. procumbens,* whereas gene *Hs2 web7* derives from chromosome 7 of *B. webbiana*.

The *Mi* gene of tomato confers effective resistance against several root-knot nematode species (Williamson 1998). It encodes a member of the plant resistance protein family characterized by the presence of a putative nucleotide-binding site and a leucine-rich repeat. Analysis of transgenic plants revealed the unexpected result that *Mi* also confers resistance to potato aphids. Tomato plant carrying *Mi* gene shows resistance to *M. arenaria, M. javanica,* and *M. incognita* but not to *M. hapla.* Although highly effective in many conditions, *Mi* fails to confer resistance at high soil temperature. About 8 resistance genes that differ from *Mi* in properties and genetic position have been identified in *Lycopersicon* spp. These genes as well as the cloned *Mi* gene provide a resource for broadening the base of root-knot nematode resistance in tomato and other crops.

Recently, *Gpa2,* a gene that confers resistance against some isolates of the potato cyst nematode, *Globodera pallida,* was cloned by a positional cloning strategy. Numerous additional sources of nematode resistance have been identified and several of the responsible genes have been genetically mapped. For example, at least eight different root-knot nematode resistance genes have been identified genetically in accessions of the wild tomato species, *L. peruvianum* (Ammati et al. 1986). The inheritance of nematode resistance in some crops, notably in soybean, is complex and includes both dominant and recessive genes, some of which have been genetically mapped. Efforts to clone several nematode resistance genes are currently in progress, and it is likely that the number of cloned genes will increase greatly over the next few years.

Although the incorporation of natural resistance is a major component of current nematode management strategies, there are many crops for which appropriate resistance loci are not available. As nematode resistance genes are cloned, it may be possible to transfer them to additional hosts; however, it is not certain that genes will function effectively in heterologous hosts. Experiments to transfer *Mi*-mediated resistance into tobacco have so far not been successful (Williamson 1998). Furthermore, acquisition of virulence by nematodes may shorten the effective utility of this approach. To fill the gap, a variety of strategies to engineer synthetic resistance are being developed (Gheysen et al. 1996).

The exploitation of nematode-responsive plant genes is important in novel nematode management methods. Strategies that combine one or more natural resistance genes with synthetic resistance may be the most effective. Investigations of cloned R genes are likely to lead to increased understanding of the molecular mechanisms by which resistance is produced. This understanding may allow development of designer R genes with faster response, broader or different target recognition, or increased durability. Such developments will need to be coupled with investigation of the mechanisms by which nematodes circumvent resistance. Understanding the interactions between host and pathogen will increase options for control strategies as our ability to use chemical pesticides decreases and the need for food production continues to increase.

In potato, a major dominant locus Gro1 conferring resistance to some pathotypes of *G. rostochiensis* was mapped by restriction length polymorphism (RFLP) to potato linkage group IX; the nearest neighboring RFLP markers are CP 51© and TG 20(a). One gene (*pMRI*) with unknown function has been isolated from potato roots undergoing infection by *G. rostochiensis*. For the most part however, few plant genes upregulated during nematode infection have thus been characterized. The availability of the cloned genes will allow introduction of these genes into selected varieties of the crops in which they have been found but also possibly other crops, further expanding their use. However, they are not the end solutions as already Mi-virulent nematode isolates have been identified in many areas of the world. The future research would concentrate on the temperature sensitivity that can be reduced by modifications in the structure, expression, or

signal transduction of Mi (Cai et al. 1997). Incorporation of Mi-3 and other resistance genes into cultivated tomato using classical or marker-assisted breeding may also broaden the basis of root-knot nematode resistance. Continued searches of germplasm are needed to identify new sources of resistance. Artificially engineered resistance based on antisense technology or expression of anti-nematode proteins may be an additional source of resistance including gene pyramiding in some instances.

13.2 Manipulation of Natural Resistance Genes

Genetic host resistance is the most cost-effective and environmentally sound method for management of plant-parasitic nematodes. Resistance to the various species of root-knot nematodes and cyst is limited to a number of host species. Resistant cultivars on infection exhibit activation of a number of inducible responses that are thought to be disease resistance mechanisms. Hypersensitive response (HR) is one such response in which there is a rapid localized necrosis of cells at the feeding site initiation limiting the parasitic ability of the nematode. It is known that many plant genes are upregulated during a resistance reaction, including genes encoding proteins such as glucanases, chitinases, and other enzymes commonly referred to as pathogenesis-related proteins.

The characterization of the natural resistance genes (R genes) provides the opportunity to understand, in molecular detail, the complex interactions that occur between a plant and a pathogen during an incompatible interaction. A common feature of R genes is their specificity for a particular pathogen, usually referred to as a gene interaction. This describes the requirement for a pathogen to express a specific avirulence (avr) gene whose product interacts, directly or indirectly, with a corresponding R gene leading to the resistance response. Failure of the pathogen to express the appropriate avr gene means it becomes a virulent pathogen capable of parasitizing the host.

13.3 Application of Recombinant DNA Technology

Recombinant DNA technology utilizes the power of microbiological selection and screening procedures to allow investigators to isolate a gene that represents as little as 1 part in a million of the genetic material in an organism. The DNA from the organism of interest is divided into small pieces that are then placed into individual cells. These can then be separated as individual colonies on plates and they can be screened through rapidly to find the gene of interest. This process is called "molecular cloning." Insertion of resistance genes into the host crop genomes is made possible by recombinant DNA technology. The understanding of nematode–host interactions using the tools of comparative and functional genomics has provided avenues for engineering specific and broad resistance against plant-parasitic nematodes. Engineering resistance can be achieved by transforming plants with protease inhibitors, lectins, toxic proteins, and dsRNA targeted against the phytonematodes (Sirohi et al. 2010).

The incorporation of resistance genes into important crop plants epitomizes effective biological disease control. Resistance is heritable and therefore inexpensive and permanently available once introduced. Also, genetically determined resistance is generally effective when plants are grown in a wide range of climates, soil type, and cropping regimes. However, problems have been encountered in obtaining resistance sources against a particular pest/pathogen and emergence of resistance breaking pathogen races or biotypes. These problems can be overcome by molecular cloning of numerous resistance genes from many different plants that are targeted against several pests and pathogens. Then it would be possible to transform various combinations of these genes into susceptible cultivars including species entirely unrelated to the plants from which the resistance genes were originally isolated. This approach would permit the use of previously unavailable resistance specificity, in particular crop plants, and should lower the

frequency with which newly virulent pathogen races become predominant.

13.3.1 Broad Classes of Transgenic Nematode Control Strategies

Nematode targets	Antifeedant and nematicidal proteins like enzyme inhibitors; disrupt essential nematode gene product with RNAi; disrupt sensory function with RNAi/peptide/plantibody; and nematicidal metabolite
Nematode–plant interface	Disrupt nematode pathogenicity factor, invasion, migration, and nematode pathogenicity factor; feeding site induction and maintenance (with RNAi, plantibody, etc.); stealth plant, repellant plant, and conversion of plant to nonhost
Plant response	Plant resistance gene/hypersensitive response is activated by nematode invasion. Feeding site-specific promoter induces cell death or other site incompatibility and conversion of plant to tolerance

13.3.2 Targets for Novel Transgenic Resistance

Strategies aimed at nematode targets include disruption of nematode intestinal function through recombinant plant expression of protease inhibitors or *Bacillus thuringiensis* (BT) toxins, expression of double-stranded RNAs (dsRNAs) that cause silencing of essential nematode genes, disruption of sensory nervous system function, and generation of nematicidal metabolites (Mc Carter 2008). Methods directed at disruption of the nematode–plant interface include expression of proteins or dsRNAs that block the function of nematode parasitism gene products involved in migration through the plant vasculature or feeding site establishment, production of molecules repellent to the nematode, or conversion of the plant to a nonhost. Approaches acting through the plant response include expression of a cloned plant resistance gene triggering a hypersensitive response, expression of gene(s) deleterious to the feeding site with a feeding site-specific promoter, and conversion of the plant from sensitive to tolerant. Degrees of resistance have been demonstrated through recombinant expression of protease inhibitors, dsRNAs, and cloned plant resistance genes. The focus of molecular plant nematology on root-knot and cyst nematodes makes it likely that transgenic technology will first be commercially applied to these sedentary endoparasites with eventual application to other species. Successful commercialization of biotechnology-derived crops with nematode resistance that result in large yield benefits for producers as well as environmental benefits will be an important milestone for the discipline of molecular plant nematology and should accelerate further progress.

Resistance manipulation is not the only basis for developing nematode resistance plants. Other approaches can be devised using proteins with biological activity that may or may not be involved in natural plant defenses against pathogens. Approaches to control can be categorized as acting on targets within the nematode, at the nematode–plant interface, and in the plant response. Strategies aimed at nematode targets include disruption of the intestine by protease inhibitors or BT toxins, triggering of RNA interference (RNAi) to cause silencing of nematode genes, disruption of sensory function, and generation of nematicidal metabolites.

Methods to disrupt the nematode–plant interface include disrupting nematode parasitism gene products involved in migration or feeding site establishment, producing repellents, or converting the plant to a nonhost. Approaches acting through the plant response include expression of a plant resistance gene triggering a hypersensitive response, generation of gene products deleterious to the feeding site with specific promoters, and conversion of the plant from sensitive to tolerant. There is, of course, some overlap between these broad classifications and categories (e.g., nematicides can be repellents, and repellents can act via nematode sensory function), and some strategies could be placed in more than one class (e.g., nonhost status may be due to events at the nematode–plant interface or due to the plant response). Nevertheless, the classification scheme provides a useful way to organize information about the numerous approaches to nematode resistance.

