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K.G. Mukerji
Editors

Integrated Management of Plant Pests and Diseases

Integrated Management of Arthropod Pests and Insect Borne Diseases

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Integrated Management of Arthropod Pests and Insect Borne Diseases

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PREFACE

This is the last volume of the IPMD series. It aims, in a multi-disciplinary approach, at reviewing and discussing recent advances and achievements in the practice of crop protection and integrated pest and disease management. This last effort deals with management of arthropods, and is organized with a first section on biological control in citrus orchards, a second one on advanced and integrated technologies for insect pest management and a last section, dealing with mites and their biological control.

A wide and exaustive literature already covers several aspects of chemical or biological control of insects and mites, but there is still a need for a more holistic vision of management, accounting for different problems and solutions, as they are applied or developed, in different regions and cropping systems, worldwide. In this series we attempted to fill this gap, providing an informative coverage for a broad range of agricultural systems and situations.

As for the other volumes of the IMPD Series, also this book is organized in chapters mainly centered on crops, with a particular emphasis given on citrus production, which by itself covers the first section. Citrus is one of the main crops in which biological control and management of pests proved successful worldwide, and the experience gained in this field may indeed result helpful for IMP efforts deployed on other crops and/or cropping systems, in other cultivated areas. Chapters grouped in Section 2 review the advancements of the integration of insect management options with other crop problems in extensive cropping systems (i.e. pecan, Chapter 6), the application of remote sensing technologies (Chapter 7), the status of knowledge about plant defense compounds and their potentials (Chapter 8). For IPM of invasive species, Chapter 9 provides an update experience gained on the field to manage and counteract the Red Palm Weevil (RPW) in Egypt, a serious invasive pest spreading in all the mediterranean regions. Long-term technological solutions are described in Chapter 10, which deals with the extensive management of forests and park areas through aerial treatments of bacterial spores. Finally, in Section 3, an updated review about current knowledge on IPM through microbial control agents of mites is provided (Chapter 11), followed by a final chapter concerning features of predatory mites.

In conclusion of this series, our sincere acknowledgements go to all the authors that provided, through their experience and dedication, a broad range of data on the management solutions thay studied and made available in different agricultural systems of the world. Thanks to their efforts, we hope we were able to contribute to spread the basic concepts related to the application of environment friendly and sustainable management practices. Our hope is that this series will result useful and helpful for interested readers and students, inspiring and supporting, at the same time, new and innovative research efforts in their daily field and laboratory work.

A. Ciancio
K. G. Mukerji †

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OBITUARY NOTICE

Prof. K. G. Mukerji

Professor Krishna Gopal Mukerji passed away suddenly, aged 75. He was born on 4th May 1934 at Lucknow as fourth child of Mr. Davendra Nath Mukerji and Mrs. Leela Mukerji. He received his early education in Lucknow and obtained his B.Sc. and M.Sc. degrees from Lucknow University in 1953 and 1955, respectively. In 1955, he started his teaching career as Lecturer in Botany at Lucknow University, from where he also obtained his Ph.D. in Botany in 1962, with a thesis entitled "Microfungi of Usar Soil of India". He was then appointed Lecturer in Botany at Banaras Hindu University in 1962, where he worked for 2 years. He then joined the Department of Botany at the University of Delhi as Lecturer (1964), to be later appointed Reader and Professor in 1969 and 1982, respectively. He became Head of the Department of Botany, University of Delhi during the years 1985–1988. During his scientific career he was actively engaged in research on different aspects of microbial ecology, with particular reference to soil fertility and plant productivity. His significant contributions concerned different areas of mycology, plant pathology, microbial ecology and biotechnology. In his research work he investigated, discovered and described several species and genera of fungi, including mycorrhizae from Indian soils. Professor Mukerji had keen interest in culture collections and received advanced training in fungal taxonomy at the Commonwealth Mycological Institute (CMI) in Kew, England (1968), where he was also offered a permanent position as mycologist, in 1968. He also worked at the Institute of Seed Pathology at Copenhagen, Denmark (1974), at the Institute of Meeresforschung, Bremerhaven, Germany (1973) and at the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands (1974).

As visiting professor or scientist, professor Mukerji visited various Institutes, Culture Collections and Laboratories throught America, Asia, Australia and Europe. His career lasted 40 years and included taxonomy and ecology of fungi from soil, photosphere and roots, as well as mycorrhizae, plant surface, organic matter and seeds. More than 200 students received their Ph.D. and M.Ph. degrees from the Delhi University, studying various aspects of mycology, plant pathology, microbial ecology and microbial biotechnology under his supervision. Professor Mukerji was a widely traveled botanist and attended several national and international conferences and symposia, presenting key notes and invited papers. He was a member of numerous societies and associations, also as Treasurer (Mycological Society of India, 1973–1976), Vice-President (Mycological Society of India, 1980–1981 and Association of Tropical Microbial Ecology, 1980–1984), President (Society for Advancement of Botany, 1987, and Society for Environment scientists, 1988–1991). He was member of several editorial boards, inlcuding the Transactions of the Mycological Society of India (1979–198l), Phytologia, the Journal of the Indian Botanical Society and the Journal of Phytology Research (since 1988). He was also Editor or Advisory Committee member for several journals, including the Indian Journal of Microbial Ecology, Frontiers in Applied Microbiology, CRC Critical Reviews on Biological Control of Plant Pests, Diseases and Weeds (since 1990). Professor Mukerji was also Councillor for the International Society of Root Research and the Nitrogen Fixing Tree Association. He authored or co-authored more than 550 research papers on various aspects of mycology, plant pathology, microbial ecology and biotechnology, and co-authored, edited and co-edited more than 45 books, dealing with various aspects of microbial ecology and biotechnology.

Professor Mukerji retired as Senior Professor from the University of Delhi in 2004 but continued his intense editorial and publication activity until this year. As one of the most distinguished mycologist and microbial ecologist from India he is still credited for his research contributions, which are recognized all over the world. His death is a great loss for India as well as for the international scientific community. Those who had the priviledge of sharing with him some work time will remember a very gentle and kind person, and a bright, experienced and outstanding scientist. We convene our sincere and heartfelt condolences at this most difficult of times to his beloved wife and son. He will be greatly missed.

A. Ciancio
Zuzana Bernhart

Section 1

IPM IN CITRUS GROVES

1

CITRUS PEST MANAGEMENT IN THE NORTHERN MEDITERRANEAN BASIN (SPAIN, ITALY AND GREECE)

JOSEP ANTON JACAS[1], FILITSA KARAMAOUNA[2], ROSA VERCHER[3] AND LUCIA ZAPPALÀ[4]

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Abstract. Main management options for arthropod pests of citrus and species recently introduced in the northern Mediterranean regions are reviewed. Available control strategies are discussed, including visual inspection practices, insect trapping methods and natural enemies release in augmentative or classical biological control. IPM practices and side effects of pesticides are also reviewed.

1. INTRODUCTION

The most widely cultivated citrus species in the Mediterranean region include orange [*Citrus sinensis* (L.) Osbeck], lemon [*Citrus limon* (L.) Burman f.], mandarin (*Citrus reticulata* Blanco), tangerine (*Citrus deliciosa* Tenore), grapefruit (*Citrus paradisi* Macfadyen), sour orange (syn. Chinese bitter orange, bigarade orange, Seville orange) (*Citrus aurantium* L.), lime [*Citrus aurantifolia* (Christm.) Swingle] and citron (*Citrus medica* L.) (Katsoyannos, 1996). Minor citrus species are pumelo (syn. shaddock) [*Citrus maxima* (Burm.) Merrill, syn. *C. grandis* (L.) Osbeck, *C.*

All authors contributed equally to this chapter.

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decumana L.], bergamot (*Citrus bergamia* Risso) and chinotto or myrtle-leaved orange (*Citrus myrtifolia* Raf.).

The total area of citrus production in the Mediterranean region sums up to 1,036,878 ha (Franco, García-Marí, Ramos, & Besrí, 2006; ISTAT, 2007; El-Otmani, Srairi, & Benhaddou, 2007; Laajimi & Ben Mimoun, 2007; MAPA, 2007; Salama Eid, Latif, & Hassan, 2007) with more than 2/3 of the entire area concentrated in Spain, Italy, Egypt and Turkey (Table 1). Integrated Pest Management is performed on a percentage of this area that varies in each country from less than 1% in France (Corsica) to 100% in Israel. In Italy, Morocco and Portugal 10–20% of the total citrus production area is under IPM, while in Turkey this management strategy is applied on 30% of this area. Integrated production (IP) is only reported in Spain, Italy, Portugal and France ranging between 0.4% (Portugal) and 10% (Italy) of the total citrus area (Franco et al., 2006).

Table 1. Total area of citrus production and percentage of integrated pest management (IPM) and integrated production (IP) in Mediterranean countries (modified from Franco et al., 2006. N.a. stands for not available).

Country	*Citrus production area (ha)*	*IPM (%)*	*IP (%)*
Spain	311,004	Most of the area	5
Italy	164,938	10–20	10
Egypt	151,075	n.a.	n.a.
Turkey	150,000	30	–
Morocco	80,000	10–20	–
Greece	57,526	Most of the area	–
Algeria	45,400	n.a.	–
Portugal	27,755	14	0.4
Tunisia	18,600	n.a.	n.a.
Israel	17,300	100	–
Georgia	11,000	n.a.	–
France (Corsica)	1,800	<1	<5
Montenegro	480	–	–

In Italy, France, Portugal and Spain citrus growers receive financial support for practicing IPM and IP as part of EU Agri-environmental measures. Requisites for a grower to receive this aid, depending on the country, include: being an associate of an IPM/IP farmer organization (Italy, Portugal); attending a course on IPM/IP (Spain, Portugal); following official IPM/IP guidelines (Italy, Spain, Portugal); keeping accurate records of pesticide applications and, in the case of IP, other cultural practices (Italy, Portugal, Spain).

IPM/IP guidelines are defined at regional level in Greece, Portugal and Turkey, and at both regional and national level in Israel, Italy and Spain. IPM/IP certification companies are reported in Italy, Spain and Portugal (Franco et al., 2006).

Guidelines for Integrated Production of Citrus have been produced by the IOBC/WPRS Commission "IP-Guidelines and Endorsement" (IOBC/WPRS, 2004), and they mainly concern standards for the IP organizations in order to develop national or local guidelines. According to these standards and in relation to integrated plant protection, all available preventive (indirect) plant protection measures must be applied before direct control techniques are used. Priority should be given to natural, cultural, biological, genetic (GMOs are generally excluded and permission may be given on a case-by-case study) and biotechnical methods of pest control and the use of agrochemicals should be minimized.

The decision for the application of direct control methods must be based on economic thresholds, wherever possible, risk assessments and forecasts, including those provided by official forecasting services. A restricted list of the key pests, diseases and weeds that require regular attention must be established by the IP organizations and their populations should be regularly monitored and recorded. In addition at least three key natural enemies in each crop must be identified in national/regional guidelines. Furthermore, the use of plant protection products toxic to these beneficial arthropods should be reduced to a minimum and always in periods of low activity of the natural enemies, or of low risk for them. Populations of key natural enemies must be preserved and incremented.

With regard to pesticides, all those locally or nationally available must be classified by the IP organizations in two lists: the "green list" including the permitted products and the "yellow list" including those pesticides permitted with restrictions. The pesticides categorization is based on several criteria (i.e. toxicity to man, toxicity to key natural enemies, toxicity to other natural organisms, pollution of ground and surface water, ability to stimulate pests, selectivity, persistence, incomplete information, necessity of use), and it is already established for certain pesticides and pesticide groups as follows:

- Not permitted: pyrethroid insecticides and acaricides, non-naturally occurring plant growth regulators (their use can only be allowed when absolutely necessary), organochlorine insecticides and acaricides, water polluting products and very persistent herbicides;

- Permitted with restrictions: dithiocarbamate fungicides (normally maximum of three applications per season and not in succession, so that predatory phytoseiid mites are not affected), fosetil-Al and phosphonate potassium (maximum of two applications per year), metalaxyl (maximum of 2 g/m^2), residual (soil) herbicides (except toxic, polluting or very persistent products) in the first 3 years after planting (maximum of one dose-equivalent per annum).

Officially recognized dose adjustment protocols must be used where available, in order to adapt dose rates to the size and density of the target trees being sprayed. The maximum volume of application per hectare must be defined according to the tree volume. A strategy of mandatory measures for minimizing the risk of resistance development of pests to pesticides (e.g. maximum number of applications per year, alternation of pesticides with different mode of action) must be set by the IP organizations. The growers/applicators must be trained in the use and the application of pesticides.

2. MAIN ARTHROPOD PESTS AND CONTROL STRATEGIES

In the citrus producing countries of the Mediterranean basin more than 140 pests and diseases are reported, including 108 insects, 10 mites, 1 nematode, 14 fungi, 2 bacteria and 8 virus and virus like diseases (Franco et al., 2006).

Among arthropods, the major pests, i.e. reported as key-pests in at least 50% of the countries, include the medfly *Ceratitis capitata* (Wiedemann) (100% of the countries), the California red scale *Aonidiella aurantii* (Maskell) (71%), the citrus leafminer *Phyllocnistis citrella* Stainton (71%) and the citrus mealybug *Planococcus citri* (Risso) (71%) (Table 2).

Quarantine pests recently introduced in the western Mediterranean area include the brown citrus aphid *Toxoptera citricida* (Kirkaldy) (Northern Spain, from Galicia to the Basque Country, Madeira island and North of Portugal), the African citrus psylla *Trioza erytreae* (Del Guercio) (Madeira and Canary islands) and the citrus snow scale *Unaspis citri* (Comstock) (Azores, Malta and France). These species are included in the EPPO lists A1–A2 and are therefore regulated as quarantine pests in the whole EPPO region (EPPO, 2007). Special attention must be paid to *T. citricida* and *T. erytreae* as they are efficient vectors of the citrus tristeza virus (CTV) and the Huanglongbing agent (Candidatus *Liberobacter*), respectively.

2.1. Sampling and Monitoring

In IPM, pest control decisions are directly dependent upon knowing the status and population trends of the most important insect pests and their natural enemies (Beardsley, AliNiazee, & Watson, 1979; Cavalloro & Prota, 1983; Katsoyannos, 1996). Sampling and monitoring are the means for acquiring this important knowledge. Simplified sampling guidelines for monitoring the main citrus insect pests in the northern Mediterranean have been advised by experts defining methods of visual inspection as well as trapping using food, chromotropic and sexual attractants for monitoring purposes (Katsoyannos, 1996) (Tables 3 and 4).

2.2. Biological Control

Biological Control has been proved very effective in management of insect pests in citrus orchards. Among 65 cases of successful biological control and 83 cases of satisfactory control of insect pests in various crops, which have been recorded all over the world, 61.5% of the first mentioned and 21.7% of the latter concern citrus (De Bach, 1964).

Several programmes of augmentative and classical biological control by means of parasitoids and predators of the main citrus pests have been conducted in most of the northern Mediterranean citrus growing countries (Table 5). The results vary, however, several successful cases have been recorded (Viggiani, 1975; Amaro, 1992; Noyes & Hayat, 1994; Katsoyannos, 1996; Tsagarakis, Kalaitzaki, Lykouressis, Michelakis, & Alexandrakis, 1999; Kalaitzaki, 2004; Siscaro, Caleca, Reina, Rizzo, & Zappalà, 2003; Siscaro, Di Franco, & Zappalà, 2008; Gomes da Silva, Borges da Silva, & Franco, 2006; Jacas, Urbaneja, & Viñuela, 2006; Malausa, Rabasse, & Kreiter, 2008; Zappalà, Siscaro, & Longo, 2008).

Table 2. Arthropod pests of citrus and rating of their pest status in the northern Mediterranean regions (modified from Franco et al., 2006).*

Group	*Order*	*Family*	*Species*	*France (F)*	*Greece (G)*	*Italy (I)*	*Montenegro (M)*	*Portugal (P)*	*Spain (S)*	*Turkey (T)*
Insects										
	Orthoptera									
		Acrididae	*Anacridium aegyptium* (L).		0	1	0	1	1	0
		Tettigonidae	*Phaneroptera nana* Fiebre		0	0	0	2	1	0
	Thysanoptera									
		Thripidae	*Frankliniella bispinosa* (Morgan)		0	0		1[a]	0	0
			Frankliniella occidentalis (Pergande)	1	1	1		2	1	2
			Heliothrips haemorrhoidalis (Bouché)		1	2[b]		2	1	0
			Pezothrips kellyanus (Bagnall)		2	2		1[b]	1	2
			Thrips australis (Bagnall)		0	0		1	0	0
			Thrips flavus Schrank		1	1		2	1	0
			Thrips major Uzel		1	0		2	1	2
			Thrips tabaci Lindeman		1	1	1	2	0	2
	Hemiptera									
		Pentatomidae	*Nezara viridula* (L.)		1	1	0	1	0	2
		Miridae	*Closterotomus trivialis* (Costa)		2	2	0	0	1,2	0
		Flatidae	*Metcalfa pruinosa* (Say)	**3**	**1**	**1**	**1**	**0**		**0**
		Cicadellidae	*Empoasca decedens* (Paoli)		0	1	1	0	2[c]	1
		Triozidae	*Trioza Erytreae* (Del Guercio)	**0**	**0**	**0**		**3**[a]	**2**[d]	**0**

Table 2 continued

Group	*Order*	*Family*	*Species*	*France (F)*	*Greece (G)*	*Italy (I)*	*Montenegro (M)*	*Portugal (P)*	*Spain (S)*	*Turkey (T)*
		Aleyrodidae	*Aleurothrixus Floccosus* (Maskell)	**3**	**2**	**2**	**1**	**3**	**2**	**2**
			Bemisia afer (Priesner & Hosny)		0	1	0	0	1	0
			Bemisia tabaci (Gennadius)							2
			Dialeurodes citri (Ashmead)	**3**	**2^{e}**	**1**	**3**	**0**	**1**	**1**
			Dialeurodes citrifolii (Morgan)		0	0	0	1^{a}	0	0
			Parabemisia myricae (Kuwana)	0	1	1	0	1	1	2
			Paraleyrodes bondari Peracchi		0	0	0	1^{a}	0	0
			Paraleyrodes citricolus Costa Lima		0	0	0	1^{a}	0	0
			Paraleyrodes minei Iaccarino		0	0	0	1	1	2
		Aphididae	*Aphis craccivora* Kock	2	1	1	2	1	1	2
			Aphis fabae Scopoli	2	1	1		1	1	0
			Aphis gossypii Glover	**3**	**2**	**2**	**0**	**3**	**2**	**1**
			Aphis spiraecola Patch		**2**	**2**	**0**	**3**	**2**	**1**
			Aulacorthum solani (Kaltenbach)	2	1	1	0	1	0	0
			Macrosiphum euphorbiae (Thomas)	2	1	1	0	1	0	0
			Myzus ornatus Laing		0	0	0	1^{a}	0	0
			Myzus persicae (Sulzer)	2	1	1		1	1	1
			Neomyzus circunflexum (Buckton)		0	0	0	0	0	0
			Rhopalosiphum maidis (Fitch)		0	1	0			0
			Toxoptera aurantii (Boyer de) (Fonscolombe)	**3**	**2**	**2**	**2**	**3**	**1**	**2**
			Toxoptera citricida (Kirkaldy)	**0**	**0**	**0**	**0**	**3^{fa}**	**2^{g}**	**0**
		Margarodidae	*Icerya purchasi* Maskell	**3**	**1**	**1**	**1**	**2**	**1**	**1**
		Ortheziidae	*Orthezia insignis* Douglas		0	0	0	1^{a}	0	0

Table 2 continued

Group	*Order*	*Family*	*Species*	*France (F)*	*Greece (G)*	*Italy (I)*	*Montenegro (M)*	*Portugal (P)*	*Spain (S)*	*Turkey (T)*
		Pseudococcidae	*Nipaecoccus nipae* (Maskell)		0	0	0	1[a]	0	0
			Phaenacoccus madeirensis Green		0	1	0	0		0
			Planococcus citri (Risso)	**3**	**3**	**2, 3**	**1**	**3**	**1, 2**	**3**
			Pseudococcus calceolariae (Maskell)		0	1	0	2	1	0
			Pseudococcus longispinus (Targioni-Tozzetti)		1	1	0	1	1	0
			Pseudococcus viburni (Signoret)		0	1	0	2	0	1
		Coccidae	*Ceroplastes floridensis* Comstock		**1**	**0**	**0**	**1**[ah]	**1**	**3**
			Ceroplastes japonicus Green		1	1	0	0		0
			Ceroplastes rusci (L.)		1	2	0	1	1	1
			Ceroplastes sinensis Del Guercio	**3**	**1**	**1**	**1**	**2**	**1**	**0**
			Coccus hesperidum L.	**3**	**1**	**1**	**1**	**2**	**1**	**2**
			Coccus pseudo-magnoliarum (Kuwana)		1	1	2	0		2
			Coccus viridis (Green)		0	0	0	1[a]	0	0
			Eucalymnatus tessellates (Signoret)		0	0	0	1[a]	0	0
			Parasaissetia nigra (Nietner)		0	0	0	1[a]	0	0
			Parthenolecanium persicae (F.)		0	1	0	1	0	0
			Protopulvinaria pyriformis (Cockerell)	0	1	1	0	1,2[a]	1	0
			Pulvinaria floccifera (Westwood)		0	1	0	0		0
			Saissetia coffeae (Walker)		0	1	0	1,2	1	
			Saissetia oleae (Olivier)	**3**	**1**	**2**	**2**	**2**	**1**	**2**
		Diaspididae	*Aonidiella aurantii* (Maskell)	**3**	**2**	**3**	**1**	**3**[i]	**3**	**3**
			Aspidiotus nerii Bouché		**1**	**3**[b]	**0**	**1**	**2**[b]	**0**
			Chrysomphalus aonidum (L.)		1	2	0	0	0	0
			Chrysomphalus dictyospermi (Morgan)	**3**	**1**	**1**	**1**	**2**	**1**	**2**

Table 2 continued

Group	*Order*	*Family*	*Species*	*France (F)*	*Greece (G)*	*Italy (I)*	*Montenegro (M)*	*Portugal (P)*	*Spain (S)*	*Turkey (T)*
			Chrysomphalus pinnulifer Maskell		0	0	0	1,2[a]	0	0
			Hemiberlesia rapax (Comstock)		0	1	0	1	1	0
			Lepidosaphes gloverii (Packard)	**3**	**0**	**2**	**0**	**1**	**1**	**0**
			Lepidosaphes beckii (Newman)	**3**	**2**	**1**	**1**	**3**	**2, 3**	**2**
			Lopholeucaspis japonica (Cockerell)		0	0	0	0		0
			Mycetaspis personata (Comstock)		0	0	0	1[a]	0	0
			Parlatoria pergandei Comstock	**3**	**1**	**2**	**0**	**2**	**2, 3**	**1**
			Parlatoria ziziphi (Lucas)	0	2	2	0	0	1	0
			Diaspidiotus perniciosus (Comstock)		0	0	0	0	0	0
			Unaspis citri (Comstock)	**0**	**0**	**0**	**0**	**3**[h]	**0**	**0**
			Unaspis yanonensis (Kuwana)	2	0	2	0	0		0
	Lepidoptera									
		Gracillariidae	*Phyllocnistis citrella* Stainton	**3**	**2**	**2**[jk]	**3**[jk]	**3**[j]	**1, 2**	**3**[j]
		Hyponomeutidae	*Prays citri* (Millière)	**3**	**2**	**2**[b]	**0**	**3**[b]	**3**[b]	**1**[b]
		Tortricidae	*Archips rosanus* (L.)		1	1	1	0		0
			Cacoecimorpha pronubana (Hübner)		1	1	1	2	1	2
		Geometridae	*Cleora fortunata* (Blachier)		0	0	0	1[a]	0	0
			Gymnoscelis pumilata Hübner		0	1	0	0		0
			Gymnoscelis rufifasciata (Haw.)		0	0	0	1	0	0
		Noctuidae	*Helicoverpa armigera* (Hübner)		0	0	0	1[b]	1	2
			Peridroma saucia (Hübner)		0	0	0	1	0	0
		Pyralidae	*Cryptoblabes gnidiella* (Millière)		1	1	0	2	1	2
			Ectomyelois ceratoniae (Zeller)		1	1	0	2	1	2

Table 2 continued

Group	*Order*	*Family*	*Species*	*France (F)*	*Greece (G)*	*Italy (I)*	*Montenegro (M)*	*Portugal (P)*	*Spain (S)*	*Turkey (T)*
		Nymphalidae	*Charaxes jasius* L.		0	1	0	0		0
	Diptera									
		Tephritidae	*Ceratitis capitata* (Wiedemann)	**3**	**3**	**3**	**2, 3**[e]	**3**	**3**	**3**[lm]
	Coleoptera									
		Curculionidae	*Asynonychus godmani* (Cratch)		0	0	0	1	0	0
			Lyxus algirus L.		0	0	0	2	1	0
			Otiorrhynchus aurifer Boheman		0	**2**[jk]	0	0		0
			Otiorrhynchus cribricollis Gyllenhall		0	**2**[jk]	0	0	1	0
			Pantomorus cervinus (Boheman)		0	0	0	2[a]	0	0
		Scarabaeidae	*Cetonia carthami aurataeformis* Curtis		0	0	0	1	0	0
			Oxythyrea funesta (Poda)		1	1	1	1	1	0
			Tropinota hirta (Poda)			1	1	1	1	0
			Tropinota squalida (Scop.)		1	1	0	1	1	0
	Hymenoptera									
		Formicidae	*Camponotus nylanderi* Emery		0	2[n]	0	0		0
			Crematogaster scutellaris (Olivier)		0	2[n]	0	0		0
			Lasius niger (L.)							
			Linepithema (=*Iridomyrmex*) *humile* (Mayr)		0	2[n]	0	2[a]	1	0
			Tapinoma nigerrimum (Nylander)		0	2[n]	0	2	1	0
			Tapinoma simrothi Krausse		0	2	0	0		0
Mites					0	0	0	2[a]	1	0
	Acariformes									
		Eriophyidae	*Aculops pelekassi* (Keifer)		2	2	2	0	0	0
			Eriophyes sheldoni (Ewing)		2	2[b]	1	2[b]	2[b]	1
			Phyllocoptruta oleivora (Ashmead)		**1**	**0**	**0**	**0**	**0**	**3**

Table 2 continued

Group	*Order*	*Family*	*Species*	*France (F)*	*Greece (G)*	*Italy (I)*	*Montenegro (M)*	*Portugal (P)*	*Spain (S)*	*Turkey (T)*
		Tarsonemidae	*Polyphagotarsonemus latus* (Banks)		1	2[b]	0	2[bk]	1	2
		Tenuipalpidae	*Brevipalpus californicus* (Banks)		1	1	0	1	1	0
			Brevipalpus phoenicis (Geijskes)		1	0	0	2	1	0
		Tetranychidae	*Eutetranychus banksi* (McGregor)		**0**	**0**	**0**	**3**[i]	**2**[o]	**0**
			Eutetranychus orientalis (Klein)		0	0	0	0	2[c]	0
			Panonychus citri McGregor	**3**	**2**	**2**	**3**	**2**	**2**	**1**
			Tetranychus urticae (Koch)	**1**	**2**	**1,2**	**1**	**1**	**1–3**	**2**

* Ratings: 3 = key pest, requires the application of control measures most of the years because of economic damage; 2 = occasional pest, may reach economic injury level; 1= potential pest, always below economic injury level; 0 = not reported on citrus. [a] = Madeira Island. [b] = on lemon. [c] = in Southern Spain. [d] = only in Canary Islands. [e] = limited areas. [f] = North of Portugal. [g] = not reported in the main citrus growing areas of Spain yet. [h] = Azores. [i] = Algarve. [j] = on young trees. [k] = in nurseries. [l] = on mandarin. [m] = on sweet orange. [n] = natural enemies disruption. [o] = in western Andalusia. Ratings of species considered key-pests in at least one country are shown in bold.

Table 3. Visual inspection practices.

