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# INTEGRATED MANAGEMENT OF ROOT-KNOT NEMATODES IN MEDITERRANEAN HORTICULTURAL CROPS

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**Abstract.** Several vegetables are grown around the Mediterranean basin for fresh consumption as a basic component of the Mediterranean diet, as climate allows cropping thorough all year. A great socio-economic and cultural diversity makes of this area a mosaic, in which large and small-scale production systems are coexisting. *Meloidogyne* spp. are the main plant parasitic nematodes causing yield losses mainly in protected crops due to climate and intensive croppings. *M. javanica*, *M. incognita* and *M. arenaria* are the most frequent species found in almost all countries. The principles of control of root-knot nematodes are changing from the use of nematicides applied to eradicate them, towards integrated nematode management, accepting the pests presence at levels that do not cause economic yield losses, according to sustainable agricultural systems. Basic information concerning biology, plant-nematode interactions, potential yield losses and value, efficacy and costs of control methods, are necessary to elaborate prediction models to support and design integrated management strategies.

## 1. INTRODUCTION

The Mediterranean region comprises the Mediterranean Sea and its coastal area, including eighteen countries: northern basin countries (Albania, Former Yugoslavia, France, Greece, Italy, Monaco, Spain) and southern basin countries (Algeria, Cyprus, Egypt, Israel, Lebanon, Libya, Malta, Morocco, Syria, Tunisia, Turkey). The Mediterranean climate is characterized by mild temperatures, with a cold period in winter, annual rainfall between 250–800 mm distributed during spring and autumn, and dry summers. The northern region is relatively more temperate and humid, whereas the southern region is warmer and drier, with endemic water shortages due to the interaction of relatively low seasonal rainfall and high evapotranspiration rates.

The Mediterranean climate predominates in the countries surrounding the Mediterranean Sea, but it also occurs in others zones of the Earth: Cape Town in South Africa, central coast of Chile, central and southern coast of California and portions of southwestern Australia. These regions share climate conditions similar to the Mediterranean ones, thus, the crops and the problems for cropping, are also similar.

*Table 1. Production of vegetable crops (tonnes) in the main producing countries of the Mediterranean basin in 2005 (FAO, 2006).*

Crop	Egypt	Italy	Spain	Turkey
Lettuce and chicory	140.000	1.010.520	920.000	375.000
Melons (inc.cantaloupes)	565.000	611.501	1.176.900	1.700.000
Strawberries	100.000	147.049	308.000	160.000
Tomato	7.600.000	7.187.016	4.651.000	9.700.000
Watermelons	1.500.000	519.463	724.900	3.800.000
Eggplants (aubergines)	1.000.000	338.803	60.000	880.000
Asparagus		43.274	47.600	11
Cucumbers and gherkins	600.000	72.572	485.000	1.725.000
Chillies and peppers, green	460.000	362.994	953.200	1.745.000
Spinach	48.700	99.367	45.000	220.000
Artichokes	70.000	469.975	188.900	30.000
Pumpkins, squash and gourds	690.000	488.083	300.000	376.000

The countries of the Mediterranean area are important producers of vegetables for fresh consumption as a basic component of the Mediterranean diet. Production and harvested area increased by about 28% and 14% between 1995 and 2005, respectively. In 2005, the harvested area was about 2.5 million hectares and production was near 73 million Tm. Production and harvested area of Turkey, Egypt, Italy and Spain are more than 75% and 70%, respectively, of the Mediterranean basin (Table 1 and Table 2) (FAO, 2006).

*Table 2. Area harvested (ha) in the main producing countries of the Mediterranean basin in 2005 (FAO, 2006).*

Crop	Egypt	Italy	Spain	Turkey
Lettuce and chicory	6.000	50.008	39.000	19.700
Melons (inc.cantaloupes)	24.000	27.815	35.200	103.000
Strawberries	3.800	6.226	7.600	10.500
Tomato	195.000	138.756	70.400	260.000
Watermelons	62.000	14.193	16.100	137.000
Eggplants (aubergines)	43.000	12.164	1.500	35.000
Asparagus		6.365	12.000	3
Cucumbers and gherkins	28.000	1.989	7.200	60.000
Chillies and peppers, green	29.000	13.787	22.500	88.000
Spinach	2.500	7.367	3.000	22.500
Artichokes	3.500	50.127	18.600	2.500
Pumpkins, squash and gourds	39.200	16.732	7.000	22.000

The most important vegetables grown in plastic houses are: tomato, strawberry, pepper, squash, eggplant, that represent 89% of production (FAO, Grupo de cultivos hortícolas, 2002).

## 2. MELOIDOGYNE

*Meloidogyne javanica*, *M. incognita*, *M. arenaria* and *M. hapla* are the most frequent root-knot nematode species present in almost all mediterranean countries (Table 3). These species are worldwide distributed and have a wide host range that includes vegetable crops. In the Mediterranean countries they often represent the main soil pathogen problems for vegetable and flower crops, especially under protected conditions (Greco & Esmenjaud, 2004). *M. incognita* and *M. javanica* are commonly found in the tropics, whereas *M. arenaria* and *M. hapla* are more common in the subtropical and temperate climates, respectively.

Other *Meloidogyne* species present in the Mediterranean basin are *M. artiella*, pathogenic to legumes and cereals in southern Europe and the Near East, as well as *M. lusitanica*, *M. baetica*, and *M. hispanica* that are not parasites of vegetables. *M. baetica* has been reported in olive trees in Portugal (Abrantes, Vovlas, & Santos, 1991) and in Spain (Nico, Rapoport, Jiménez-Díaz, & Castillo, 2002). *M. baetica* has been detected on *Pistacia lentiscus*, and *Aristolochia baetica* in Spain (Castillo, Vovlas, Subbotin, & Troccoli, 2003), whereas *M. hispanica* has only been reported in peach orchards in south-eastern Spain (Hirschmann, 1986).

Finally, the other important root-knot nematode specie able to parasitise vegetables, *M. chitwoodi*, is not present in the Mediterranean basin although it has been detected in some temperate countries in Europe, and in South-Africa, but not in Asia (EPPO, 2006).