Disruption of antifeedant/nematicidal proteins, disruption of essential nematode gene products with RNAi, disruption of sensory function, nematicidal metabolites, disruption of nematode pathogenicity factors, repellent or stealth plants, conversion of plant to nonhost, plant resistance gene/hypersensitive response activated by nematode invasion, feeding site-specific promoter inducing cell death or other site incompatibility, and conversion of plant to tolerance are some of the major approaches.

13.3.3 Targeting Feeding Cells Required by Nematodes

Much of the research for possible use of transgenic plants to control plant-parasitic nematodes has been concentrated to three genera: *Meloidogyne, Heterodera*, and *Globodera.* Although feeding sites of root-knot nematode giant cell complexes differ in many respects from those of cyst-forming nematodes (syncytia), the end function remains the same, i.e., to mediate the effective transfer of the nutrients from the plants to the feeding nematodes. The active involvement of the host's own genes in the production of giant cells or syncytia has been proposed. The molecular cloning of host genes and promoters involved in the formation of feeding sites could facilitate the production of antisense constructs to inhibit expression of key genes. A well-established basis for achieving this is to insert a part of gene expressing the genes or a part of gene expressing the targeted mRNA in a reversed direction, so providing antisense, RNA. The antisense RNA is thought to base pair with its target RNA forming double-stranded RNA. This duplex formation may inhibit mRNA maturation and/or translation, or it may lead to rapid mRNA degradation. Antisense RNA has now been used to regulate the expression of numerous plant genes including chalcone synthetase, polygalacturonase, and phosphinothricin acetyltransferase. In principle, careful selection and downregulation of the most appropriate host gene or genes may prevent the normal development of nematode feeding sites while minimizing adverse effects on the plants. Alternatively part of the gene may be introduced in the same orientation to achieve "co-suppression" of expression of the natural gene and introduced transgene.

These approaches result in lowered level of the sense mRNA and hence of the translated protein. A different approach is to express a phytotoxic gene product specifically in the feeding cells causing either specific cell death or limiting nematode feeding by attenuating the feeding cell. These need to be nematode responsive so that they don't drain on the plant sources unnecessarily. Some resistance to both *M. incognita* and *H. schachtii* has been reported using this approach.

13.3.4 Targeting the Nematode Directly

A number of targets for disruption via transgene products can be proposed on current knowledge of nematode physiology, biochemistry, and the interface with the host plant. *Meloidogyne* spp. invade primarily between cells rather than by more damaging intracellular route favored by cyst nematodes such as *Heterodera* spp. Hence, an effect or molecule active toward a target involved in the invasion process, such as locomotion or chemoreception, must be exported to the apoplast to facilitate control of Meloidogyne. The plant cells die soon after they are entered by a nematode such as *Heterodera* spp. *Globodera* spp., *Pratylenchus* spp., or *Radopholus* spp. presumably due to traumatic physical damage. The defense protein should be present at effective levels before invasion, thus making constitutive expression or a rapid local systemic response a necessity.

Another approach to designing nematicide plants is to transform plants with nematode genes that may disrupt nematode development if expressed in the feeding cells. Collagenase is one such possible molecule that could be utilized in this manner. Collagen is the major structural component of nematode cuticle which serves as the structural exoskeleton of the organism and also forms the stylet and lining of the esophagus and intestine. If genes encoding nematode collagenases could be isolated, they may be useful to design transgenic

plants. Collagenase expressing transgenic plants would then certainly disrupt the feeding and molting process at least of end parasitic nematodes. Alterations in the nematode collagen molecular structure too would give similar result.

13.3.5 Molecules for Transgenic Expression

A defense strategy that delivers a protein such as proteinase inhibitor with efficacy against nematodes has distinct advantages. Effect or molecules can be envisaged that are without phytotoxic effects. This enables promoters that provide expression in cells that are not attacked by nematodes to be of value. Also such a defense strategy may prove effective against a wide range of nematodes irrespective of their feeding type, and this has a number of commercial advantages as most crops are parasitized by more than one economically important nematodes.

13.3.6 Plantibodies

A plantibody is an antibody produced by genetically modified crops. Antibodies are part of animal immune systems and are produced in plants by transforming them with antibody genes from animals. Although plants do not naturally make antibodies, plantibodies have been shown to function in the same way as normal antibodies. One of the most remarkable aspects of recombinant DNA technology is when a protein belonging to the exclusive realm of animals can be successfully used in plants to help them fight against pathogens that are difficult to control (Fermin-Munoz 2000). The expression of nematode-specific antibodies *in planta*, and hence the term "plantibody," is a promising new avenue for controlling plant pathogens (Gibbs 1997). A strategy has been devised against the major phytonematode, *Meloidogyne* spp. (Baum et al. 1996).

Engineering resistance against various diseases and pests is hampered by the lack of suitable genes. Monoclonal antibodies will inhibit the biological activity of molecules that are essential for the pathogenesis. Schots et al. (1992) obtained resistance by transfecting plants with genes encoding monoclonal antibodies against pathogen-specific proteins. Potato cyst nematode was chosen as a model and it was thought that monoclonal antibodies are able to block the function of the saliva proteins of this parasite. These proteins are, among others, responsible for the induction of multinucleate transfer cells upon which the nematode feeds. It is well documented that the ability of antibodies to bind molecules is sufficient to inactivate the function of an antigen, and in view of the potential of animals to synthesize antibodies to almost any molecular structure, this strategy should be feasible for a wide range of diseases and pests. Antibodies have several desirable features with regard to protein engineering. The antibody (IgG) is a Y-shaped molecule, in which the domains forming the tips of the arms bind to antigen and those forming the stem are responsible for triggering effector functions (Fc fragments) that eliminate the antigen from the animal. Domains carrying the antigen-binding loops (Fv and Fab fragments) could be used separately from the Fc fragments without loss of affinity. The antigen-binding domains could also be endowed with new properties by fusing them to toxins or enzymes. Antibody engineering was also facilitated by the polymerase chain reaction. A systematic comparison of the nucleotide sequence of more than 100 antibodies revealed that not only the 3′ ends but also the 5′ ends of the antibody genes were relatively conserved. They designed a small set of primers with restriction sites for forced cloning, which allowed the amplification of genes encoding antibodies specific for the saliva proteins of *Globodera rostochiensis.* Complete heavy- and light-chain genes as well as single-chain Fv fragments (scFv), in which the variable parts of the light (VL) and heavy chain (VH) were linked by a peptide, were transferred to potato plants.

In root-knot nematodes, cellulases from *M. incognita* and other *Meloidogyne* species, in addition to other stylet-secreted proteins, have

been selected as targets for this strategy (Baum et al. 1996). Cellulases in plant-parasitic nematodes are important in the initial steps of pathogenesis. The rationale of selecting cellulases as the target of plantibodies is that upon contact between the anti-cellulase plantibody and the nematode cellulase, the migration of the nematode inside the plant will be stopped or diminished. If successful, this strategy will allow farmers to avoid using highly toxic nematicides, soil sterilants, or fumigants. Other useful targets under analysis are the proteins involved in the initiation of the cell cycle that leads to the generation of giant cells that support the feeding nematodes in infected roots (Vrain 1999).

The expression of specific intracellular antibodies in plants could confer resistance to particular plant pathogen including plant-parasitic nematodes by binding proteins that are essential for disease process. The expression of antibodies that are specific to nematode esophageal gland secretion may be an alternative route to engineering resistance. Monoclonal antibodies specific to esophageal gland products from *M. incognita* and *H. glycines* have been produced. Antibodies of 110 kDa with reactivity to secretory granules of *G. rostochiensis* have also been expressed in potato. The antibody should bind to a target of animal or plant origin in a way that disrupts parasitism. Antibodies such as these will aid our understanding of the nature and role of the glands and their products in specific host–parasite relationship, and expression of these antibodies in transgenic plants may also interfere with the development of feeding sites or disrupt normal feeding processes. Secretions of nematodes into plants are obvious targets limited only by difficulty of demonstrating antibody efficacy before plant transformation.

13.3.7 Proteinase Inhibitors

Proteinase inhibitors (Pls) are an important element of natural plant defense strategies. Protein inhibitors of a range of proteinase classes are widely expressed in plants where they are often induced by wounding and herbivory. They are already consumed in many plant foodstuffs, such as rice seeds, potato tubers, and cowpea. Toxicological studies have demonstrated the lack of harmful effects of serine Pls in mammalian systems.

The potential of plant PIs as anti-nematode effectors was first explored using the serine PI cowpea trypsin inhibitor (CpTI) (Fuller et al. 2008). The cowpea trypsin inhibitor, CpTI, expressed in transgenic potato influences the sexual fate of newly established *G. pallida* more toward becoming male, but doesn't reduce the fecundity of the females so formed. In contrast, CpTI reduces fecundity of females of *M. incognita* without influencing their fate. A modified cystatin (cystatins are protein inhibitors of cysteine proteinases) from rice, Oc-IAD86, limits growth of both *H. schachtii* and *M. incognita* when expressed in *Arabidopsis*. The effect of a single PI on members of two different principal groups of economically important nematodes is a demonstration of the potential of a broad range resistance strategy for control of nematode pests of a target crop.