Plant parts	*Purpose – target pest*
During the growing season/ warm periods of the year	Twigs are shaken by hand and the number of adults of whiteflies taking flight is noted (every week)
Twigs	Observations are made on the presence of honeydew, sooty mould and ants, which are associated with the presence of aphids, whiteflies and soft scales as well as on cottony egg masses which are associated with mealybugs. Parasitism should be also recorded
	Detection of the citrus leafminer
Apical twigs of…	Monitoring the presence of aphids (every week) and *Closterotomus trivialis* (spring)
Green twigs	Monitoring the development of armoured scales (every 2 weeks), soft scales (every 2–3 weeks) and 1st and 2nd instar nymphs of *Icerya purchasi*
Previous flushing twigs	Detection of *Eriophyes sheldoni*-affected organs in spring and mid-summer
Flowers	Detection of the citrus moth
Fruits	Monitoring the development of diaspidid scales (every 2 weeks) and locating foci of infestation Fruits and fruit stem inspections for mealybugs, white cottony egg mass, sooty mould developed on secreted honeydew and ants, which are associated with mealybugs (every 2 weeks) Detection of the citrus moth Detection of *Tetranychus urticae* during the summer-early autumn Detection of *Ceratitis capitata* as colour change begins In the packinghouse, presence of diaspidid scales should be checked
Young leaves	Detection of whitefly and soft scales foci of infestation in the orchard Observations are made on the presence of sooty mould and ants, which are associated with whiteflies, soft scales and mealybugs (every 2–3 weeks) Detection of the citrus mite, *Panonychus citri*, at the end of summer
Cold periods of the year	Detection of *T. urticae* from May to October
Twigs	Detection of 3rd instar nymphs and pre-ovipositing females of *I. purchasi*

[a] Visual inspections also allow monitoring coccinellid predators on trees.

Table 4. Insect pests trapping methods.

Type of trap	*Purpose – target pest*
Yellow water-pan traps	Monitoring aphids: Moericke pan-traps, both square (60 × 60 × 10 cm) and round (30 cm in diameter), painted canary yellow inside and containing water with a spoonful of added detergent up to a depth of 3–4 cm are commonly used; should be placed in the citrus orchards in mid-spring, 2–5 traps/ha, 70 cm above the ground, to be checked 1–2 times/week during the growing season
Suction traps of 12 m high	Monitoring aphids
Yellow sticky traps	Catching newly emerged whitefly adults: traps should be placed in the lower outside canopy of the south or southeast quadrant of the tree Catching males of *Aonidiella aurantii* and other armoured scales Monitoring parasitoids and detection of leafhoppers (*Empoasca* spp.)
Sex pheromone-baited traps	Attracting adult males of *A. aurantii* [pheromone (3Z, 6R)-3-methyl-6-isopropenyl-3.9-decadien-l-yl acetate]; 2–5 traps/ha at 1.8–2.5 height above the ground, to be checked twice a week from early spring to mid-late autumn Attracting adult males of *Planococcus citri*: various designs of traps available, the yellow or white sticky trap with pheromone [(1R-CIS)-3-isopropenyl-2.2-dimethylcyclobutyl-methyl acetate] dispenser being most effective; 2–5 traps/ha; catches to be correlated with shifts in the population densities of female mealybugs Monitoring of the citrus moth, *Prays citri*
White traps coated with glue mixed with trimedlure as well as other sex and food attractants	Catching adults of *Ceratitis capitata*; 10 traps/ha
Transparent sticky band traps	Measuring the density of crawlers of *A. aurantii* in order to determine the timing of chemical treatment; the traps are tightly fixed around heavily infested twigs from mid spring onwards For other diaspidids and soft scales
Beating branches of trees with a rubber-covered stick over a 1 m^2 cloth screen	Monitoring coccinellid predators

Table 5. Parasitoids/predators associated with augmentative or classical biological control (ABC and CBC respectively) of citrus insect pests in northern Mediterranean countries (modified from Katsoyannos, 1996).

		*Country of release / Frequency of occurrence / Success of control**					
Insect pests	*Parasitoids/Predators*	*France*	*Greece*	*Italy*	*Portugal*	*Spain*	*Turkey*
Flatid planthoppers (Flatidae)							
Metcalfa pruinosa	*Neodryinus typhlocybae* (Ashmead) (Dryinidae)	CBC, 1996: E, Fr		CBC, 1987 (on other crops in Veneto): E, Fr; CBC, 2004 (on citrus in Sicily): E		CBC, 2007: (on other crops)	
Whiteflies (Aleyrodidae)							
Aleurothrixus floccosus	*Cales noacki* Howard (Aphelinidae)	CBC, 1971: E, vFr	CBC, 1991: E, vFr	CBC, 1980s: E, vFr	CBC, 1978: E, vFr	CBC, 1970: E, Fr, SC	
	Amitus spiniferus (Brèthes) (Platygastridae)	CBC, 1973: E; CBC, 1980-82: TE, F (Corsica)		CBC, 1981: E (Liguria) CBC, 1983: E, P (Sicily)		CBC, 1971: E, MrC	
	Delphastus pusillus (LeConte) (Coccinellidae)	CBC, 1994: F					
Dialeurodes citri	*Encarsia* (= *Prospaltella*) *lahorensis* (Howard) (Aphelinidae)	CBC, 1976: E, Fr CBC, 1984: NR (Corsica)	CBC, 1976: E (Corfu)	CBC, 1973: E, vFr CBC, 1978: E, vFr (Sicily); CBC, 1980: NR (Sardinia)		CBC, 1992: NR	CBC, 1976: NT
	Serangium montazerii Fürsch (= *Serangium parcesetosum* Sicard) (Coccinellidae)	CBC, 1986: E, Re (Corsica) CBC, 1987-88: E, Re					

Table 5 continued

Insect pests	*Parasitoids/Predators*	*Country of release / Frequency of occurrence / Success of control**					
		France	*Greece*	*Italy*	*Portugal*	*Spain*	*Turkey*
Parabemisia myricae	*Eretmocerus debachi* Rose & Rosen (Aphelinidae)		CBC, 1989-91: NR (Crete)	CBC,1991: E; CBC, 1991: NR (Sicily)		CBC, 1995: E, Fr, SC	CBC, 1986: E, Fr
Aphids							
	Lysiphlebus testaceipes (Cresson) (Braconidae)	CBC, 1973-74: E, Fr		Accidental introduction, 1977: Fr		CBC, 1976: E, Fr, PC	
	Harmonia axyridis Pallas (Coccinellidae)		CBC, 1994: F (continent, Chios & Crete)				
Fluted scales (Margarodidae)							
Icerya purchasi	*Rodolia* (= *Novius*) *cardinalis* (Mulsant) (Coccinellidae)	CBC, 1912: E, Fr	CBC, 1913: Fr (Chios)	CBC, 1901: E, Fr	CBC, 1988: E, Fr	CBC, 1922: E, Fr; CBC, 1997: F	CBC, 1912: E, Fr
Mealybugs (Pseudococcidae)							
Planococcus citri	*Leptomastix dactylopii* Howard (Encyrtidae)	CBC, 1953: F CBC, 1972: E, MdC		CBC, 1956: E, Fr (Sicily); CBC, 1960s: F (Procida island); CBC, 1975: NR; CBC, 1989-90: NR; ABC, 1979-81: PC (Sardinia)	CBC, 1991: F	CBC, 1951: NR; CBC, 1977: E, Fr, PC; CBC, 1981: NR; ABC, 1978: PC	CBC, 1969: E, MdC

Table 5 continued

Insect pests	*Parasitoids/Predators*	*Country of release / Frequency of occurrence / Success of control**					
		France	*Greece*	*Italy*	*Portugal*	*Spain*	*Turkey*
Planococcus citri	*Cryptolaemus montrouzieri* Mulsant (Coccinellidae)	CBC, 1918: E, Fr	CBC, 1933, 1964-5, 1969: F (continent); CBC, 1977: NR; ABC, 1977: SC (Crete); ABC, 1991-92: SC (continent)	CBC, 1908, 1988: E; CBC, 1965: E (Sicily); ABC, 1965: SC (Sicily); ABC, 1979-81: SC (Sardinia)	CBC, 1918-29: E	CBC, 1926, 1974: NR; ABC, 1927: E, Fr, PC	CBC, 1965: NR
	Nephus reunioni Fürsch (Coccinellidae)		CBC, 1977: NR (Crete); ABC, 1970s: PC (Crete);	CBC, 1970s: NR (Sicily)	CBC, 1984: E		
	Nephus (*Sidis*) *anomus* (Mulsant & Rey) (Coccinellidae)		ABC, 1991-92: PC (continent); CBC, 1992: NR (continent)				
	Nephus quadrimaculatus (Herbst) (Coccinellidae)		ABC, 1991-92: PC (continent)				
	Coccidoxenoides perminutus Girault (= *Pauridia peregrina* Timberlake) (Encyrtidae)			CBC, 1959: F (Procida island)			
Soft scales (Coccidae)							
Coccus hesperidum	*Coccophagus ceroplastae* (Howard) (= *Aneristus ceroplastae* Howard) (Aphelinidae)	CBC, 1973-75: E					
Coccus pseudomagnoliarum	*Rhyzobius forestieri* (Mulsant) (Coccinellidae)		CBC, 1981: E, Fr (Chios); ABC, 1983-92:				

Table 5 continued

Insect pests	*Parasitoids/Predators*	Country of release / Frequency of occurrence / Success of control*					
		France	*Greece*	*Italy*	*Portugal*	*Spain*	*Turkey*
Saissetia oleae	*Microterys nietneri* (Motschulsky) (Encyrtidae)	CBC, 1969:E, MdC; CBC, 1971:	CBC, 1962: E, MdC	CBC, 1970: E, MdC (Sardinia); CBC, 1971: E, MdC	CBC, 1977-78: E, Fr	CBC, 1921: F	
	Metaphycus helvolus (Compere) (Encyrtidae)	E, MdC (Corsica)				CBC, 1976: E, MdC	
	Metaphycus lounsburyi (Howard) (= *Metaphycus bartletti* Annecke & Mynhardt) (Encyrtidae)	CBC, 1970s E, MdC; CBC, 1980: E, MdC (Corsica)	CBC, 1970s: TE (Crete)	CBC, 1979: E, MdC		CBC, 1979: E, MdC; CBC, 1988: E, PC	
	Metaphycus swirskii Annecke & Mynhardt (Encyrtidae)	CBC, 1979: E	CBC, 1970s: TE (Crete);	CBC, 1977: E			
	Diversinervus elegans Silvestri (Encyrtidae)	CBC, 1972-75: E, PC; CBC, 1979: NR (Corsica)	CBC, 1973-79; TE (Crete)	CBC, 1977: NR			
	Rhyzobius forestieri (Mulsant) (Coccinellidae)	CBC, 1986: E, Fr	CBC, 1981: E, Fr (Chios); ABC, 1983: SC (Chios)				
Armoured scales (Diaspididae)							
Aonidiella aurantii	*Aphytis melinus* DeBach (Aphelinidae)		CBC, 1962: E, MdC E, MrC (Crete)	CBC, 1964: E, MdC (Sicily)		CBC, 1976: E, Fr, PC	
	Aphytis lingnanensis Compere (Aphelinidae)		CBC, 1963: F	CBC, 1966: NR (Sicily)		CBC, 1976: E, PC	CBC, 1968: NR

Table 5 continued

Insect pests	*Parasitoids/Predators*	*Country of release / Frequency of occurrence / Success of control**					
		France	*Greece*	*Italy*	*Portugal*	*Spain*	*Turkey*
Aonidiella aurantii	*Aphytis coheni* DeBach (Aphelinidae)		CBC, 1962: F			CBC, 1936:F; CBC, 2000:F	
	Encarsia (= *Prospaltella*) *perniciosi* (Tower) (Aphelinidae)		CBC, 1969: NR				
	Comperiella bifasciata Howard (Encyrtidae)	CBC, 1960: E, Fr	CBC, 1921, 1969, 1972: NR; CBC, 1970s: E, MrC (Crete)	CBC, 1939:NR CBC, 1989:E, Fr (Sicily)			CBC, 1987:NR
	Rhyzobius (= *Lindorus*) *lophanthae* (Blaisdell) (Coccinellidae)	Accidental introduction, 1908: Fr		CBC, 1908: E, Fr, P		CBC, 1908: E, Fr, PC	
	Chilocorus nigritus (Fabricius) (Coccinellidae)						CBC, 1980: NR
Aspidiotus nerii	*Aphytis chilensis* Howard (Aphelinidae)		CBC, 1979: E, Fr (Crete)	ABC, 1972: NR (Sicily)		CBC, 1976: NR	
	Aphytis melinus DeBach		CBC, 1979: E, Fr (Crete)			CBC, 1976: NR	
Chrysomphalus dictyospermi	*Aphytis melinus* DeBach	CBC, 1966: E	CBC, 1962: E, Fr	CBC, 1964: E, Fr (Sicily)		CBC, 1967: E, Fr	
	Encarsia (= *Aspidiotiphagus*) *lounsburyi* (Berlese & Paoli) (Aphelinidae)			CBC, 1916: E			
Lepidosaphes beckii	*Aphytis lepidosaphes* Compere (Aphelinidae)	CBC, 1973: E, Fr	CBC, 1962: E, Fr		CBC, 1976-77: E, Fr		
Lepidosaphes gloverii	*Encarsia herndoni* (Girault) [=*Encarsia elongata* (Dozier)] (Aphelinidae)	CBC, 1987-88: E, Fr (Corsica)		CBC, 1988: E, Fr (Sicily)		CBC, 1979: E, Fr, SC	
Unaspis yanonensis	*Aphytis yanonensis* DeBach & Rosen (Aphelinidae)	CBC, 1984: E; ABC, 2000: MdC					
	Coccobius fulvus (Compere & Annecke) (Aphelinidae)	CBC, 1984: E, Fr					

Table 5 continued

Insect pests	*Parasitoids/Predators*	*Country of release / Frequency of occurrence / Success of control**					
		France	*Greece*	*Italy*	*Portugal*	*Spain*	*Turkey*
Moths							
Phyllocnistis citrella (Gracillariidae)	*Ageniaspis citricola* Logvinovskaya (Encyrtidae)		CBC, 1996: F (Crete)	CBC 1995-96: F	CBC, 1998: F (Madeira)	CBC1995-96: E, SC (Canary Islands)	
	Semielacher petiolatus (Girault) (Eulophidae)		CBC, 1996-99: E (Crete, Peloponnese)	Accidental introduction, 1998: vFr, SC	Accidental introduction, 2003: vFr	CBC, 1995: E, PC	
	Cirrospilus ingenuus Gahan [=*Cirrospilus quadristriatus* (Subba Rao & Ramamani)] (Eulophidae)		CBC, 1996: F (Crete)			CBC, 1995: F	
	Quadrastichus sp. (now *Quadrastichus citrella* Reina & LaSalle) (Eulophidae)			CBC, 1996: F		CBC, 1995: F	
	Quadrastichus sp. (Eulophidae)		CBC 1996-1999: E (Crete) F (Peloponnese)				
	Galeopsomyia fausta LaSalle (Eulophidae)					CBC. 1997: F	
	Citrostichus phyllocnistoides (Narayanan) (Eulophidae)		CBC 1996-99: E, vFr	CBC 1998-99: E, vFr, SC	Accidental introduction, 2003: Fr	CBC, 1998-99: E, Fr,SC	
Fruit flies (Tephritidae)							
Ceratitis capitata	*Aceratoneuromyia indica* (Silvestri) (= *Syntomosphyrum indicum* Silvestri) (Eulophidae)			CBC, 1909: F			
	Dirhinus giffardi Silvestri (Chalcididae)			CBC, 1913: NR			
	Diachasma fullawayi Silvestri (Braconidae)					CBC, 1931: F	
	Diachasmimorpha tryoni (Cameron) (Braconidae)					CBC, 1931: F	
	Psyttalia incisi (Silvestri) (= *Opius incisi* Silvestri) (Braconidae)					CBC, 1931: F	
	Tetrastichus giffardianus Silvestri (Eulophidae)					CBC, 1960: F (Canary Islands)	
	Diachasmimorpha longicaudata (Ashmead) (Braconidae)					CBC, 1979: F	

* Fr = Frequent; vFr = very frequent; E = Established; TE = Temporarily established; F = Failed; NR = Not reported; Re = Recorded; Po = Positive; SC = Substantial control; MdC = Moderate control; MrC = Minor control; PC = Partial control.

2.3. Chemical Control

Although many classical or augmentative biological control trials of arthropod pests have been successful in citrus, chemical control is still used. The recently finalized EU Review Programme on the inclusion of old active substances of plant protection products (registered in the EU up to 1993) in the positive list (Annex I) of the Directive 91/414/EEC (CEC, 1991), based on agreed and harmonised criteria for evaluating the safety of pesticides, resulted in a modification of the availability of insecticides, acaricides and insect attractants authorized for citrus pest management in the various member states of the E.U. (Tables 6 and 7) (Hellenic Ministry of Rural Development and Food, 2008; MAPA, 2008; MiPAAF, 2008). Some of these pesticides were also tested to evaluate their side effects on beneficial arthropods (Table 8).

Table 6. Insecticides, acaricides and insect attractants (active substances) registered for use on citrus in Spain, Italy and Greece (June 2008).

Country	*Insecticides/acaricides/insect attractants (active substances)*
Spain	Abamectin (O, L, M, G), acetamiprid (O, L, M, G), alpha-cypermethrin (O, L, M, G), azadirachtin (O, L, M, G), *Bacillus thuringiensis* var. *kurstaki* (O, L, M, G), benfuracarb (O, L, M, G), bifentrin (O, L, M, G), buprofezin (O, L, M, G), carbosulfan (O, L, M, G), chlorpyrifos (O, L, M, G), chlorpyrifos-methyl (O, L, M), cihexatin (O, L, M, G), clofentezine (O,L,M,G), cypermethrin (O, L, M, G), deltamethrin (O, L, M, G), diazinon (O, L, M, G), dichlorvos (O, L, M, G), dicofol (O, L, M, G), diflubenzuron (O, M, G), dimethoate (O, L, M, G), etofenprox (O, M, G), etoxazol (O, M), fenazaquin (O, M, G), fenbutatin oxide (O, M, G), fenitrothion (O, L, M, G), fenoxycarb (O, M, G), fenpyroximate (O, L, M, G), flufenoxuron (O, M, G), hexythiazox (O, L, M, G), imidacloprid (O, L, M, G), kaolin (O, M), lambda-cyhalothrin (O, L, M, G), lufenuron (O, L, M, G), malathion (O, L, M, G), methomyl (O, L, M, G), methoxyfenozide (O, M), mineral oil (O, L, M, G), oxydemeton-methyl (O, L, M, G), phosmet (O, L, M, G), piridaben (O, L, M, G), pirimicarb (O, L, M, G), pirimiphos-methyl (O, L, M, G), potassium salts of vegetable fatty acids (O, L, M), propargite (O, L, M, G), pymetrozine (O, L, M, G), pyriproxifen (O, L, M, G), spinosad (O, L, M, G), tau-fluvalinate (O, L, M, G), tebufenozide (O, L, M, G), tebufenpyrad (O, L, M, G), trichlorfon (O, L, M, G)
Italy	Abamectin (O, L, M), acrinathrin (O, L, G), alfamethrin (O, L, M), azadirachtin (O, L, M, G), *Bacillus thurigiensis* var. *aizawai* (O, L, M), *Bacillus thurigiensis* var. *kurstaki* (O, L, M), *Beauveria bassiana* (O, L, M), bifentrin (O, L, M), buprofezin (O, L, M), calcium polysulfur

Table 6 continued

	(O, L, M), chlorpyrifos (O, L, M), chlorpyrifos-methyl (O, L, M, C), clofentezine (O, L, M, Cl, G, B), cypermethrin (O, L), deltamethrin (O, L, M), diazinon (O) **(1)**, dicofol (O, L, M), dimethoate (O, L, M) **(2)**, ethoprophos (O, L, M), etofenprox (O, L, M, Cl, B, SO, G, P, T, C), etoxazol (O, L, M, Cl, B, SO, G, P, T, C), fenazaquin (O, L, M, Cl), fenbutatin oxide (O, L, M), fenpyroximate (O, L, Cl), flufenoxuron (O, M, Cl), fluvalinate (O, M), hexythiazox (O, L, M), imidacloprid (O, L, M, Cl), lambda-cyhalothrin (O), lufenuron (O, L, M, Cl), malathion (O, L, M, Cl, B, SO, G, P, T, C) (**1**), methomyl (O, L, M), methoxyfenozide (O, M, Cl), mineral oil (O, L, M, Cl), phosalone (O, L, M) **(3)**, phosmet (O, L, M), pirimicarb (O, L, M), pirimiphos-methyl (O, L), propargite (O, L, M, G, Cl), pymetrozine (O, L, M, Cl), pyrethrines (O, L, M), pyridaben (O, L, M, Cl, T), pyriproxifen (O, L, M), rotenone (O, L, M), spinosad (as bait) (O, L, M, Cl, SO, C, G, B, T), spirodiclofen (O, L, M, Cl, G, B, C, SO), tebufenozide (O, L, M), tebufenpyrad (O, L, M, Cl, G, C, T, B), thiamethoxam (O, L, M, Cl), trichlorfon (O, L, M) **(4)**, zeta-cypermethrin (O, L)
Greece	Acetamiprid (O, L, M, and nurseries, G, C), azadirachtin (O, L, M, G), *Bacillus thurigiensis* var. *aizawai* (O, L, M, G, C), *Bacillus thuringiensis* var. *kurstaki* (O, L, M, G, C), *Beauveria bassiana* (M), buprofezin (O, L, M, G, SO, C), chlorpyrifos (O, L, M, G, P), chlorpyrifos-methyl (O, L, M), cypermethrin (O, M,G), deltamethrin (O, L, M, G, C, P), diflubenzuron (O, L, M, G, P), fatty acid potassium salt (O, L, M, G, C), fenoxycarb (O, L, M), flucythrinate (O, L, M, G), flufenoxuron (O, L), imidacloprid (O, L, M, G), methomyl (O, L, M, P), methoxyfenozide (O, M, G), mineral oil (O, L, M, G, SO, P), phosmet (O, L, M, G), pirimicarb (O, L, M, G, P), pymetrozine (O), pirimiphos-methyl (M), pyrethrins (O, L, M, G), pyriproxyfen (O, L, M), tau-fluvalinate (O, L, M, G), tebufenozide (L, M), thiamethoxam (O, L, M) Insect attractants: farnesol (O, L, M, G), nerolidol (O, L, M, G)

O: Orange, L: Lemon, M: Mandarin, G: Grapefruit, SO: Sour orange, C: Citron, P: Pomelo, Cl: Clementine, T: Tangerine, B: Bergamot. 1 = Active substance not included in the Annex I of the directive 91/414/EC, the commercial plant production products are revoked from 6 December 2007 and the stocks were commercialized and used until 6 December 2008. 2 = Use allowed only on nonproductive orchards. For some commercial plant production products the extension of the authorization for use on citrus to control aphids has been approved with a pre-harvest interval of 100 days. 3 = Active substance not included in the Annex I of the directive 91/414/EC. The authorization of the commercial plant production products containing phosalone is revoked from 23 June 2007. The stocks were commercialized and used until 22 June 2008. 4 = Active substance not included in the Annex I of the directive 91/414/EC. The authorization of the commercial plant production products containing this a.i. is revoked from 21 November 2007. The stocks were commercialized and used until 21 November 2008.

Table 7. Insecticides, acaricides and insect attractants (active substances) registered for use against the main arthropod pests of citrus in Spain, Italy and Greece (June 2008).

Pest	*Insecticides/acaricides (active substance)*[a]
Thrips (Thysanoptera)	Acrinathrin (I), chlorpyrifos (G), fatty acid potassium salt (G), malathion (I), pirimiphos-methyl (I), rotenone (I)
Whiteflies (Aleyrodidae) *Aleurothrixus floccosus* *Dialeurodes citri*	Acetamiprid (S), azadirachtin (S, G), buprofezin (S, G), carbosulfan (S), chlorpyrifos (S), cypermethrin (I), deltamethrin (I), dimethoate (S), etofenprox (S), fatty acid potassium salt (G), fenazaquin (S, I), fenpyroximate (S), imidacloprid (S, I, G), lufenuron (S, I), malathion (S, I), methomyl (S), mineral oil (G), phosmet (S), piridaben (S), pyrimiphos-methyl (S), rotenone (I), zeta-cypermethrin (I)
Aphids (Aphididae) *Aphis gossypii* *Aphis spiraecola* *Toxoptera aurantii* *Toxoptera citricida*	Acetamiprid (S, G), alpha-cypermethrin (S), azadirachtin (S, G), benfuracarb (S), bifentrin (S), carbosulfan (S), chlorpyrifos (S, G), chlorpyrifos-methyl (G), cypermethrin (S, I, G), deltamethrin (S, I), dimethoate (S), etofenprox (S), fatty acid potassium salt (G), fenitrothion (S), flucythrinate (G), fluvalinate (I), imidacloprid (S, I), lambda-cyhalothrin (I), malathion (I), methomyl (S), mineral oil (S, G), oxamyl (G), oxydemeton-methyl (S), phosmet (S), pimetrozine (S, I), pirimicarb (S, I,G), pyrimiphos-methyl (S, I), potassium salts of vegetable fatty acids (S), pymetrozine (G), pyrethrines (G), rotenone (I), tau-fluvalinate (S), thiamethoxam (I, G), zeta-cypermethrin (I)
Armoured scales (Diaspididae) *Aonidiella aurantii* *Aspidiotus nerii* *Chrysomphalus dityospermi* *Lepidosaphes beckii* *Parlatoria pergandei* *Parlatoria ziziphi* *Unaspis citri*	Azadirachtin (S), buprofezin (S, G), chlorpyrifos (S, G), chlorpyrifos-methyl (G), cypermethrin (I, G), flucythrinate (G), dimethoate (S), fenitrothion (S), fenoxycarb (S), fenpyroximate (S), malathion (S, I), methomyl (S, I), mineral oil (S, I, G), phosmet (S, I, G), pyrimiphos-methyl (S), pyriproxifen (S, I, G), rotenone (I)
Soft scales (Coccidae) *Ceroplastes sinensis* *Coccus hesperidum* *Saissetia oleae*	Azadirachtin (S), buprofezin (S), chlorpyrifos (S, G), chlorpyrifos-methyl (G), cypermethrin (S, I, G), deltamethrin (I), dimethoate (S), fenitrothion (S), fenoxycarb (S, G), fenpyroximate (S), flucythrinate (G), imidacloprid malathion (S), malathion (I), methomyl (S, I), mineral oil (S, I, G), phosmet (S, I), pyriproxifen (S, I, G), pyrimiphos-methyl (S), rotenone (I), tau-fluvalinate (S)

Table 7 continued

Mealybugs (Pseudococcidae) *Planococcus citri*	Azadirachtin (S), buprofezin (S, G), chlorpyrifos (S, G), cypermethrin (I, G), dimethoate (S), fenitrothion (S), flucythrinate (G), malathion (S, I), methomyl (S, I), mineral oil (S, I, G), phosmet (S, I, G), pyrimiphos-methyl (S, I), rotenone (I)
Moths *Phyllocnistis citrella* (Gracillariidae) *Prays citri* (Hyponomeutidae)	Abamectin (S, I: *P. citrella*), acetamiprid (G: *P. citrella* nurseries), alpha-cypermethrin (S), azadirachtin (S, G: *P. citrella*), *Bacillus thuringiensis* var. *aizawai* (G: *P. citri*), *Bacillus thuringiensis* var. *kurstaki* (S, G: *P. citri*), benfuracarb (S), buprofezin (*P. citrella*), carbosulfan (S), chlorpyrifos (S, G), cypermethrin (S, I: *P. citri,* G), deltamethrin (S), diazinon (S), dichlorvos (S), diflubenzuron (S) dimethoate (S), etofenprox (S), fenitrothion (S), fenoxycarb (S), fenpyroximate (S), flucythrinate (G: *P. citri*), flufenoxuron (S, I, G: *P. citrella*), imidacloprid (S, I, G: *P. citrella*), lufenuron (S, I), malathion (S, I), methomyl (S, I), methoxyfenozide (I, G: *P. citrella*), mineral oil (G), phosmet (S, I), pyrimiphos-methyl (S, I), rotenone (I: *P. citri*) tau-fluvalinate (S), tebufenozide (S, I, G: *P. citrella*), thiamethoxam (I, G: *P. citrella*)
Fruit flies (Tephritidae) *Ceratitis capitata*	Azadirachtin (S), *Beauveria bassiana* (G), cypermethrin (I, G), deltamethrin (I), dichlorvos (S), etofenprox (I), flucythrinate (G), imidacloprid (S), lambda-cyhalothrin (S, I), lufenuron (S), malathion (S, I), phosmet (S, I, G), pyrimiphos-methyl (I), rotenone (I), spinosad bait (I), trichlorfon (S, I), zeta-cypermethrin (I)
Mites *Eutetranychus banksi* *Panonychus citri* *Phyllocoptruta oleivora* *Tetranychus urticae*	Abamectin (S, I: *T. urticae*), acrinathrin (I), bifentrin (S), buprofezin (S), clofentezine (S, I), dicofol (S, I, G), etoxazol (S, I, G), fenazaquin (S, I, G), fenbutatin oxide (S, I, G), fenitrothion (S), fenpyroximate (S, I), flufenoxuron (S, I), hexythiazox (S, I), malathion (S), mineral oil (S, I, G), oxamyl (G), oxydemeton-methyl (S), propargite (S, I, G), pyridaben (S, I), pyrimiphos-methyl (S, I), spirodiclofen (I), tebufenpyrad (S, I, G)

[a] G = Greece; I = Italy; S = Spain.