### 2.1. Symptoms

Galls in roots are the most characteristic symptom shown by plants infected by the most important *Meloidogyne* species. The size of galls is variable depending on quantity of inoculum and plant species. Low nematode density produces individual or scattered galls induced by one or few females and an egg mass, related to individual females, can be observed on the root surface. As density of nematodes increases, galls develop closer to each other and roots become deformed, their size increase considerably. In this case, only few egg masses can be seen on the root surface, as the majority of them are inside the root.

Plant species affects gall size, ranked from more to less discrete galls: *Alliaceae*, *Cruciferae*, *Asteraceae*, *Chenopodiaceae*, *Apiaceae*, *Fabaceae*, *Cucurbitaceae* and *Solanaceae*. However, plants from the same family do not have the same sensibility to root-knot nematodes. For example, galls on pepper are smaller than on aubergine or tomato, or galls on carrot are smaller than on celery. The severity of diseased roots for the same vegetable crop and for the same initial population density can also differ according to the time of the year. For instance, lettuce roots are more severely galled when the crop is grown in summer than in late

autumn. Symptoms in the aboveground depend on disease severity, they can range from no symptoms, when initial population density is lower than the tolerance limit, to dead plants, at higher population densities. Damping-off can occur when seeds are planted in heavily infested soils. Nutrient and water absorption is affected in plants with severely galled roots. As a consequence, plant growth is retarded, leaves show nutrient deficiency, wilt, yellowing and necrosis, flowering can be reduced or flowers become dry, in addition, the number of fruits is reduced or fruit size does not attain marketable standards.

*Table 3. Distribution of Meloidogyne javanica, M. incognita, M. arenaria and M. hapla in countries from the Mediterranean basin.*

Country	<i>Meloidogyne</i> species			
	<i>M. javanica</i>	<i>M. incognita</i>	<i>M. arenaria</i>	<i>M. hapla</i>
Albania		Shepherd and Barker, 1990		
Algeria	Ibrahim, 1985	Scotto La Massese, 1961; Ibrahim, 1985	Sellami, Lonici, Eddoud, and Besenghir, 1999	Sellami et al., 1999
Cyprus	Phillis, 1983; Ibrahim, 1985	Phillis, 1983		
Egypt	Ibrahim, Ibrahim, and Rezk, 1972; Ibrahim and Rezk, 1988	Ibrahim et al., 1972; Ibrahim and Rezk, 1988	Taylor, Sasser, and Nelson, 1982	
Former Yugoslavia	Shepherd and Barker, 1990; Grujicic, 1974	Grujicic, 1974	Grujicic, 1975	Grujicic and Paunovic, 1971
France		Shepherd and Barker (1990)	Dalmasso, 1980	Berge, Dalmasso, and Ritter, 1972
Greece	Pyrowolakis, 1975	Kyrou, 1976	Koliopanos, 1982	Pyrowolakis, 1975
Israel	Tarjan, 1953	Tarjan, 1953; Orion, Nessim-Bistrisky, and Hochberg, 1982		Minz, 1956
Italy	Ibrahim, 1985	Ibrahim, 1985	Ibrahim, 1985	Ambrogioni, 1969
Jordan	Hashim, 1979	Hashim, 1979	Karajeh, Abu-Gharbieh, and Masoud, 2005b	
Lebanon	Saad and Tanveer, 1972	Saad and Tanveer, 1972	Macaron, Laterrot, Davet, Makkouk, and Revise, 1975	
Libya	Dabaj and Jenser, 1987	Khan and Dabaj, 1980; Dabaj and Jenser, 1987	Khan, 1982	Dabaj and Jenser, 1987

Malta	Ibrahim, 1985	Ibrahim, 1985		
Morocco	Ibrahim, 1985	Ibrahim, 1985	Taylor et al., 1982	
Spain	Jiménez-Millan, Bello, Arias, and López Pedregal, 1964	Jiménez-Millan et al., 1964	Jiménez-Millan et al., 1964	Jiménez-Millan et al., 1964
Syria	Ibrahim, 1985	Ibrahim, 1985	Tayar, 1982	
Tunisia	Waldmann, 1971	Moens, 1985		
Turkey	Ibrahim, 1985	Bora, 1970	Taylor et al., 1982	

The sequence of symptoms is faster in summer than in other seasons since plant requirements are greater. In vegetables cultivated for their tubers or roots, *Meloidogyne* can cause deformations that result in severe losses in quality. Damage caused by *Meloidogyne* can be more severe when synergistic relationships with fungi or bacteria occur producing additional root-rot that accelerates the sequence of symptoms.

### 2.2. Biology and Ecology

*Meloidogyne* can survive without a host plant as juvenile of 1st (J1) or 2nd (J2) stage inside the egg, in the egg mass or as J2 in soil. When a crop is planted, J2 penetrate the root directly, just behind the root tip. Migration into the vascular cylinder is intercellular and non destructive. The J2 infects roots establishing a feeding site within the developing vascular cylinder if there is a compatible response, and if conditions are conducive to it. After infection, J2 moults three times before reaching maturity. In optimal conditions, juveniles of *Meloidogyne javanica*, *M. incognita*, or *M. arenaria* develop to females. Reproduction is by parthenogenesis, and each female produces an egg mass containing from 500 to 1500 eggs. Below optimal conditions, some juveniles develop to males, which do not feed on the plant, in order to regulate nematode population densities and avoid intraspecific competition.