Transgenic expression of the sweet potato serine PI, sporamin, inhibited growth and development of female *H. schachtii* parasitizing sugar beet hairy roots (Urwin et al. 2000). Here, the severity of the effect was clearly correlated to the level of trypsin inhibitory activity detected in the transformed root line. Inhibitory activity of a potato serine PI (PIN2) expressed in transgenic wheat also showed a positive correlation with plant growth and yield following infestation with *Heterodera avenae.* The potential of cysteine proteinase inhibitors (cystatins) to provide nematode resistance has been explored in more depth. Initially the efficacy of a rice cystatin (Oc-I) was enhanced by protein engineering, using crystallographic data, to produce a PI with greater inhibitory activity. Expression of the engineered variant (Oc-IΔD86) in root cultures conferred higher amounts of resistance against *G. pallida* than the unaltered molecule. In the first demonstration of a transgenic technology working against both major groups of economically important nematodes, *viz.,* root-knot and cyst nematodes, the modified cystatin was expressed

in transgenic *Arabidopsis* plants and uptake of the cystatin was correlated with loss of nematode cysteine proteinase activity. No females of either *H. schachtii* or *M. incognita* developed to egg production during growth on the cystatin-expressing plants. The same plants also showed resistance against *R. reniformis,* with higher levels of PI expression again correlated with reduced reproductive success. The number of female nematodes was reduced by 35 % and the fecundity of each by 69 %, corresponding to 81 % resistance in terms of reduced overall reproductive success, a common method of expressing nematode resistance.

Cystatins have also been used to protect other plant species against a range of nematodes with diverse feeding strategies. Rice plants expressing a low level of the OcIΔD86 cystatin under the control of a short CaMV35S promoter reduced reproductive success of *M. incognita* by 55 % (Fuller et al. 2008). Cavendish dessert bananas expressing the same cystatin from the maize ubiquitin promoter show 70 ± 10 % resistance to the migratory endoparasite *Radopholus similis* and similar technology also benefits East African Highland banana. The development of PI-mediated nematode resistance culminated in successful field trials of transgenic potatoes expressing a cystatin. The best transgenic lines of the fully susceptible potato cv. Desiree were shown to have commercially useful resistance (Urwin et al. 2002). Engineered potato plants in which the expression of the cystatin is limited mainly to the roots, and in particular the unique feeding structure induced by the nematode within the plant root, have been trialed. These plants were shown to have similar resistance to that achieved with constitutive expression for both *G. pallida* and *M. incognita.* The design of proteinase inhibitor-based biotechnology has been directed by information about the target nematode proteinases. cDNAs encoding serine and cysteine digestive proteinases have been cloned and their developmental expression profiles determined. Two cysteine proteases encoding cathepsin L-like enzymes have been isolated from feeding female *H. glycines* and homologues have been identified in *G. pallida* (Urwin et al. 2002) and *M. incognita.*

A peptide that disrupts chemoreception of nematodes without a lethal effect was reported by Green et al. (2012), which provided resistance to *G. pallida* both in a containment and a field trial, when precisely targeted under control of a root tip-specific promoter. DNA barcoding and quantitative PCR were combined to recognize nematode genera from soil samples without microscope-based observation and use the methods for nematode faunal analysis. Thus, they established that the peptide and a cysteine proteinase inhibitor that offer distinct bases for transgenic plant resistance to *G. pallida* do so without impact on the nontarget nematode soil community. Atkinson and Hepher (1996) developed a method of conferring nematode resistance on a plant, comprising modifying or transforming said plant to express a gene or other DNA coding for a proteinase inhibitor and selecting said modified or transformed plant which expresses said proteinase inhibitor such that said modified or transformed plant exhibits nematode resistance.

Proteinase inhibitors of various plants have been used to control the proteolysis of root-knot nematodes by inhibiting gut serine proteinases (Prasad et al. 2011). The 3D models of serine proteinases as well as proteinase inhibitors were generated using Modeller 9v8 and I-Tasser server. These models were testified using several validation methods including Procheck, What-IF, ProSA, Errat, and Verify-3D. All predicted structure validation score showed that geometric quality of the backbone conformation, residue interaction, residue contact, and energy profile of the structure were well within the limits accomplished for reliable structures. Protein–protein interacting surface patches were identified by PPi-Pred. Site-specific docking was performed by using ZDOCK server, and backbone refinement of protein complex was performed in FiberDock server. Protein complexes having minimum energy was again docked in using RosettaDock server. Docking output parameter like energy score, number of forming hydrogen bonds, Van der Waals interaction, and electrostatic potential, inferring that serine proteinase was inhibited by proteinase inhibitors. The energy

score, electrostatic potential map, key hot spot residues, and hydrogen bonding show that some serine proteinase inhibitors were potent inhibitor in comparison to others. This study has been further helpful in understanding the mechanism of proteolysis by inhibition of serine proteinase and selecting potent inhibitor for transgenic plant development.

13.3.8 Promoters

Well-defined synthetic plant promoters that direct controlled local gene expression in response to pathogens are available (Rushton et al. 2010). These promoters could be used to help define signaling pathways, to isolate novel mutants using "targeted genetics" (Hooley 1998), and to engineer plants with increased disease resistance. The control regions of plant genes are modular and contain a number of *cis*-acting elements, each of which may contribute to one or more aspects of a complex expression profile. One strategy to overcome this complexity is to produce synthetic promoters containing only defined individual elements, thereby reducing expression profile complexity. However, although there are numerous reports of synthetic promoters being inducible by elicitors in transient expression systems, in most cases, it is not known to what extent individual *cis*-acting elements retain their functionality in planta when removed from their native promoter context and whether we can use these individual "modules" to make synthetic promoters that direct a desired expression pattern.

13.3.8.1 Constitutive Expression Promoters

Most information on this type of promoter has been with respect to the cauliflower mosaic virus, CaMV35S. Goddijn et al. (1993) used the enzyme B-glucuronidase (GUS) as a reporter gene to study downregulation of a version of CaMV35S promoter following root invasion by *M. incognita* and *H. schachtii*. It was found that there was progressive downregulation of CaMMMV35S in the syncytium induced by *H. schachtii* well after the final molt for females. Consequently the effect does not prevent the feeding cell from providing sufficient proteinase inhibitor to disrupt the growth of *H. schachtii* in prototype transgenic resistance. CaMV35S provides lower levels of expression on monocotyledons than in dicotyledons. However, it has been shown to have sufficient activity in rice to detect efficacy of a PI against *M. incognita*. Increased constitutive expression can be obtained in monocotyledons using other promoters such as that from maize ubiquitin gene. The principal advantage of constitutive promoters such as CaMMMMMMV35S and ubiquitin for both monocots and dicots is that they have been well characterized and provide a high degree of expression. In addition, they allow the expression to be detected using tissues collected from sites away from nematode feeding. The main disadvantage is that they ensure the effector molecule is expressed in the yield of the transgenic crop. This may limit the commercial acceptability of the crop.

13.3.8.2 Root-Specific Expression Promoters

Roots are rarely of commercial value. Promoters that express only in roots protect this part of the plant from nematodes and avoid concerns over expression in the yield. Several root-specific promoters have been patented by Liley and Atkinson (1997). A gene with root-specific expression in tobacco, TobRB7, has been proposed to encode a putative water channel protein. It shows substantially specific expression at root meristems in healthy plants, but it also remains active in the giant cells induced by *Meloidogyne*. Field trials of a transgenic root-knot nematode-resistant tobacco plant having tobacco root-specific gene TobRB7 promoter nematode-responsive element showed substantial reduction in galling and egg production.

13.3.8.3 Wound-Response Promoters

Promoter wun-1 responds to invasion by *G. rostochiensis* but not *M. incognita*. The invading second-stage juvenile (J2) of *G. pallida* induces expression in potato roots that extends in a locally systemic manner up to several cells from site of the invading nematode. It is not

activated by invasion by *M. incognita* presumably because this species does not cut plant cell walls during its intercellular invasion. Promoters that respond to nematode attack may be of value for directing defenses against nematodes that migrate intracellularly. If so, expression of the transgenic defense in cells ahead of the nematode may be necessary to ensure an effective expression level prevails before the cell is penetrated and killed. These promoters have particular value against the nematodes like *Pratylenchus* and *Radopholus*.

13.4 Nematode Ecdysteroids

Ecdysteroids are polyhydroxylated AT-6-ketosteroids with complete side chains, i.e., polyhydroxylated derivatives of cholesterol containing a C-7 double bond and a keto-group at C-6, and are involved in hormonal regulation of molting and other developmental processes. Ecdysteroids regulate molting in insects and hence a good amount of work has been carried out on these compounds. Molting in nematodes is regulated by ecdysteroids and juvenile hormones; effort has been placed on isolating these and similar compounds from nematodes. They occur in very low concentration in nematodes compared to insects. Cholesterol is the major sterol of the few vertebrate-parasitic nematodes analyzed thus far (Barrett et al. 1970). Significant amounts of sitosterol, campesterol (24~–methylcholesterol), cholestanol, stigmastanol (24a-ethylcholestanol), and campestanol occur in *Ascaridia galli* and *Ascaris suum*. The presence of 24 alkylsterols in these digestive tract parasites is probably due to occurrence in the host diet. Several attempts have been made to isolate certain compounds from other animals which behave as hormones. Effects of ecdysteroids on various activities of nematodes have been documented (Table 13.2).