Table 8. Side effects of pesticides (active substances), registered for the control of citrus pests, on beneficial arthropods[a].

Active substance	*Rodolia cardinalis*	*Cryptolaemus montrouzieri*	*Euseius stipulatus*	*Lysiphlebus testaceipes*	*Leptomastix dactylopii*	*Cales noacki*
Abamectin	1	3–4	2–3			3–4
Azadirachtin	3–4	1	1	1	3–4	
Bacillus thuringiensis var. *kusrtaki*	1	1	1	1	1	1
Benfuracarb			1			2–3
Bifentrin	3–4		3–4		1	
Buprofezin	1–2	3	1–2	1	1–2	1
Carbosulfan	1–2		1–2	1		2–3
Chlorpyrifos	1–2	2	2	3	3	2–3
Chlorpyrifos-methyl	1	1	3	3	2–4	1–2
Clofentezine	1	2	1–2			1
Cypermethrin	4	4	4	1		3–4
Deltamethrin	4	3–4	4	1	4	3
Diazinon	3		2	3		1–2
Dicofol	1	1–4	3–4	1	3–4	2
Diflubenzuron			1			1–2
Dimethoate	1	4	2–3	1–2	4	2
Fenazaquin	4	2	4			3
Fenbutatin oxide		1	2	1	1	1
Fenitrothion	1–2		3	1–2	4	3
Fenoxycarb		4	1–2			2
Flucythrinate		2				3
Flufenoxuron			2–3			1–2
Fosalone	2			1		3
Hexythiazox	1		1	1		
Imidacloprid	4		2–3	1	4	3
Lambda-cyhalothrin						3
Lufenuron	4	1	1			1
Malathion	2–3	4	2	3	4	3–4
Methomyl	4	4	4	2		3–4
Mineral oil	1	1–2	1–2	2	1	1–4
Oxydemeton-methyl	1	3	2	1	3	1–2
Phosmet	4	4	2–3	1	3	4
Piridaben			4		1	
Pirimicarb	1–2	2	1–2	1	1	1
Pyrimiphos-methyl	1–2	1–2	1–4		4	3–4
Propargite			4	1		2–3
Pyriproxifen	4	4	1		1–2	2–3
Spinosad	1	1		4	3–4	

Table 8 continued

Tau-fluvalinate	4	3	3–4	1	3	2
Tebufenozide			1			
Tebufenpyrad		2				
Trichlorfon	2–3	1	1	3	1–2	2
Zeta-cypermethrin	4	4	4	1		3–4

[a] Classification according to the IOBC WG "Pesticides and Beneficial Organisms" standards: 1 = harmless; 2 = slightly harmful; 3 = moderately harmful; 4 = harmful (sources: Jacas & García Marí, 2001; Pascual-Ruíz & Urbaneja, 2006; Urbaneja et al., 2008; Suma, Zappalà, Mazzeo, & Siscaro, 2009).

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2

MAIN ARTHROPOD PESTS OF *CITRUS* CULTURE AND PEST MANAGEMENT IN GREECE

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Abstract. The key arthropod pests in the citrus producing areas in Greece comprise the Mediterranean fruit fly *Ceratitis capitata*, the California red scale *Aonidiella aurantii* and the citrus mealybug *Planococcus citri*. Outbreaks of the whiteflies *Aleurothrixus floccosus* and *Dialeurodes citri*, the scales *Ceroplastes rusci* and *Saissetia oleae* as well as the Tetranychidae mites *Panonychus citri* and *Tetranychus urticae* and the Eriophyiidae mites *Aculops pelekassi* and *Aceria sheldoni* may occur locally. The citrus leafminer *Phyllocnistis citrella*, aphids (*Aphis spiraecola*, *A. gossypii*, *Toxoptera aurantii*) and thrips (*Heliothrips haemorrhoidalis*, *Pezothrips kellyanus)* are of minor importance. The problems due to major and minor citrus pests and control measures in Greece are reviewed and Integrated Pest Management (IPM) strategies are recommended.

1. INTRODUCTION

The total citrus-producing area in Greece is 57,525.6 hectares (ha). The most widely cultivated *Citrus* species include orange *Citrus sinensis* (L.) Osbeck (40,054 ha), lemon *Citrus limon* Burman f. (10,497 ha), mandarin *Citrus reticulata* Blanco (6,514 ha), grapefruit *Citrus paradisi* Macfadyen (340 ha), citron *Citrus medica* L. (100 ha), bergamot *Citrus bergamia* Risso (12 ha), bitter orange *Citrus aurantium* L. (7.82 ha) and pumelo *Citrus maxima* (Burm) Merrill (0.78 ha) (Hellenic Ministry of Rural Development and Food, 2006, Unpublished data).

Major arthropod pests in terms of importance in the citrus-growing regions of the country comprise the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), the California red scale *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) and the mealybug *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae). However, outbreaks of the woolly whitefly *Aleurothrixus floccosus* Maskell (Hemiptera: Aleyrodidae), the citrus whitefly *Dialeurodes citri*

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(Ashmead) (Hemiptera: Aleyrodidae), *Ceroplastes rusci* L (Hemiptera: Coccidae), the Mediterranean black scale *Saissetia oleae* (Bernard) (Hemiptera: Coccidae) and Tetranychidae and Eriophyiidae mites are recorded at small (local) scale. The citrus leafminer *Phyllocnistis citrella* (Stainton) (Lepidoptera: Gracillariidae) and the citrus infesting aphids and thrips are of minor importance (Hellenic Ministry of Rural Development and Food and Regional Plant Protection Services, 2007, unpublished data).

2. PEST STATUS AND CONTROL MEASURES

An idea for the present status of the citrus arthropod pests in the citrus-producing areas of Greece can be obtained from an empirical risk assessment of the Hellenic Ministry of Rural Development and Food, which is based on data from the Regional Plant Protection Services of the Ministry (2007). A rank scale from 1 to 3 is used (1 being the most serious pest to 3 being the less serious one) so that the citrus arthropod pest status is as follows: the Mediterranean fruit fly *Ceratitis capitata* (1–2); the California red scale *Aonidiella aurantii* (1–3); the mealybug *Planococcus citri* (1–3); the woolly whitefly *Aleurothrixus floccosus* (1–3); the citrus whitefly *Dialeurodes citri* (Ashmead) (Hemiptera: Aleyrodidae) (1) locally on the island of Crete; *Ceroplastes rusci* (1) locally in Argolis-Peloponnese; the Mediterranean black scale *Saissetia oleae* (1) locally in Argolis; the Tetranychidae mites *Panonychus citri* and *Tetranychus urticae* (1–3) locally in Peloponnese; the Eriophyiidae mites *Aculops pelekassi* and *Aceria sheldoni* (1–3) locally in Argolis–Peloponnese and the western mainland; the citrus flower moth *Prays citri* Milliére (Lepidoptera: Hyponomeutidae) (2); the purple scale *Lepidosaphes beckii* (Newman) (Hemiptera: Diaspididae) (2) locally in Argolis; the rose tortrix moth *Archips rosanus* (Lepidoptera: Tortricidae) locally in Argolis; the citrus leafminer *Phyllocnistis citrella* (2–3); *Citrus* infesting aphids (2–3) and thrips (3) locally in Achaia-Peloponnese (Hellenic Ministry of Rural Development and Food and Regional Plant Protection Services, 2007, unpublished data).

A forecasting/warning system concerning infestation by the main insect pests of the most important crops in the country, including *Citrus*, operates by the Regional Plant Protection Services of the Hellenic Ministry of Rural Development and Food. Forecasting of infestation is based on meteorological data, monitoring by trapping or sampling, historical and other data sources (literature, agronomists/consultants/farmers files). Instructions for preventive plant protection measures are provided to the citrus growers when there is an issue of warning (Table 1).

A review of the problems and the control measures for the main citrus arthropod pests in Greece i.e. the Mediterranean fruit fly *Ceratitis capitata*, scale insects (especially *A. aurantii* and *P. citri*), whiteflies (especially *A. floccossus)*, the citrus leafminer *P. citrella*, aphids, the citrus flower moth *P. citri*, thrips and mites will be presented in the following sections.

Table 1. Citrus arthropod pests for which plant protection instructions are provided by the Regional Plant Protection Services in conventional citrus culture in Greece.

Arthropod pest	*Meteo data*	*Monitoring by trapping/ sampling*	*Historical data*	*Other data*[a]
Ceratitis capitata	Y	Y/Y	Y	Crete: Lit, A, F
Aonidiella aurantii	Y	Y/Y	Y	Achaia: Population Prediction Model Crete: Lit, A, F
Planococcus citri	Y	Y/Y	Y	Achaia: Population Prediction Model Crete: Lit, A, F
Aleurothrixus floccosus	N except Crete	N/Y	Y in most areas	Crete: Lit, A, F
Dialeurodes citri[a]	Y	N/Y	Y	Crete: Lit, A, F
Ceroplastes rusci[a]	N	N/Y	Y	N
Saissetia oleae[a]	N	N/Y	Y	N
Mites	N except Argolis	N/Y	N except Argolis	N
Prays citri	Y in some areas	Y in some areas/Y	Y	N
Lepidosaphes beckii[a]	Y	N/Y	Y	N
Citrus aphids	N except Crete	N/Y	N except Crete	Crete: Lit, A, F
Phyllocnistis citrella	N except Crete	N/Y	Y in some areas	Crete: Lit, A, F
Thrips[b]	N	N/Y	N	N

[a] Y = Yes; N = No; Lit = Literature, A = Agronomists, F = Farmers. Source: Hellenic Ministry of Rural Development and Food and Regional Plant Protection Services (2006).

[b] The pests cause problems in certain regions and data refer to those regions.

2.1. The Mediterranean Fruit Fly, Ceratitis capitata

The Mediterranean fruit fly (medfly) *Ceratitis capitata* was first recorded in Greece by Papageorgiou in 1915 on infested citrus orchards in Attica and on the island of Aegina. Until today the presence of the medfly is reported almost all over Greece, except the highlands of the continental country and the lowland of the northwestern region. It infests mainly citrus fruits, pears, apples, peaches, apricots and figs and can cause serious yield losses (Tzimos, 1961; Mourikis, 1965;

Michelakis, 1992; Zervas, Kateva, & Christopoulos, 1995; Papadopoulos & Economopoulos, 1997a; Katsoyannos, Kouloussis & Carey, 1998; Papadopoulos, 1999; Papachristos, 2007).

Adults of medfly are active from late spring until early winter and they are very abundant from the beginning of August until the end of October; they remain inactive or below detectable levels throughout the rest of the year (Zervas et al., 1995; Katsoyannos et al., 1998; Papadopoulos, 1999; Papachristos, 2007). However, in the southern part of the country (the island of Crete) the adults are active during winter (Mavrikakis, Economopoulos, & Carey, 2000). Larvae inside the infested fruits and pupae in the soil are the main stages of overwintering in the north whereas in Crete it appears that the medfly overwinters in all development stages (Mourikis, 1965; Zervas et al., 1995; Papadopoulos, Carey, Katsoyannos, & Kouloussis, 1996; Katsoyannos et al., 1998; Papadopoulos, 1999; Mavrikakis et al., 2000). Moreover, patterns of fluctuation of adult population size may be varying significantly even in the same region depending on the host plant composition and availability (Katsoyannos et al., 1998). Although the precise determination of *C. capitata* generations is difficult because of a high overlapping between them, it is considered that *C. capitata* completes 5–7 generations depending on the region and the year (Mourikis, 1965).

Infestation of citrus fruits starts as soon as they begin to ripen and continues until they are completely ripe. Among the main citrus species, the most susceptible one is bitter orange, followed by orange, whereas lemon seems to be immune (Katsoyannos et al., 1998; Mavrikakis et al., 2000; Papachristos, 2007). In most areas of Greece the late autumn ripening oranges escape heavy infestation because the medfly is not active at the particular season (Katsoyannos et al., 1998; Papachristos & Papadopoulos, 2009).

Today the control of the medfly is mainly carried out with the use of pesticides (Economopoulos, 1996). Mass trapping as well as the sterile insect technique have also been applied with success (Zervas, Christopoulos, & Kateva, 1997; Economopoulos et al., 1996).

2.1.1. Monitoring

Detection and population monitoring of *C. capitata* is based on trapping of adults. A wide variety of traps and trophic, optical and sexual attractants are used in trapping of the medfly, which serves for monitoring of population or for pest control.

Jackson traps baited with the male specific parapheromone trimedlure are specialized for capturing males of *C. capitata* but these traps have low accuracy in low population densities (Katsoyannos et al., 1998). The International Pheromone Plastic McPhail trap (IPMT), baited with a water solution of a protein hydrolysate (9%) and borax (3%) added as a preservative, is the most common method for capturing female and male adults (Katsoyannos, 1994). Another trapping method using the IPMTs and the food attractants ammonium acetate (AA), 1,4 diaminobutane (putrescine) and trimethylamine (TMA) (Katsoyannos, Papadopoulos, Hearth, Hendrichs, & Kouloussis, 1999a, 1999b) have been proved effective even at low and medium population levels of the medfly. In recent experiments it was shown that putrescine may be excluded from the traps when monitoring established populations of *C. capitata* (Heath, Epsky, Midgarden, & Katsoyannos, 2004).

Monitoring of *C. capitata* by adult trapping is advised to be supported with systematic fruit collection especially by the most suceptible citrus species. Fruit sampling is considered to be an efficient warning system for early detection of the fly (Katsoyannos et al., 1998).

2.1.2. Mass Trapping

The method of mass trapping has been applied successfully in orange orchards (Zervas et al., 1997). Traps of reversed cup type (Zervas, 1994) baited with parapheromone trimedlure were used to attract males and the modified McPhail trap IPMT, baited with 9% food lure (hydrolyzed protein, *Dacus* bait) in water with the addition of 3% borax were used to attract females. Attracted medflies were killed by the insecticide methomyl. Male capturing traps were placed on orange trees at the first fortnight of September (one trap every second tree) whereas the female capturing traps were placed on orange trees 10 days later (one trap every third tree).

Aiming at the reduction of cost at the use of traps in mass trapping, Zervas (1994) developed three types of low cost and easy to handle medfly traps using a local market material. Efficacy of the new traps was similar to the commonly used traps. Two types of these traps were based on the utilisation of reversed plastic cups with trimedlure on cotton rolls as a male attractant and sugar mixted with the insecticide methomyl as a killing agent. In the third type a transparent bottle was used with a food lure (9% water solution of protein hydrolysate and 2% borax). Due to the easy manufacture and low cost, the last trap type is used with various modifications (i.e. in a lot of cases the fertilizer sulfur ammonium is placed instead of protein hydrolysate as a food attractant) by a lot of farmers for the mass trapping of medfly.

Moreover, a variety of traps (yellow plastic spheres, flat yellow plates of plastic or wood, delta, glass McPhail, plastic McPhail, paper folded traps, paper envelope etc) combining visual, food and sexual stimuli have been evaluated in commercial citrus orchards with promising results (Michelakis, 1988; Liaropoulos et al., 2003; Katsoyannos & Papadopoulos, 2004).

2.1.3. Biological Control

Despite the long history of medfly in Greece, not enough data exist regarding the presence of native parasitoids or the establishment of exotic parasitoids that have been introduced in commercial citrus orchards for biological control. Older efforts for introduction and release of parasitoids were unsuccessful. Specifically, *Dirhinus giffardii* Silvestri (Hymenoptera: Chalcididae) was introduced but was not recovered (Argyriou, 1969). Test release of *Opius concolor* Szepligeti (Hymenoptera: Braconidae) on citrus showed that the parasitoid did not parasitize medfly in the field although it developed on medfly under laboratory conditions (Argyriou, 1969).

Recently the presence of the parasitoid *Aganaspis* (*Trybliographa*) *daci* (Weld) (Hymenoptera: Eucoilidae) was recorded on infested figs on the island of Chios (Papadopoulos & Katsoyannos, 2003, 2007). The parasitoid exhibits high parasitism levels on medfly pupae in the field. Until today it is not known whether *A. daci* parasitizes *C. capitata* larvae in plant hosts other than figs or whether it is established in other areas of Greece. No parasitoids were found in samplings of medfly pupae in sweet oranges in Chios (Papadopoulos & Katsoyannos, 2007). Nevertheless, parasitism of *C. capitata* by *A. daci* can be an important factor for medfly control in citrus orchards in Greece as figs are one of the most important hosts contributing to the built up of *C. capitata* populations in late summer and early autumn (Katsoyannos et al., 1998; Papadopoulos, Katsoyannos, Carey, & Kouloussis, 2001).

2.1.4. Cultural Practices

The succession of host fruits and their availability throughout the year is of high importance in determining overall population levels of the med fly. Thus collecting and destroying infested fruits fallen on the ground is advised as a control strategy (Katsoyannos, 1996a). In citrus orchards, the presence of bitter orange and fig trees, which are found scattered inside or at the periphery of the orchards, seem to have an important role in *C. capitata* population dynamics (Katsoyannos, 1983; Katsoyannos et al., 1998; Papachristos, 2007). Early in the season (May to August), the rate of population built-up depends on the availability of mature bitter oranges whereas later in the season on the presence of figs. In addition, bitter oranges and figs are highly attractive for *C. capitata* and seem to keep females from attacking nearby hosts and dispersal (Katsoyannos, 1983; Katsoyannos et al., 1998). Elimination of these fruits or their utilization as traps on the trees can contribute in an integrated management programme of *C. capitata*.

2.1.5. Biotechnical Methods

The sterile insect technique (SIT) is considered as an environmental friendly and effective control method for Tephritids fruit flies. An attempt to control *C. capitata* with SIT was performed in the valley of Fodele – Crete with encouraging results (Economopoulos et al., 1996; Papadopoulos & Economopoulos, 1997b).

2.1.6. Chemical Control

Cover and bait sprayings are the main control methods of medfly in Greece (Economopoulos, 1996). The sprays may be applied empirically based on the time of the year (Liaropoulos et al., 2003) or on systematic observations and monitoring of insect populations. Usually the bait sprays are applied from the ground using conventional sprayers and cover part of each tree or part of each two or three trees.

A food attractant, mainly a compound that releases ammonia, is added in the pesticide solution.

The timing of spray applications is determined by monitoring medfly population with traps, which are hanged on citrus trees a few weeks before fruits begin to mature. The monitoring of adult population may take place at field or region scale. Trap networks for monitoring *C. capitata* population have been installed and operate in some areas of Greece by the Regional Plant Protection Services so forecasting of infestation is possible based on trap captures and environmental conditions and warnings are released to the growers when necessary. Although treatment application thresholds against medfly have not been established at national level, those suggested by Katsoyannos (1996a) are applied in many cases (20 adults/trap/week in autumn on clementines approaching ripeness, 40–50 adults/trap/week in autumn on oranges (pre-ripe and ripe stages), 10 adults/trap/week in spring on late ripening varieties).

When no monitoring is performed, sprayings are performed when the fruits reach maturity whereas the number of applications depends on the region, the season and the variety of citrus fruits. A spray program for orange and mandarin, which was recommended by the Ministry of Rural Development and Food in the past and can be applied even today, if using other insecticides, involves the following actions: (a) For bait spraying, the first application is carried out 15 days before fruit ripening and is repeated at 5–7 days intervals. The spray solution consists of 2% protein (as an attractant) and 0.3% dimethoate or fenthion or 0.5% malathion. It is applied on hedges and bushes in the perimeter of the orchard, on the interior and the upper part of the trees, mainly on branches that do not bare fruits (b) For cover spaying, the first application is carried out at the beginning of ripening and spraying may be repeated after 20 days depending on the prevailing enviromental conditions and the time of fruit harvest. The spray solution consists of 0.03% dimethoate or fenthion or other organophosphorate insecticides (Anonymous, 1973).

In most of the orange cultivated areas in Greece the bait sprayings applied for the control of *Bactrocera oleae* (Gmelin) also contribute to a significant reduction of the medfly populations (Economopoulos, 1996). The same attractants (salt of protein hydrolysates) as those for the medfly have been used in bait sprays for *B. oleae* together with organophophorate insecticides. Applications start early in the summer and they are continued until the end of autumn (Broumas, 1994).

In some cases, spray solutions are applied on an artificial medium such as a gunny placed on a parallelepiped plastic plate which is hanged on trees branches (Economopoulos, 1996). Laboratory experiments by Mavrikakis, Remboulakis, and Economopoulos, (2003) showed that a type of a paper surface (Vioryl) baited with a water solution of protein hydrolysate (6%) had a better “attract and kill” performance on medfly than the gunny surface baited with the same food lure. The insecticide (active ingredient) used as a killing agent was spinosad in a proportion of 0.04% v/v and it was effective for a time period more than 5 weeks.

Some of the former mentioned insecticidal active substances used for the control of medfly have been withdrawn after being re-evaluated (Directive 91/414/EEC) in the E.C. (fenthion, malathion). Currently, the insecticides which are authorized for use against medfly in Greece include *Beauveria bassiana* (mandarin), cypermethrin (lemon, grapefruit, mandarin, orange), flucythrinate (lemon, grapefruit, mandarin, orange) and phosmet (lemon, grapefruit, mandarin, orange) (Authorized Plant Protection Products Data Base of the Hellenic Ministry of Rural Development and Food, 2008).

2.1.7. Recommended IPM Strategies

In small size citrus groves, integration of cultural and chemical control of medfly is recommended whereas the biological, chemical and SIT methods may be integrated at citrus-growing areas in large scale. Collection and destroying of infested fruits fallen to the ground, fruits remaining on trees after harvest as well as fruits without commercial value (such as bitter oranges) are cultural practices of significant value in order to prevent medfly built-up population. Early population detection is crucial for a successful control thus monitoring with IPMT traps baited with AA and TMA supported by systematic fruit inspection especially of the most preferred host (bitter oranges) are required. Bait sprayings should be preferred to cover sprayings. The timing of bait spray application should be based on medfly monitoring and on treatment application threshold. As the available treatment application threshold is practically empirical there is still a demanding need for its accurate determination.

2.2. Scale Insects

Scale insects have always been on the top of the list of the economically important insect pests in citrus in Greece. Half of the most common insect pests in the Greek citrus orchards belong to the superfamily Coccoidea with the California red scale *Aonidiella aurantii* and the citrus mealybug *Planococcus citri* being the most frequently found ones among all. In addition, a number of less important species are known to cause local outbreaks. In the past, the Mediterranean black scale *Saissetia oleae* was considered to be a major pest but after the introduction and successful establishment of several exotic parasitoids, its population is kept below the economic injury level. At present, the Chinese wax scale *Ceroplastes floridensis* and the cottony-cushion scale *Icerya purchasi* can cause local outbreaks in specific regions in Peloponnese (Stathas, personal communication) whereas high populations of *Ceroplastes rusci*, *Lepidosaphes beckii* and *Saissetia oleae* are occasionally recorded too (Hellenic Ministry of Rural Development and Food, 2007, unpublished data).

Although considerable effort has been imposed to develop sustainable control methods of scale insects based on the use of biological control agents and the minimization of the use of chemical pesticides, spraying with insecticides is still used for the control of scale insects in citrus. Currently the most common practice in control of main scales of citrus is spray applications following the forecasts and instructions issued by the Regional Plant Protection Services, which are based on population monitoring with traps and/or visual observations.

2.2.1. Sampling and Monitoring

An efficacious monitoring system is prerequisite for having a successful forecasting/warning system for citrus scale insects. A number of monitoring tools are available for citrus scales, which are used in Greece. Sex pheromone traps are utilized for the detection, monitoring and forecasting outbreaks of the California red scale *A. aurantii*. White sticky sex-pheromone baited traps are used for monitoring purposes of other armoured scales. Pheromone traps are also available for the citrus mealybug *P. citri*. Field observations and sampling are necessary for the detection of other citrus scales. Various detailed sampling schemes have been proposed for this area of concern (Katsoyannos, 1996a).

2.2.2. Biological Control

An extended trial has been directed toward classical biological control of citrus scales in the past so Greece is a good example of a country where native scale insects have been controlled by exotic natural enemies, both parasitoids and predators. Eleven parasitoid species have been introduced, seven of them providing sufficient control whereas several predators associated with classical biological control of scale insects are frequently present in the Greek citrus orchards.

Five parasitoid species, *Aphytis melinus* DeBach, *A. lingnanensis* Compere, *A. coheni* De Bach Compere (Hymenoptera: Aphelinidae), *Encarsia perniciosi* (Tower) (Hymenoptera: Aphelinidae) and *Comperiella bifasciata* Howard (Hymenoptera: Encyrtidae) were introduced and released in field trials against *A. aurantii* between 1962 and 1970. *A. melinus* is the leading parasitoid targeting the California red scale but its efficacy is reduced in cases of outbreaks. *Comperiella bifasciata* had a minor establishment whereas *A. lingnanensis* and *A. coheni* failed to be established (Katsoyannos, 1996a).

Aphytis lepidosaphes Compere and *Aphytis melinus* DeBach were established after introduction in 1962 and they sufficiently controlled *L. beckii* and *Chrysomphalus dictyospermi* Morgan (Hemiptera: Diaspididae) respectively, which were major pests of citrus in the past (Katsoyannos, 1996a).

The parasitoid *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae) was released in Crete twice (in 1975 and 1981) for the control of the citrus mealybug *P. citri* but failed to get established (Katsoyannos, 1996a). Moreover, three coccinellid predators were introduced, *Nephus reunioni* (1977), *Nephus sidi* (1992) and *Cryptolaemus montrouzieri* Mulsant (several attempts: 1933, 1964, 1965, 1969, 1977). Establishment of the *Nephus* spp. was not reported, however, *C. montrouzieri* Mulsant, which was the most effective of the three species, could not overwinter in most regions (Katsoyannos, 1996a).

Four parasitoid species, *Metaphycus helvolus* Compere, *M. bartletti* Annecke & Mynhardt, *M. swirskii* Annecke & Mynhardt (Hymenoptera: Encyrtidae) and *Diversinervus elegans* Silvestri (Hymenoptera: Encyrtidae) targeted the control of *Saissetia oleae* with relatively sufficient results (Argyriou, 1986). The coccinellid predator *Rhyzobius forestieri* (Mulsant) was released 25 years ago on the island of Chios and it is now the most abundant coccinellid found in citrus orchards. The predator preys mainly on soft scales, such as *S. oleae* and *Coccus pseudomagnoliarum* (Kuwana) (Hemiptera: Coccidae), which maintain at acceptable levels (Katsoyannos, 1997).

Populations of the cottony-cushion scale *Icerya purchasi* Kaussari (Hemiptera: Margarodidae) were regulated by the introduced coccinellid predator *Rhodolia cardinalis* Mulsant (Katsoyannos, 1996a). Localized outbreaks of the pest have been occurring until recently but nowadays the predator is available to the growers for augmentative releases.

Besides the classical biological control, naturally occurring biological control is a widespread phenomenon in Greek citrus orchards. Many indigenous parasitoids and predators are found to parasitize and prey on citrus scale insects. Three indigenous parasitoids [*Aphytis chrysomphali* (Mercet), *Aphytis chilensis* Howard and *Encarsia citrina* (Craw)] and three predators [the coccinellids *Rhyzobius lophanthae* (Blaisdell) and *Chilocolus bipustulatus* (L.) and the nitidulid *Cybocephalus fodori* (Endrodi-Younga)] are the most common species that contribute to the suppression of the armoured scales in citrus, with the predator *R. lophanthae* being the most important one (Katsoyannos, 1996a).

A considerable number of indigenous natural enemies are related to the control of the citrus mealybug; the parasitoids *Anagyrus pseudococci* (Girault) and *Leptomastidea abnormis* (Girault) (both Hymenoptera: Encyrtidae) along with the predators *Nephus includens* (Kirsch) and *N. bisignatus* (Boheman) (Coleoptera: Coccinellidae) contribute substantially to its control (Katsoyannos, 1996a; Kontodimas, Eliopoulos, Stathas, & Economou, 2004). Among the various parasitoid species associated with soft scales in citrus, *Tetrastichus ceroplastae* (Girault) (Hymenoptera: Eulophidae) and *Scutellista cyanea* Motschulsky (Hymenoptera: Pteromalidae) are the most frequent species that parasitize *Ceroplastes rusci* and *C. floridensis* Comstock (Katsoyannos, 1996a; Stathas, Kavallieratos, & Eliopoulos, 2003).