Although *Meloidogyne* can occur wherever a plant can develop (Sasser & Carter, 1985), survival and development of root-knot nematodes are conditioned by the host plant and the environmental conditions in soil. Thus, a host plant allows build-up of nematode population densities, whereas a poor host hinders the build-up that, finally, doesn't take place if the plant is a non-host. Vegetables such as tomato, cucumber, lettuce, aubergine, or melon are hosts for root-knot nematodes, while vegetables such as cabbage and onion are poor or non-host (Netscher & Luc, 1974). However, there exists inter (Sasser, 1954) and intraspecific (Taylor & Sasser, 1978; Southards & Priest, 1973; Sasser, 1966; Riggs & Winstead, 1959) variability in the reproductive capacity of root-knot nematodes in selected hosts. This aspect suggested the existence of physiological races that can be recognized by the use of differential hosts (Hartman & Sasser, 1985). Nevertheless, more races can be differentiated when new hosts are included in the test (Noe, 1985; Southards &

Priest, 1973). Therefore, this test is not a practical tool to design plant rotations for managing nematode population densities.

In addition, the plant host influences the length of the life cycle of *Meloidogyne* (Godfrey & Oliveira, 1932) and length of embryogenesis (Bafokuzara, 1983).

Soil temperature is the most important environmental factor that regulates the life cycle of *Meloidogyne*. The length of the life cycle can be expressed as accumulated temperature over a minimal threshold temperature, this is the thermal time. The thermal time requirement of plant-parasitic nematodes and its ecological significance have been reviewed extensively (Trudgill, 1995a; Trudgill & Perry, 1994). *Meloidogyne* needs between 11,500 and 13,000 heat units to complete its life cycle. A heat unit is one degree centigrade over the minimal threshold temperature (10°C) acting for an hour (Tyler, 1933). Ferris, Roberts, and Thomason, (1985) reported that the nematode needs between 600 and 700 degree days over 10°C to complete one generation. Thermal time requirements for the complete life cycle differ between species and populations. The length of one generation of *M. hapla*, *M. javanica*, and *M. incognita* was 554, 343, and 400°C-days over 8.25, 13.1, and 10.1°C, respectively (Ploeg & Maris, 1999; Madulu & Trudgill, 1994; Lahtinen, Trudgill, & Tiilikkala, 1988).

Dao (1970) found that a population of *M. incognita* from the Netherlands was able to infect and reproduce at about 5°C lower than a Venezuelan population, reason for which he suggested the existence of thermotypes defined as nematode population with a fixed difference in temperature requirements. Nematode survival in the absence of a host is also conditioned by temperature, in general the optimum temperatures for survival of eggs and juveniles ranged from 10 to 15°C (Thomason, Van Gundy, & Kirkpatrick, 1964; Bergeson, 1959). Knowledge of thermal of time requirements of each species allows predictions the time necessary to reach certain events (Trudgill, 1995b) and to develop management strategies (Van Gundy, 1985).

The second-stage juvenile of *Meloidogyne* spp. lives free in the soil, hatching in this second-stage from eggs. These juveniles move in the moist soil searching for roots of a possible host plant, then penetrate through the growth zones and induce differentiation of plant cells into specialized feeding cells. Symptoms on roots are: root swellings called galls and general alteration of the root vascular system.

### 2.3. Yield Losses of Economic Importance

*Meloidogyne* spp. can cause yield losses of over 30% in various vegetable crops (Netscher & Sikora, 1990). In experimental conditions, yield reductions in aubergines caused by *M. incognita* were higher than 80% (Di Vito, Greco, & Carella, 1986). In northeastern Spain, an initial population density of 4,750 juveniles/250 cm<sup>3</sup> soil of *M. javanica* caused a 36% and a 61% reduction of yield in lettuce and tomato cropped in summer in plastic-houses, respectively (Verdejo-Lucas, Sorribas, & Puigdomènech, 1994). Cucumber yield loss caused by an initial population density of 1,100 juveniles/250 cm<sup>3</sup> soil of *M. javanica* was 60% (Ornat, Verdejo-Lucas, & Sorribas, 1997).

Maximum yield loss of tomato cropped in plastic-houses from March to July has been estimated at 36% in northern Spain (Sorribas, Ornat, Verdejo-Lucas,

Galeano, & Valero, 2005a), and 21% in the Balearic Islands (unpublished data). Differences in yield losses could be mainly explained by differences in initial population densities, environmental conditions, and crop management. Furthermore, yield losses could increase when *Meloidogyne* interacts with other plant pathogens such as *Fusarium oxysporum* or *Rhizoctonia solani*, producing a disease complex (Back, Haydock, & Jenkinson, 2002; Hussey & McGuire, 1987; Webster, 1985; Taylor, 1979) therefore increasing their severity of attack or overcoming plant resistance.

Economic importance of *Meloidogyne* on vegetables crops for a specific area can vary depending on the frequency of infestation and the population levels. For example, in northeastern Spain, 50% of 66 plastic-houses, and 27% of 59 open fields were infested by *Meloidogyne*. *M. javanica* was the most abundant species in plastic houses (41% of the sites) whereas *M. incognita* was the main species in open fields (50% of the sites). Nematode population densities at planting the spring crop ranged from 1 to 590 juveniles/250 cm<sup>3</sup> soil, and from 1 to 2,100 juveniles/250 cm<sup>3</sup> soil when planting the summer crop (Ornat, 1998; Sorribas, 1996). The tolerance limit, defined as the maximum density of inoculum that does not cause yield loss, for *M. javanica* on tomato, was 2 juveniles/250 cm<sup>3</sup> sandy soil in commercial plastic-houses (Sorribas, 1996). In pot experiments, tolerance threshold to *M. incognita* for eggplant, tomato, artichoke, pepper, and cabbage was 0.054, 0.55, 1.1, 0.3 and 0.5 eggs and juveniles/cm<sup>3</sup> soil, respectively (Di Vito, Cianciotta, & Zaccheo, 1992; Sasanelli, Di Vito, & Zaccheo, 1992; Di Vito et al., 1986). Tolerance limits have to be determined for each specific growing area since they are affected by plant nematode interaction, soil type, environmental conditions, and crop management.

### 3. ROOT-KNOT NEMATODES MANAGEMENT

Vegetable production systems in the Mediterranean basin are diverse and depending on the economy of the farm, similarly to other growing areas in tropical and subtropical climates (Sikora & Fernandez, 2005). Vegetables are grown in plastic houses and in open field in both, large and small-scale production systems. Large-scale production systems are managed by large enterprises or cooperatives for both export and national markets, are highly specialized, have access to technology and have permanent technical assistance. On the other hand, small-scale producers are family enterprises which produce and commercialize mainly for local markets, are moderate to scarcely specialized, have limited access to technology and occasionally they benefit from technical assistance.