Among phytoparasitic nematodes, the only sterols detected in *Ditylenchus triformis* and *D. dipsaci* were cholesterol and lathosterol (cholest-7-enol), except for traces of phytosterols in the latter species (Cole and Krusberg 1967). Their hosts contained only 24 alkylsterols. Curiously, the sedentary plant parasites examined to date contain greater relative proportions of phytosterols than the migratory *Ditylenchus* spp. It is not known whether the more highly evolved parasitism of the sedentary plant parasites has included an adaptation of these organisms to utilize or store substantial amounts of plant sterols. The relative lack of stanols in *Heterodera zeae* compared to *Globodera solanacearum* (Orcutt et al. 1978) is another interesting difference among

Table 13.2 Effect of ecdysteroids on nematodes

Dose	Effect	Nematode
0.01 M	Supernumerary molting	*Heterodera schachtii*
3.5×10^{-4} M	Egg hatch inhibited	*Heterodera glycines*
Precocene II	Toxic	*Caenorhabditis elegans*
100 μg/ml	Toxic	*Steinernema feltiae*
50 μg/ml	Inhibit development	*C. briggsae*
0.1 μg/ml	Retarded growth	Free-living nematode, *Cephalobus* sp.
50 μg/ml	Inhibited growth and reproduction	*C. elegans*
0.01 ng/ml	Stimulated molting	*Nematospiroides dubius*
0.05 ng/ml	Increased length	Animal parasite, *Ascaris suum*
464 μg/ml	Inhibited development	Trichostrongylid, *Haemonchus contortus*
15 μg/ml	Decreased length, promoted molting	Animal parasite, *Trichinella spiralis*
480 μg/ml	Inhibited development	*Haemonchus contortus*
0.05 ng/ml	Increased length	Animal parasite, *Ascaris suum*
5 ng/ml	Promoted molting	*A. suum*

plant-parasitic nematodes. The possibility that the two major genera of cyst nematodes have characteristically different sterol compositions has interesting phylogenetic ramifications.

Initially, steroids may appear to be complex, but they differ from each other in only a few ways: the presence of a side chain on the tetracyclic ring system (or steroid nucleus) and the presence and location of double bonds and methyl, ethyl, keto, or hydroxyl substituents. Systematic nomenclature of steroids is based on the structure of cholestane. Steroids with a complete side chain and a single hydroxyl group are called sterols; they function primarily as integral structural components of cell membranes. Most sterols contain double bonds, usually at C-5 (a A~– bond); however, some sterols do not contain a double bond, and they are referred to as stanols (e.g., cholestanol).

A number of 4-desmethylsterols with a *trans-A/B* ring configuration can satisfy the steroid nutritional requirement in *C. elegans,* but sterols with a *cis-A/B* ring configuration or *trans-A/B* sterols with a 4-methyl group cannot (Chitwood et al. 1986). *C. elegans* removes methyl or ethyl substituents at C-24 of the plant sterols sitosterol, campesterol, stigmasterol, stigmastanol, and 24-methylenecholesterol to produce various sterols with structures partially dependent upon that of the dietary sterol. Additional metabolic steps in *C. elegans* include reduction of A *~– and An-bonds, C-7 dehydrogenation, isomerization of an AT-bond to a As0*-bond, and 4a-methylation. An azasteroid and several long-chain alkylamines interfere with the dealkylation pathway in *C. elegans* by inhibiting the 2-sterol reductase; these compounds also inhibit growth and reproduction in various phytoparasitic and animal-parasitic nematodes. Sterols also serve as metabolic precursors to steroid hormones, which in vertebrates typically lack a side chain (e.g., testosterone, estrone).

An azasteroid 25-azacoprostane was located by Bottjer et al. (1984) in *C. briggsae* and *P. redivivus,* who observed the inhibition of development and reproductive ability in these worms. Nematodes treated with azasteroid possessed morphological abnormalities, and addition of cholesterol to the medium reversed the inhibition due to azacoprostane in the development in *N. dubius, N. brasiliensis*, and *C. briggsae* but not *P. redivivus.* Compounds like N,N-dimethyldodecanamine are toxic or inhibitory to nematodes like *M. incognita, Bursaphelenchus xylophilus, C. elegans, P. redivivus*, and *Ostertagia ostertagi*. Various abnormalities including reduced survival, motility, egg production, and less emergence of adults were observed in *Ostertagia ostertagi* (Lozano et al. 1985). Many long-chained alkylamines have been shown to inhibit sitosterol dealkylation in nematodes like *C. elegans*, which might alter sites of nematode steroid metabolism, as it happens in insects.

The occurrence of ecdysteroids in nematodes, albeit at low concentrations, has been firmly established (Barker and Rees 1990). In addition to apparently stimulating molting in a few species, exogenously applied ecdysteroids have been shown to have interesting biological effects on meiotic reinitiation in oocytes and on microfilarial production in filariae, although such effects demonstrate the feasibility of influencing nematode physiology with exogenously applied ecdysteroids. However, it remains to be confirmed whether ecdysteroids are truly endogenous nematode hormones or merely represent compounds with strong biological activity. Nonetheless, there are indications that interference with the ecdysteroid system might be exploitable in the development of novel approaches to control of nematodes.

Compared to other organisms, nematodes have been the subject of relatively few investigations into their steroid biochemistry (Chitwood 1999). Nutritional experiments have clearly demonstrated a dietary requirement for sterol that results from the inability of nematodes to biosynthesize steroids de novo. Although the specificity of the nutritional requirement varies somewhat among nematodes, most 4-desmethylsterols can be directly utilized by nematodes or else metabolized to sterols better suited for nematode growth and development. Free and esterified sterols of eggs of the root-knot nematode, *Meloidogyne incognita* races 2 and 3 and *M. arenaria* race 1, were isolated and identified by gas–liquid

chromatography–mass spectrometry (Chitwood et al. 1987). The major sterols of eggs of each race were 24-ethylcholesterol (33.4–38.8 % of total sterol), 24-ethylcholestanol (18.3–25.3 %), 24-methylcholesterol (8.6–11.7 %), 24-methylcholestanol (7.7–12.5 %), and cholesterol (4.6–11.6 %). Consequently, the major metabolic transformation performed by *Meloidogyne* females or eggs upon host sterols appeared to be saturation of the sterol nucleus.

A phytoecdysteroid, 20-hydroxyecdysone (20 E) was traced by Soriano et al. (2004), which is a major molting hormone in nematodes. The effects of direct application on nematodes were assessed by treating *Heterodera avenae* juveniles with concentrations of 20E from 8.2×10^{-8} to 5.2×10^{-5} M before applying to *Triticum aestivum* growing in sand. *H. avenae*, *Heterodera schachtii* (sugar beet cyst nematode), *Meloidogyne javanica*, and *Pratylenchus neglectus* were treated with 5.2×10^{-5} 20E and incubated in moist sand. To test the protective effects of 20E in plants, the latter three nematodes were applied to *Spinacia oleracea* in which elevated concentrations of 20E had been induced by methyl jasmonate. Abnormal molting, immobility, reduced invasion, impaired development, and death occurred in nematodes exposed to 20E either directly at concentration above 4.2×10^{-7} M or in plants. Phytoecdysteroid was found to protect spinach from plant-parasitic nematodes and may confer a mechanism.

The animal-parasitic nematodes, such as *Haemonchus contortus*, *Ascaris suum*, and *Trichinella spiralis*, were found to contain compounds similar to the ecdysteroids (Hitcho and Thorson 1971). The occurrence of ecdysones 20-hydroxyecdysone and 20, 26-dihydroxyecdysone in the dog heartworm, *Dirofilaria immitis*, was established with high-performance liquid chromatography (HPLC) in conjugation with RIA. The ecdysone performs particular function in nematodes at a specific dose; if there is an alteration in the dose, then it will have negative effect on the nematodes. The azasteroids and long-chained alkylamines possess the potential for development of certain compounds which may come in the way of life cycle of phytonematodes through novel site of action, i.e., nematode steroid metabolism.

13.5 Inhibition of Sterol Metabolism

Specific information about the hormone-related events in nematode has been obtained through the basic studies of nematode sterol metabolism. Structurally sterols contain a tetracyclic ring system, a hydroxyl group at C-3, and an aliphatic side chain. Sterols are major components of cell membrane and metabolic precursor to steroid hormones and many other important compounds (Ness and Mackean 1977). Nematodes possess a nutritional requirement for sterols that results from their inability to biosynthesize sterols. The interference with uptake, metabolism, or utilization of sterols by the nematodes offers a means for disruption of membrane function or hormonal regulation (Table 13.3). Typical animal sterols, such as cholesterol, are present in plants (sitosterol or 24 α-ethyl cholesterol and campesterol or 24 α-methyl cholesterol). Azasteroids and related nonsteroidal amines have been shown to dramatically affect sterol metabolism in *C. elegans*.

An analogous series of dimethylalkyl compounds, consisting of four amines, an amide, and a phosphonate ester, inhibited motility and reproduction of *Caenorhabditis elegans*. Dimethylamines with straight-chain lengths of 12, 14, or 16 carbon atoms were equally active nematicides, causing greater than 80 % population growth inhibition at a concentration of 25 ppm (Lozano et al. 1984). The C12 straight-chain amine and its corresponding amide produced similar inhibition and were much more potent than either the corresponding C12 phosphonate or a C12 branched-chain amine. Inhibition of the delta 24-sterol reductase system was exhibited by all four amines, but not by the amide or phosphonate, in the following order of activity: C12 branched-chain amine greater than C12

Table 13.3 Effect of different sterol inhibitors on nematodes

Dose	Effect	Nematode
5 μg/ml (azasteroid)	Inhibited reproduction	*C. elegans*
1 μg/ml	Toxic	*C. elegans*, *Meloidogyne* spp.

straight-chain amine greater than C14 amine greater than C16 amine. The C12 branched amine also blocked the C-24(28)-dehydrogenase system in the conversion of sitosterol to fucosterol, the initial step in sitosterol dealkylation.