In general citrus-infesting scale insects are adequately retained at low levels by the activity of introduced and/or native parasitoids and predators. However, in cases where locally outbreaks occur due to various factors, further action is needed to suppress the infestation below the economic injury level. Augmentative releases of natural enemies were performed in the past against the citrus mealybug *P. citri* and the soft scales *S. oleae* and *C. pseudomagnoliarum* using the coccinellid predators *Cryptolaemus montrouzieri* and *Exochomus quadripustulatus* (L.) respectively (Katsoyannos, 1996a). Presently augmentative releases are performed sporadically against *P. citri* using the coccinellid predators *Nephus includens* and *N. bisignatus* with very satisfying results, especially in organic citrus orchards. Growers occasionally use *Rhodolia cardinalis* to suppress local outbreaks of *I. purchasi*; the predator has been commercially available in the Greek market since 2006.

2.2.3. Cultural Practices

Cultural techniques involve a series of modifications of the standard management practices in order to prevent or make the environment less favorable for the reproduction, dispersal and/or survival of scale insects. Reduced fertilizers input and irrigation regimes are conidered to moderate the susceptibility of the trees to scale insects infestation. Since scale insects are favored by low light and high humidity conditions, which are favoured by dense foliage, pruning of trees in spring should be regulated to enhance air movement and sunlight infusion into the canopy and hence to minimize survival and establishment of high populations of the pests in the orchards (Katsoyannos, 1996a).

2.2.4. Chemical Control

Chemical control of citrus scale insects following monitoring of the population with traps and visual observations is a common practice in Greece. Insecticides (active substances) authorized for use against the California red scale and the citrus mealybug in Greece include buprofezin (citron, lemon, grapefruit, mandarin, orange; only *A. aurantii*: bitter orange), chlorpyrifos (lemon, grapefruit, mandarin, orange, pumelo), cypermethrin (lemon, grapefruit, mandarin, orange), flucythrinate (lemon, grapefruit, mandarin, orange), paraffin oil (lemon, grapefruit, mandarin, orange, pumelo; only *A. aurantii*: bitter orange), petroleum oil (lemon, grapefruit, mandarin, orange), phosmet (lemon, grapefruit, mandarin, orange), pyriproxyfen (only *A. aurantii*: lemon, mandarin, orange) and white oil (lemon, grapefruit, mandarin, orange). Some of these active substances are registered for the control of other armoured scales and soft scales in citrus whereas chlorpyrifos-methyl and fenoxycarb are added in the pesticide list against *Aspidiotus nerii* (only chlorpyrifos methyl) and *Saissetia oleae* (Authorized Plant Protection Products Data Base of the Hellenic Ministry of Rural Development and Food, 2008).

2.2.5. Recommended IPM Strategies

A good monitoring system that provides the necessary information for early scale detection and the status of natural enemies is essential for the selection of the sufficient control measures. In general, natural enemies provide adequate control of the citrus-infesting scales except in cases that outbreaks *A. aurantii* occur; the lack of an efficient predator against the California red scale makes it difficult to suppress the pest at high population levels. Therefore, conservation of existing natural enemies and augmentative releases of insectary-reared parasitoids and predators are very important in maintaining the scales population at low levels. Accessory cultural techiques (i.e. pruning in spring and moderate use of fertilizers and irrigation in summer) contribute in keeping the scale numbers low. In cases insecticide application is required (e.g. when widespread increase of the California red scale populations or the citrus mealybug outbreaks occur) one or two well-timed cover sprays may be applied by using selective insecticides. Less selective chemicals should be applied only spotted or in extreme situations on heavily infested areas of the grove (aiming also at the protection and conservation of natural enemies).

2.3. Whiteflies

Three exotic whitefly species have invaded Greece during the last three decades, the citrus whitefly *Dialeurodes citri* in 1973 (Pappas & Viggiani, 1979; Pappas, 1981), the Japanese bayberry whitefly *Parabemisia myricae* (Kuwana) (Hemiptera: Aleyrodidae) in 1989 (Michalopoulos, 1989; Michelakis & Alexandrakis, 1989) and the woolly whitefly *Aleurothrixus floccosus* in 1991 (Katsoyannos, 1991), which caused considerable economic yield losses in citrus orchards especially in the early years after the invasion. Among the three species the woolly whitefly caused the most serious problems to the citrus growers. Woolly and citrus whiteflies are still considered to be main insect pests locally (Kalaitzaki, Alexandrakis, Varikou, & Pervolarakis, 2003; Kontodimas et al., 2005). However, all three species are excellent examples of efficient biological control in practice.

2.3.1. Sampling and Monitoring

The Japanese bayberry whitefly prefers to oviposit on incompletely expanded new leaves whereas woolly and citrus whiteflies prefer completely expanded but not yet dark green young leaves. In any case, sampling of infested young leaves and their examination under a stereoscopic microscope is necessary in order to assess the infestation level and the efficiency of any natural enemies (mainly the rate of parasitism). At the same time visual observation should be done in order to record the relative infestation intensity of the citrus trees. In addition, yellow sticky traps could considerably help to record the first flights of the whiteflies' adults as well as to monitor the annual fluctuation of the population of whiteflies and their natural enemies.

2.3.2. Biological Control and Cultural Practices

All the whitefly species were exotic, hence their biological control was based on the introduction of their natural enemies, especially parasitoids (classical biological control).

The control of *Dialeurodes citri* was achieved by the introduction of the parasitoid *Encarsia lahorensis* (Howard) (Hymenoptera: Aphelinidae) on the island of Corfu in 1976 (Pappas & Viggiani, 1979). By 1994, the parasitoid was well established and was found in almost all citrus orchards in the mainland (Katsoyannos, 1996a); it was also imported and released on the island of Crete in 2003 (Kalaitzaki et al., 2003).

Control of *Parabemisia myricae* was attempted with the parasitoid *Eretmocerus deBachi* Rose and Rosen (Hymenoptera: Aphelinidae), which was introduced and released in Crete in 1990 and 1991, but no data regarding its establishment are available (Katsoyannos, 1996a). However, the presence of *P. myricae* is rare after the introduction of *E. deBachi.*

In case of *Aleurothrixus floccosus*, the parasitoid *Cales noacki* Howard (Hymenoptera: Aphelinidae) was introduced in 1991 and augmentation of its population in the insectary followed. During the years 1992–1995 more than 30 million individuals of the parasitoid were released in more than 300 sites of citrus-growing areas of the country. In most of these areas the control of the woolly whitefly was achieved during 1995 (infestation level <1 nymph/cm^2 and parasitism by *C. noacki* up to 99%) (Katsoyannos & Kontodimas, 1996; Katsoyannos, Kontodimas, & Stathas, 1998; Kontodimas et al., 2005). In the following years, *C. noacki* was established in all citrus-growing areas and controlled successfully *A. floccosus*. Moreover, when occasional and local outbreaks of *A. floccosus* occur, the parasitoid population recovers by the end of the same year and suppresses the whitefly population (Kontodimas et al., 2005).

The presence of the native coccinellid predators *Clitostethus arcuatus* (Rossi) and *Oenopia (Synharmonia) conglobata* (L.) is also noticeable in considerable numbers on citrus trees infested by *A. floccosus*. Specifically, *C. arcuatus* appears to be an effective egg predator of woolly whitefly (Katsoyannos, Ifantis, & Kontodimas, 1997; Kontodimas, Stathas, & Martinou, 2008).

Cultural practices for the control of *A. floccosus* should focus in the protection of its natural enemies and especially the parasitoid *C. noacki.*

2.3.3. Chemical Control

A few insecticides (active substances) are registered for use against whiteflies in citrus in Greece at present i.e. azadirachtin (*A. floccosus*, *P. myricae*), buprofezin (*A. floccosus*, *D. citri*, *P. myricae*), fatty acid potassium salts (*A. floccosus*), imidachloprid (*A. floccosus*), paraffin oil (*A. floccosus*, *D. citri)* (Authorized Plant Protection Products Data Base of the Hellenic Ministry of Rural Development and Food, 2008). However, in order to protect the natural enemies of the whiteflies in citrus orchards, one application with potassium salts of fatty acids is recommended only in cases of heavy infestation of new seedlings and strictly before any release of beneficials.

2.3.4. Recommended IPM Strategies

Normally satisfactory control of the wooly whitefly *A. floccosus* is accomplished by the established parasitoid *C. noacki.* Therefore an IPM strategy for the control of the pest should focus on the conservation of the population of the exotic parasitoid and the native predators by avoiding chemical control of other insect-pests.

In case that chemical application cannot be avoided, a selective insecticide, if possible, should be used and the treatment should be restricted to the area infested by the second pest. Release of 400.000 *C. noacki* individuals/ha for a rapid suppression of a new high infestation (> 1 nymph/cm^2) of *A. floccosus* is recommended (Katsoyannos et al., 1998).

Rearing of *C. noacki* in insectaries is suggested for augmentative biological control of *A. floccosus*, which in addition could serve as a useful deposit in case of invasion of the quarantine pest citrus blackfly *Aleurocanthus woglumi* Ashby (Hemiptera: Aleyrodidae) (A1 EPPO List).

2.4. The Citrus Leafminer, Phyllocnistis citrella

The citrus leafminer *Phyllocnistis citrella* was first recorded in Greece on the island of Rhodes (southeastern Greece) and in Crete in June 1995 (Anagnou-Vernoniki, 1995; Michelakis & Vacante, 1997). More recordings of the pest followed on other Greek islands (Cos, Astipalea, Lesvos, Chios, Samos and Paros) and in the mainland (Attica and Laconia-Peloponnese) in July and August, 1995 (Anagnou-Vernoniki, 1995). After a few months the citrus leafminer was found in almost all citrus growing areas of Greece (Tsagarakis, Kalaitzaki, Lykouressis, Michelakis, & Alexandrakis, 1999).

Upon introduction of the pest, quarantine and other regulatory measures were enforced but they had a very limited impact on the spread of the pest which very rapidly invaded all citrus cultivated areas in the state. The growers were advised not to overuse and/or misuse insecticides as experience from other countries had shown that the chemical control was a short term and expensive solution due to the long vegetative period of citrus and the large number of generations of citrus leafminer per year. Furthermore, chemicals would have unfavorable side effects on the existing effective biological control of other citrus pests and there was a possibility of resistance development of the citrus leafminer to insecticides.

Nevertheless, many growers tried to apply insecticides but soon they had to admit that chemicals were an inadequate solution to the problem. Moreover, chemical control using broad spectrum insecticides was indeed putting at risk the Integrated Pest Management of citrus insect pests, such as scales and aphids as well as the successful biological control of the woolly whitefly *Aleurothrixus floccosus* by disruption of the newly established exotic parasitoid *Cales noacki*. Biological control, on the other hand, was known to be the most effective method of managing the citrus leafminer in commercial orchards (Hoy et al., 1995; Neale, Smith, Beattie, & Miles, 1995; Smith & Beattie, 1996; Argov & Rössler, 1996). Therefore the impact of the native natural enemies was studied and classical biological control by introduction and release of the most promising parasitoid species in two sites (Crete and Peloponnese) was attempted (Kalaitzaki, 2004). In addition cultural measures to prevent dispersal were strongly recommended by the agronomists/consultants to the citrus growers (Michelakis & Vacante, 1997).

Early studies after the implementation of the classical biological control programme of the citrus leafminer in Crete showed that the number of immature individuals of the citrus leafminer/leaf fluctuated from 0.1–3.9 to 0.01–1.06 on orange trees and mandarin trees, respectively (with mandarin being more resistant to infestation) (Kalaitzaki, 2004). Five peaks of the live immature individuals of the citrus leafminer/leaf were recorded on orange trees (two in summer: end of May and mid June and three in autumn: end of September, end of October and mid November) and four peaks on mandarin trees (mid June, end of September, end of October, beginning of December) (Kalaitzaki, 2004). Lower levels of infestation (0.017–1.76 live immature individuals/per leaf) were recorded in Argolis-Peloponnese, the second area of the parasitoids' release (Tsagarakis et al., 1999). Later studies (2004 and 2005) on the islands of Lesvos and Chios and in western mainland revealed low infestation of the citrus leafminer i.e. 0.06–0.58, 0.08–0.72 and 0.04–0.82 immature individuals/leaf respectively. As a final point the citrus leafminer causes problems particularly on young citrus trees and overgraftings in nurseries but its damage on adult trees under Mediterranean conditions lacks economic importance (Garcia-Marí, Granda, Zaragoza, & Agusti, 2002).

2.4.1. Sampling and Monitoring

Sampling for monitoring infestation by the citrus leafminer involves collection of new shoots and leaves from growth flushes and observation of mines. The economic threshold for a chemical application on citrus leafminer has been estimated by several researchers; in China it is 0.74 larvae/leaf (or percentage of leaf damage over 20%) (Huang & Li, 1989); in Australia it is fixed at 25% of shoots with leaves smaller than 3 cm exhibiting mines of L1 (Beattie & Smith, 1993); in Florida it is set at 30% of young shoots baring mines with live larvae (sampling should be performed when half of the trees in the orchard have young shoots) (Knapp et al., 1995).

2.4.2. Biological Control

Five exotic hymenopteran parasitoid species were introduced from Cyprus in 1996 and were subsequently mass reared in the insectary of the Institute of Subtropical Crops and Olive (National Agricultural Research Foundation) in Chania-Crete. The introduced species were the encyrtid *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae) and the eulophids *Cirrospilus quadristriatus* (Subba Rao and Ramamani), *Citrostichus phyllocnistoides* (Narayanan), *Quadrastichus* sp. and *Semielacher petiolatus* (Girault) (Hymenoptera: Eulophidae). A small number of all parasitoids were released in 1996 but cultures of *Ageniaspis citricola* and *Cirrospilus quadristriatus* could not be kept after the first year and hence these species did not establish (Kalaitzaki, 2004).

Citrostichus phyllocnistoides, *Quadrastichus* sp. and *Semielacher petiolatus* were released systematically (100–250 individuals/species/10 days from June to November) in orange and mandarin orchards in Crete (Chania) and the impact of both native and introduced parasitoids was studied from 1997 to 1999. Similar releases were also performed in Peloponnese (Argolis, Korinthia, Lakonia) (Tsagarakis et al., 1999).

All of the released parasitoid species established in Crete and contributed to the reduction of the citrus leafminer population, especially *Citrostichus phyllocnistoides* which dispersed very rapidely and far from the release sites and it achieved the highest percentage parasitism (mean 15.2–20.8% and maximum 45.8–51.1% on mandarin and orange trees, respectively).

Only a few native parasitoid species were found to parasitize the citrus leafminer in Crete i.e. *Pnigalio pectinicornis* L., *Neochrysocharis formosa* (Westwood) and *Cirrospilus pictus* (Nees) (all Hymenoptera: Eulophidae) in very small numbers and with small contribution in percentage parasitism of the citrus leafminer (Kalaitzaki, Lykouressis, & Michelakis 1999; Kalaitzaki, 2004). Among them, *P. pectinicornis* was the most abundant one (Kalaitzaki et al., 1999; Kalaitzaki, Lykouressis, Perdikis, &

Alexandrakis, 2007). Results of the second release site differ. The exotic parasitoids *C. phyllocnistoides* and *S. petiolatus* were recovered whereas *Quadrastichus* sp. was not recovered (Tsagarakis et al., 1999). Although the population of *C. phyllocnistoides* increased with time after the release, the exotic parasitoid did not displace the native species *N. formosa* and *P. pectinicornis*. *Neochrysocharis formosa* was the most abundant parasitoid for a period of 3 years after the release (Tsagarakis & Lykouressis, 2002; Tsagarakis, Kalaitzaki, Lykouressis, Michelakis, & Alexandrakis, 2003). *Citrostichus phyllocnistoides* has been found in almost all citrus producing areas in Greece (Kalaitzaki, 2004).

The parasitoids *Pnigalio* sp., *Cirrospilus* sp., *N. formosa* and *C. phyllocnistoides* were collected at the areas of Marathonas-Attiki, Galatas-Trizinia and Skala-Lakonia in 1999–2000 (Anagnou-Veroniki, Doukas, & Kontodimas, 2002), *Pnigalio pectinicornis* and *Cirrospilus* sp. were collected on the islands of Lesvos and Chios and in western mainland, as were *N. formosa* and *C. phyllocnistoides* (Papanikolaou, 2005).

The list of native parasitoids expanded with the first recording of *Pnigalio soemius* (Walker) (Hymenoptera: Eulophidae) (Papanikolaou, 2005; Papanikolaou, Kavallieratos, Kontodimas, & Tomanovic, 2006) and *Semielacher silvicola* Boucek (Hymenoptera: Eulophidae) in Attica, in 2005 (Vamvakas, Anagnou-Veroniki, & Kontodimas, 2005).

2.4.3. Cultural Practices

Cultural measures advised involve some changes of the standard management practices in order to prevent or make the environment less favorable for the pest reproduction, dispersal and/or survival. Decrease of fertilization and irrigation inputs should moderate the susceptibility of the new flush to the citrus leafminer during summer when the pest is very active. Moreover, fertilization, irrigation and pruning should be regulated to enhance discrete flush patterns over large areas, breaking generations overlap (Anagnou-Veroniki, Volakakis, & Gianoulis, 1995; Michelakis & Vacante, 1997).

2.4.4. Chemical Control

The application of insecticides to control the citrus leafminer is proved to be a short term solution especially for plant nurseries and newly grafted trees which suffer more from the pest infestation.

The insecticides (active substances) which were initially recommended were fenoxycarb and diflubezuron in combination with summer oils. Only the external part of the trees, where the flushes and hence the citrus leafminer infestation occurs, should be sprayed with the solutions. Fenoxycarb showed no killing effects on *C. noacki*, the parasitoid of *A. floccosus* (Buchelos & Foudoulakis, 2000). Later on more selective insecticides i.e. chlorfenapyr, abamectin, flufenoxuron, azadirachtin, summer oils etc. were used (Michelakis & Vacante, 1997).

Other insecticides added in the list were acephate, diazinon, dimethoate, methomyl, phospanidon, fenvalarate whereas their side effects on the natural enemies of the pest were illustrated (Anagnou-Veroniki et al., 1995).

Today the list with the registered insecticides for use against the citrus leafminer in Greece includes acetamiprid, (in nurseries for lemon, mandarin and orange), azadirachtin (grapefruit, lemon, mandarin, orange), buprofezin (bitter orange), flufenoxuron (lemon, orange) imidacloprid (grapefruit, lemon, mandarin, orange), methoxyfenozide (mandarin, orange), paraffin oil (bitter orange), tebufenozide (mandarin) and thiamenthoxam (lemon, mandarin, orange) (Authorized Plant Protection Products Data Base of the Hellenic Ministry of Rural Development and Food, 2008).

2.4.5. Recommended IPM Strategies

Manipulation of the timing and quantities of irrigation and fertilizers provided and pruning in order to separate the main growth flushes (spring–summer–autumn) and decrease the susceptibility of the summer flush, when the citrus leafminer is active, contributes to maintenance of the pest populations at low levels (Anagnou-Veroniki, 1995; Katsoyannos, 1996a, 1996b; Michelakis & Vacante, 1997). Conservation of the established exotic parasitoids and native beneficials is essential for the control of the citrus leafminer in the productive orchards. Chemical treatments using selective insecticides for the control of the pest should be restricted in the nurseries.

2.5. Aphids

Commonly, aphids can be very important pests in citrus-growing areas under favourable environmental conditions due to their high reproductive potential which results in several generations per year and the production of alate adults which can spread very quickly and migrate to great distances (Kavallieratos et al., 2005; Athanassiou, Kavallieratos, Tomanović, Tomanović, & Milutinović, 2005). Nevertheless, aphids infesting citrus in Greece and generally in the northern Mediterranean countries are most often kept in low numbers under biological control by several indigenous parasitoids and predators (Katsoyannos, 1996a).

Nine aphid species have been reported to infest citrus trees in Greece, which refer to *Aphis craccivora* Koch, *Aphis gossypii* Glover, *Aphis spiraecola* Patch, *Aulacorthum solani* (Kaltenbach), *Brachycaudus helichrysi* (Kaltenbach), *Macrosiphum euphorbiae* (Thomas), *Myzus persicae* (Sulzer), *Rhopalosiphum maidis* (Fitch) and *Toxoptera aurantii* (Boyer de Fonscolombe) (Kavallieratos & Lykouressis, 1999). The exotic *Aphis spiraecola* and the indigenous *Toxoptera aurantii* and *Aphis gossypii* are the most important aphid species on citrus in the region (Argyriou, 1969).

Strict monitoring and quarantine procedures must be followed for continued exclusion from the EPPO region of *Toxoptera citricida* (Kirkaldy) (EPPO A2 List, 2004), which is the highest potency vector for *Citrus Tristeza Closterovirus* (CTV) causing the homonymous destructive virus disease of citrus.

Aphis gosypii is also an efficient vector of many isolates of CTV, whereas *T. aurantii* is a less efficient vector. The prevention of aphids' population outbreaks is primarily based on conservation and, possibly, stimulation of the activity of their natural enemies (Katsoyannos, 1996a).

2.5.1. Sampling and Monitoring

During spring, yellow water-pan traps are used and visual inspections are carrried out for monitoring citrus aphids. Moericke pan-traps, both square (60 × 60 × 10 cm) and round (30 cm in diameter), painted canary yellow inside and containing water up to depth of 3–4 cm with a spoonful of added detergent, are commonly used. They are placed in the citrus groves in mid-spring (density 2–5 traps/ha at a height of 70 cm above the ground) and they are checked once or twice per week during the growing season. Information about citrus aphids populations provided by 12 m high suction traps, if available, might also be useful (Katsoyannos, 1996a).

Inspections, especially on the apical twigs of new growth flushes, are made weekly during the growing season. Curled newer leaves, honeydew, sooty mould and the presence of ants are signs aiding the detection of foci of aphid infestation in an orchard. Rates of parasitism are monitored by examination of aphids on sampled leaves and twigs.

Aphidophagous coccinellid population levels are monitored using visual inspections and by heating branches of trees with a rubber-covered stick over a 1 m^2 cloth screen and recording the numbers of adults and larvae of the beetles thus dislodged (Katsoyannos, 1984).

2.5.2. Biological Control

Control of citrus aphids is mainly based on natural enemy manipulation (conservation) in pest management. Aphids have several natural enemies which most often suppress the pests below levels of economic concern. Indigenous parasitoids which have been reported to parasitize aphids in Greece include the hymenopteran Aphidiinae parasitoids *Aphidius colemani* Viereck, *Aphidius matricariae* Haliday, *Aphidius urticae* Haliday, *Diaeretiella rapae* (M'Intosh), *Ephedrus persicae* Froggat, *Lysiphlebus confusus* Tremblay and Eady, *Lysiphlebus fabarum* (Marshall), *Lysiphlebus testaceipes* (Cresson), *Praon volucre* (Haliday), *Binodoxys acalephae* (Marshall) and *Binodoxys angelicae* (Haliday) (Kavallieratos & Lykouressis, 1999; Kavallieratos et al, 2001; Kavallieratos, Stathas, & Tomanović, 2002; Kavallieratos & Lykouressis, 2004).

Alloxysta spp., *Asaphes vulgaris* Walker, *Asaphes* spp., *Dendrocerus* spp., *Pachyneuron aphidis* (Bouché), *Pachyneuron* spp., *Phaenoglyphis* spp. and *Syrphophagus aphidivorus* (Mayr) have been reported as hyperparasitoids that attack primary parasitoids of aphids infesting citrus (Santas, 1979; Kavallieratos & Lykouressis, 1999).

Coccinellidae predators *Coccinella septempunctata* L., *Adalia bipunctata* L., *Propylea quatuordecimpunctata* L., *Hippodamia variegate* (Göeze), *Oenopia (Synharmonia) conglobata* L., *Adalia decempunctata* L. and *Scymus (Pullus) subvillosus* (Göeze) are frequent in Greece. Other aphid predators belong to the Syrphidae such as *Epistrophe baiteata* (De Greer), *Paragus albifrons* Meigen, *Paragus majaranae* Rondani, *Scaeva albomaculata* Macquart, *Syrphus latefasciatus* Macquart, and *Sphaerophoria* sp. The green lacewing *Chrysoperla* *carnea* (Chrysopidae) Stephens is a notable aphid predator in Greece too (Katsoyannos, 1996a).

Conservation of these agents includes management practices e.g. avoiding chemical treatment, especially between mid-spring and early summer. Long-term positive effects on the naturally-occurring biological control of citrus aphids are obtained by preserving existing habitats, which function as reservoirs of the natural enemies, or by creating new ones where needed.

In general, preserving sufficient plant diversity in the agroecosystem is desirable, since it ensures the continuous presence of other aphid species which serve as alternative prey of coccinellids during the summer scarcity of citrus aphids. Other important measures include preserving hibernation sites and preventing ants (Formicidae) from tending aphid colonies and disturbing the aphids' natural enemies. Although, these methods are indirect and their effect is difficult to be evaluated, they are cost effective and easy to implement (Katsoyannos, 1996a).

A classical biological control case involved the release of the predator *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) in citrus in experimental fields in Marathon (Attica), on Chios island, in Leonidion (Peloponnese) and in Chania (Crete) in 1994. The predator was highly efficient against aphid population outbreaks (Katsoyannos, Kontodimas, Stathas, & Tsartsalis, 1995). However, when sampling was contacted between 1995 and 1999 there was no evidence for the establishment of *H. axyridis* in the release sites (Kontodimas et al., 2008). Only small colonies of overwintered adults (<50 individuals) were observed in Attica region. Later samplings (2000–2007) revealed no presence of *H. axyridis* in any of the orchards where the predator had been released.

2.5.3. Chemical Control

Insecticides (active substances) which are registered for use against aphids in Greece include acetamiprid (grapefruit: *T. aurantii*, nurseries for lemon, mandarin and orange), azadirachtin (grapefruit, lemon), chlorpyrifos (grapefruit, lemon), chlorpyrifos-methyl (lemon, orange) cypermethrin (grapefruit, lemon), fatty acid potassium salt (citron, grapefruit, lemon), flucythrinate (grapefruit, lemon), oxamyl (grapefruit, lemon), paraffin oil (grapefruit, lemon), petroleum oil (grapefruit, lemon), pirimicarb (grapefruit, lemon), pymetrozine (orange), pyrethrines (orange), thiamethoxam (orange), and white oil (grapefruit, lemon) (Authorized Plant Protection Products Data Base of the Hellenic Ministry of Rural Development and Food, 2008).

2.5.4. Recommended IPM Strategies

A generalized strategy is needed for citrus aphids' control. This strategy must be based on thorough monitoring of aphids' and their natural enemies' population levels. Biological control measures must be combined with spot treatment (on new growth flushes) using selective insecticides, applied when needed, and also with cultural practices. Conservation of aphidophagous insects is achieved mainly by avoiding the use of selective insecticides, especially in overall cover-spray treatments (Katsoyannos, 1996a).

2.6. The Citrus Flower Moth, Prays citri

The citrus flower moth *Prays citri* usually completes three generations in Greece whereas development time is estimated to range from 15 to 19 days in the region of Achaia-Peloponnese (Buchelos et al., 1963; Tzanakakis & Katsoyannos, 2003). The adults of the first generation appear in August and those of the second generation in October–November. Maximum infestation on lemons is observed at the end of their main florescence whereas the next generations of the pest infest the flowers and fruits of the subsequent florescences of multiple-flowering lemon trees (Buchelos, Sueref, & Tsoka-Thanasoulopoulou, 1963). The major damage concerns the citrus flowers and the newly formed fruits and it is more severe in lemons and citrons. In addition, serious damages have been reported on new overgraftings at warm regions in autumn (Tzanakakis & Katsoyannos, 2003).

Control of the pest depends largely on cultural practices and methods directed against the adult moths, since the larvae mining within the flower tissues are not normally vulnerable to insecticides and insect pathogens.

2.6.1. Sampling and Monitoring

Monitoring of infestation is possible by sampling and examination of flowers and newly formed fruits (Cavalloro & Protta, 1983). Synthetic sex pheromone Z-7-tetradecenal is available in the US for monitoring (Mineo, Mirabello, del Busto, & Viggiani, 1983; Benfatto, 1984) or mass-trapping (120 traps/ha) of males (Sternlicht, Barzakay, & Tamim, 1990).