*Meloidogyne* damage is greater in protected crops than in open field because of susceptibility of main crops, cropping intensity, and environmental factors. In protected crops, root knot nematode management is mainly based on fumigants and nematicides because implementation of variations in their predefined plans is difficult due to market requirements. However, restrictions or banning of some of the most effective nematicides have been led to the use of other techniques such as plant resistance, solarization and/or biofumigation, and cultural practices, mainly in ecological and integrated production systems. In open fields, the number of

vegetables growing in rotation is higher than in plastic houses, environmental factors are less conducive to disease and in consequence the nematode can be managed more efficiently.

### 3.1. Plant Resistance

In Nematology, resistance is the ability of a plant to suppress development or reproduction of nematodes (Roberts, 2002). The use of resistant cultivars is an elegant, economical and environmentally safe method for controlling root knot nematodes (Netscher & Sikora, 1990; Netscher & Mauboussin, 1973). In addition, plant resistance is particularly useful for organic farming or integrated production since these systems do not allow, or restrict, the use of chemical control, respectively. In commercial resistant cultivars yield is not significantly affected when cropped in nematode infested soils because of tolerance is coupled with resistance (Sorribas, et al., 2005a; Roberts, 2002; Rich & Olson, 1999; Ornat et al., 1997; Philis & Vakis, 1977). However, commercial resistant cultivars are only available for tomato and pepper, despite the fact that sources of resistance have been reported for other vegetables for example: complete resistance to *M. javanica* within *Solanum melongena* and *S. torvum* (Boiteux & Charchar, 1996), to *Meloidogyne hapla* in inbred lines of processing carrot (Wang & Goldman, 1996), and to *M. arenaria* and *M. javanica* in *Cucumis sativus* (Walters, Wehner, & Barker, 1996, 1999).

In Solanaceae, the expression of plant resistance to *Meloidogyne* spp. is characterized by a hypersensitive reaction, which consists in localized plant-cell necrosis around the nematode's head (Kaplan & Keen, 1980). Tomato is the most important vegetable cropped in the Mediterranean basin and commercial resistant cultivars and rootstocks are available. Resistance in tomato is conferred by the single dominant gene Mi, which was introgressed from the wild relative of tomato *Lycopersicon peruvianum* (Smith, 1944) and is present in all resistant commercial cultivars. The Mi-resistance gene confers resistance, but not immunity, to *Meloidogyne incognita*, *M. javanica* and *M. arenaria* (Roberts & Thomason, 1989). However, expression of resistance is affected by some factors such as soil temperature, species and populations of *Meloidogyne*, Mi-dosage, and tomato genetic background. The efficient use of resistance to manage root knot nematodes must take into consideration the following factors. First, soil temperatures higher than 28°C suppress resistance expression (Dropkin, 1969). This limitation, due to temperature, suggests that, in the Mediterranean basin, the use of these varieties may have to be restricted to spring planting, when soil temperatures are lower.

Second, resistant tomatoes have a high level of resistance to populations of *M. incognita* and *M. arenaria*, but are less resistant to *M. javanica* (Ornat et al., 2001b; Sorribas & Verdejo-Lucas, 1999; Busquets, Sorribas, & Verdejo-Lucas, 1994). Considering that *M. javanica* is the most common species of root-knot nematode in the Mediterranean region (Verdejo-Lucas, Ornat, Sorribas, & Stchiegel, 2002; Ornat & Verdejo-Lucas, 1999; Sellami et al., 1999; Eddaoudi, Ammati, & Rammah, 1997; Tzortzakakis & Gowen, 1996; Sorribas & Verdejo-Lucas, 1994; Ibrahim, 1985; Philis, 1983; Lamberti, 1981) it will be necessary to combine resistance with other



management techniques if resistance durability is to be assured. In addition, some *Meloidogyne* populations can overcome resistance.

Virulence, defined as the ability of nematodes to reproduce on a host plant that possesses one or more resistance genes, occurs naturally in *Meloidogyne* populations on tomato apparently without previous exposure to, or selection by, the Mi-resistance gene (Prot, 1984; Netscher, 1976). In Spain, over 30 root-knot nematode populations examined only one population of *M. javanica* was virulent to the Mi-resistance gene, occurring naturally without previous exposure to the resistance gene (Ornat et al., 2001). Virulent nematode populations may also be selected after repeated exposure to tomatoes with Mi-gene resistance (Roberts, 1995; Castagnone-Sereno, Bongiovanni, & Dalmasso, 1993; Netscher, 1976) or suddenly (Williamson, 1998).

In the Mediterranean region, virulent populations to the Mi gene have been reported: *M. javanica* in Greece (Tzortzakakis & Gowen, 1996), Jordan (Karajeh, Abu-Gharbieh, & Masoud, 2005a), Morocco (Eddaoudi et al., 1997), and Spain (Ornat et al., 2001); and *M. incognita* in both Greece (Tzortzakakis, Adam, Blok, Paraskevopoulos, & Bourtzis, 2005), and Spain (Robertson et al., 2006).

Finally, Mi-gene dosage also influences resistance expression. Mi-gene can be in homozygosis (Mi Mi) or heterozygosis (Mi mi) in tomato cultivars. Tzortzakakis, Trudgill and Phillips (1998) reported that tomatoes carrying the Mi-gene in homozygosis were more resistant than in heterozygosis. However, in addition to gene dosage, genetic background especially in heterozygous condition could affect expression of resistance (Jacquet, Bongiovanni, Martinez, Verschave, & Wajnberg, 2005). Some experiments carried out in controlled conditions in Spain showed different expression of the resistance in heterozygous commercial tomato cultivars with reproduction indexes (Final population density/Initial population density) that ranged from 0 to 3.1 in the tomato cultivars Bandera and Carpy, respectively (Sorribas & Verdejo-Lucas, 1999).