Nematode cells may not possess a specific requirement for sterol as a structural component of membranes, except for cells needed to produce regulatory metabolites of sterols, such as steroid hormones (Silberkang et al. 1983). Perhaps this lack of a major structural role for sterols in nematode membranes is reflected in the small quantities of sterol (0.01–0.06 % of dry weight) in many phytoparasitic nematode species.

Steroid metabolism in insects and nematodes presents a unique area of biochemical differences from vertebrates and plants, since neither insects nor nematodes are able to biosynthesize the steroid nucleus (Svoboda and Chitwood 1992). Both insects and nematodes rely on dietary sources of sterol for normal growth and development and are capable of considerably altering their dietary sterols, particularly C_{28} and C_{29} phytosterols, to produce cholesterol as well as a large number of other sterols. Further, cholesterol is essential for the production of molting hormones (ecdysteroids) in most insects. Thus, the disruption of uptake, transport, or metabolism of dietary sterols in insects or nematodes could be fatal to the organism and could lead to new, specific control technology. A variety of compounds have been developed that effectively inhibit sterol metabolism in insects and nematodes and demonstrate that this is a vulnerable area of biochemistry in these organisms to exploit.

Inhibition of motility and reproduction of *C. elegans* were observed by an analogous series of dimethylalkyl compounds, consisting of 4 amines, an amide, and a phosphonate ester (Patterson 1984). Dimethylamines with straight-chain lengths of 12, 14, or 16C atoms were equally active nematicides, causing greater than 80 % population growth inhibition at a concentration of 25 ppm. The C12 straight-chain amine and its corresponding amide produced similar inhibition and were much more potent than either the corresponding C12 phosphonate or a C12 branched-chain amine. Inhibition of the delta 24-sterol reductase system was exhibited by all 4 amines, but not by the amide or phosphonate, in the following order of activity: C12 branched-chain amine > C12 straight-chain amine > C14 amine > C16 amine. The C12 branched amine also blocked the C-24(28)-dehydrogenase system in the conversion of sitosterol to fucosterol, the initial step in sitosterol dealkylation.

13.6 Phytoecdysteroid

As nematodes are placed in a clade of molting metazoans, the Ecdysozoa, it is likely they all have similar hormonal regulation of ecdysis (Aguinaldo et al. 1997). Since ecdysteroids possess biological activity in free-living and animal nematodes, phytoecdysteroids may also provide an important plant defense against nematodes and, thus, may serve as a basis for the development of resistant cultivars.

The phytoecdysteroid 20-hydroxyecdysone (20E) is a major molting hormone of invertebrates, possibly including nematodes (Soriano et al. 2004). As 20E is inducible in spinach, the defensive role against plant-parasitic nematodes was investigated. The effects of direct application on nematodes were assessed by treating cereal cyst nematode, *Heterodera avenae*, juveniles with concentrations of 20E from 8.2×10^{-8} to 5.2×10^{-5} M before applying to *Triticum aestivum* growing in sand. *H. avenae*, *H. schachtii*, *M. javanica*, and *Pratylenchus neglectus* were treated with 5.2×10^{-5} 20E and incubated in moist sand. To test the protective effects of 20E in plants, the latter three nematodes were applied to *Spinacia oleracea* in which elevated concentrations of 20E had been induced by methyl jasmonate. Abnormal molting, immobility, reduced invasion, impaired development, and death occurred in nematodes exposed to 20E either directly at concentration above 4.2×10^{-7} M or in plants. Phytoecdysteroid was found to protect spinach from phytonematodes and may confer a mechanism for nematode resistance.

13.7 Juvenile Hormone (JH) in Nematode Management

The insect juvenile hormones epoxy farnesoic acid methyl ester derivatives are known to possess many bioregulatory roles and also involved in inhibiting/affecting various activities in nematodes (Table 13.4). The extracts of juveniles of *H. contortus* were found to contain JH activity (Rogers 1973), compared to the ecdysteroids, a greater number of JH and analogs have been evaluated for bioactivity toward nematodes and have been found to inhibit nematode development to a greater extent than the ecdysteroids. Fodor et al. (1982) observed a high adult mortality due to precocene II and a drastic growth reduction of growing *Caenorhabditis elegans* larvae. Symptoms caused by precocene II were partly reversible by the insect juvenile hormone analog methoprene, suggesting a physiological role of juvenile hormones in nematodes.

These compounds are known to possess many other bioregulatory roles. Rogers found extracts of juveniles of *H. contortus* to contain JH activity in an insect bioassay. Failure to detect radiolabeled farnesol, an intermediate in JH biosynthesis in insects, in adult *B. pahangi* and *D. immitis* fed [^{14}C] mevalonate suggested that adult filarial worms cannot synthesize JH. Compared to ecdysteroids, a greater number of JH and JH analogs have been evaluated for bioactivity toward nematodes and have been found generally to inhibit nematode development to a greater extent than ecdysteroids (Chitwood 1987). High (0.01 M) concentrations *of* farnesyl diethylamine *or* farnesyl methyl ether resulted in supernumerary molting and prodigious gonadal enlargement in *H. schachtii*; the latter compound at 0.0001 M inhibited molting in *T. spiralis* juveniles and at 10^{-7} M retarded formation of male copulatory appendages.

In C. *elegans,* 7 of 10 JH or JH analogs inhibited growth and reproduction at 25–50 mg/ml. Four JH or analogs inhibited development *of* *C. briggsae* at 50 mg/ml and were directly toxic to the insect associate *Steinernema feltiae* at 20–100 mg/ml. Farnesyl methyl ether and JH-I inhibited ecdysis in *P. decipiens* at 10^{-6} M when added to medium in which molting normally occurs but stimulated ecdysis normally in non-molt-promoting saline solution when added 2.5 days after the onset of incubation in *H. contortus*; JH-I and several analogs inhibited egg hatch at 0.001 M and JH-I also inhibited the final molt in *N. dubius* at 3.3×10^{-11} M. At 10–100 mg/ml, JH-3 and the majority of 22 related juvenoids were directly toxic and/or inhibited molting in *H. contortus*-infective juveniles. Finally, six of seven JH or analogs inhibited *N. brasiliensis* egg production in vivo following exposure of juveniles to 2×10^{-4} M; at 5.6×10^{-4} M, several retarded development in *X. brasiliensis* juveniles in vitro; and several at 3.5×10^{-4} M inhibited egg hatch in the soybean cyst nematode, *Heterodera glycines*. Precocene II, which disrupts JH biosynthesis in insects, was toxic to C. *elegans* at 100 mg/ml; effects were partially reversed by application of the JH analog methoprene. The previously described cautions in interpretation of effects *of exogenous ecdysteroids* are equally appropriate to JH. Because JH from nematodes *have been* chemically

Table 13.4 Effect of juvenile hormone on nematodes

Dose	Effect	Nematode
0.01 M	Supernumerary molting	*Heterodera schachtii*
0.0001 M	Inhibited molting	Animal parasite, *Trichinella spiralis*
10^{-7} M	Retarded formation of male copulatory organ	Animal parasite, *Trichinella spiralis*
50 μg/ml	Inhibited growth and reproduction	*Caenorhabditis elegans*
50 μg/ml	Inhibit development	*C. briggsae*
100 μg/ml	Toxic	*Steinernema feltiae*
Precocene II	Toxic	*C. elegans*
3.5×10^{-4} M	Egg hatch inhibited	*Heterodera glycines*

characterized only by bioassay and TLC and because of the nonspecificity of many of the described effects, hormonal activity of juvenoids in nematodes has not yet been established. The toxicity or inhibition of development of many JH or JH analogs indicates the potential for these compounds as potent inhibitors of nematode growth and development.

13.8 Vertebrate Steroid Hormones

Vertebrate steroid hormones are metabolites of cholesterol lacking the sterol side chain present in ecdysteroids and cholesterol. These vertebrate hormones and ecdysteroids are produced from the cholesterol by the two divergent metabolic pathways with no interconnections. These hormones have been reported to affect nematodes in some ways (Table 13.5). A few reports of mammalian hormones in nematodes have appeared, such as identification of progesterone in *P. redivivus* (Willet 1980). One of the few major biochemical differences between vertebrate and phytonematodes and their hosts is that nematodes are incapable of de novo sterol biosynthesis and hence possess a nutritional requirement for sterol. Cholesterol and lathosterol are the major nematode sterols, whereas the plant tissue sterols are 24-ethylcholesta-7, 22-dienol, and 24-ethyllathosterol. Similarly, because *Rotylenchulus reniformis* contained 24-ethylcholesterol and cholesterol as its major sterols but host roots (cotton) contained 24-ethylcholesterol and 24-ethylcholesta-5, 22-dienol as the major sterols, *R. reniformis* dealkylates plant sterols at C-24 to produce cholesterol (Chitwood 1999). Proteins that bind testosterone, estradiol, and progesterone have been identified in *T. colubriformis* and *N. brasiliensis*. In the latter species, several steroid hormones or analogs inhibited the binding.