Although no correlation exists between trap catch and level of flower damage (Mineo et al., 1983; Benfatto, 1984) as males of overlapping generations of the pest are caught throughout the growing season, both samples and trap captures are taken into account for accurate timing of chemical application on citrus (mainly lemon and citron) when required.

The threshold of flower damage by *P. citri* for chemical control is estimated at >50% of flowers infested whereas in the case of fruits when 3% are affected (Cavalloro & Protta, 1983). A percentage of 20–30% of healthy flowers is sufficient for a satisfying production in lemons (Katsoyannos, 1996b).

2.6.2. Biological Control and Cultural Practices

Prays citri has several natural enemies, principally parasitoids e.g. *Ageniaspis fuscicollis* (Dalman) subsp. *praysincola* Silvestri (Hymenoptera: Encyrtidae) and *Elasmus flabellatus* Boyer de Fonscolombe (Hymenoptera: Eulophidae), which are not always effective in contoling the pest (Tzanakakis & Katsoyannos, 2003).

In twice-flowering lemons, good control of *P. citri* can be obtained by forcing early flowering in spring and summer, before the adult flight peaks of the pest in each season are observed (Calabretta & Nucifora, 1985).

2.6.3. Chemical Control

Registered insecticides (active substances), which can be used against *P. citri* in Greece, include *Bacillus thuringiensis* var. *aizawai* (citron, grapefruit, lemon, mandarin, orange), *B. thuringiensis* var. *kurstaki* (citron, grapefruit, lemon, mandarin, orange), chlorpyrifos (grapefruit, lemon, mandarin, orange, pumelo), cypermethrin (grapefruit, lemon, mandarin, orange), flucythrinate (grapefruit, lemon, mandarin, orange), paraffin oil (grapefruit, lemon, mandarin, orange, pumelo) and petroleum oil (grapefruit, lemon, mandarin, orange) (Authorized Plant Protection Products Data Base of the Hellenic Ministry of Rural Development and Food, 2008).

2.6.4. Recommended IPM Strategies

Regulation of flowering before the adult flight peaks of the pest in second-flowering lemons is recommended. Selective chemicals could be applied, if necessary, after monitoring of infestation by sampling of flowers and newly formed fruits.

2.7. Thrips

Thrips species infesting citrus in Greece include the greenhouse thrips *Heliothrips haemorrhoidalis* (Bouché) (Thysanoptera: Thripidae) (Katsoyannos, 1996a; Tzanakakis & Katsoyannos, 2003) and the Kelly's citrus thrips *Pezothrips kellyanus* (Bagnal) (Thysanoptera: Thripidae) which was first recorded in Peloponnese (Korinthos) in 1981 (Zur Strassen, 1986; Palmer, 1987). The greenhouse thrips *H. haemorrhoidalis* infests mainly citron and lemon whereas the Kelly's citrus thrips *P. kellyanus* infests mainly lemon and orange with grapefruit following and mandarin being almost invulnerable (except the mandarin variety Minneola (tangelo) in Chania-Crete) (Varikou, Tsitsipis, Alexandrakis, & Mound, 2002).

In samplings performed in Chania – Crete in spring 2003, *P. kellyanus* was the only thrips species collected in lemon and mandarin orchards and the most abundant one collected in orange and grapefruit orchards. Some *Thrips* spp. were also found on mandarin and grapefruit in lower numbers whereas *Frankliniella occidentalis* was a minor species found solely in mandarin samples (Varikou, 2006).

In general, thrips are considered as a minor pest of citrus causing qualitative damage (scarring or escharosis) of the fruits (Katsoyannos, 1996a; Varikou et al., 2002; Tzanakakis & Katsoyannos, 2003; Hellenic Ministry of Rural Resources and Food, 2006, unpublished data).

However, escharotic fruits due to the feeding of the Kelly's citrus thrips reached 70% of the total produce in Chania-Crete and they were rejected for export during the selection process at packaging in 2001 (Varikou, 2006). Baker et al. (2005) refer that feeding of Kelly's citrus thrips on young and mature fruit causes scarring (halo), marking and rind bleaching which typically encircles the apex of the fruit in the immediate vicinity of the calyx.

Heliothrips haemorrhoidalis is a polyphagous parthenogenetic species which can complete six generations on citron and overwinters as an adult on citrus trees and on poaceous weeds. This is the reason why weed control by ploughing, in February, was recommended together with chemical applications in March by Anagnostopoulos (1939).

The Kelly's citrus thrips *P. kellyanus* is arrhenotokous (Varikou, 2006) and is not known to breed on any indigenous plant species of the Mediterranean region except *Citrus* sp. (Webster, Cooper, & Mound, 2005a). However, *P. kellyanus* can use the pollen of plant species other than citrus as an extra food resource. Biology of *P. kellyanus* was first studied by Varikou, Tsitsipis, Alexandrakis, and Hoddle, (2009a) and it was found that its lower development threshold is 10.2°C and its thermal constant 204.6 day degrees [whereas 504 day degrees for *H. haemorrhoidalis* (Rivnay, 1935)]. Therefore the Kelly's citrus thrips is present throughout the year in Crete (Chania) with its population increasing during citrus flowering in spring as pollen is essential for its reproduction and development in the citrus orchards (Varikou, 2006; Varikou, Tsitsipis, & Alexandrakis, 2009b).

Lemon is the best host among citrus for the survival and maintenance of the thrips population due to the numerous flowerings during the year. *Pezothrips kellyanus* is mostly attracted by blue colour traps (compared to yellow and white) at the fruiting period but not during flowering or the rest of the year (Varikou, 2006).

Entomophagous arthropods of the Kelly's citrus thrips have not been studied in Greece. A predatory mite, *Iphiseius (Amblyseius) degenerans* (Berlese) (Phytoseiidae), has been found in citrus orchards with high thrips populations in Italy but its efficacy to control the thrips has not been not proved (Conti et al., 2001). Hence, chemical control is applied at present.

Registered insecticides (active substances) for the control of thrips in citrus in Greece include chlorpyrifos, fatty acid potassium salt and oxamyl (Authorized Plant Protection Products Data Base of the Hellenic Ministry of Rural Development and Food, 2008).

2.8. Mite Pests

Citrus are infested by a large number of mite species in Greece i.e. the tetranychids *Panonychus citri* (Koch) and *Tetranychus urticae* Koch (Tetranychidae) and the eriophyiids *Aculops pelekassi* (Keifer) and *Aceria sheldoni* (Ewing) (Eriophyidae), which can cause serious damage on fruit production when outbreaks of the population occur (Papaioannou-Souliotis, 1985, 1991, 1996; Papaioannou-Souliotis, Tsagarakou, & Dermatas, 1992). However, the damages by mites are usually occasional and locally restricted as the natural enemies maintain the pest numbers under economic injury level in the citrus orchards.

Panonychus citri was first recorded on *Citrus* spp. in Greece in 1986 (Emmanouel & Papadoulis, 1987). It exists throughout the year and can complete many generations but the population is high in spring and fall (Papaioannou-Souliotis et al., 1992; Emmanouel, Papadoulis, Karkazi, & Papadima, 1994). *Tetranychus urticae* was first recorded in the country in 1962 (Pelekassis, 1962). Its population densities are higher during summer whereas in citrus orchards near the coast it is present throughout the year (Papaioannou-Souliotis, 1995).

Aculops pelekassi was first found in 1958 and since then its presence is frequent all over the country (Papaioannou-Souliotis, 1985; Papaioannou-Souliotis, Ragusa di Chiera, & Tsolakis, 1996). It is active during mild winters and can develop more than five generations per year. In population outbreaks it can cause up to 60% loss of yield (Papaioannou-Souliotis, 1985). *Aceria sheldoni* has been found in all Greek citrus-growing regions, causing damage mainly in lemons, which can be significant only during years with high population (Papaioannou-Souliotis, 1985). Sampling for monitoring infestation involves collection of leaves and counting the number of mites.

2.8.1. Biological Control and Cultural Practices

Many phytoseiid predatory species are found in citrus orchards such as *Euseius stipulatus* (Athias-Henriot), *Typhlodromus athenas* Swirski and Ragusa, *Amblyseius andersoni* (Chant) and *Iphiseius degenerans* Berlese. *Euseius stipulatus* is the most frequent phytoseiid predator and is the majority (80%) of the phytoseiid population in citrus groves (Papaioannou-Souliotis, 1991). Moreover it does not diapause and all the developmental stages may be present during winter (Papaioannou-Souliotis, Tsagkarakou, & Nomikou, 1997).

Generalist predators like *E. stipulatus* can control the phytophagous mite populations at low densities (McMurtry, Morse, & Johnson, 1992) and especially the tetranychids when other food resources (e.g. pollen) are also available for their good development (Bouras & Papadoulis, 2005). Collection of the infested fruits is a practice which may decrease the population of mites.

2.8.2. Chemical Control

Low toxicity selective acaricides are used to control *P. citri* with applications in spring (when the first symptoms appear) and late October. The number of applications depends on the acaricide but more than two applications per year are a common practice (Papaioannou-Souliotis, 1985; Papaioannou-Souliotis, 1991; Papaioannou-Souliotis et al., 1992). Selective acaricides can be applied against *A. pelekassi* in summer (beginning of June) and autumn (mid September to mid November) and against *A. sheldoni* in spring and at the beginning of June (Papaioannou-Souliotis, 1985).

Table 2. *IPM strategies recommended for each citrus insect pests overall throughout the year* (**underline show duration**).

Insect pest/Month	*Control actions*											
	J[a]	F	M	A	M	J	J	A	S	O	N	D
All scales			Pruning			Moderate use of nitrogen fertilizers & irrigation						
Aonidiella aurantii	Sufficient control with *Aphytis melinus* - If preventive augmentative release then up to 10 individuals/tree before the beginning of male scale's flight											
			1-2 chemical applications using selective insecticides in outbreaks									
Planococcus citri					Releases of beneficials (*Leptomastix dactylopii*, *Nephus includens* and *N. bisignatus*) – Application using summer oils or selective insecticides in outbreaks							
Other Diaspididae				Summer oils								
Ceratitis capitata									Bait sprayings			
Aleurothrixus floccosus	Sufficient control with *Cales noacki* in commercial orchards–Release of 400.000 individuals/ha in outbreaks (>1 nymph / cm^2) In nurseries and new seedlings 1application using fatty acids potassium salts strictly before release of *C. noacki*											
Phyllocnistis citrella						Sufficient control with the parasitoids in commercial orchards. Chemical application using selective insecticides only in nurseries						
Aphids						Spotted sprayings						
Thrips			Pruning/weed management			Spraying after booming for Kelly's citrus thrips'						
Panonychus citri	Sufficient control with phytoseiid predatory mites											
	Spraying in outbreaks									Spraying in outbreaks		

[a] J-D: January–December.

Source: Lykouressis (1991), Katsoyannos (1996a), Katsoyannos (1996b), and Alexandrakis, Stathas, Lykouressis, and Michelakis (2001).

Registered acaricides (active substances) for control of citrus mite pests include acrinathrin (*P. ulmi*), azocyclotin (*P. ulmi* and eriophyiids), clofentezine (*P. ulmi*), dicofol (all species), etoxazol (tetranychids), fenazaquin (tetranychids), fenbutatin oxide (all species), fenpyroximate (*P. ulmi*), paraffin oil (all species), petroleum oil (eriophyiids), propargite (tetranychids), pyridaben (*P. ulmi*), tebufenpyrad (tetranychids) and white oil (eriophyiids) (Authorized Plant Protection Products Data Base, Hellenic Ministry of Rural Development and Food, 2008).

2.8.3. Recommended IPM Strategies

Usually the phytoseiid predators (mainly *E. stipulatus*) keep the tetranychid mites at low populations on citrus. Monitoring of the mite population in citrus groves is very important for the early detection of mite infestation and hence the limited use of acaricides. Furthermore the use of selective acaricides highly contributes to conservation of the phytoseiid predatory mites.

3. ARTHROPOD IPM IN CITRUS THROUGHOUT THE YEAR

Following the IPM strategies recommended for each citrus insect pest separately, the outline of an overall IPM programme referring to these pests throughout the year is presented in Table 2 (Lykouressis, 1991; Katsoyannos, 1996a; Katsoyannos, 1996b; Alexandrakis et al., 2001).

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BIOLOGICAL CONTROL IN CITRUS IN SPAIN: FROM CLASSICAL TO CONSERVATION BIOLOGICAL CONTROL

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Abstract. The status of citrus pest management in Spain and of biological control, including classical biological control strategies, is reviewed. The augmentative versus inoculation-based control, and the use of invertebrate biological control agents in citrus orchards and nurseries are described. Fortuitous and conservation biocontrol strategies, as well as exploitation of resident species, either native or naturalized, are discussed. Pesticide side-effect testing on natural enemies, presence of alternative hosts and use of banker plants are described, together with ground cover mangement strategies.

1. INTRODUCTION

Spain is one of the largest producers of citrus for the fresh market worldwide (5,129,110 Mg in 2004; MAPA, 2007), mainly oranges, mandarins and lemons. Many potential pests are kept under excellent or satisfactory natural control by either exotic or indigenous natural enemies [e.g. *Panonychus citri* (McGregor) (Acari: Tetranychidae) by *Euseius stipulatus* (Athias-Henriot) (Acari: Phytoseiidae)] (Table 1). Exotic biological control (BC) agents were imported during the last century into Spain following classical (= inoculative) BC programs and are now naturalized in our country [e.g. *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae)] (Table 2). However, the fact that most of Spanish citrus production goes to the fresh market (84.1%; MAPA, 2007) has important consequences on the status of some citrus pests which directly damage the fruit. Because these species are subjected to cosmetic thresholds, which are commonly very low (Hare, 1994), BC is considered

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insufficient on a limited, but important, number of pests, such as scales [e.g. *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae)] or spider mites (*Tetranychus urticae* Koch) (Acari: Tetranychidae). Furthermore, because of the quarantine pest status of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), thresholds are nil for fruit exported to *C. capitata*-free countries (Jacas, Palou, Beitia, & del Rio, 2008). Hence efforts to improve the BC of these species are underway. On the one hand, classical BC is a very powerful tool against exotic pests of an exotic crop such as citrus is in the Mediterranean basin, and this is one of the reasons why this type of BC has been so widely used in the region (Jacas, Urbaneja, & Viñuela, 2006). In recent years, though, concerns about the potential non-target effects of these exotic species on indigenous food webs have arisen worldwide (Lenteren, van Bale, Bigler, Hokkanen, & Loomans, 2006; Lynch & Thomas, 2000; Lookwood, Howarth, & Purcell, 2001; Stiling, 2004) and citrus is not an exception (Michaud, 2002).

Table 1. Status of citrus pests in Spain and its relation to biological control (BC).

Pest species	*Natural control*[a]	*Key mortality factor*[b]	*BC strategy*[c]
Icerya purchasi	E	Imported NE	C
Insulaspis gloverii	E	Imported NE	C
Aleurothrixus floccosus	S	Imported NE	C
Ceroplastes sinensis	S	Climate-Native NE	C
Chrysomphalus dyctiospermi	S	Native NE	C
Coccus hesperidium	S	Climate-Native NE	C
Panonychus citri	S	Native NE	C
Phyllocnistis citrella	S	Imported NE	C
Planococcus citri	S	Imported NE	A
Saissetia oleae	S	Climate-Native NE	C
Aonidiella aurantii	I	Native & imported NE	A
Aphis gossypii	I	Native & imported NE	C, A
Aphis spiraecola	I	Native & imported NE	C, A
Ceratitis capitata	I	Native & imported NE	I, C
Cornuaspis beckii	I	Native & imported NE	C
Parlatoria pergandii	I	Native & imported NE	C
Tetranychus urticae	I	Indigenous NE	C, A
Toxoptera aurantii	I	Native & imported NE	C, A

[a] E: excellent; S: satisfactory; I: insufficient.
[b] NE: natural enemies.
[c] C: Conservation; A: augmentation; I: inoculation.

A European Union (EU)-harmonized legislation on Invertebrate Biological Control Agents (IBCA) is expected to change the current situation of import and release of exotic IBCAs in the EU. As a consequence, classical BC will probably lose its prevalence in the European citrus industry in favor of other BC strategies focused on existing IBCAs (either indigenous or naturalized) and their management.

In this scenario both augmentative and conservation strategies aimed at increasing the impact of these natural enemies on citrus key pests will become the cornerstone of future Integrated Pest Management (IPM) in Spain.

Table 2. Classical BC Programs developed in Spain against citrus pests.

Target pest	*Year*	*Natural enemy*	*Establishment*	*Success*[a]
Diaspididae	1908	*Rhyzobius lophanthae*	Yes	P
C. dictyospermi	1936	*Comperiella bifasciata*	No	–
Icerya purchasi	1922	*Rodolia cardinalis*	Yes	C
	1997	*Cryptochaetum iceryae*	No	–
Saissetia oleae	<1921	*Microterys nietneri*	No	–
	<1971	*Metaphycus helvolus*	Yes	P
	<1978	*Metaphycus lounsburyi*	Yes	P
Planococcus citri	1927	*Cryptolaemus montrouzieri*	Yes	P
	1977	*Leptomastix dactylopii*	Yes	P
Ceratitis capitata	1931	*Diachasma fullawayi*,	No	–
		Diachasmimorpha tryoni,	No	–
		Psyttalia incisi	No	–
	1960	*Tetrastichus giffardianus*	Yes[b]	No
	1979	*Diachasmimorpha longicaudata*	No[c]	–
	2002	*Fopius arisanus*	No[c]	–
Cornuaspis beckii	1970	*Aphytis lepidosaphes*	Yes	P
Aleurothrixus floccosus	1970	*Cales noacki*, *Amitus spiniferus*	Yes	S
	1971	*Amitus spiniferus*	Yes	P
Aonidiella aurantii	1971	*Encarsia perniciosi*	Yes	P
	1976	*Aphytis lingnanensis*, *A. melinus*	Yes	P
	2000	*Comperiella bifasciata*	No	–
Aphis gossypii	1976	*Lysiphlebus testaceipes*	Yes	P
Insulaspis gloverii	1979	*Encarsia herndoni*	Yes	C
Parabemisia myricae	1982	*Eretmocerus debachi*	Yes	S
Tetranychus urticae	1985	*Galendromus occidentalis*	No	–
Dialeurodes citri	1992	*Encarsia lahorensis*	No	–
Aleyrodidae	<2001	*Encarsia strenua*	Yes	P
Phyllocnistis citrella	1995	*Ageniaspis citricola*,	Yes[b]	C
		Cirrospilus ingenuus,	No	–
		Semialacher petiolatus	Yes	P
	1996	*Quadrastichus citrella*	No	–
	1997	*Galeopsomyia fausta*	No	–
	1999	*Citrostichus phyllocnistoides*	Yes	S

[a] P = partial; S = substantial; C = complete.
[b] In the Canary Islands.
[c] Program in progress.

2. THE ORIGINS: CLASSICAL BIOLOGICAL CONTROL IN CITRUS

Classical BC has been profusely practiced in Spanish citrus orchards (Jacas et al., 2006). Up to 20 programs have been developed during the last century (Table 2). Citrus are an imported crop group in the Mediterranean basin and most of the pest species associated with them originate from Australasia, the area of origin of citrus. These are the typical conditions for pests to become the target of classical BC programs (Barbosa & Segarra-Carmona, 1993). Therefore, this strategy has been repeatedly applied not only in Spain, but in most Mediterranean countries (e.g. Cohen, 1975; Garrido & Ventura, 1993; Katsoyannos, 1996), as well as in most other citrus growing areas under Mediterranean climate, such as California (UC, 1991), Australia (Smith, Beattie, & Broadley, 1997) or South Africa (Charleston et al., 2003).

Scales are predominant among the target pests (Table 2) and this can not be considered an accident. Coccoidea represent 50.0% (n = 8) of them, and Homoptera as a whole represent 81.3% (n = 13). Well-protected insects, such as most homopterans, and those presenting concealed ways of life, like leaf miners, borers, etc. are often more likely to be successfully amenable by BC than free living ones (Hall & Bennett, 1994; Hespenheide, 1991; Khan, Overholt, & Ng'eny-Mengech, 2003; Knipling, 1995). This could partly explain why the citrus leaf miner, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), ranks first, together with the Mediterranean fruit fly, or Medfly, *C. capitata*, according to the number of natural enemies introduced against each of them (n = 6).

Whereas the Medfly is considered one of the world's most damaging fruit pests (IAEA, 2003; White & Elson-Harris, 2004), *P. citrella* is considered a secondary citrus pest. The case of this leaf miner can be considered a paradigm. As in similar situations (Michaud, 2002), the appraisal of urgency when *P. citrella* was detected in Spanish citrus orchards in 1993 prompted funding agencies to prioritize BC projects based on the rearing and release of imported natural enemies. Therefore, *P. citrella* became an automatic target for the classical BC approach without a critical evaluation if such an approach was useful. Pre-introduction studies (Lenteren & Woets, 1988; Barbosa & Segarra-Carmona, 1993; FAO, 1996; EPPO, 1999, 2000) were very limited (Urbaneja, Llácer, Tomás, Garrido, & Jacas, 2000; Urbaneja, Llácer, Garrido, & Jacas, 2003) and 6 different parasitoids (Table 2), were introduced in less than 5 years. The host specific *A. citricola* successfully established on the Canary Islands, but from the remaining eulophids, only *C. phyllocnistoides* finally succeeded in establishing on the mainland (Karamaouna et al., 2009).

Although the introductions of exotic natural enemies focused on *C. capitata* began in 1931 (Servicio Fitopatológico Agrícola, 1933), no success has been achieved so far. A classical BC program against this pest is being carried out at present (Jacas et al., 2006) and both *Fopius arisanus* (Sonan) and *Diachasmimorpha longicavdata* (Ashmead) (Hymenoptera: Braconidae) will be probably released from quarantine confinement during 2010.

Success has been higher for introductions aimed at regulating homopteran pests (Table 2). In fact, some of these natural enemies, like *Rodolia cardinalis* (introduced against *I. purchasi*), *Cales noacki* Howard (Hymenoptera, Aphelinidae) [imported in 1971 against *Aleurothrixus floccosus* (Maskell) (Hemiptera: Aleyrodidae)] and

Lysiphlebus testaceipes (Cresson) (Hymenoptera, Braconidae) [introduced in 1977 against *Aphis gossypii* Glover and *A. spiraecola* Pagenstecher (Hemiptera: Aphididae)], are considered nowadays key natural enemies in Spanish citrus orchards (Urbaneja et al., 2008).

3. AUGMENTATION VERSUS INOCULATION

A few IBCAs primarily introduced in classical BC programs, namely the parasitoids *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) and *Leptomastix dactylopii* (Howard) (Hymenoptera: Encyrtidae) and the predator *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) failed to satisfactorily establish in Spain and consequently do not usually reach numbers high enough to naturally regulate their target pests below economic thresholds (Table 2).

Table 3. IBCAs used in augmentative BC strategies in Spanish citrus orchards and nurseries.

Natural enemy	*Target pest*	*Strategy*
Cryptolaemus montrouzieri	*Planococcus citri*	From end of April – July, if gravid females are present, release 3–10 individuals per tree Repeat at 2–3 week intervals.
Leptomastix dactylopii, *Anagyrus pseudococci*	*Planococcus citri*	From May – July, when third instar nymphs and young females are present, release 10–20 individuals per infested tree Repeat at 2–3 week intervals
Aphytis melinus	*Aonidiella aurantii*	From the end of winter, if parasite susceptible stages (NII, males and young females) are present, release 50–150 · 10^3 wasps/ha in 5 – 8 releases, 15 days apart from each other In severely infested orchards, releases should be combined with petroleum spray oils[a].
Neoseiulus californicus, *Phytoseiulus persimilis*	*Panonychus citri,* *Tetranychus urticae*	In nurseries, hot spot releases of 10 – 30 individuals per infested young tree[b].
Phytoseilus persimilis	*Tetranychus urticae*	Hot spot releases of 100 – 500 individuals per tree when economic threshold (20% occupied leaves) is exceeded[c].

[a] Summer releases should be carefully considered due to hot temperatures and low relative humidity. Releases after summer are only recommended in orange orchards.
[b] Against *P. citri*, only recommended in nurseries if *Euseius stipulatus* is not present.
[c] Experimental strategy under development.

Since the cost of production of these natural enemies is relatively low, the current use of these IBCAs involves augmentative releases against their respective target pests (Table 3). These strategies are also in use in other Mediterranean countries (Katsoyannos, 1996), as well as in areas with a similar climate, such as Australia, California, or South Africa (Charleston et al., 2003; UC, 1991; Smith et al., 1997).

As shown in Table 3, augmentative releases include both exotic and native natural enemies. Consistent with latest trends in EU agriculture, which emphasizes the role of indigenous natural enemies, *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae), a native parasitoid of the citrus mealybug, *Planococcus citri* Risso (Hemiptera: Pseudococcidae), has been recently studied and could successfully substitute the exotic *L. dactylopii* in the future (Campos & Martínez-Ferrer, 2003). Similarly, studies are in progress to establish conditions for using the native predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) against the two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) another native mite which is considered a key pest for clementine mandarins in Spain (Aucejo, Gómez-Cadenas, & Jacas, 2004; Ansaloni, Pascual-Ruiz, Hurtado, & Jacas, 2008). Likewise, releases of *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) and *P. persimilis* have also been proposed against the citrus mite, *Panonychus citri*, and *T. urticae*, respectively, in nurseries (Table 3) (Abad-Moyano, Pina, Pérez-Panadés, Carbonell, & Urbaneja, 2009).

4. FORTUITOUS BIOLOGICAL CONTROL

Anagyrus pseudococci is not the only example of an indigenous natural enemy having a significant impact on an exotic pest. Although such pests are usually the target of the Classical BC strategy, indigenous natural enemies can sometimes result in excellent BC, which is then called fortuitous BC.

The native predator *E. stipulatus* provides a good example of such a situation in Spain. This species is the most abundant Phytoseiidae in Spanish citrus (Ferragut et al., 1988). If undisturbed, its populations are usually able to regulate *Panonychus citri* populations below their economic threshold (Ripollés, Marsá, & Martínez, 1995). Therefore *E. stipulatus* is considered as one of the most relevant natural enemies in Spanish citrus orchards and its conservation is a key factor for IPM success (Urbaneja et al., 2008). Most other indigenous natural enemies do not have such a dramatic impact on their host/prey pests (Table 1). However, both the increasing legal restrictions for importing exotic natural enemies into the EU (Bigler et al., 2005) and the disappearance of many pesticides in the EU following the process of re-evaluation of all pesticide active ingredients under EU Directive 91/414/EEC, have lead to a renewed interest on these native species and their conservation (Table 1).

5. THE CORNERSTONE: CONSERVATION

Conservation biological control exploits resident, either native or naturalized, natural enemies. This strategy is especially useful in permanent ever-green crops (Barbosa, 1998; Landis, Wratten, & Gurr, 2000), such as citrus, where both pests and their natural enemies are active and abundant throughout the year (Garrido &

Ventura, 1993). One of the most popular tactics used for the conservation of natural enemies in the Spanish citrus industry has been the use of pesticides with a reduced impact on beneficial arthropods by exploiting either their intrinsic or their ecological selectivities (Croft, 1990). The use of reservoir plants and, more recently, studies focused on both the management of the ground cover and the use of banker plants to enhance the performance of resident natural enemies are providing citrus growers new tools for implementing conservation BC in their orchards.

5.1. The First Step: Pesticide Side-Effect Testing

Citrus IPM has since long recognised the need for the evaluation of the impact of pesticides on the most relevant natural enemies. Pesticide side-effect testing was routinely done by Spanish researchers for many years for advisory purposes. As a consequence, in 2001, a database including around 270 records referred to 6 important citrus IBCAs and 80 different pesticides was published (Jacas & García-Marí, 2001). The natural enemies were the parasitoids *C. noacki*, *L. dactylopii*, *L. testaceipes* and the predators *C. montrouzieri*, *E. stipulatus* and *R. cardinalis*. Some of the products tested up until that moment are no longer permitted in the EU whereas some new active ingredients have been registered. Therefore, there is a need for regularly updating that list. Pascual-Ruiz and Urbaneja (2006) recently revised the database.

Results specifically dealing with acaricides have been recently published (Urbaneja et al., 2008). Products recommended for IPM in citrus orchards in the Region of Valencia have been listed in Table 4. When available, their residual toxicity on the selected IBCAs is provided. These products are effective against their target pests whereas their effects on IBCAs are usually low (*Bacillus thuringiensis*, Fenbutatin oxide, Hexithiazox, Mineral oils, Pirimicarb, Spinosad) and/or short-lasting (Mineral oils, Chlorpyrifos). In a few cases, some harmful products are allowed provided that ecological selectivity is exploited. This is the case of imidacloprid, which is prohibited from April till July to protect *R. cadinalis* which typically invades citrus orchards at that time.