In pepper (*Capsicum* spp.), a resistance to root-knot nematode is conferred by a dominant gene N (Thies & Fery, 2000; Di Vito & Saccardo, 1979; Hare, 1956) and a minimum of five dominant genes (Me1 to Me5) from accessions PM 127 and PM687 (Hendy, Pochard, & Dalmasso, 1985). Genes Me confer the same broad resistance spectrum as Mi as well as stability at high temperature (Djian-Caporalino et al., 1999). Peppers carrying the Me1 gene are resistant to both Mi-virulent and avirulent populations of *M. arenaria*, *M. incognita* and *M. javanica*, although Mi-virulent populations of *M. arenaria* and *M. incognita* can parasitize peppers containing the Me3 gene (Castagnone-Sereno, Bongiovanni, & Djian-Caporalino, 2001).

Although plant resistance is an economical, environmental safety and healthy control method, sensory characteristics not always are accepted by the market. When it occurs, grafting plants on resistant rootstocks could be an alternative. In addition, grafting could be a method to manage other important soil-born pathogens and a source conferring resistance in vegetables for which no commercial resistant cultivars are available. Its use in vegetable crops has increased in the last decade, mainly for tomato, cucurbits, pepper and eggplant (MBTOC, 2006). In 2003–2004 the use of grafted tomato in 2003–2004 was 45, 30, 28 and 12 millions plants in

Spain, Morocco, France, and Italy, respectively (Besri, 2005). Nevertheless grafted plants are more expensive than non-grafted ones, they give an extra production: i.e. grafted tomato in Morocco and Spain yielded 53 and 70 T per Ha more, respectively, than non-grafted ones (Besri, 2005; Verdejo-Lucas, Buñol, Sorribas, & Ornat, 2004). Grafting have to be used in an integrated manner to avoid the selection of virulent populations, i.e. the use of sweet pepper rootstocks resistant to *Phytophthora capsici* and *Meloidogyne incognita* selected virulent root-knot nematode populations, but not of *P. capsici* (Ros et al., 2005).

### 3.2. Heat

Thermal control is generally aimed at inducing internal injuries that will lead to death over a short period of time (Lagüe, Gill, & Pélouquin, 2001). Steaming is the introduction of water vapour in soil to kill soilborn pests with the latent heat release when steam condenses into water (Bungay, 1999). Treatments consist in increasing soil temperature to 70°C for at least half an hour (Runia, 2000). Death of *M. incognita* occurs when exposed for a few minutes to temperatures above 48°C (Noling, 1997). Negative pressure steaming or sheet steaming can be used for soil sterilization purposes. Negative pressure steaming allows treatment at more soil depth than sheet steaming, and uses almost half the fuel of sheet methods (Runia, 2000). Steaming requires high amounts of water, power or fuel (Crump, 2001) therefore this method is restricted to high value crops.

Soil solarization consists in increasing temperature of wet soil by covering the area with a transparent film that traps solar radiation. This tactic was developed by Katan and co-workers in Israel in the mid 70s (Katan, Greenberger, Laon, & Grinstein, 2007). Solarization can control many soil born pests, pathogens and weeds when extended over a period of 6–8 weeks under intense solar radiation. In the Mediterranean basin, the best conditions for pest control through soil solarization are given during the hot, dry summer, mainly in plastic houses which are not cultivated in most areas due to high temperatures.

The efficacy of solarization to control root-knot nematodes is variable e.g. Greco, Brandonisio, and Elia (1992) reported a 99% efficacy, despite the fact that it had not been effective in a previous experiment in Italy (Greco, Brandonisio, & Elia, 1985). The variability of results can be attributed to the complex mode of action and the influence of environmental conditions (Stapleton, 2000). To predict the effectiveness of this method, information is required on the survival of nematodes as affected by a range of temperatures and exposure time (Greco & Esmenjaud, 2004). Survival rate of *Meloidogyne arenaria*, *M. incognita* and *M. javanica* is inversely related to accumulated soil temperature. Degree days (°C) of 950, 1,200 and 1,900 were needed to reduce initial population densities of *M. javanica*, *M. incognita* and *M. arenaria*, respectively, to 90%. (Sorribas et al., 2005b). Solarization can provide excellent control under conducive conditions and proper use, but under marginal environmental conditions it would be more effective to use new technologies, and or combining it with other tactics in an integrated manner as organic amendments, nematicides, antagonists, cover crops, and plant resistance (MBTOC, 2006).

### 3.3. *Soil-less Cultivation*

Soil-less cultivation is a technique used for bypassing soil pathogens. However, this tactic has not resulted in the elimination of problems caused by plant-parasitic nematodes. The most probable sources of nematode infestation are: infested plant material, infested soil carried into soil-less system by wind, equipment, animals and humans and infested water (Hallmann, Hänisch, Braunsman, & Klenner, 2005). All commonly used substrates are suitable for nematode infestation (Stapel & Amsing, 2004).

### 3.4. *Crop Rotation*

Crop rotation is the most important cultural method to control plant parasitic nematodes. The main aim of crop rotation for nematode management is the reduction of the initial population level of damaging nematode species to levels that allow the following crops to become established and complete early growth before being heavily attacked (Nusbaum & Ferris, 1973). However, some factors can restrict its use: On one hand, the occurrence of polyphagous nematodes or nematode communities can restrict the selection of suitable host plants. On the other hand, crop value also affects the use of rotation for nematode management because growers tend to produce the most economically important crops demanded by markets (e.g. tomato, pepper, cucumber, melon), and mainly fumigants and nematicides are used to control them. However, when the availability of nematicides is restricted or their use banned, rotation is often an important option (Duncan, 1991).

The aim of any rotation is to allow a sufficient interval of time between susceptible crops to reduce nematode population densities to a level that allows to grow and yield at an acceptable rate the next susceptible crop (Trivedi & Barker, 1986). Thus, vegetable species that are non-host, poor host, or resistant may be included in the rotation sequences. Crop rotation would be effective when a susceptible crop is planted once every four growing seasons and following a non-host or resistant crop when nematode densities in soil are low (Bridge, 1996).