Table 13.5 Effect of vertebrate steroid hormones on nematodes

Dose	Effect	Nematode
0.05 ng/ml	Promoted growth	*Ascaris suum*
25 μg/ml	Inhibited reproduction	*C. elegans*
16 μg/ml (cortisol)	Stimulated molting	*Trichinella spiralis*
0.95 ng/ml (estradiol)	Inhibited molting	*N. nubius*

Progesterone, testosterone, estrone, and estriol were identified with GC-MS and nuclear magnetic resonance spectrometry (NMR) in samples consisting of mixed sexes of an intestinal parasite from goats, *Trichostrongylus colubriformis* (Chung et al. 1986). Because the amounts of progesterone and testosterone isolated from nematodes were correlated with the sex of the host, the suggestion was that the nematode obtained the steroids from its host. Although host intestinal contents were devoid of the four steroids, radiolabeled cholesterol was not converted to them by nematode homogenate supernatants. The supernatants did hydroxylate progesterone. In a related study, progesterone, testosterone, and estrone were identified in *Turbatrix aceti* by GC-MS and NMR. Proteins that bind testosterone, estradiol, and progesterone have been identified in *T. colubriformis* and *N. brasiliensis.*

13.9 DAPG (2,4-Diacetylphloroglucinol)

The antibiotic 2,4-diacetylphloroglucinol (DAPG) is produced by some isolates of the beneficial bacterium *Pseudomonas fluorescens*. DAPG is toxic to many organisms, and crop yield increases have been reported after application of DAPG-producing *P. fluorescens.* Production of the antibiotic 2,4 diacetylphloroglucinol contributes to biological control activity of many beneficial strains of the bacterium *Pseudomonas fluorescens*. However, studies by Meyer et al. (2009) indicated that DAPG is not toxic to all nematodes and did not affect the tested species of beneficial bacterial-feeding nematodes. Augmentation of DAPG-producing *P. fluorescens* populations for nematode biocontrol could be targeted to specific nematode species known to be affected by this compound and by other antibiotics produced by the bacteria, or these bacteria could be used for other possible effects, such as induced plant resistance.

DAPG is active against numerous organisms, including plants, fungi, viruses, bacteria, and nematodes, and production or increased production of DAPG has been associated with enhanced activity against plant pathogens. This study was conducted to determine whether DAPG is toxic to selected nematodes. The plant-parasitic nematodes *Heterodera glycines, Meloidogyne incognita, Pratylenchus scribneri*, and *Xiphinema americanum* and the bacterial-feeding nematodes *Caenorhabditis elegans, Pristionchus pacificus,* and *Rhabditis rainai* were immersed in concentrations ranging from 0 to 100 mg/ml DAPG. Egg hatch and viability of juveniles and adults were determined. DAPG was toxic to *X. americanum* adults, with an LD50 of 8.3 mg/ml DAPG. DAPG decreased *M. incognita* egg hatch, but stimulated *C. elegans* hatch during the first hours of incubation. Viability of *M. incognita* J2 and of *C. elegans* J1 and adults was not affected. There were no observed effects on the other nematodes. The study indicated that DAPG is not toxic to all nematodes and did not affect the tested species of beneficial bacterial-feeding nematodes. Augmentation of DAPG-producing *P. fluorescens* populations for nematode biocontrol could be targeted to specific nematode species known to be affected by this compound and by other antibiotics produced by the bacteria, or these bacteria could be used for other possible effects, such as induced plant resistance.

Pseudomonas fluorescens F113, which produces 2, 4-diacetylphloroglucinol, was investigated as a potential biocontrol agent against *G. rostochiensis* (Cronin et al. 1997). Exposure of nematode cysts to the pseudomonad, under in vitro conditions or in soil microcosms, almost doubled the ability of the eggs to hatch. The percentage of mobile juveniles was reduced threefold following their incubation in the presence of the pseudomonad, both in vitro and in soil. Results obtained with a transposon-induced DAPG-negative biosynthetic mutant of F113 and its complemented derivative with restored DAPG synthesis showed that the ability of strain F113 to produce DAPG was responsible for the increase in hatch ability and the reduction in juvenile mobility. Similar effects on egg hatch ability and juvenile mobility of *G. rostochiensis* were obtained in vitro by incubating nematode cysts and juveniles, respectively, in the presence of synthetic DAPG. DAPG-producing *P. fluorescens* F113 is proposed as a potential biocontrol inoculant for the protection of potato crops against the potato cyst nematode.

13.10 Avermectins

The avermectins are a series of 16-membered macrocyclic lactone derivatives with potent anthelmintic and insecticidal properties. These naturally occurring compounds are generated as fermentation products by *Streptomyces avermitilis*, a soil actinomycete. Eight different avermectins were isolated in 4 pairs of homologue compounds, with a major (a-component) and minor (b-component) component usually in ratios of 80:20 to 90:10.

Other anthelmintics derived from the avermectins include ivermectin, selamectin, doramectin, and abamectin. Ivermectin (22, 23-dihydroavermectin B_{1a}+22, 23-dihydroavermectin B_{1b}) is a broad-spectrum antiparasitic avermectin. Abamectin is a mixture of avermectins containing more than 80 % avermectin B1a and less than 20 avermectin B1b. It is widely used as a nematicide.

13.10.1 Mechanism of Action

The avermectins block the transmittance of electrical activity in nerves and muscle cells by stimulating the release and binding of gamma-aminobutyric acid (GABA) at nerve endings. This causes an influx of chloride ions into the cells leading to hyperpolarization and subsequent paralysis of the neuromuscular systems. GABAergic receptors are found at the neuromuscular junctions and the central ventral cords in nematodes, whereas in mammals they are found primarily in the brain. Ivermectin does not readily cross the blood–brain barrier in mammals at therapeutic doses. The efficacy of avermectins in the control of *Meloidogyne incognita* infesting tomato is by maximizing their low water solubility and short residual time in the soil (Garabedian and Van Grundy 1983).

The effectiveness of abamectin and emamectin benzoate in combination with a surfactant was proved by Jansson and Rabatin (1997), when applied as a root dip and as an injection into the pseudostem for controlling *Radopholus similis* on banana. However, avermectin therapy is not without its drawbacks. Resistance to avermectins has been reported, which suggests use in moderation. Research on ivermectin, piperazine, and dichlorvos in combinations also shows potential for toxicity. Avermectin has been reported to block LPS-induced secretion of tumor necrosis factor, nitric oxide, prostaglandin E2, and an increase of intracellular concentration of CO_2.

In recent years, environmental and economic considerations have led to a search for more effective inexpensive and safe compounds. In this context, avermectins have been proved to be nematicidal and to be effective in reducing the nematode population both in soil and root. But the disadvantages of these compounds are low water solubility and prone to rapid decomposition by soil microorganisms. But this will not affect its efficacy, and because of its rapid decomposition, it will not contaminate the soil environment and groundwater. Biotechnology has a great role to play in many present nematode management programs involving classical breeding for plant resistance to nematodes. A number of powerful DNA-based approaches to the identification and quantification of genetic variation have been developed and successfully adapted in nematology.

A laser-capture microscopy was developed (Taylor et al. 2008); cells from both the nematode and the nematode-induced feeding site were identified, captured, and examined using bioinformatics for possible new gene targets. These gene targets were used to design gene-specific inhibitors that inhibit important biological functions in the invading nematode or prevent the nematode from organizing and obtaining nutrients from the feeding site. Through a more refined approach to gene discovery, new methods of nematode management have been developed that are nematode specific, exhibit low phytotoxicity, and are environmentally benign.

13.11 Lectins

Lectins simply are “all plant proteins possessing at least one non-catalytic domain, which binds reversibly to a specific mono- or oligosaccharide.” This definition is far less restrictive than all previous ones and comprises a broad group of active glycoconjugate-binding proteins, including fusion proteins, buildup of a carbohydrate-binding domain, randomly arrayed with a catalytic domain. Plant lectins are proteins that possess at least one non-catalytic domain that binds reversibly to a specific mono- or oligosaccharide. In plants, seven different classes of lectins are defined according to their structure and evolution. They function mainly in the storage of nitrogen and in recognition processes. Some plant lectins are involved in defense mechanisms against insects (Peumans and Van Damme 1995).

Lectins have been used, mainly to characterize sugar moieties on nematode surfaces or in secretions via binding experiments. They have been shown to bind to several sedentary parasitic nematodes at the excretion pore, the cuticle, the amphids, or the head region in general, as well as at the spicules and the vulva (Aumann et al. 1991). The behavior of *Radopholus similis* and *Pratylenchus coffeae* was affected by lectins or lectin-related proteins, *viz*., egg hatching, chemotaxis, infection, and reproduction of adult females and the mobility of the nematodes at stages intermediate to these crucial steps (Peumans and Van Damme 1995).

The lectin may exert its effect either through interaction with glycoproteins present in the intestine or through binding to glycoproteins present externally on the nematode (Fuller et al. 2008). Interaction with glycoproteins associated with chemoreception, on the amphid sensory organs themselves or in their secretions, could disrupt sensory perception and compromise the nematode’s ability to establish feeding sites. The present experimental evidence would seem to support this hypothesis. *M. incognita*, for example, feeds only once it is sedentary inside plant roots, yet the application of Con A as a soil drench resulted in a reduction in the amount of galling on tomato roots (Marban-Mendoza et al. 1987).

Binding sites for Con A were found to be associated with the anterior amphids of the mammalian-parasitic nematode *Strongyloides ratti*, and disruption of its chemokinetic response occurs in the presence of lectins. Glycoproteins present on the amphids may have important roles as chemosensors.

Transgenic expression of lectins has some potential as an anti-nematode defense. The broad-spectrum activity of lectins such as GNA against a variety of phytopathogens makes their agronomic application appealing. The detectable amount of lectin expressed does not correlate with the degree of pathogen resistance, complicating the selection of lines for assay. There is also evidence that the protective effect of lectins does not apply to all nematodes. In vitro bioassays using root segments showed that treatment with a number of lectins, including Con A and soybean agglutinin, enhanced the penetration of the burrowing nematode *Radopholus citrophilus* (Kaplan and Davis 1991). This could have obvious detrimental effects in an agricultural environment where complex populations of nematodes are present.