5.2. Alternative Hosts and Banker Plants

There are two well-known examples of conservation BC in Spanish citrus orchards consisting of providing alternative hosts to natural enemies. One of them exploits *Nerium oleander* L. (Gentianales: Apocynaceae), a bush native to the Mediterranean basin, as a reservoir for aphid natural enemies. *Aphis nerii* Boyer de Fonscolombe (Hemiptera: Aphidae) is a stenophagous aphid feeding on *N. oleander* which can not survive on citrus. Because both *A. nerii* and citrus aphids, such as *A. gossypii* and *A. spiraecola*, are attacked by the same guild of natural enemies (different aphidiine parasitoids and predators like syrphids, cecidomyids and coccinellids), Spanish citrus growers have long used *N. oleander* to establish wind breaks and hedgerows in their orchards. However this strategy should not be used in lemon orchards because *N. oleander* hosts a key pest for this crop, the oleander scale, *Aspidiotus nerii* (Bouché) (Hemiptera: Diaspididae).

Table 4. Residual toxicity of active ingredients allowed under IP label in the Region of Valencia (CAPA, 2004). Classification according to the IOBC WG "Pesticides and Benefial Organisms" standards (1: harmless; 2: slightly harmful; 3: moderately harmful; 4: harmful).

Active ingredient	*Target pests*	*R. cardinalis*	*C. montrouzieri*	*E. stipulatus*	*L. testaceipes*	*L. dactylopii*	*C. noacki*
Abamectin	Mites, leaf miner	1	3–4	2–3			3–4
Acetamiprid[a]	Whiteflies, aphids, leaf miner						
Azadirachtin	Leafminer	3–4	1	1	1	3–4	
B. thuringiensis	Citrus moth	1	1	1	1	1	1
Benfuracarb	Aphids			1			2–3
Buprofezin	Diaspididae, whiteflies	1–2	3	1–2	1	1–2	1
Chlorpyrifos	Coccoidea, citrus moth, aphids	1–2	2	2	3	3	2–3
Chlorpyrifos-methyl	Medfly, coccoidea, citrus moth	1	1	3	3	3–4	1–2
Clofentezine	Mites	1	2	1–2			1
Dicofol	Mites	1	1–4	3–4	1	3–4	2
Etoxazol[a]	Mites						
Fenazaquin	Mites	4	2	4			3
Fenbutatin oxide	Mites		1	2	1	1	1
Fenperoximate[a]	Mites						
Hexithiazox	Mites	1		1	1		
Imidacloprid	Leaf miner	4		2–3	1	4	3
Mineral oil	Mites, coccoidea, whiteflies	1	1–2	1–2	2	1	1–4
Piridaben	Mites			4		1	
Pirimicarb	Aphids	1–2	2	1–2	1	1	1
Pirimiphos-methyl	Coccoidea, whiteflies	1–2	1–2	1–4		4	3–4
Propargite	Mites			4	1		2–3
Pymetrozine[a]	Aphids						
Pyriproxifen	Coccoidea	4	4	1		1–2	2–3
Spinosad	Medfly	1	1		4		
Tebufenpyrad	Mites		2				

[a] Products allowed under IP label whose side effects on citrus natural enemies are yet to be described.
Source: Jacas and García Marí (2001), Pascual-Ruíz and Urbaneja (2006) and Urbaneja et al. (2008).

A similar situation applies to *Oxalis pes-caprae* L. (Oxalidales: Oxalidaceae). This is an herbaceous plant indigenous of South Africa which was introduced long time ago into Spanish citrus orchards. It is an annual plant which produces a bulb that insures its survival. It usually dries during the dry Mediterranean summer, but reappears as the fall rainy season starts. *Petrobia hartii* (Ewing) (Acari: Tetranychidae) is an *O. pes-caprae* inhabitant which can not feed on citrus. Because this mite can serve as an alternative prey for the same Phytoseiid mites feeding on other phytophagous mites occurring on citrus, such as *P. citri* or *T. urticae*, the conservation of *O. pes-caprae* in citrus orchards was included in some citrus IPM guidelines (Aucejo et al., 2003). More recently, studies are underway to ascertain the usefulness of banker plants as a source of aphid parasitoids in citrus, using the same strategy already in use in protected crops (Calvo & Urbaneja, 2004). Barley plants infested with cereal-specific aphids, such as *Rhopalosiphum padi* (L.), parasitized by aphidiine parasitoids, such as *Aphidius colemani* Viereck, are produced by commercial insectaries and introduced into the orchards before citrus aphid populations peak during early spring. This strategy could prove very useful when grassy covers [e.g. *Festuca arundinacea* Schreb (Poales: Poaceae), see below] providing food to these specific aphids are also used.

5.3. Ground Cover Management Strategies

Spanish citrus orchards are quite commonly grown on bare soil by either use of herbicides or mechanical means. This is not the ideal situation and the use of a cover crop as an ecological infrastructure (Boller, Häni, & Poehling, 2004) is encouraged by IPM guidelines. However, little is still known about the fauna inhabiting this *stratum* in citrus and the ecological relationships occurring between the ground and the tree arthropodofaunas. Therefore, the ground cover management is being investigated at this moment as a means of conserving ground-dwelling natural enemies and enhancing their impact on some citrus pests (top-down control), as well as a means of providing bottom-up control of these pests. This is the case of both *T. urticae* and *C. capitata*.

Tetranychus urticae is a serious problem in clementine mandarins in the Eastern coast of Spain. Infestations downgrade fruit and, because *T. urticae* can feed on more than 900 plant species (Bolland, Gutiérrez, & Flechtmann, 1998), cover crop management can dramatically affect the dynamics of *T. urticae* populations on the trees. In 2003, a survey of the acarofauna associated to the most common weeds appearing in citrus orchards showed that Poaceae presented the lowest ratio *T. urticae*/Phytoseiidae from the 45 weed species studied (Aucejo et al., 2003). Therefore, a cover of *Festuca arundinacea* Schreb (Poales: Poaceae), a grass that had been previously selected as a citrus ground cover for other agronomic favorable characteristics, has been compared to a wild cover and to bare soil during the last 2 years (2006–2007).

The results obtained so far show that the *F. arundinacea*-sown cover has resulted in the lowest populations of *T. urticae* on the trees and could consequently be recommended to growers. The mechanisms explaining these results could be related both to a host-feeding specialization by *T. urticae* (bottom-up control) and to the composition of the beneficial acarofauna associated to the ground cover (top-down control), which resulted more diverse and balanced on both *F. arundinacea* and the trees grown on that particular cover than on both the wild cover and the bare soil systems (Aguilar-Fenollosa, Pascual-Ruiz, Hurtado-Ruiz, & Jacas, 2008, 2009). In addition to *T. urticae*, other citrus pests spend part of their life cycle on the ground cover, such as aphids, or in the soil, like *C. capitata*, which pupates in it. In recent years, different groups of ground-dwelling predators have been catalogued in Spanish citrus orchards (Monzó et al., 2005; Urbaneja et al., 2006). These studies showed that rove beetles (Coleoptera: Staphylinidae) were the most abundant-active group representing about 38.6% of the total number of predators collected, followed by spiders (Arachnida: Araneae) (28.9%), earwigs (Dermaptera) (18.0%), ground beetles (Coleoptera: Carabidae) (12.7%) and tiger beetles (Coleoptera: Cicindelidae) (1.8%). A recent study (Monzó, Urbaneja, Sabater-Muñoz, Castañera, 2007; Monzó, Mollá, Castañera, & Urbaneja, 2009) indicates that the wolf spider *Pardosa cribata* Simon (Araneae: Lycosidae), the most abundant ground-dwelling spider in the Valencian citrus orchards, could play an important role in controlling those pests.

CONCLUSIONS

Biological control has been and will definitively be an increasingly important part of citrus crop protection practices. Because of the present European limitations on the importation of exotic IBCAs, straightforward protocols to evaluate candidate species are urgently needed. Simultaneously, studies focused on the role of indigenous IBCAs in the citrus agrosystem and the conservation of both native and naturalized natural enemies, have to be emphasized. Augmentative and conservation BC will probably play a gradually more important function in the Spanish citrus industry and the first changes are already happening.

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4

CITRUS INTEGRATED PEST MANAGEMENT IN ITALY

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Abstract. Main insect pests of citrus in Italy are presented, with details on their biology and main natural enemies. They are the California red scale, *Aonidiella aurantii*, the Oleander scale, *Aspidiotus nerii*, the Citrus mealybug, *Planococcus citri*, the Citrus leafminer, *Phyllocnistis citrella* and the Mediterranean fruit fly, *Ceratitis capitata*. Management of *A. aurantii* is generally based on integrated control strategies supported by a monitoring system through pheromone traps. Natural enemies include predatory beetles and endoparasitoids. *Aspidiotus nerii* is controlled by predatory beetles and Aphelinid parasitoids. Management relies on pruning, chemical control and monitoring through sticky tapes. Several predators (spiders, true bugs, lacewings and ants) feed on *P. citrella*, with over 90 parasitoid species. Natural enemies of *P. citri* include predators and endoparasitoids, which provide satisfactory control. *Ceratitis capitata* is the main pest of citrus; biological control with parasitoids had limited success. Its populations are limited by microorganisms and occasional predators, cultural methods, chemicals and attractive traps. Management and control of secondary pests of citrus, like whiteflies, some scales and moths, are also discussed.

1. INTRODUCTION

In Italy citrus are cultivated on around 170, 000 ha, the majority of which is represented by sweet orange (60%), followed by lemon (19%), clementine (13.8%), mandarin (6.1%) and minor species (bergamot, grapefruit, citron and chinotto) (1%).

The pests reported on this crop are nearly 100 but among them only around 30, mainly sap-sucking species, may require control. In the last 30–40 years the picture of the main arthropod pests deeply changed. The major reason of this modification can be found in the repeated applications of large spectrum pesticides that altered the bio-ecological equilibrium of this complex agro-ecosystem. However, the changes are also related to the effect of modifications in cultural techniques (fertilization, use of phytoregulators, irrigation, pruning, weeding, new varieties,

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etc.) that modified the cultivated ecosystem. Besides, an additional cause of modifications in the citrus orchard fauna is represented by the accidental introduction of new exotic pests. Among these, the most significant in the last 30–40 years have been *Aphis spiraecola* Patch, *Aleurothrixus floccosus* (Maskell), *Dialeurodes citri* (Ashmead), *Parabemisia myricae* (Kuwana), *Coccus pseudomagnoliarum* (Kuwana) and other scales [such as *Aonidiella citrina* (Coquet), *Pseudococcus calceolariae* (Maskell) and *Unaspis yanonensis* (Kuwana)], *Phyllocnistis citrella* Stainton, *Pezothrips kellyanus* (Bagnall) and the red spider mite *Panonychus citri* (McGregor). Recently (April 2008) a new record was added to the list of citrus pests in Italy: the Orange spiny whitefly *Aleurocanthus spiniferus* Quaintance (Porcelli, 2008). This species, which is included in the "*EPPO A1 List of pests recommended for regulation as quarantine pests*" and in the EU Annex II/A1: "*Pests known not to occur in the EU, whose introduction into, and/or whose spread within, all EU Member States is prohibited, with reference to specific plants or plant products*", is a further example of fortuitous introduction of exotic species on citrus.

The reasons of all these introductions are not easily explicable but they're most probably related to increased commercial exchanges, as well as to continuous changes in climate. The flow of exotic arthropods towards the Mediterranean basin in general and Italy in particular is unceasingly active. There are several potential invasive species, among which the Brown citrus aphid *Toxoptera citricidus* (Kirk.), the most efficient vector of the Citrus Tristeza Virus, represents a real risk, considering its recent establishment in some limited areas of Spain and Portugal (Madeira island and Northern region). Other species that are most likely to be introduced or spread out in Italy are the whiteflies *Aleurocanthus woglumi* Ashby, *Aleurodicus dispersus* Russell, *Aleuroclava jasmini* (Takahashi) (which are all present in the Middle East); the psyllid *Diaphorina citri* Kuwayama (vector of the Greening disease and present in Saudi Arabia and in Madeira island); the scales *Ceroplastes floridensis* Comstock (recorded in the Middle East) and *Chrysomphalus aonidum* (L.) (up to now only occasionally reported on citrus in Italy), as well as the longhorned beetle *Anoplophora chinensis* (Forster) (reported in Northern Italy on other host plants).

The introduction of exotic species is not only detrimental because of their direct damage but most of the time because of the measures adopted to control their populations, often chemical treatments, that may in fact disrupt comprehensive integrated control strategies carefully developed over the years. However, many of the newly introduced species have been the object of classical biological control programs with importation of natural enemies from the areas of origin of the pests and the majority of them achieved substantial results ensuring effective control.

2. MAIN PESTS AND THEIR CONTROL METHODS

In Italian citrus orchards the key arthropod pests are presently the California red scale *Aonidiella aurantii* (Maskell), the Oleander scale *Aspidiotus nerii* Bouché (on lemon), the Citrus mealybug *Planococcus citri* (Risso), the Citrus leafminer *P. citrella* (on young trees and in nurseries), the Mediterranean fruit fly *Ceratitis* *capitata* (Wiedemann) and the Two spotted spider mite *Tetranychus urticae* (Koch) (on lemon) (Barbagallo, 2000). Together with these species, some others such as the Citrus green bug *Closterotomus trivialis* (Costa), the Cotton or Melon aphid *Aphis gossypii* (Glover), the Chaff scale *Parlatoria pergandii* Comstock, the Olive black scale *Saissetia oleae* (Olivier), the Fig wax scale *Ceroplastes rusci* (L.) and the Leafroller *Archips rosanus* (L.), have been showing recrudescent infestations over the last decades.

2.1. Aonidiella aurantii (Maskell)

The armoured scale *A. aurantii* (Hemiptera: Diaspididae), commonly known as California red scale, is native to South-Eastern Asia (Southern China and Indochinese peninsula) but is spread almost worldwide.

The armour of the mature female is almost round in shape (1.6–2.1 mm), clear brown almost translucent with the nymphal exuviae located at the centre. Generally the scale appears reddish in colour because of the red body visible through the cover. The shield remains tightly attached to the substrate when the scales are moulting or reproducing. A characteristic, well developed, whitish ventral coating isolates the body of the female from the plant tissues. Before mating the body of the female is pear-shaped, pale yellow and its cover assumes a greyish colour; soon after mating, due to the production of eggs, the cephalothoracic portion of the body rapidly develops and reaches the apex of the abdomen. The crawlers that progressively emerge from under the female, move around to find a suitable place to settle and begin to produce a white cottony circular cover ("white cap" stage). Starting from the second instar, males begin to develop in a different way forming an elongated and usually clearer cover. The adult male (1.6–1.7 mm) emerges after four moults and is yellowish-orange, with some brown sclerified portions on the dorsal region.

Figure 1. Aonidiella aurantii *colonies on Tarocco orange.*

Aonidiella aurantii is extremely polyphagous, it has been recorded on more than one hundred hosts including agricultural (almond, avocado, carob, grape, jujube, loquat, mango, mulberry, olive, peach, pear, walnut, etc.), forest (alder, conifer, eucalyptus, maple tree, oaks, etc.) and ornamental plants (acacia, aralia, araucaria, bougainvillea, boxwood, camellia, crossvine, dracaena, euonymus, magnolia, oleander, palm, privet, etc.). However, its preferential host plants are citrus (Fig. 1), in decreasing susceptibility order: lemon, grapefruit, orange and mandarin, on which it can certainly be still considered as one of the key pests in arid and semiarid regions worldwide (Moreno & Luck, 1992; Franco, García–Mari, Ramos, & Besri, 2006; Grafton-Cardwell, 2006). This is related to the direct damage to the trees, due to the infestations on all aerial parts of the plant from which the scale sucks sap and inoculates toxic saliva causing leaf yellowing (Fig. 2), deformation and drop, dieback of twigs and limbs, cortical lesions on branches and trunk, sometimes with production of gum, but also mainly because of the commercial damage linked to fruit downgrading caused by the simple presence of instars on the peel (Walker, Zareh, & Arpaia, 1999).

Figure 2. Leaf yellowing caused by Aonidiella aurantii *on citrus.*

The general difficulty in chemically controlling armoured scales, the easy development of resistance by *A. aurantii* to chemical compounds (Forster, Luck, & Grafton–Cardwell, 1995; Grafton-Cardwell, Ouyang, Striggow, Christiansen, & Black, 2004; Martínez Hervás, Sots, & García–Marí, 2006) and the spread of integrated and organic citriculture, led to the search for alternative control methods.

Management of this pest is generally based on integrated control strategies supported by a monitoring system by means of pheromone traps. The captures allow identifying the flights of male scales, which correspond to the generations annually performed by the scale in the field, and also indicate which orchards or areas of the orchard have higher levels of scale population. The flight data together with the Degree-Days calculation, help to determine the right time for chemical or biological control. Pheromone traps have to be placed in the field in February-beginning of March. The sticky cards are then changed weekly and the pheromone dispensers monthly through October. Two to four pheromone traps per uniform 4-ha block are used, adding two traps for each additional 4 ha.

Narrow range petroleum oil sprays can be used to reduce scale populations, with reduced negative effects on the complex of the natural enemies. Other commonly used chemicals, such as chlorpyrifos, imidacloprid or insect growth regulators (buprofezin, pyriproxifen), can negatively impact ladybeetle and lacewing populations and their use is therefore incompatible with a rational integrated management of the orchard. Pheromone traps, however, are not reliable predictors of red scale populations when insect growth regulators are used, since males are more sensitive than females to these compounds. In this case the traps may underestimate the scale population (Rill, Grafton–Cardwell, & Morse, 2007).

The complex of natural enemies feeding on California red scale includes the predatory beetles *Chilocorus bipustulatus* (L.), *C. kuwanae* Silv., *C. nigritus* (F.), *Exochomus quadripustulatus* (L.), *Rhyzobius lophanthae* (Blaisdell) (Coleoptera: Coccinellidae), *Cybocephalus rufrifrons* Reitter (Coleoptera: Cybocephalidae), the dipteran *Lestodiplosis aonidiellae* Harris (Diptera: Cecidomyidae), and the mite *Typhlodromus cryptus* Athias-Henriot (Acari: Phytoseiidae). The scale is also controlled by the endoparasitoids *Encarsia perniciosi* (Tower) (Hymenoptera: Aphelinidae) and *Comperiella bifasciata* Howard (Hymenoptera: Encyrtidae), and by the ectoparasitoids *Aphytis chrysomphali* (Mercet), *A. lingnanensis* Compere, *A. melinus* DeBach and *A. proclia* (Walker) (Hymenoptera: Aphelinidae).

Figure 3. Aphytis melinus *adult female.*

Although numerous natural enemies are associated with California red scale and their relative importance varies according to the differences in the climatic conditions as well as the host stage preferences, some of them are considered more effective and employed in biological control programs. In particular the aphelinid ectoparasitoid *Aphytis melinus* (Fig. 3) is the most commonly used biocontrol agent of *A. aurantii* in Italy as well as worldwide through augmentative releases (Furness, Buchanan, George, & Richardson, 1983; Moreno & Luck, 1992; Forster et al., 1995; Luck, Forster, & Morse, 1997; Rizqi, Nia, Abbassi, & Nadori, 2001; Rizqi, Bouchakour, Aberbach, & Nia, 2006). The technique consists in releasing about 200,000 parasitoids/ha/year starting in February–March (when the average temperature is around 18°C or after the first captures of males on the pheromone traps) and following a bi-weekly interval. Half of the total amount of parasitoid adults should be released before mid-June, then the releases can be suspended for 1 or 2 months (depending on the climatic conditions) when second and third instar scale are not available and then completed generally through mid-November.

The effectiveness of biocontrol agents depends on careful monitoring and use of selective insecticides. Besides, a critical point is represented by the control of ants which can severely disrupt red scale parasites while protecting and maintaining this species and honeydew-producing pests, such as soft scales or mealybugs. In the last years some trials were carried out in Southern Italy (Tumminelli et al., 2000, 2006a; Mazzeo, Benfatto, Palmeri, & Scazziotta, 2004) which gave inconsistent results and therefore the effectiveness of *A. melinus* releases has not been clearly demonstrated. The explanation of these results can be searched in the mutual relationship between the biology and behaviour of the parasitoid and its host, in the methodology of release, in the difficulty to involve uniform areas and in the low quality of the parasitoids used. In 2005–2006 a further trial was carried out (Zappalà et al., 2008), trying to eliminate some of these elements of uncertainty, therefore conducting the experiment in a uniform integrated citrus orchard, releasing *A. melinus* locally produced by the insectary of the Regional Phytosanitary Services, regularly submitted to quality control tests (Zappalà Siscaro, Saraceno, Palmeri, & Raciti, 2006). Evenly distributed release points were used, according to a scheme supported by a parallel trial on the dispersal capacity of *A. melinus* (Palmeri, Campolo, Grande, Siscaro, & Zappalà, 2008). The data obtained suggest that *A. melinus* contributes to the control of California red scale infestations, but cannot be considered as the key solution, at least in Sicilian conditions. In any case the results obtained highlighted that the elimination of chemical treatments in the released plots as well as in the surroundings, restored a biological equilibrium ensuring a consistent presence of fundamental natural enemies (Zappalà et al., 2008).

Further investigations presently focus on the evaluation of the actual role played by endoparasitoids, namely *C. bifasciata*, which was recovered several years after its first introduction (1988–1994). This species showed to be well adapted in Eastern Sicily where it has colonized a wide area, 50 km, on average, far away from the first release site. The presence of *C. bifasciata* both in organic and conventionally managed orchards is of particular interest and could be of great help in the quick diffusion of the encyrtid, already successfully started, in all citrus growing areas of Southern Italy (Siscaro, Di Franco, & Zappalà, 2008). Future studies will also regard the interactions between *A. aurantii*, its natural enemies and the most common species of ants in Sicilian citrus orchards. Interesting hints could come from the evaluation of the effect of joint releases of predators, such as for example *C. bipustulatus*, which has an impressive "cleaning effect" on dense colonies, mostly on branches and trunk, and is less sensitive to high temperatures.

2.2. Aspidiotus nerii Bouché

Commonly known as Oleander scale, *A. nerii* (Hemiptera: Diaspididae) is almost worldwide distributed on citrus and in the Mediterranean basin it normally completes 3 generations per year. It mainly overwinters as virgin female and immature male on branches of various size. The reproduction is sexual or parthenogenetic and each female produces an average of 100 eggs with the parthenogenetic biotypes performing a lower fecundity. This scale insect is extremely polyphagous and attacks citrus (mainly lemon), acacia, asparagus, carob, ivy, jojoba, kiwi, mulberry, oleander, olive, palm, peach, pear, plum.

The armour of the female is pale brown in colour, about 1.5–2.5 mm in diameter, round shaped. The body of the insect is yellowish. The nymphal exuviae are yellow and located centrally or slightly laterally. The ventral shield is white and very thin. The pygidium has 3 pairs of lobes, the central ones have a sclerified basal region while the external ones are poorly developed; dorsal ducts are short and spiracles are not provided with glands.

Male armour is slightly smaller, white and almost oblong in shape, 1–1.5 mm in length. The adult male (1 mm in length) has only one pair of wings, like all the other scales, and is characterized by a long aedeagus. It is yellow in colour with blackish-brown appendages.

The pest infests branches, leaves and fruits. Heavy attacks may cause loss of vigour, deformation of infested plant parts, chlorotic spots on leaves and leaf drop. On the fruits the feeding sites of the scales remain green and do not develop normally while the rest of the rind changes colour at maturity and grows evenly.

The Oleander scale is controlled by the predatory beetles *Chilocorus bipustulatus*, *Exochomus quadripustulatus*, *Rhyzobius lophanthae* and *Scymnus* spp. (Coleoptera: Coccinellidae). The species is also parasitized by the Hymenoptera Aphelinidae *Aphytis chilensis* How., *A. chrysomphali*, *A. diaspidis* (How.), *A. hispanicus* (Mercet) and *Encarsia citrina* (Craw).

Regular pruning represents an important tool in controlling this scale as well as all the other scales. Chemical control can be performed using mineral oils before the scale moves on the fruits and, in case of heavier infestations, the use of an IGR (buprofezin) can be considered. The timing of treatments is made harder by the absence of an efficient monitoring system. Pheromone traps were tried in the field but they were not commercially developed. A method which proved effective in monitoring the scale populations, and particularly in placing the chemical treatments, was based on wrapping sticky tape around 1-year-old branches that have both old and new wood and are infested by live female scales, in order to capture the crawlers as they emerge from the females and move across the twigs (Tumminelli, Perrotta, Raciti, & Colazza, 2006b). The economic threshold varies considerably, depending on the market and crop yield, but it normally corresponds to 3–5% of fruits infested with more than 10 scales in the fall. If this threshold is exceeded at harvest, monitoring crawlers the following spring, together with visual inspection of fruit, may be a valid method to correctly place a treatment. This should be avoided at petal fall on the peak of crawlers of the first generation (around May) because it may increase fruit drop and be toxic to honeybees. It could instead be applied on the peak of crawlers of the second generation which normally takes place in July (Tumminelli et al., 2006b).

2.3. Phyllocnistis citrella (Stainton)

The Citrus leafminer (CLM), *P. citrella* (Lepidoptera: Gracillariidae), native to South-East Asia, has spread worldwide, throughout almost all citrus growing areas in the last decades (Hoy & Nguyen, 1997; CAB International, 2003; Grafton-Cardwell, Godfrey, Headrick, Mauk, & Peña, 2008). In Italy it was first reported in 1994 (Benfatto, 1995).

Adults are about 2 mm long with a wingspan of about 4 mm. They have narrow long fringed wings; the anterior pair is silvery and shining white with brown and white markings as well as a distinct black spot on each wing tip. Hind wings are whitish and extremely slender, both costal and inner margins have long setae. Antennae are filiform and made of about 30 segments.

The egg (0.35 × 2 mm) is lenticular and the chorion is smooth and transparent. The larvae are pale yellow from the first to the third instar with the last abdominal segment bifurcate. Their size is 1.7 – 2 × 0.3 – 0.4 mm. The fourth instar larva or prepupa is yellow in colour, cylinder shaped and without bifurcation on the last abdominal segment. It stops feeding but remains quite active. It forms a silken cocoon within the mine and as the silk dries the leaf curls over the pupal cell. The pupa is yellow to light brown, turning darker with age. It is characterized by the presence of a cephalic spine which is used to make an opening at the anterior of the chamber, to let the adult emerge. The last two abdominal segments are fused in the female pupa while they are distinct in the male.

Figure 4. Phyllocnistis citrella *larva and mine on a citrus leaf.*

Leafminers are most active from dusk to early morning. Soon after emergence the female emits a sex pheromone that attracts males. Females lay 30–70 eggs during their 2–12 days life span, deposited singly along the midrib on the lower surface of new tender terminal leaves. Eggs hatch within 2–3 days at 26–27°C; however, it may take up to 12 days at lower temperatures. Newly born larvae begin feeding immediately in shallow, winding mines under the leaf cuticle. As the larva increases in size, the mine becomes more visible because of the air that penetrates and because of the central frass trace within the mine (Fig. 4). Larvae molt 4 times, each larval stage lasting about 1 day, while the pupal stage lasts 7–10 days at 26°C. Mature larvae pupate within the mine inside a silken chamber under the rolled edge of the leaf. From egg to adult the life cycle takes about 15–17 days to complete at 26°C and 70–80% humidity, but it may last up to 7 weeks depending on temperature and humidity conditions. Up to 13 generations per year occur in tropical areas. The population dynamics and the activities of the Citrus leafminer vary in relation to differences in climatic conditions and flushing of citrus trees. In Italy the spring flushing escapes *P. citrella* infestation since temperatures are not suitable for the leafminer development. The attacks of the pest occur during summer-fall.

Several predators, mainly spiders, bugs, lacewings and ants, have been observed feeding on the pest (Browning & Peña, 1995). Besides, over 90 hymenopterous parasitoid species, belonging to the families Braconidae, Encyrtidae, Eulophidae, Eurytomidae, Eupelmidae and Pteromalidae have been reported (Heppner, 1993; Hoy & Nguyen, 1997; Schauff, Lasalle, & Wijesekara, 1998). Among these species, about 70 are considered as primary parasitoids and only 6 are classified as preferentially living on *P. citrella*. Indigenous natural enemies, mainly parasitoids, detected on the CLM never reached an effective control in all newly infested citrus areas, such as Florida (Hoy & Nguyen, 1997), Israel (Argov & Rössler, 1996), Spain (Garrido Vivas, 1995), Turkey (Uygun et al., 1996) and Italy (Barbagallo et al., 1998; Caleca & Lo Verde, 1998; Giorgini, Pedata, & Viggiani, 1998).