Different sequences of vegetable rotation with susceptible host – poor host – poor host – susceptible host, that are normally conducted in commercial open fields in north-eastern Spain show that nematode population densities remain at similar levels at planting the next susceptible crop two years later. However, in plastic-houses, the choice of vegetables that can be cropped in the rotation sequence is reduced due to economic reasons.

In Crete, the option of vegetables in the rotation sequence for cultural management is reduced to resistant solanaceous crops in rotation with susceptible ones of the same family or cucurbits. In this context, growing a resistant tomato or pepper for one cropping cycle in a site infested by *M. javanica* and followed by a susceptible tomato resulted in final nematode population similar to those produced by fenamiphos applications to a sequence of two susceptible crops (Tzortzakakis et al., 2000). Cucumber cropped after a previous resistant tomato cultivar yielded 60% more than after a susceptible tomato in plastic-house infested by *M. javanica* in

Spain, (Ornat et al., 1997). Similar results have been reported for cantaloupe (Rich & Olson, 2004) or cucumber (Colyer, Kirkpatrick, Vernon, Barham, & Bateman, 1998; Hanna, Colyer, Kirkpatrick, Romaine, & Vernon, 1994) after resistant tomato, and cucumber and squash after resistant pepper (Thies et al., 2004).

### 3.5. Trap Crops

Trap cropping consists in planting a good host for a short period of time, enough to ensure high nematode penetration and initial development to a non-motile growth stage. After that, roots have to be removed or destroyed in order to kill nematodes before achieving reproduction (Sikora, Bridge, & Starr, 2005). This method is more attractive to growers if they can use a profitable short cycle vegetable included in their common crop rotation. For example, lettuce can act as a trap crop in the northeastern Spain when it is planted in October or November instead of September (Ornat et al., 2001). Lettuce and radish are used in organic peri-urban production in Cuba (Cuadra, Cruz, & Fajardo, 2000). Arugula (*Eruca sativa* L.) allows nematode infection but restricts its development and reproduction and can be used as a biofumigant when incorporated into the soil (Melakeberhan, Xu, Kravchenco, Mennan, & Riga, 2006).

### 3.6. Fallowing and Tillage

*Meloidogyne* is an obligate parasite that needs a host plant to complete its life cycle. Therefore, during the lack of a host plant the nematode has to consume their own reserves and could die by starvation. In the absence of a host plant, environmental conditions such as temperature and moisture are the main factors affecting survival rate of nematodes (Bergeson, 1959; Roberts, Van Gundy & McKinney, 1981; Towson & Apt, 1983; Goodell & Ferris, 1989). Soil tillage during the fallowing periods eliminates weeds and volunteer plants to prevent increases on nematode densities since *Meloidogyne* can reproduce on a wide range of weeds (Table 4).

Soil desiccation and direct heat from the sun may have an immediate impact on population decline. Decrease of population densities depends on the length of the fallow period related to the accumulated soil temperature during this period. In intensive agriculture the fallow period is limited to a few weeks. However when coupled with root destruction, even short-term fallowing has a significant impact on nematode populations (Verdejo-Lucas, 1999). For instance, fallowing during 8 weeks in summer, the hottest and driest season, reduced root knot nematode population about 50%, this reduction was about 80% when soil was tilled at the end of the crop just before fallowing (Ornat, Verdejo-Lucas, Sorribas & Tzortzakakis, 1999).

Table 4. Weed host species for *Meloidogyne incognita* (Mi), *M. javanica* (Mj) and *M. arenaria* (Ma) associated with vegetable crops in northeast of Spain (Ornat, 1998; Barceló, Sorribas, Ornat, & Verdejo-Lucas, 1997).

Botanic family	Species	
Amarantaceae	<i>Amaranthus albus</i> L.	Mi, Mj
	<i>A. blitum</i>	Mj
	<i>A. graecizans</i> L. <i>sylvestris</i> (Vill.)	Mi
	<i>A. hybridus</i>	Mi
	<i>A. retroflexus</i> L.	Mi, Ma
Caryophyllaceae	<i>Stellaria media</i> (L.) Vill.	Mi, Mj, Ma
Chenopodiaceae	<i>Atriplex patula</i> L.	Mi
	<i>Chenopodium album</i> L.	Mi, Mj
	<i>Ch. murale</i> L.	Mj
Compositae	<i>Cirsium arvense</i> (L.) Scop.	Mj
	<i>Erigeron</i> L. spp.	Mi, Mj
	<i>Galisongia parviflora</i> Cav.	Mi
	<i>Senecio vulgaris</i> L.	Mi, Mj
	<i>Sonchus oleraceus</i> L.	Mi, Mj
	<i>S. tenerrimus</i> L.	Mi, Mj, Ma
	<i>Xanthium strumarium</i> L.	Mi
Convolvulaceae	<i>Convolvulus arvensis</i> L.	Mi, Mj
Cruciferae	<i>Capsella bursa-pastoris</i> (L.) Medicus	Mi, Mj, Ma
	<i>Coronopus didymus</i> (L.) Sm.	Mi, Mj
	<i>Lepidium draba</i> L.	Mj
Cyperaceae	<i>Cyperus rotundus</i> L.	Mi
Euphorbiaceae	<i>Mercurialis annua</i> L.	Mj
Geraniaceae	<i>Geranium molle</i> L.	Mi
Gramineae	<i>Bromus willdenowii</i> Kunth	Mi
	<i>Cynodon dactylon</i> (L.) Pers.	Mi
	<i>Digitaria sanguinalis</i> (L.) Scop.	Mi, Mj, Ma
	<i>Lolium perenne</i> L.	Mi
	<i>Poa annua</i> L.	Mi, Ma
	<i>Setaria verticillata</i> (L.) Beauv.	Mi, Mj, Ma
	<i>Sorghum halepense</i> (L.) Pers.	Mi
	<i>Labiatae</i>	<i>Lamium amplexicaule</i> L.
Leguminosae	<i>Mentha</i> L. spp.	Mj
	<i>Medicago arabica</i> (L.) Hudson	Mj, Ma
	<i>Trifolium</i> L. spp.	Mi, Mj.
	<i>Vicia sativa</i> L.	Mi
Malvaceae	<i>Malva sylvestris</i> (L.)	Not identified
Oxalidaceae	<i>Oxalis corniculata</i> (L.)	Mj
	<i>O. corymbosa</i> DC.	Ma
Polygonaceae	<i>Polygonum aviculare</i> L.	Mi
	<i>Rumex crispus</i> L.	Mj, Ma
Portulacaceae	<i>Portulaca oleracea</i> L.	Mi, Mj
Primulaceae	<i>Anagallis arvensis</i> L.	Ma
Rosaceae	<i>Potentilla reptans</i> L.	Mi
Scrophulariaceae	<i>Veronica hederifolia</i> L.	Mi
Solanaceae	<i>Solanum nigrum</i> L.	Mi, Mj
Urticaceae	<i>Urtica urens</i> L.	Mi