Lectins have the potential to act as anti-nematode proteins in strategies to control both sedentary and migratory endoparasitic nematodes (Wuyts et al. 2001). It has been shown in the past that lectins bind to the surface of sedentary nematodes. They studied the binding of three types of lectins, concanavalin A of *Canavalia ensiformis*, wheat germ agglutinin of *Triticum aestivum*, and *Helix pomatia* lectin, to the surface of the migratory endoparasite *Radopholus similis*, which is the most important and damaging nematode parasite of banana. Binding occurred in the head region, at the excretion pore, the pores of the reproduction system, and those of the phasmids. These results indicate an interaction between lectins and *R. similis* that could have toxic effects or inhibit chemoreception of host root signals. It was also shown that the secretions of *R. similis* can be stained and visualized with the protein-specific dye Coomassie Brilliant Blue R. Secretions appear at the amphids, the excretion pore, the vulva, the spicules, and the phasmids. This technique can be applied in experiments to test the effect of lectins on the production of secretions by *R. similis* and other migratory and sedentary nematodes.

Lectins can block recognition of chemotactic factors by nematodes and thereby modify responses to chemoattractants (Marban-Mendoza et al. 1987). A temporary inhibition of attraction of the nematode to the filtrates was observed following exposure to concanavalin A (Con A) and limulin (LPA) and two enzymes, mannosidase and neuraminidase. The lectins or enzymes did not affect nematode development or locomotion at the concentrations applied, thereby supporting the premise that the effects were due to binding to, or to conformational changes of, nematode chemoreceptors which mediate the recognition function. In a similar approach, Bottjer et al. (1985) found a significant inhibition of the feeding stimulus of the animal-parasitic nematode, *Trichostrongylus colubriformis*, following exposure to the glucose–mannose-specific lectin *Lens culinaris* agglutinin and concluded that this effect was due to lectin binding to nematode receptors.

In contrast to the previously mentioned anti-nematode proteins, lectins are readily available and relatively easily applicable on a laboratory scale. They can be used in control strategies at two levels: expression inside the host root and expression and secretion into the plant's rhizosphere. It is assumed that lectins, by analogy with their effect in insects, have an intestine-associated activity in nematodes: the permeability of the digestive tract increases after ingestion of lectins. This has not previously been tested in vitro because of the lack of artificial diets for phytophagous nematodes. Some lectins disrupt orientation of nematodes presumably by binding to glycoconjugates associated with chemoreceptor, for example, concanavalin a, which has been reported to suppress *M. incognita* when used as a soil amendment in a tomato crop. To date transgenic plants expressing two lectins have been used to challenge nematodes. Pea lectin (a-mannosides) expressed transgenically in potato didn't influence either invasion or growth of *G. pallida* to the same extent as CpTI. Snowdrop lectin (a-mannosides) gave a variable effect on *Pratylenchus neglectus* and no effect on *H. schachtii.*

13.12 Endotoxins of *Bacillus thuringiensis* (Bt)

The effects of *Bacillus thuringiensis* as a nematicidal biocontrol agent have been investigated for free-living nematodes, animal-parasitic nematodes, insect-parasitic nematodes, and phytonematodes. The strains *Bt. israelensis, Bt. kurstaki*, and *Bt. morrisoni* have shown considerable variability with respect to lethality for animal-parasitic and free-living nematodes (Bottjer et al. 1985). Braun (2000) reported that the crystal proteins encoded by Cry genes have been classified as CryI to CryVI depending on a host specificity and amino acid homology. The homology group CryI to CryV specified to insects as a host and CryVI specified to nematode as a host. El-Nagdi and Youssef (2004) tested the soaking of faba bean seeds or treating their soil with the bio-agent (strain of *B. thuringiensis*) and compared the results with the nematicide oxamyl. The biocontrol agent significantly reduced the population density of *M. incognita* with increasing the measured plant growth. On this basis, seed soaking in such biocontrol agent was recommended as an economical method for managing *M. incognita.*

The mode of action of bacterial toxin on second-stage juveniles of *M. incognita* was due to bacterial endotoxin, the efficacy of endotoxin as a nematicidal effect based on the morphological structure of crystal toxin (Abd El-Moneim and Massoud 2009). The spherical crystal toxin gave the highest reduction in nematode population because they can easily pass through the nematode mouth part. The toxin produced by the four studied isolates of *B. thuringiensis* were classified into three groups based on the toxin structure, which observed by light microscope. Two bacterial isolates (AI and AII) produced typical spherical crystal shape, and one (AIII) isolate produced a pyramidal crystal, while the other produced a bipyramidal crystal (AIV). These results were confirmed by using SDS-PAGE. The crystals from isolates AI and AII contained a protein band very close to each other (128 and125 KDa, respectively). These two isolates gave highly significant reduction in second-stage juveniles and galls on tomato plants comparing with the untreated plants. The other two isolates (AIII and AIV) recorded significant reduction in nematode population on tomato plants comparing with plant check, but in less values than those recorded in the presence of isolates AI and AII. The high percent of increase in plant performance values (plant shoot dry weight (g) and plant height (cm)) were found in the presence of bacterial isolates AII, AI, then AIII, and AIV at concentrated level of crystal toxin $1 \times 10^8/1$ ml comparing with untreated plant.

Bt has been used as a bioinsecticide for many years. It is a Gram-positive bacterium, found in soil and insect-rich environments. The bacterium probably produces several toxic metabolites including exotoxins and endotoxins. But it is the endotoxins that have attracted most attention as insecticidal or nematicidal genes. Genes encoding *Bt* endotoxins have been introduced into crops including tomato, cotton, maize, rice, and potatoes. The anti-nematode properties have been studied mainly with animal-parasitic nematodes and some with phytonematodes. The nematicidal effects of several commercial preparations have been tested on a variety of plant- and animal-parasitic nematodes.

The results of a glasshouse-based assay in which commercial *Bt* preparations were added to nematode-infected soil revealed that the final population levels and egg viability of *M. incognita* on tomato and *Tylenchulus semipenetrans* on citrus were reduced in treated soil. The number of egg masses produced by root-knot nematode on the transgenic tomato plants expressing *Bt* (CR-450) endotoxin "CryIab" was reported by Waele. Results showed that transgenic plants expressing the transgene recorded the less number of egg masses compared to control plants.

Nematode-resistant transgenic plants can be designed by various approaches using the available information and knowledge. The simplest would be to introduce a nematode toxin gene under a constitutive promoter. This strategy has been adopted to design transgenic plants carrying the *Bt* gene that are resistant to insect pests. *Bt* is a source of two types of toxins effective against insects, namely, the B-exotoxins and b-endotoxins. There are many b-endotoxins encoded by cry group of genes which show high specificity to

insects on oral ingestion. B-exotoxins have been found to have some effects against nematodes. The b-endotoxins cannot be ingested by plant-parasitic nematodes because of their size. If a suitable toxin gene could be identified, this very approach can be used to construct nematicide transgenic plants. However, a number of toxins known to be active against vertebrates also act upon nematodes. TxP-1 toxin from female mites of the species *Pyemotes tritici* is extremely potent against wide range of insect species causing immediate muscle paralysis upon exposure to very low doses. Nematode natural enemy can serve as a source for these toxin molecules.

Bacillus thuringiensis crystal proteins are pore-forming toxins, used as insecticides around the world (Wei et al. 2003). Previously, the extent to which these proteins might also target the invertebrate phylum Nematoda has been mostly ignored. Seven different crystal toxin proteins were expressed from two largely unstudied *Bt* crystal protein subfamilies. By assaying their toxicity on diverse free-living nematode species, it was demonstrated that four of these crystal proteins are active against multiple nematode species and that each nematode species tested is susceptible to at least one toxin. Toxicity in nematodes correlates with damage to the intestine, consistent with the mechanism of crystal toxin action in insects. Structure–function analyses indicate that one novel nematicidal crystal protein can be engineered to a small 43-kDa active core, which demonstrated that at least two Bt crystal protein subfamilies contain nematicidal toxins.

The protein toxins produced by *Bacillus thuringiensis* are the most widely used natural insecticides (Marroquin et al. 2000). Despite successful and extensive use of these toxins in transgenic crops, little is known about toxicity and resistance pathways in target insects since these organisms are not ideal for molecular genetic studies. To address this limitation and to investigate the potential use of these toxins to control parasitic nematodes, they studied *Bt* toxin action and resistance in *Caenorhabditis elegans* and demonstrated for the first time that a single Bt toxin can target a nematode. When fed with *Bt* toxin, *C. elegans* hermaphrodites undergo extensive damage to the gut, a decrease in fertility and death, consistent with toxin effects in insects. Ten recessive mutants were screened and isolated that resist the toxin's effects on the intestine, on fertility, and on viability. These mutants defined five genes, indicating that more components are required for *Bt* toxicity than previously known. They observed that a second, unrelated nematicidal *Bt* toxin may utilize a different toxicity pathway. The study indicated that *C. elegans* can be used to undertake detailed molecular genetic analysis of *Bt* toxin pathways and that *Bt* toxins hold promise as nematicides.