Since in the native areas of the leafminer the host-specific enemies represent the main biological mortality factor of the pest (Binglin & Mingdu, 1996; Morakote & Nanta, 1996; LianDe, MinSheng, Jin, & Qing, 1999), a classical biological control program has been started in 1995 in Italy by introducing exotic natural enemies. Three Hymenoptera parasitoids [*Ageniaspis citricola* Logvinovskaya (Encyrtidae), *Quadrastichus* sp. (now *Quadrastichus citrella* Reina & La Salle) and *Citrostichus phyllocnistoides* (Narayanan) (Eulophidae)] have been introduced, reared and released. Moreover, in 1998 the Australasian ectoparasitoid *Semielacher petiolatus* (Girault) (Hymenoptera: Eulophidae) was recorded for the first time in Italy. The species probably spread naturally after its introduction in other countries of the Mediterranean basin (Mineo, Caleca, & Massa, 1998; Siscaro, Longo, Maugeri, Reina, & Zappalà, 1999a).

Ageniaspis citricola (Fig. 5) is a poliembryonic koinobiont endoparasitoid of *P. citrella* eggs and young larvae (Edwards & Hoy, 1998; Zappalà & Hoy, 2004). The species shows several biological features which characterize effective parasitoids, such as host-specificity, high reproductive rate (more than 180 eggs/female, female-biased sex ratio and short cycle), host discrimination capacity, high dispersal and searching rate (Hoy & Nguyen, 1997; Zappalà & Hoy, 2004). Therefore, the encyrtid *A. citricola*, native to Taiwan, Thailand and Vietnam, has been used in biological control programs in several citrus regions: Algeria, Argentina, Australia, Bahamas, Brazil, Cyprus, Colombia, Florida, Greece, Honduras, Israel, Louisiana, Morocco, Mexico, Oman, Peru, Syria, Spain, Texas, Tunisia, Turkey and Venezuela (Berkani & Mouats, 1998; Schauff et al., 1998; Siscaro, Longo, & Mineo, 2000).

Figure 5. Ageniaspis citricola*, ovipositing adult female.*

In Italy, the encyrtid has been introduced and reared since 1995 (Siscaro, Barbagallo, Longo, & Patti, 1997; Siscaro & Mazzeo, 1997). Nearly 15,000 adults have been released in Eastern Sicily and Calabria during 1996–2000 (Siscaro, Longo, & Mineo, 2000). The species was recovered in some coastal lemon orchards where it overwintered in 1998 (Siscaro et al., 1999a) and this strain, collected in the field, has been reared and released in the following years, although without any permanent establishment (Siscaro, Barbagallo, Longo, Reina, & Zappalà, 1999b). The encyrtid is, however, one of the major CLM antagonists in several countries (Argentina, Australia, Bahamas, Brazil, Canary Islands, Florida, Honduras, Louisiana and Venezuela), where it has permanently established. Therefore *A. citricola* appears to be climatically adapted to humid tropical and subtropical climates (Hoy & Nguyen, 1997).

Laboratory observations have shown that the species, at the pupal stage, has a great resistance to low temperatures (5–10°C); adults survive longer at 10°C while individuals exposed to temperatures higher than 25°C die in less than 24 h. High mortality of adults at 25–35°C suggests that the parasitoid is unsuitable to the biological control of *P. citrella* in Mediterranean citrus orchards (Zappalà & Siscaro, 2004).

Quadrastichus citrella is an ectoparasitoid of CLM second and third instar larvae. Its biological cycle lasts about 20 days at 20°C and R.H. > 80%. At the same temperature the adults survive up to 40 days (Argov & Rössler, 1998; Llácer, Urbaneja, Jacas, & Garrido, 1998). The parasitoid, native to China, Japan, Taiwan and Thailand, has been introduced in Morocco (Smaili, Afellah, Aarab, & Zrida, 1999), Cyprus, Greece, Spain and Israel (Schauff et al., 1998; Kalaitzaki, 2004), with no evidence of establishment (Argov, 2000), except in Spain where the species temporarily established in the Valencia area (Vercher, García–Mari, Costa–Comelles, Marzal, & Villalba, 2003). In 1996 this eulophid was introduced in Southern Italy and about 3,000 specimens were released in more than 30 sites. Although preliminary observations indicated that the ectoparasitoid seemed to have a good adaptability to Italian citrus areas (Longo & Siscaro, 1997), it has not overwintered in any release site (Barbagallo, Longo, Siscaro, Reina, & Zappalà, 2000).

Citrostichus phyllocnistoides is reported as larval ectoparasitoid of *P. citrella* (Subba Rao & Ramamani, 1965; Bouček, 1988; Neale, Smith, Beattie, & Miles, 1995). Nevertheless it has been recovered in India on *Trioza obsoleta* Buckton (Homoptera: Psyllidae) feeding on *Diospyros melanoxylon* (Roxb.) (Dash & Das, 1997). Further studies (Massa, Rizzo, & Caleca, 2001; Massa & Rizzo, 2001; Lo Duca, Massa, & Rizzo, 2002) have shown that *C. phyllocnistoides* parasitizes also Lepidoptera Nepticulidae (*Acalyptris minimella* (Rebel) on *Pistacia lentiscus* L., *Stigmella* sp. on *Rubus ulmifolius* Schott and an unidentified nepticulid on *Salix alba* L.). It prefers second and third instar CLM larvae both for ovipositing and host feeding, while first instar larvae are selected only for host feeding (Reina & Siscaro, 2004). The female lays one or more eggs (up to 5), but only one will complete its development (Subba Rao & Ramamani, 1965). Its cycle lasts 12–13 days at 22–26°C (Ding, Li, & Huang, 1989). Sex ratio is female-biased, 80% of females is obtained from third instar larvae, while 70% of males from second instar larvae. The eulophid is reported on *P. citrella* in Afghanistan, China, India, Indonesia, Japan, Oman, Pakistan, South Africa, Sudan, Swaziland, Taiwan and Thailand (Schauff et al., 1998). It has been introduced in Australia, Cyprus, Greece, Israel (Schauff et al., 1998) and Spain (García-Marí et al., 2000). Its permanent establishment has been recorded in all these areas with the exception of Australia (Argov, 2000; García-Marí et al., 2000). Moreover, in Portugal the accidental immigration and establishment (ecesis) of this species was reported in 2003 (Gomes da Silva, Borges da Silva, & Franco, 2006).

Figure 6. Semielacher petiolatus *adult female.*

In Italy *C. phyllocnistoides* was introduced in 1999 (Mineo & Mineo, 1999a) and in the same year about 600 specimens were released in Western Sicily (Mineo, Mineo, & Sinacori, 2001). In 2000–2001 more than 3,000 specimens were released in Eastern Sicily (Conti, Raciti, Campo, Siscaro, & Reina, 2001). The eulophid was recovered in all the release sites (Conti et al., 2001) and also overwintered far from them (Mineo et al., 2001). Observations carried out in the following years showed its permanent establishment with a contribution to parasitization which increased from around 5% in 1999–2000 to almost 60% in 2001–2007.

Semielacher petiolatus (Fig. 6) is a solitary ectoparasitic wasp which lays eggs on *P. citrella* second and third instar larvae (Fig. 7), although it frequently parasitizes also prepupae (Bouček, 1988; Argov & Rössler, 1998; Mineo & Mineo, 1999b; Ateyyat, 2002; Lim & Hoy, 2005). It has been observed developing on alternative hosts, such as Diptera Agromyzidae *Agromyza hiemalis* Becker on *Urtica* spp., *Chromatomyia horticola* (Goureau) on *Sonchus* spp. and *Liriomyza* sp. on *Merculiaris annua* L. and Lepidoptera *Cosmopterix pulchrimella* Chambers (Cosmopterigidae) on *Parietaria diffusa* M. & K., *Stigmella aurella* (Fabr.) (Nepticulidae) on *Rubus ulmifolius* Schott and *Dialectica scalariella* Zeller (Gracillariidae) on *Echium* sp. (Massa & Rizzo, 2000; Massa et al., 2001).

Figure 7. Semielacher petiolatus *egg* (yellow arrow*) laid close to a CLM larva.*

The parasitoid, after various larval instars, pupates in host mines (Fig. 8) and parasitized CLM larvae will not complete their development. The life cycle is completed in 10 days at 25°C. Host feeding was observed in the laboratory (Argov & Rössler, 1998). *Semielacher petiolatus* has been recovered on *P. citrella* in Australia (Bouček, 1988) and in Solomon Islands (Schauff et al., 1998). The eulophid has been introduced in Cyprus, Israel, Morocco, Oman, Syria, Tunisia, Turkey (Schauff et al., 1998), Egypt (Hamed, Reckhaus, Mahrous, Soliman, & Gassert, 1999), Greece (Michelakis, 1997) and Spain (García-Marí et al., 1997). In 2003 adults of *S. petiolatus* were imported from Italy to Florida but after the evaluation in the quarantine facilities in order to verify the potential effectiveness of

S. petiolatus as a natural enemy of the Citrus leafminer in Florida it was decided not to release it in the field (Lim & Hoy, 2005; Lim, Zappalà, & Hoy 2006). In Italy the parasitoid has been detected for the first time on *P. citrella* in 1998 (Mineo et al., 1998), performing interesting parasitism activity (Caleca, Lo Verde, Blando, & Lo Verde, 1998). Further observations have shown its spontaneous diffusion in all citrus orchards in Southern Italy (Viggiani, 2001). This record together with Algerian (Schauff et al., 1998) and Jordanian ones (Mineo, 1999), reveals its dispersal capability in Mediterranean citrus areas (Siscaro et al., 2000).

Figure 8. Semielacher petiolatus *larva* (left) *and pupa* (right). *The leaf cuticle has been removed to reveal the parasitoid instars.*

As reported for *C. phyllocnistoides*, also for *S. petiolatus* accidental immigration and establishment was observed in Portugal in 2003 where the parasitoid is now very frequently present (Gomes da Silva et al., 2006). In 1999–2000 *S. petiolatus* contribution to the total CLM biological control in Italy was around 90% (Mineo & Mineo, 1999b; Conti et al., 2001). In summer 2001 the eulophid was still the most efficient *P. citrella* parasitoid, showing an incidence on the total parasitization activity near 80%. Nevertheless during fall of the same year, the main biological control was also due to *C. phyllocnistoides*. In the following years the role of this last species greatly increased (up to 60%) being still more active in the final part of the CLM infestation season (September–October), while *S. petiolatus* is mainly active in early summer.

Several indigenous Hymenoptera parasitoids belonging to the family Eulophidae have been obtained from samples collected in Italian citrus groves infested by *P. citrella*. The main species were *Cirrospilus pictus* (Nees) and *Pnigalio agraules* (Walker) and, as observed in other Mediterranean citrus growing areas (Garrido

Vivas, 1995; Argov, Rössler, & Rosen, 1995), the incidence of the first one on the parasitization has reached 80–90% (Caleca et al., 1998; Caleca & Lo Verde, 1998; Conti et al., 2001). Moreover, the following species have been occasionally detected in Sicily: *Apotetrastichus postmarginalis* (Bouček), *A. sericothorax* (Szelényi), *Asecodes delucchii* (Bouček), *A. erxias* (Walker), *Neochrysocharis formosa* (Westwood) and *Ratzeburgiola incompleta* Bouček. Finally, other eulophids have been rarely recovered: *Aprostocetus* spp., *Baryscapus* sp., *Chrysocharis pentheus* (Walker), *Cirrospilus diallus* Walker, *C.* nr. *lyncus* (Nees), *C. vittatus* Walker, *Diglyphus isaea* (Walker) and *Pnigalio soemius* (Walker) (Viggiani & Giorgini, 1995; Benfatto, 1996; Caleca, Lo Verde, & Massa, 1996; Liotta, Peri, Salerno, Di Cristina, & Manzella, 1996; Caleca et al., 1998; Caleca & Lo Verde, 1998; Giorgini et al., 1998; Lo Pinto & Salerno, 1998; Mineo, 1999; Conti et al., 2001).

Starting from 1999 to 2002 the exotic eulophids *C. phyllocnistoides* and *S. petiolatus* have progressively substituted almost all the indigenous parasitoids previously detected on *P. citrella*, inducing a decrease of their parasitism to less than one third of what had been recorded, up to 1998.

The high degree of specificity reached by *P. citrella* parasitic complex represents an important element in the biological control of this pest. The data collected in the main Italian citrus growing areas from 1996 up to now reveal an activity of the parasitoids (including both parasitization and host feeding) that has grown from 32% (1996–1998) up to 65% in more recent years (Siscaro & Reina, 2005), mainly thanks to the biocontrol activity of the 2 exotic eulophids *S. petiolatus* and *C. phyllocnistoides*. These species permanently established in Sicilian citrus groves showing a contribution of 90% to the total parasitization (Siscaro, Caleca, Reina, Rizzo, & Zappalà, 2003). Besides, a seasonal alternation in their activity was highlighted: *S. petiolatus* parasitization is in fact mainly concentrated in the first months of CLM infestation (June–August), while *C. phyllocnistoides* activity is more intense in the second part of the season (September–October) (Siscaro et al., 2003).

The establishment of the introduced eulophids is most likely related to the presence of alternative hosts (Massa et al., 2001; Massa & Rizzo, 2001; Lo Duca et al., 2002), and their seasonal alternation could be partly explained by the different biological and ecological attitudes the two species showed on hosts of native flora (Rizzo, 2003). Therefore it is important to maintain a rich biodiversity in citrus groves in order to provide alternative food and shelter to CLM parasitoids, mainly in winter and spring, when CLM populations are at their minimum levels.

Biological control, together with cultural techniques based on reducing irrigation and fertilization with the aim of containing excessive vegetation, has proved effective in containing *P. citrella* infestations in adult citrus orchards. The situation is different on young and re-grafted trees, as well as in nurseries where damage caused by the leafminer may be more serious. In these cases chemical treatments can be applied using cytotropic or systemic insecticides, or insect growth regulators which, for their mode of action, should be employed sooner than the previous compounds. Mineral oils also have a repellent action on egglaying females and a reduced impact on the ecosystem. They can be used at a dose of 0.5–1 l/hl which protects the plants for 6–10 days, without rain, and the treatments must be applied sooner than the other larval insecticides in the infestation season (Siscaro & Zappalà, 2004).

2.4. Planococcus citri (Risso)

This mealybug (Hemiptera: Pseudococcidae), commonly known as Citrus mealybug, is reported as citrus pest in the Mediterranean basin together with five other mealybug species. It has uncertain origins, but recent findings on its main parasitoids indicate that it has spread from central Africa (Franco, Suma, Borges de Silva, Blumberg, & Mendel, 2004) and is now present almost worldwide.

The adult female (1.6–3 mm long) has a convex body covered with a waxy secretion and is surrounded by 18 pairs of lateral filaments, with slightly increasing length from the head to the abdomen. Antennae and legs are well developed. A ventral circulus is present on the ventral part of the abdomen. The ovipositing female produces a white ovisac that covers the eggs. The light yellow crawlers are highly mobile.

It is a very polyphagous species which can infest almost all species and varieties of citrus with a different degree of susceptibility (Franco et al., 2004), as well as several other agricultural and ornamental crops such as ficus, gardenia, jasmine, oleander, persimmon, pothos, pittosporum, rhododendron, etc.

It mainly overwinters as immature female but also younger instar nymph, which can be found in crevices on trunks and branches. It performs several generations per year (more than 5) and is favoured by warm and humid climate. Small colonies of the mealybug start settling in early summer, near the calyx of little fruits, in the contact points between them or, for some varieties, in the navel. High population levels are reached in late summer-fall. Several agronomic factors can favour the presence of *P. citri*, such as particularly dense and dark canopy, clusters of fruits, presence of navel, excess in nitrogen fertilization and irrigation. A very important role in the growth and dispersal of *P. citri* colonies is played by ants, which usually breed the mealybug, carrying it in good feeding sites in order to increase the production of honeydew which they feed on. Ants also protect *P. citri* from its natural enemies, thus disrupting biological control.

Planococcus citri infestations can produce a reduction in plant growth and in fruits size, as well as fruit downgrading, caused by the presence of colonies with chlorotic areas surrounding the mealybug feeding sites and sooty mould developing on honeydew. High infestations can also cause defoliation, fruit splitting and fruit drop. Besides, some secondary pests such as the Honeydew moth *Cryptoblabes gnidiella* (Millière) (Lepidoptera: Pyralidae), may develop on fruits infested by the Citrus mealybug.

Several species of indigenous natural enemies are reported, such as the predators *Sympherobius* spp. (Neuroptera: Hemerobiidae), *Scymnus* spp. (Coleoptera: Coccinellidae), *Dicrodiplosis* spp. (Diptera: Cecidomyiidae) and *Leucopis* spp. (Diptera: Chamaemyiidae), and the parasitoids *Anagyrus pseudococci* s.l. and *Leptomastidea abnormis* (Girault) (Hymenoptera: Encyrtidae). They all actively contribute to the control of the Citrus mealybug, although this is essentially due to the activity of the two encyrtids which can reach parasitization levels respectively of up to 60% *A. pseudococci* (Raciti, Barraco, & Conti, 2001) and 18–60% *L. abnormis* (Viggiani, 1974; Longo, 1985). The first species is already present in June, even though at very low densities, and it shows peaks of activity in August–September (Raciti et al., 2001).

The control of *P. citri* represents a very good example of application of IPM strategies. Satisfactory results have been achieved with inoculative releases of the endoparasitoid *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae). This species is native to Central America and was introduced in Italy for the first time in 1955 for the biological control of *P. citri* (Zinna, 1960). Later, it was re-introduced in almost all Italian citrus growing regions (Longo, Mazzeo, & Siscaro, 1994). However, in the climatic and ecologic conditions of North-Mediterranean citrus growing areas, *L. dactylopii* is not able to overwinter and therefore needs to be reared and periodically inoculated in the field through releases replicated every year (Katsoyannos, 1996; Longo et al., 1994). The releases must be preceded by the localization of infested trees, on which 20–30 adult parasitoids per tree will be inoculated around May–June. In this period the average temperature is higher than 15°C and the first young Citrus mealybug females start moving from the trunks to the fruits or the initial small colonies begin settling (Longo et al., 1994). The releases can be repeated, if necessary, in June–July up to a total of 2,000 specimens/ha (Raciti et al., 2001). However, the encyrtid has proved effective in controlling the Citrus mealybug, reaching parasitization levels in some cases higher than 90% (Mineo & Viggiani, 1976; Longo & Benfatto, 1982; Spicciarelli, Battaglia, & Tranfaglia, 1994; Fronteddu, Basoni, Canu, Fancello, & Nanni, 2000).

Other exotic species that were introduced to control the Citrus mealybug are the predators *Cryptolaemus montrouzieri* Mulsant and *Nephus reunioni* Fürsch (Coleoptera: Coccinellidae) and the parasitoid *Coccidoxenoides perminutus* Girault (Hymenoptera: Encyrtidae). *Cryptolaemus montrouzieri* was first introduced in Italy in 1908 and established in Italian citrus orchards but in case of unfavourable climatic conditions or irrational chemical treatments its populations can be strongly reduced and need to be reintegrated by means of inoculative releases. *Nephus reunioni* was instead introduced in Italy in 1985 but didn't manage to establish and its predatory activity was unsufficient to control *P. citri* populations (Longo & Benfatto, 1987). The parasitoid *C. perminutus* established, but occurs in the field at very low densities (Franco et al., 2004).

Pheromone traps may be used to monitor the pest population dynamics in the field but, given the inconsistent correlation between the number of captures and the levels of infestation on fruits (Franco et al., 2001; Franco, Gross, Silva, Dunkelblum, & Mendel, 2002), this technique should be joined by the direct observation of fruits (10 fruits on 10% of the trees) to verify the levels of infestation in relation to the economic threshold (5–10% of infested fruits).

However, the traps, if placed early in May, are a very useful tool to detect the beginning of the mealybug activity in order to plan eventual releases of natural enemies after evaluating the presence of overwintering females on the trunk and branches, identifying the infested trees to be used as release spots and evaluating the occurrence and the activity of ants. These may in fact protect honeydew producing pests from their natural enemies thus disrupting their control action (Delabie, 2001; Eubanks, Blackwell, Parrish, Delamar, & Hull-Sanders, 2002; Kaplan & Eubanks, 2002). The negative role played by ants in biological control programs suggests to manage their populations using one of the various available techniques, including cultural methods such as canopy pruning to avoid contact with weeds and soil tillage to disturb their nests, as well as chemical or mechanical methods using insecticide-treated baits, sticky or insecticide-treated bands placed around the trunk and ground, trunk or foliar treatments with insecticides (Tumminelli, Saraceno, & Conti, 1997; Benfatto, 1999; Franco et al., 2004). In Italian citrus orchards the most common ant species that are known to have interactions with honeydew-producing insects, thus potentially disrupting the control activity of their natural enemies, are *Lasius alienus* (Förster), *Tapinoma nigerrimum* (Nylander) and *Camponotus nylanderi* Emery (La Pergola, Alicata, & Longo, 2008). *Pheidole pallidula* Nylander is also quite abundant in citrus orchards and although it is a typical terricolous species, it has been seen foraging on colonies of sapsucking insects (La Pergola, 2008).

Chemical control of *P. citri* should be applied in case of high infestations, using IGRs, such as buprofezin, in summer-fall or, in the fall, organophosphates such as chlorpyrifos-methyl. Good results can also be obtained with mineral oils.

Present researches focus on the enhancement of the activity of native parasitoids (namely *A. pseudoccocci* s.l.) by using semiochemicals (Franco et al., 2008).

2.5. Ceratitis capitata (Wiedemann)

This species, commonly known as Mediterranean fruit fly, is widely distributed in Sub-Saharian Africa (from where it probably originated), in the Mediterranean basin (including France and the Balkans up to Hungary), in the Middle East, in Saudi Arabia, in Central and South America, in Western Australia and in Hawaii. In Germany it has been observed several times attacking fruit but it is not permanently established. In Mexico, Florida and California its occurrence has been faced through eradication programs, however hypothesis of permanent establishment have been formulated.

The adult is 3.5–5 mm long, yellowish with brown tinge, especially on abdomen and legs. Eyes are reddish-purple and ocellar bristles are present. Male has a pair of bristles with enlarged spatulate tips next to the inner margins of the eyes. Thorax is creamy white to yellow, with characteristic pattern of black blotches. Light areas with very fine white bristles are present as well as humeral bristles. Scutellum is enlarged and shiny black. Abdomen is oval with fine black bristles scattered on dorsal surface and 2 narrow transverse light bands on basal half. Ovipositor, when extended, is 1.2 mm long. Wings, usually held in a drooping position on live flies, are broad and hyaline with black, brown and brownish yellow markings. One longitudinal orange-yellowish band crosses middle of wing, two similar transversal bands are present on the caudal part of the wings.

The egg is shiny white, elongate, slightly curved, tapering and 1 mm long, with micropylar region distinctly tubercular. The mature larva is 7–9 mm long, yellowish-white, slender, elongate, tapering anteriorly. The pupa is reddish-brown, 4.5 mm long. Some strains have pupae of different colour in the two sexes.

The Mediterranean fruit fly is known to attack more than 260 different fruits, flowers, vegetables and nuts. Thin-skinned, ripe succulent fruits are preferred (apple, apricot, coffee, fig, Indian fig, loquat, peach, pear, persimmon, strawberries, etc.). Although it may be a major pest of citrus (Fig. 9), often it is a more serious pest of some deciduous fruits, such as peach, pear, and apple.

Using the oviscapt the female lays eggs in clusters of 1–15 (in relation to the size of the fruit), about 2–5 mm deep under the skin of fruits. Oviposition may take from 2 to 20 min. Although each female after oviposition normally labels the fruit with deterrent substances, several females may lay eggs in the same fruit and up to 80 eggs may be found. This might be related to the decreased deterring efficacy on large fruits. One single female may lay as many as 22 eggs/day and as many as 800 during her lifetime (usually about 300). The females are attracted by visual and olfactory cues and normally choose the same kind of fruit during each oviposition cycle. Females usually die soon after they stop laying eggs. When the eggs hatch, the larvae promptly begin feeding on the pulp of fruits and about 15 days at a mean temperature of 25°C are necessary to complete their development.

Figure 9. Ceratitis capitata *adult female on a citrus fruit.*

Larvae pass through 3 instars. In cooler regions the species usually overwinters as pupa (while in warm climates it develops without interruptions), buried a few centimetres deep in the soil. In southern areas, a small number of individuals may also survive on late-season oranges. The pupa does not survive if temperatures are lower than 2°C for a week.

Development of this fruit fly is mainly dependant on temperature. The optimum is around 32°C, which allows a generation to be completed in 2 weeks. Females will not oviposit when temperatures drop below 16°C, except when exposed to sunlight for several hours. Development stops at 10°C. Pupae carry the species through unfavourable conditions. During warm weather eggs hatch in 1.5–3 days. The duration of the egg stage is considerably increased by lower temperatures. Larval life may be as short as 6–10 days when the mean temperatures average 25-26.1°C. The kind and ripening conditions of the fruit often influence the length of the larval stage. In citrus fruits, especially limes and lemons, it appears to be longer. Thus, larvae require 14–26 days to reach maturity in a ripe lemon, as compared with 10–15 days in a green peach. Mature larvae, which are able to jump, leave the fruit in largest numbers at or just after daybreak and pupate in soil. Minimum duration of the pupal stage is 6–13 days when the mean temperature ranges from about 24.4 to 26.1°C.

The largest numbers of adults emerge early in the morning during warm weather and more sporadically during cool weather. They can actively fly short distances, although they can cover longer distances carried by the wind. Newly emerged adults are not sexually mature. Males often show sexual activity 4 days after emergence. They produce a sexual pheromone which attracts females but is also perceived by other males and even by human olfaction. Both sexes are sexually active throughout the day. When the daily mean temperature averages 24.4–25.6°C most females are ready to mate from 6 to 8 days after eclosion. Mating lasts from 2 to 4 h; males usually mate several times, while 60% of females mate only once.

The Mediterranean fruit fly is one most noxious fruit pests in the world. Because of its worldwide distribution, its ability to tolerate colder climates better than most other species of fruit flies, and its wide range of hosts, it is ranked first among economically important fruit fly species. In some of the Mediterranean countries, only the earlier varieties of citrus are grown, because the flies develop so rapidly that late season fruits are too heavily infested to be marketable. Harvesting before complete maturity also is practiced in Mediterranean areas generally infested with this fruit fly.

The damage caused by *C. capitata* is considerable, particularly in summer and autumn. Infestation is indicated on the fruit by a variously coloured area, depending on the host, surrounding the oviposition puncture. Rotting of the underlying tissue causes a depression on the surface and the fruit drops prematurely. Even if a fruit has only been pierced for egg laying (without actual presence of living instars), it is totally unsaleable. On citrus fruits the oviposition punctures cause acceleration in the ripening process of the external tissues and the subsequent early drop of the fruits, also because of the occurrence of secondary infestations and infections.

The constant global exchanges of goods and people may greatly facilitate transportation of the Mediterranean fruit fly in areas where it is not yet present, therefore making the efforts to contain it within its current distribution almost useless. Once the species is established, eradication efforts may be extremely difficult and expensive. In addition to reduction of crop yield, infested areas have the additional expense of control measures and costly sorting processes for both fresh and processed fruit and vegetables. Besides, the presence of *C. capitata* may strongly affect exportation to those countries where the species is not present or permanently established.

Several species of entomophagous have been reported in the entire area of distribution of this fruit fly. Some of them have been imported from Africa and

Australia. Effective parasitoids of *C. capitata* are *Psyttalia concolor* (Szlep.) (Hymenoptera: Braconidae) and *Pachyneuron vindemmiae* (Rond.) (Hymenoptera: Pteromalidae). Efforts have been made to develop biological control programs using *P. concolor*, but with very limited success. This is mainly due to the high intrinsic rate of increase of the Mediterranean fruit fly and also to the behaviour of larvae, which feed and develop inside the fruits and therefore easily escape parasitization by braconid wasps, especially when infesting large fruits. In the Mediterranean basin, natural control is mainly carried out by microorganisms and occasional predators (Coleoptera Carabidae and Staphylinidae, Hymenoptera Formicidae of the genus *Crematogaster*), mostly active in soil against larvae and pupae.