### 3.7. Biological

Several fungi antagonistic to nematodes have been detected in the Mediterranean area. *Pochonia chlamydosporia* var. *chlamydosporia*, *P. chlamydosporia* var. *catenulata*, *Fusarium oxysporum*, *F. solani*, *Fusarium* spp., *Acremonium strictum*, *Gliocladium roseum*, *Cylindrocarpon* spp., *Engiodontium album*, *Dactylella oviparasitica*, and other fungi non identified were isolated from *Meloidogyne* in two areas of vegetable production in Mediterranean coast of Spain (Verdejo-Lucas, et al., 2002).

*Pochonia chlamydosporia* was assessed as biological control agent of *Meloidogyne* in plastic house experiments in Greece and Spain. In Greece, *P. chlamydosporia* had a variable establishment in soil and did not control the nematode (Tzortzakakis & Petsas, 2003; Tzortzakakis, Phillips, & Trudgill, 2000). In Spain, the same fungal isolate used in Greece, consistently reduce root galling in tomato but reduction in eggs per gram root was only achieved when a native isolate was used in multiple fungal applications (Sorribas, Ornat, Galeano, & Verdejo-Lucas, 2003; Verdejo-Lucas, Sorribas, Ornat, & Galeano, 2003). However, in an open field experiment carried out in Italy, the same isolate of *P. chlamydosporia* showed encouraging results (Ciancio, Leonetti, & Alba, 2002).

The bacterial obligate parasite *Pasteuria penetrans*, has been studied extensively as a control agent. In Spain, *P. penetrans* was detected in 7% of the 93 sampled fields adhered to cuticle of *Meloidogyne*, as well as other phytoparasitic nematodes. In natural conditions, the percentage of *Meloidogyne* juveniles with spores fluctuated between 16 and a 50% (Verdejo-Lucas, Español, Ornat, & Sorribas, 1997). In plastic house experiments in Crete, *Pasteuria penetrans* applied at 20,000 or 25,000 spores/g of soil was able to parasitize 65%–75% of juveniles + females (Tzortzakakis, Verdejo-Lucas, Ornat, Sorribas, & Goumas, 1999; Tzortzakakis & Gowen, 1994).

Currently, biological control of nematodes is difficult because of the complexity of the soil biology and environment to promote or establish antagonists in soils that effectively suppress nematode populations (Starr, Bridge, & Cook, 2002). For more information see chapters 2, 3, 10 and 15 in this book and Lopez-Llorca, Jansson, Macià, and Salinas (2006), Sikora (1992) and Stirling (1991).

### 3.8. Biofumigation

The term biofumigation has been applied to the process where volatile toxic gases are released in the degradation of organic amendments, plant roots, and tissues and where such gases control diseases, nematodes, and weeds. Brassicaceae are commonly researched as biofumigant due to the production of glucosinolates and their isothiocyanate (ITC) derivates, which have herbicidal, fungicidal, insecticidal, and/or nematicidal properties (Bello, 1998; Kirkegaard & Sarwar, 1998; Gamliel & Stapleton, 1993). No suppression of nematodes or inconsistencies among studies are attributed to different concentrations of glucosinolates derivates (Zasada & Ferris, 2004; Kirkegaard & Sarwar, 1998). Biofumigation combined with solarization can improve its effectiveness and have been used successfully in the production of

tomatoes, melons, peppers, and other vegetables (Bello, 1998; Sanz, Escuer & López-Pérez, 1998). For more information see Chapters 11 and 12 in this volume.

### 3.9. Chemical

Two major groups of nematicides are distinguished by the manner in which they spread through the soil. Soil fumigants are volatile chemicals that have to be applied before planting due to their phytotoxicity. Non-fumigant nematicides are liquids or solids that act by contact, or by plant systemic action, and can be applied at planting and after planting, depending on their degradation. Their distribution in soil depends on the soil water solution.

Methyl bromide was the most used fumigant by its effectiveness against soil born pathogens, pests and weeds. Nowadays, their use has been banned or restricted due to its effect on ozone depletion layer. The phase out for non Article 5 countries (developed countries) was in 2005, and will be in 2015 for Article 5 countries (developing countries). Alternatives to Methyl bromide are being assessed by the Methyl Bromide Technical Options Committee (MBTOC, 2006). Fumigants nematicides (1,3-dichloropropene, metham-sodium, chloropicrin) are more effective in the control of root-knot nematodes than non-fumigant nematicides (fenamiphos, cadusaphos, oxamyl). Fumigants could be adopted as an alternative to methyl bromide, but some of them could be banned in a short-term in some countries (see EC directive 91/414/CEE). Non-fumigants nematicides can lack their efficacy by microbial degradation due to repeated applications, i.e. ethoprophos and fenamiphos (Mojtahedi, Santo, & Pinkerton, 1991; Davis, Johnson, & Wauchope, 1993; Karpouzas, Giannakou, Walker, & Gowen, 1999; Karpouzas, Hatziapostolou, Papadopoulou-Mourkidou, Giannakou, & Geogiadou, 2004). Consequently, alternation of active compounds is required to prolong their efficacy (Sikora et al., 2005).