Nematoxicity has been observed in a secretion of a fungal endoparasite, *Nematoctoms* spp., during spore germination that causes rapid paralysis and death of the nematode. *Seinura,* the predatory nematode, injects a toxin into the nematode prey, causing immobilization within seconds. More studies are needed to decipher the nature of these molecules which are potentially useful in designing nematicide transgenic plants. If the toxin is a protein, it would be possible to incorporate it into plants to function in a manner similar to *Bt*-transformed plants. Constitutive expression of any toxin gene may place upon the pest population very strong selective pressures for resistance. Secondly peptide toxins useful in this approach typically are narrow is their toxic spectra, as in the case of *Bt* toxins. Another disadvantage of this approach is the negative energy balance the host plant suffers when there is no pest pressure.

Finally, the global constitutive expression of toxin genes threatens that nontarget species, including humans, would be exposed to the protein products.

13.13 Bio-fumigation

Bio-fumigation refers to the process by which soilborne pests and pathogens are suppressed by naturally occurring biocides released in soil when tissues of brassicaceous plants decompose in soil (Kirkegaard 2000). Bio-fumigation is based on incorporating soil amendment like fresh plant mass or manure into the soil, which will release chemical substances, known as isothiocyanates (ITCs), able to suppress nematodes, apart from

Table 13.6 Major commercially available bio-fumigant plants

Sl. No.	Bio-fumigant plant
1.	Fodder radish (*Raphanus sativus)*
2.	Indian mustard (*Brassica juncea*)
3.	Rape (*B. napus*)
4.	Turnip (*B. campestris*)
5.	White mustard (*Sinapis alba*)
6.	Ethiopian mustard, Abyssinian mustard *(B. carinata)*
7.	Garden Rocket, Roquette, Arugula *(Eruca sativus)*

acting as a soil heater to enhance biological activities. Plants from *Cruciferae* family (cabbage, radish, cauliflower, etc.) release large amount of these toxic substances to soilborne pests and diseases in the soil and are considered the best material for bio-fumigation.

It is recommended to alternate the use of horticultural residues with green manure, especially from brassica, using 5–8 kg/square meters of green matter, although combinations of legumes and grass can be applied. In the case of the use of green manure cultivated in the same field, fast growing plants should be used to be incorporated at least 30 days after having been planted, to avoid the increase of pathogen populations. Planting mustard after bio-fumigation can serve as bioindicator of possible phytotoxicity, because the germination of their seeds is sensitive to phytotoxic substances. At the same time they are very sensitive to nematodes and permit the detection of areas in the crop where bio-fumigation is not effective. They act like trap plants and like bio-fumigants when incorporated into the soil.

When tissues of crucifers (radish, cabbage, cauliflower, broccoli, calendula, basil, *Tagetes,* chrysanthemum, rapeseed, barley, Sudan grass, velvet bean, mustards, oil seed radish, sorghum species such as Sudan grass, etc.) or green manure crops decompose in soil, ITCs are released, when glucosinolates (GSLs) in the tissues are hydrolyzed (Table 13.6). Such crops are rich in organic substances called glucosinolates. Under certain conditions, they become isothiocyanates during decomposition (Halbrendt 1996). Sorghums produce a cyanogenic glucoside compound called "dhurrin" that breaks down to release toxic cyanide when the plant tissue is damaged.

Glucosinolates are sulfur- and nitrogen-containing compounds that occur naturally in brassicas and closely related families. In rapeseed (*Brassica napus*), there are aliphatic glucosinolates which include 3-butenyl (gluconapin), R-2-hydroxy-3-bunenyl (progoitrin/glucorapiferin), and 4-pentenyl (glucobrassicanapin) which mainly occur within the foliage and aromatic glucosinolates such as 2-phenylethyl (gluconasturtiin) which mainly occur in the roots (Lewis and Papavizas 1975). The occurrence of specific glucosinolates is not uniform throughout the brassica family, and some species have different types and concentrations of molecules. Species such as Indian/brown mustard (*Brassica juncea*), black mustard (*Brassica nigra*), white mustard (*Sinapis alba*), and oil radish (*Raphanus sativus*) are known to have a higher glucosinolate content. Glucosinolates and the enzyme myrosinase are naturally partitioned from one another in living cells. However, tissue disruption such as that caused during insect feeding, mechanical damage, or infection facilitates the mixing of myrosinase and glucosinolates. Myrosinase degrades glucosinolates through hydrolysis to produce a variety of volatile products including isothiocyanates, organic cyanides, ionic thiocyanates, and oxazolidinethiones, which appear to have some potentially exploitable biological activity. Indeed, there is an increasing body of work on the use of the "glucosinolate–myrosinase system" in crop protection.

It is recommended to alternate the use of horticultural residues with green manure, especially from brassica, using 5–8 kg/sq.m of green matter, although combinations of legumes and grass can be applied. In the case of the use of green manure cultivated in the same field, fast growing plants should be used to be incorporated at least 30 days after having been planted, to avoid the increase of pathogen populations. Planting brassica after bio-fumigation can serve as bioindicator of possible phytotoxicity, because the germination of these seeds is sensitive to phytotoxic substances. At the same time they are very sensitive to nematodes and permit the detection of areas in the crop where bio-fumigation is not effective. They act like trap

plants and like bio-fumigants when incorporated into the soil.

In suitable conditions, the bio-fumigation technique is able to efficiently produce a number of important substances. In the above plant families, one of the most important enzymatic defensive systems is the myrosinase–glucosinolate system. With this system, tissues of these plants can be used as a soft, eco-compatible alternative to chemical fumigants and sterilants. In a number of countries over the past few years, several experiments have been carried out to evaluate the effectiveness of the myrosinase–glucosinolate system, in particular using the glucosinolate-containing plants as a biologically active rotation and green manure crop for controlling several soilborne pathogens and diseases.

13.13.1 General Procedure of Bio-fumigation

Incorporate the fresh mass into the soil, which can be done directly if the mass is coming from grown crop or with mass taken from other side and brought into the plot or field. If the mass is transported to the field, the soil should be well prepared before the incorporation. During transportation and storage of these organic materials in the field, care must be taken not to lose the gases produced from biodegradation, by covering the piles of the bio-fumigant with plastic until the time of application. A dose of 50 t/ha is recommended, although when problems with nematodes are very serious, 100 t/ha should be applied, a dose that can be reduced by choosing cultivation techniques such as application in furrows. The bio-fumigant should be distributed uniformly, so that no concentration of pathogens will appear that could create problems for the crop. Once the bio-fumigant is distributed, it should be incorporated immediately into the soil.

Water the field, if possible by sprinkling, until the soil is saturated, although watering can be done by flooding, or drip irrigation can be installed. Cover the soil surface tightly with a transparent plastic film for at least two weeks to retain the gases produced from the biodegradation of the organic matter. This could be the same plastic as the one used for soil solarization. The film is removed 3–4 weeks after and the soil slightly removed in order to permit the gases to escape from soil. Planting of the interested crop can be done 24 h later.

In addition to managing soilborne pests, bio-fumigation has also been shown to improve soil chemical, physical, and biological characteristics. In their mineralization in the soil, they release chemical compounds similar to methyl isothiocyanate – a toxin that is produced by degradation of metham sodium. Bio-fumigation can be used individually or in combination with solarization. At 4 months after incorporation of the sorghum green manure into the soil and application of methyl bromide, but prior to planting peach trees, ring nematode populations were greatest ($P < 0.05$) in the unfumigated soil than in sorghum green manure under plastic, sorghum green manure without plastic, and methyl bromide-fumigated plots (Nyczepir et al. 1999). Abragan et al. (2006) observed significant reduction in the root-knot nematode population with mixed crucifers (86.7 %) followed by radish (85.4 %), broccoli (82.2 %), cauliflower (71.3 %), and cabbage (70.9 %).

Three *Brassica juncea* lines (Nemfix, Fumus, and ISCI99) containing high concentrations of 2-propenyl glucosinolate were found most effective, causing over 95 % mortality of encysted eggs of *Globodera pallida* in polyethylene-covered soil (Lord et al. 2011). The toxic effects of green manures were greater in polyethylene-covered than in open soil. Toxicity in soil correlated with the concentration of isothiocyanate-producing glucosinolate but not total glucosinolate in green manures.

Significant suppression of phytonematodes could be achieved using *Brassica* green manures, and this arose from a combination of two separate mechanisms. The first mechanism was short-term suppression (over 2–3 days) related to the release of isothiocyanates (ITCs) from the brassica tissues. To maximize the impact of this mechanism, strategies to increase ITC release from the plant tissues and their residence time in the soil were developed into a “best-bet” approach. This

involved the use of brassica tissues which release high concentrations of toxic ITCs (e.g., mustard), incorporating around 5 kg/m2 (5 % W/W) of fresh tissue into the soil, adequate maceration of the tissues at a cellular level to release the ITCs, adequate water to facilitate hydrolysis of ITCs, rapid and thorough incorporation for complete mixing of ITCs through the soil, watering or covering to retain the volatile ITCs in the soil, and targeting light-textured soils with low organic matter to reduce inactivation of ITCs.

Bio-fumigation is also an alternative to chemical methods for soil disinfection in greenhouses and outdoors. It includes the use of plant material and organic supplements, viz., manure and chicken fertilizer, which being incorporated in the soil undergo decomposition and produce volatile substances. Bio-fumigant green manures should not host the nematodes. Although most of the brassicas are poor to moderate hosts of *Meloidogyne* spp., providing potential for population increase, radish (*Raphinus sativus)* is relatively resistant to nematodes. Radish is less costly, readily available, and has a large seed which can establish well and grow rapidly. Radish leaves also represent a waste product that growers can utilize. It offers the most promising option as a bio-fumigant green manure where nematode populations are high.

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