## 4. TOOLS FOR DECISION IN INTEGRATED PEST MANAGEMENT

All available management methods should be used in an integrated manner considering the biology of root-knot nematodes, nematode-plant interactions, agronomical practices, environmental characteristics, and socio-economic aspects of the specific growing area. The management of *Meloidogyne* with only one tactic may be partially effective (cultural practices) or may be no durable due to negative environmental effects (depletion of ozone layer, water contamination), lack of persistence (fast degradation in soil of some non-fumigant nematicides), occurrence of virulent populations or shift of nematological problems (plant resistance). Integrated nematode management (INM) is a strategy that uses all available tactics in a complementary and environmental safe manner to maintain nematode population levels below economic threshold.

Although guidelines for integrated pest management (i.e. IOBC/WPRS) and overviews for integrated nematode management (Sikora et al., 2005) have been

published to help technicians and vegetable growers it is necessary to validate and adapt them for each specific growing area.

Economic threshold, defined as the nematode population density at which the value of the damage caused equals the cost of control (Ferris, 1978), is a basic requirement to develop integrated nematode management strategies (INM). Estimation of the economic threshold is based on Seinhorst nematode damage function (1965), that relates nematode densities in preplanting to relative yield, crop value, cost of control, and the control cost function (Ferris, 1978). To design INM strategies for specific cropping sequences it is needed to construct nematode damage functions for each *Meloidogyne* spp.-vegetable combinations, and to know the fluctuation of root-knot nematode population considering the particular agrosystem. Thus, sampling plans are required to estimate frequency and abundance of plant parasitic nematodes (McSorley & Parrado, 1982; McSorley & Dickson, 1991; Prot & Ferris, 1992). In addition, the use of accurate methods to extract plant parasitic nematodes is essential (see Hooper & Evans, 1993). Knowledge of all this information allows the development of predictive models to make decisions on nematode management (McSorley & Phillips, 1993; Ferris & Noling, 1987).

#### *4.1. Management of Meloidogyne javanica with Rotation in Plastic Houses in Northeastern Spain: an Example*

In the vegetable production area of northeastern Spain two to three crops are usually cropped at the same site from spring through winter. Tomato and lettuce are the most frequently cultivated vegetables for fresh market in plastic houses. Tomato is growing from March to July, and lettuce is growing from mid September, October or November to December, January or February. Between crops, there is a fallow period. Fluctuation of nematode population densities are shown in Fig. 1. Control of root-knot nematodes in the area was mainly based on fumigants or non-fumigants nematicides. However, a change has been produced when growers accepted and implemented sustainable production systems that restrict or ban the use of nematicides. In this context, more accurate information was needed to design strategies to manage nematode problems.



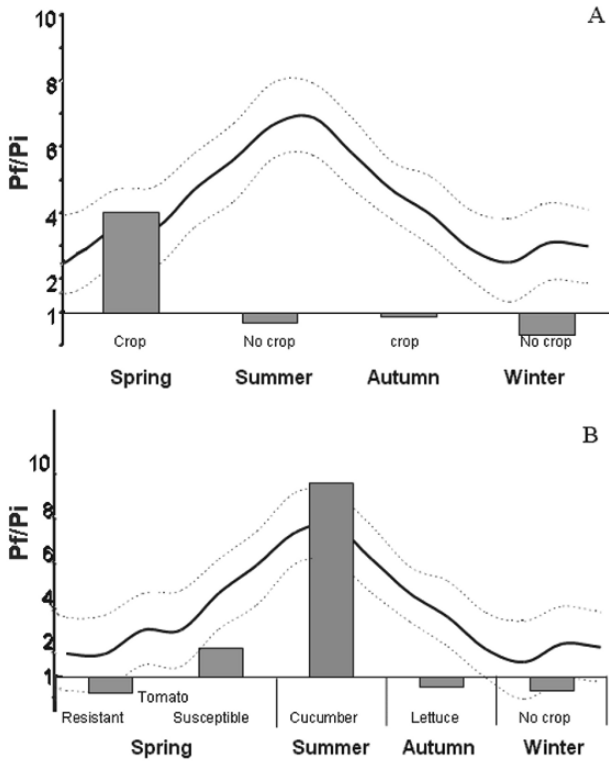


Figure 1. Average reproduction index of *Meloidogyne* in a cropping season in plastic house, (A) two crops per season, (B) three crops per season.

Considering two crops per season, a Seinhorst nematode damage function for *M. javanica*-tomato was obtained. Maximum yield losses and tolerance limit were estimated in 34% and 2 J2/250 cm<sup>3</sup> soil, respectively. Nematode achieves three generations during the tomato crop according to their thermal time model. At the end of the tomato crop, if plants are uprooted, nematode densities in soil decrease about 50% during the period between crops, and an additional 30% reduction occurs if soil is tilled. Conversely, nematode density does not decrease if the aboveground plants are cut and roots are left in soil. The following crop of lettuce can act as trap crop when it is planted in middle October or November reducing nematode densities between 50 and 20%, respectively, because the nematode can infect roots but does not accumulate enough degree days to reproduce at the end of the commercial crop.

Considering the usual range of nematode densities at the end of the susceptible tomato crops founds in this area (8 000 and 28 000 J2/250 cm<sup>3</sup> soil), the estimated increase in tomato yield using both agronomical methods, tillage and lettuce as trap crop, can range between 22 and 13%. The efficacy of these methods could be

increased using other economically interesting methods to growers as resistant tomato cultivars, that prevent increases in nematode population densities, and/or increasing accumulated temperature during fallowing periods by solarization alone, because survival of root-knot without host is inversely related to accumulated soil temperature, or combined with biofumigation.

#### ACKNOWLEDGEMENT

Authors thank the support of Comision Interministerial de Ciencia y Tecnología (CICYT), project AGL2004-01207/AGR

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