Cultural control methods, such as reducing the favourable host plants consociations, eliminating the infestation site and destroying infested fruits, are fundamental elements in field programs aiming at reducing the Mediterranean fruit fly populations.

Chromotropic and chemotropic traps are used to attract adults. Males of *C. capitata* are known to be attracted by essential oils from *Angelica archangelica* L., which contain several sesquiterpene hydrocarbons (α-copaene, α-ylangene, etc.). The same kind of molecules are also contained in coriander, mint, thyme and citrus essence. However, none of these compounds found practical application, differently from what happened for esters of the methylcyclohexanecarboxylic acid: siglure, medlure, trimedlure. Other attractants commonly used are also ammonium compounds (diammonium phosphate) and hydrolyzed proteins. These compounds attract both males and females (while parapheromones attract only males) within a range of 20 m, but they mainly attract females looking for proteins. However these attractants are less selective towards beneficials.

A possible control method is the “attract and kill” technique which is based on the use of devices pre-treated with a pyrethroid (mainly deltamethrin or lambda-cyhalothrin) and activated with trimedlure or with hydrolyzed proteins. This technique is suggested on orchards with a minimum extension of 5 ha, unless they are isolated enough.

Chemical control can be performed using deltamethrin, etofenprox, phosmet and spinosad bait which has been recently authorized on citrus in Italy. In order to contain the negative secondary effects on the biocenosis it is highly recommended to perform localized treatments adding protein baits to the toxic compound. The treatments should be started in mid-July and repeated every 25–30 days, spraying trees only partially on one every two–three rows, and using around 200 l of solution per hectare. The treatments should be extended to the whole orchard when 20 or more adults are captured on the traps and/or after the first punctures on the fruits are observed. The results of trials carried out to evaluate in the field the repellent and oviposition deterrent effect of clays and copper products, that can also be used in organic groves, showed that kaolin can be applied to reduce the percentange of infested fruits (showing *C. capitata* punctures) at harvest (Caleca, Lo Verde, Palumbo Piccionello, & Rizzo, 2008).

In several countries severe quarantine measures are applied, strongly affecting importation, in order to avoid the introduction and the permanent establishment of the Mediterranean fruit fly. Infested or potentially infested fruits are rejected or treated with fumigants or with low temperatures (2 weeks at 1°C), in order to eliminate all living instars present inside the fruits.

3. SECONDARY PESTS

Whiteflies, and namely the Woolly whitefly *A. floccosus*, are among the arthropod pests whose infestations in Italy greatly decreased over the last decades. Initially *A. floccosus* was considered of great economic importance because of the serious damage it can cause, mainly due to the very large amount of phloem sap sucked out from all parts of the tree both by the adults and the nymphs and the consequent production of huge amounts of honeydew. In case of strong infestations by this whitefly, blacken of citrus plants by sooty moulds is conspicuous and in such situations, honeydew, sooty mould and waxy secretions tend to mix up together, forming a continuous and hardly permeable cover on the lower surface of the leaves. Moreover, this whitefly has a strict relation with ants which protect the colonies interfering with the activity of natural enemies of this and also of other pests.

However, biological methods have proved effective in controlling this species. In particular after the arrival of *A. floccosus* in Italy (in the 1970s), the parasitoid *Cales noacki* Howard (Hymenoptera: Aphelinidae) was introduced. This species, native to South America, had already been imported in France in the 1970s (Onillon & Onillon, 1972) and was then introduced in Southern Italy in 1980 (Liotta & Maniglia, 1983; Longo, 1985). The beneficial insect was reared on its natural host, feeding on citrus trees both in insectaries and in the field. From these "bank plants" branches bearing parasitized whitefly colonies were collected and used to spread out the parasitoid in the field, directly by farmers. The parasitoid showed an intense activity reaching levels of parasitization higher than 80%, and managed to permanently establish also feeding on other hosts. It did not require further inoculations, except in orchards submitted to several chemical treatments (Longo et al., 1994). In the citrus growing areas where the climatic conditions are not unfavourable to the parasitoid (it is mainly affected by high summer temperatures) and where integrated pest management is performed with rational use of pesticides, the Woolly whitefly is almost always kept under adequate control by the aphelinid, without requiring further specific control treatments.

Together with *C. noacki*, another parasitoid, the platygastrid *Amitus spiniferus* (Bréthes) was introduced, reared and released following the same technique used for the aphelinid. Although this second entomophagous was at first only found in low numbers and immediately after the releases, it is now quite widespread in all citrus growing areas and contributes to the control of *A. floccosus* infestations. Chemical control is therefore normally not necessary against the Woolly whitefly, at least in areas where natural enemies are protected and their action is promoted.

Similarly, two other exotic whitefly species, *D. citri* and *P. myricae*, have been successfully contained in the framework of classical biological control programs. The first species, native to India, was first recorded in Italy in 1965 and initially caused very heavy infestations. Presently it has almost disappeared, thanks to the effective control performed by the parasitoid *Encarsia lahorensis* (Howard)

(Hymenoptera: Aphelinidae) introduced in Italy from California in 1973 (Viggiani & Mazzone, 1978). The entomophagous was distributed throughout the main citrus growing areas of Southern Italy using plants or parts of plants bearing Citrus whitefly parasitized colonies. It permanently established and managed to effectively control the populations of the pest which rapidly became a species of minor importance in Italian citrus orchards (Longo et al., 1994).

In 1990 the Japanese bayberry whitefly, *P. myricae*, was reported for the first time in Italy (Rapisarda, Siscaro, Leocata, & Asero, 1990). This polivoltine, polyphagous homopteran, with a strong tendency to parthenogenesis, is highly efficient in colonizing new areas. It mainly develops on new shoots and frequently lays eggs along the leaf margin which at maturity show a typical serrate aspect. Several parasitoids, such as the aphelinids *C. noacki* and *Encarsia meritoria* Gahan, adapted to develop on this whitefly without, however, successfully containing its populations. Therefore, in order to enlarge the parasitic complex, the aphelinid *Eretmocerus debachi* Rose & Rosen was imported from Israel in several citrus growing regions. The newly introduced entomophagous established, rapidly reached parasitization levels of 20–30% and showed a remarkable spreading capacity. Thanks to these characteristics the endoparasitoid managed to successfully control the whitefly as it had already happened in other Mediterranean citrus growing areas (Rose, De Bach, & Woolley, 1981). The Japanese bayberry whitefly is now only very rarely present in citrus orchards and no chemical treatment is required, because of the effective control performed by its natural enemies.

Finally, other pests, which are normally considered as secondary since they are kept under control by a rational agronomic management of the orchard, by a reduced use of pesticides and by an effective natural enemies complex, are: the soft scales *Ceroplastes rusci*, *Coccus hesperidum* L. and *Saissetia oleae*, the armoured scale *Parlatoria pergandii*, the moths *Prays citri* Mill. and *Archips rosanus*. Three species of aphids [*Aphis spiraecola*, *A. gossypii* and *Toxoptera aurantii* (B.d.F.)] are considered particularly noxious to young and regrafted trees. However, the direct damage caused by these pests is by far less important than the indirect damage related to their capacity of transmitting viral diseases and namely the Citrus Tristeza Virus. In particular *A. gossypii* is considered, in the Mediterranean basin as well as in other citrus growing regions of the world, the main vector of this virus in those areas where its most efficient specific aphid vector (*Toxoptera citricidus*) is still absent (Bar-Joseph & Loebenstein, 1973; Yokomi, Joost, & Backus, 2005).

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5

ENTOMOPATHOGENIC NEMATODE ECOLOGY AND BIOLOGICAL CONTROL IN FLORIDA CITRUS ORCHARDS

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Abstract. Biological control through augmentation of entomopathogenic nematodes (EPNs) in soil is an important component of integrated pest management (IPM) of the root weevil, *Diaprepes abbreviatus*, in Florida citrus orchards for over 20 years. However, to improve the effectiveness of EPNs for weevil control substantial information is needed about the post-application biology of EPNs as well as the ecology and importance of endemic species for weevil management. Current status of EPNs augmentation as a weevil control tactic, their role in soil food webs in different habitats, and the biocontrol potentials of endemic EPN communities, are reviewed. We also discuss molecular approaches to assess EPN population distribution and dynamics, and how these techniques could contribute to our understanding of nematodes ecology to enhance EPNs in biocontrol. Basic and applied study of EPNs increased during the past half-century, accelerating awareness of limitations for many conventional management practices. Understanding the EPNs population biology is necessary to discover and exploit new ways to increase their efficacy and reliability for biological control in managed ecosystems.

1. INTRODUCTION

Since the era of synthetic pesticide development accelerated in the mid-twentieth century, the management of citrus groves worldwide has generally evolved from a heavy reliance on prophylactic insecticide use to a more rational application of these

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chemicals within integrated pest management (IPM) programs. IPM typically reduces pesticide use by monitoring both pest and beneficial organisms, and predicting crop loss as the basis for management decisions (Timmer & Duncan, 1999). Nevertheless, the quantities of synthetic pesticides used in orchards remain significant and pose serious threats to the environment, especially natural biological control processes, wildlife, groundwater contamination, resource depletion, and human health and safety (Edwards, 1993; Pimentell et al., 1993). Recognition of these concerns has led to increased interest in organic methods for crop and pest management in citriculture; and biological control, whether conducted as classical, augmentation or conservation biological control, necessarily plays a pivotal role. When considering the costs and benefits of various management paradigms, it is noteworthy that increased chemical inputs do not necessarily result in increased output per unit area. In the Mediterranean Basin, Spanish and Italian citrus growers use 4-fold and 15-fold greater quantities of pesticides than do Greek growers, but obtain similar citrus production per hectare (Gutiérrez et al., 2005). Nevertheless, pest management is of frequent and critical concern in all agricultural endeavours, and the development of sustainable pest management systems requires a fundamental understanding of how populations of pests and their natural enemies behave in specific crop habitats (Hoy & Herzog, 1985; Flint & Dreistadt, 1998; Rechcigl & Rechcigl, 2000; Horowitz & Ishaaya, 2004).

In Florida citrus orchards, pesticide use during the last quarter of the twentieth century decreased steadily as a succession of pests were shown to be manageable through biological control by endemic or introduced natural enemies. Growers learned that fruit destined for juice processing could usually be produced without the use of insecticides and that fruit grown for the fresh market often required no more than a few well-timed pesticide treatments to manage mites and fungi that cause rind blemishes. Copper sprays and petroleum oils were the primary pesticides used during this period, and serious outbreaks of scale and other soft body pests were rare and almost always associated with disruption of biological control through unnecessary use of pesticides.

Unfortunately, the limited use of pesticides and widespread success of biological control in Florida citrus groves came to an abrupt halt when the devastating bacterial disease known as “Huanglongbing” or “citrus greening” caused by the bacterium *Candidatus Liberibacter asiaticus*, was detected in the state for the first time in 2005. Citrus greening is vectored by the Asian Citrus Psyllid, *Diaphorina citri*, infects all known citrus cultivars, spreads rapidly, and is lethal to trees. Growers attempt to slow the spread of the disease by removing infected trees and suppressing the vector with frequent applications of systemic and topical insecticides. Consequently and predictably, many secondary pests are increasing in Florida citrus orchards, and psyllid resistance to most classes of insecticides is becoming widespread. Moreover, the increased production costs and diminishing effectiveness of these intensive pest management practices are coinciding with lower citrus prices, largely due to the increased availability of other fruit juices, to significantly lower the profitability of Florida citriculture. In view of this crisis, the future of the Florida citrus industry is uncertain. However, fundamental research to understand and control citrus greening is being heavily supported by federal, state and private sources, and hopefully will lead to new and more effective strategies to deal with this problem.

Prior to the introduction of citrus greening and its psyllid vector into Florida, the Diaprepes Root Weevil, *Diaprepes abbreviatus*, was considered the most important insect pest of citrus, and it remains a serious problem (Graham, McCoy, & Rogers, 1996; Graham, Bright, & McCoy, 2003; Duncan, Shapiro, McCoy, & Graham, 1999; McCoy, 1999). Like citrus greening, the weevil can kill trees and cause entire orchards to become non-profitable in just a few years (Fig. 1) but, fortunately unlike greening, the most severe devastation appears limited to certain areas and grove conditions. For nearly 20 years, biological control through the augmentation of entomopathogenic nematodes (EPNs) to citrus soils has been an important component of weevil IPM in Florida and it continues to be an effective strategy in many groves. Moreover, recent research to improve the effectiveness of EPNs for weevil control has revealed substantial information about their post-application biology as well as the ecology and potential importance of endemic EPN species for weevil management. In this chapter, we review the current status of EPN augmentation as a weevil control tactic in Florida citrus groves, the role of EPNs in soil food webs of different habitats in Florida, and possibilities for better exploiting the biocontrol potential of endemic EPN communities.

Figure 1. Twenty-four-year-old citrus trees in flatwoods soil exhibiting typical symptoms of Diaprepes/Phytophthora *pest-disease complex.*

2. IPM OF THE *DIAPREPES-PHYTOPHTHORA* COMPLEX

2.1. Diaprepes *Economic Importance, Biology and Management*

Diaprepes abbreviatus is highly polyphagous and a major economic pest not only of citrus, but of numerous ornamentals, vegetables, sugarcane, and other crops. It is native to the Caribbean where it has long been considered a serious agricultural pest (Wolcott, 1936). It was first detected in Florida in 1964 (Woodruff, 1964) and has spread to all citrus producing areas of the state. In 1997, the weevil was known to infest over 13,000 ha of commercial citrus and 50, 000 ha of other crops in 20 Florida counties (Lapointe, Shapiro, & Bowman, 1999). Over the past 40 years, many citrus growers have experienced devastating losses of trees and entire groves due to *D. abbreviatus*. Annual losses and cost of control for the Diaprepes root weevil in Florida citrus were estimated at $72 million, whereas losses in ornamentals and vegetables were estimated at $2 million (Pena & Amalin, 2000). *Diaprepes abbreviatus* recently became established in Texas and California where it is considered a major threat to agriculture and is the subject of quarantine and eradication programs.

The life cycle of this weevil is marked by lack of synchrony and extensive variability, characteristics that make it particularly difficult to control (McCoy, 1999). In Florida, adult *D. abbreviatus* emerge from soil throughout the year with a significant peak in spring and sometimes autumn (Stansly, Mizell, & McCoy, 1997; Duncan, McCoy, Stansly, Graham, & Mizell, 2001). The adults feed on new foliage, mating occurs in the canopy, and eggs are laid in masses glued between leaves by the ovipositing female. Individual females can produce more than 20,000 eggs during a lifetime that can last almost a year. The larvae hatch, drop to the soil, and burrow down to the roots where they begin feeding. As they grow, the larvae feed on larger roots, and pupation occurs in the soil after 9–11 larval instars. Typically, a broad range of instars occur in the soil simultaneously (Woodruff, 1985; Quintela, Fan, & McCoy, 1998; McCoy, 1999; McCoy, Stuart, & Nigg, 2003; Nigg et al., 2003).

Diaprepes abbreviatus feeding damage to the roots of citrus trees can be extensive (Fig. 2). Major structural roots are often girdled and killed; and, if the crown is girdled, then the tree dies. However, tree damage is greatest in the presence of the plant pathogenic oomycetes *Phytophthora nicotianae* or *P. palmivora*, which invade the roots at weevil feeding sites. The pest-disease complex is particularly severe in poorly drained soils that favor infection by the fungal zoospores (Graham et al., 1996; Duncan et al., 1999; McCoy, 1999).

Control measures for *D. abbreviatus* include chemical insecticides, which can be applied as adulticides, egg sterilants, or soil barrier treatments for neonates (McCoy et al., 2009). The efficacy of all treatments is short-lived; and, therefore, in order to kill the maximum number of insects, growers are advised to monitor adult weevils as they emerge from soil and occupy the tree canopy. Pesticides should be applied at the onset of egg-laying, approximately 10 days following peak adult emergence. Nevertheless, because of the non-synchronous life cycle of this weevil, pesticides must be applied repeatedly to be effective, and such repeated applications can disrupt natural enemies and lead to additional pest problems. Moreover, the present chemical controls are only marginally effective compared to the organochlorine soil pesticides that were used previously and are now banned for environmental reasons (Duncan et al., 1999; McCoy, 1999).

At present, the only recommended control for *D. abbreviatus* larvae in soil is the application of EPNs twice per year (Bullock, Pelosi, & Kidler, 1999; Duncan & McCoy, 1996; Duncan, McCoy, & Terranova, 1996, Duncan et al., 2007; McCoy, 1999; McCoy, Shapiro, Duncan, & Nguyen, 2000). In the absence of pesticides, EPN augmentation twice annually in orchards on coarse sandy soil was shown to reduce adult weevil populations by more than half (Duncan et al., 2003, 2007).

Figure 2. Excavated root system of 3-year-old citrus tree heavily damaged by Diaprepes abbreviatus *larval feeding. Note the deep channels in major roots which provide infection courts for* Phytophthora *spp.*

2.2. EPN Biology and Use in IPM Programs

Entomopathogenic nematodes in the genera *Steinernema* and *Heterorhabditis* are obligate lethal parasites of insects and are an attractive alternative to chemical insecticides because they kill insect pests very quickly, are safe to mammals, and have little or no direct effect on nontarget organisms (Bathon, 1996; Georgis, Kaya, & Gaugler, 1991). These nematodes typically occur in the soil and have an environmentally-resistant non-feeding infective juvenile (IJ) stage that actively seeks out and kills insect hosts (Kaya, 1990; Lewis, Gaugler, & Kaya, 1992; Grewal & Georgis, 1998). The nematodes kill their hosts with the aid of symbiotic entomopathogenic bacteria in the genera *Photorhabdus* and *Xenorhabdus* for

Heterorhabditis and *Steinernema*, respectively. The bacteria are carried in the nematode intestine and released into the host upon infection (Poinar, 1990). Insect death usually occurs within 72 h of infection, and the nematodes feed, develop to adults, and reproduce within the host, often completing 2–3 generations, before producing a new generation of specialized IJs, which emerge from the cadaver and seek new insect hosts (Poinar, 1990). Because of their established safety to non-target organisms, EPNs are exempt from pesticide registration with the Environmental Protection Agency in the United States and are similarly exempt in many other countries.

EPNs have been used effectively in classical biological control programs. For example, *Steinernema scapterisci* was discovered in Uruguay, near the presumed center of origin of the mole cricket (*Scapteriscus* spp.). The nematode was introduced into Florida where it is now established and, in combination with introduced parasitoids, provides effective biological control of several invasive mole cricket species (Nguyen & Smart, 1990; Adjei, Smart, Frank, & Leppla, 2006). Most commonly, however, EPNs are used for augmentation biological control where they function as biopesticides and treatment efficacy is expected to be of short duration, typically a matter of 1–2 weeks (McCoy et al., 2000; Duncan et al., 2003, 2007). In such programs, EPNs function in the same manner as non-persistent chemical pesticides and are applied repeatedly, as needed.

Surveys have shown that some naturally occurring EPNs are more abundant in undisturbed compared to intensively farmed habitats (Campos-Herrera et al., 2008), and that use of various animal manure mulches can increase the prevalence of some EPNs (Bednarek & Gaugler, 1997; Duncan et al., 2007). Such observations suggest the possibility of developing conservation biological control tactics by which agricultural or other managed habitats are modified in ways that enhance natural control of arthropods by EPNs. However, reliable and effective conservation biological control tactics are as yet unknown and remain a matter of speculation (Lewis, Campbell, & Gaugler, 1998; Stuart, Barbercheck, Grewal, Taylor, & Hoy, 2006).

In the Florida citrus industry, *Steinernema carpocapsae* was the first nematode to be developed commercially for root weevil control (Schroeder, 1987; Figueroa & Roman, 1990; Smith, 1994) but a further discovered species, *S. riobrave* (formerly *S. riobravis*) (Cabanillas, Poinar, & Raulston, 1994), was found to cause greater *D. abbreviatus* mortality (Schroeder, 1994; Duncan et al., 1996; Bullock et al., 1999) with some field studies reporting ~90% suppression (Duncan & McCoy, 1996; Duncan et al., 1996; Bullock et al., 1999). However, estimates of the efficacy and profitability of using EPNs for weevil control in citrus vary widely and probably reflect variation in factors such as product quality, application rates, suitability of edaphic conditions for EPNs, and experimental methods (Adair, 1994; Duncan et al., 1996; Duncan, Graham, & Zellers, 2002; Duncan et al., 2003; 2007; Bullock et al., 1999; Stansly et al., 1997; McCoy et al., 2000; McCoy, Stuart, Duncan, & Nguyen, 2002). Currently, *S. riobrave* is marketed in Florida under the brand name Bio Vector 355 (Becker Underwood Inc., Ames, IA) and, in 1999, approximately 19,000 ha of citrus were treated with this product to control citrus root weevils (Dimock, personal communication).

Despite the evident and long-recognized potential of EPNs for insect pest management and the commercial development of effective EPN products for augmentation, their market penetration and incorporation into broadly applied IPM programs has been disappointing. In some crop-pest systems for which EPNs appeared promising, use of chemical pesticides or transformation of crop cultivars to express *Bacillus thuringiensis* toxins provided cheaper management options (Lewis et al., 1998; Shapiro-Ilan, Gouge, & Koppenhöfer, 2002). However, the greatest impediment to the use of EPNs is that they often appear less reliable and less effective than available chemical pesticides (Lewis et al., 1998). Extreme variability of soil physical and biological properties, even at a small scale, is probably one of the major causes of inconsistent performance of EPNs and most other soilborne biological control agents. Indeed, it would be remarkable if a single organism was able to provide consistently high pest control in the myriad soil habitats encountered in most crops. Moreover, although EPNs can be isolated from most soil habitats, EPN distributions are typically patchy and their equilibrium densities low, and these patterns tend to be quickly reestablished following an augmentation event (McCoy et al., 2000; Duncan et al., 2007). Habitats that support EPN equilibrium densities high enough and uniform enough to be suppressive to arthropod pests are apparently rare but, when identified, could reveal important traits and environmental conditions amenable to a conservation biocontrol approach involving EPNs. Advantages of such an approach could include an increase in the duration of EPN efficacy and, consequently, a reduction or elimination of the need for periodic augmentation of commercially formulated EPNs or use of chemical pesticides.

2.3. Spatial Relationships Between Soils, Root Weevils and Endemic EPNs

Damage to citrus caused by the *Diaprepes* root weevil is related to regional and local variation in orchard soils. Trees growing in finer textured soils or in low, wet areas are often more heavily damaged than trees growing in well drained, coarser textured soils. This undoubtedly is due at least in part to a higher incidence of *Phytophthora* spp. in finer, wetter soils. However, soil conditions also appear to have a large effect on weevil abundance. A 3-year survey of six orchards in which weevil abundance was measured weekly revealed a 10-fold greater weevil abundance in finer textured compared to coarser textured soils (Futch, Duncan, & Zekri, 2005). Florida citrus orchards are planted in regions characterized by different soil profiles. The “central ridge” is composed of very deep, well drained, uniformly sandy (>96% sand) soils. In contrast, the coastal and inland “flatwoods” regions have greater variation in soil texture and shallow water tables that require tree rows to be planted on raised beds for drainage and adequate rooting volume. Many flatwoods soils are sandy (80–95% sand), but the particle sizes of the sand fractions are smaller on average than those that characterize soils on the central ridge. Thus, weevil-infested orchards on the central ridge often exhibit little damage by *D. abbreviatus*, whereas orchards on fine-textured, poorly drained flatwoods soils are sometimes abandoned as unprofitable, due to an inability to manage the large weevil populations.

Relationships between spatial patterns of *D. abbreviatus* and specific abiotic soil factors have been reported within and among sites (Li et al., 2003; Li, Syvertsen, McCoy, Stuart, & Schumann, 2004a; Li et al., 2007), but experimental evidence of direct causal relationships is lacking (Li et al., 2004b). Nonetheless, a growing body of evidence suggests that soils influence *D. abbreviatus* populations indirectly by affecting the community composition and predatory efficacy of endemic EPN species. Surveys that measured numbers of *Diaprepes* larvae falling from the canopy to the soil and numbers of adult weevils emerging from the soil over the course of 2 years concluded that the net survival rate from hatched egg to teneral adult is ~ 0.7–1.6% (McCoy et al., 2003).

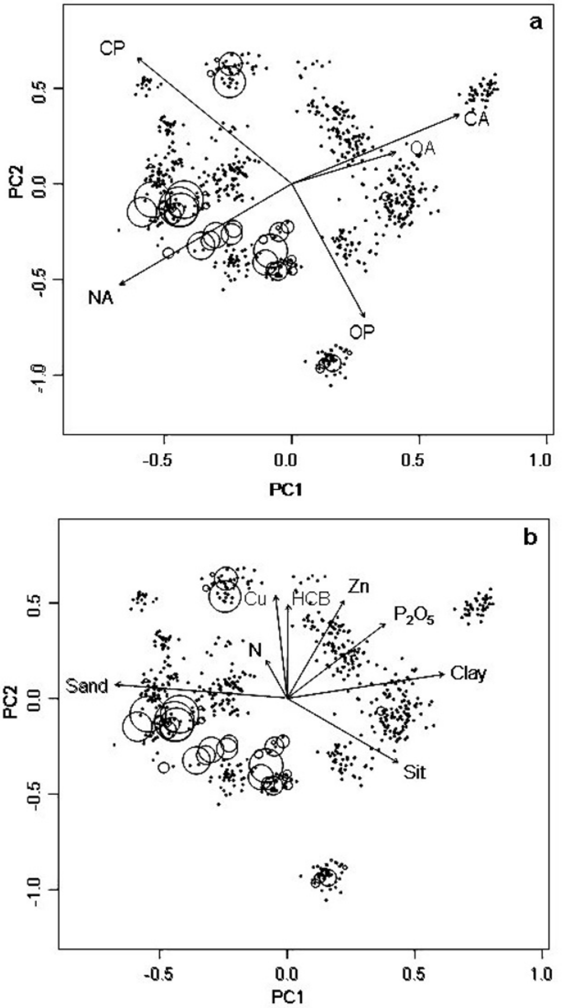


Figure 3. Representation of EPN population density distribution using a biplot in which vectors show the loading factors of the original variables on the associated factorial axes. (**a**) *Association with cultural practices: NA, natural area, OPC, organic perennial crop, CPC, conventional perennial crop, OAC, organic annual crop, CAC, conventional annual crop.* (**b**) *Association with sand, silt and clay contents, available water, and organic N, Zn and Cu concentrations.* Circles *show EPN population density by increasing the diameter as population size increases* (from Campos-Herrera et al., 2008).

Therefore, if EPNs are important weevil larva predators, relatively small reductions in the rate at which EPNs prey on weevils could have a disproportionately large effect on increasing the weevil survival rate and, hence, the numbers of egg-laying adults in an orchard. Duncan et al. (2003, 2007) found that caged weevil larvae buried in orchards on the central ridge are killed, primarily by endemic EPNs, at an average rate of 53% per week (range 38–82%) compared to <10% per week in some flatwoods orchards with finer soil texture. Numerous field surveys and controlled experiments report direct relationships between sand content or soil porosity and EPN prevalence, movement, or infectivity (Kung, Guagler, & Kaya, 1990; Hara, Guagler, Kaya, & Le-Beck, 1991; Zhang, Yang, Zhang, & Jian, 1992; Rueda, Osawaru, Georgi, & Harrison, 1993; Liu & Berry, 1995; Portillo-Aguilar, Villani, Tauber, Tauber, Nyrop, 1999; Hazir, Keskin, Stock, Kaya, & Özcan, 2003; Campos-Herrera et al., 2008) as well as negative effects related to high clay content of soils (Kung et al., 1990; Barbercheck, 1992; Portillo-Aguilar et al., 1999; Mráček, Bečvář, Kindlmann, & Jersákova, 2005; Koppenhöfer & Fuzy, 2006). For example, Campos-Herrera and Gutiérrez (2009) found LC_{90} values of 220, 753 and 4,178 IJs/cm^2 of soil surface for *S. feltiae* on *Spodoptera littoralis* in loamy sand, sandy loam, and sandy clay loam soils, respectively, from Spain. Similarly, in samples positive for EPNs, the abundance of EPNs in a survey of La Rioja was highly positively correlated with the percentage sand fraction of the soil (Campos-Herrera et al., 2008; Fig. 3).

In addition to soil effects on EPN efficacy, EPN species vary in efficacy against the *Diaprepes* root weevil. The likelihood that a *Diaprepes* larva is preyed upon by EPNs depends not only on a soil's conduciveness for host-finding and penetration by EPNs, but also on the relative abilities of the particular EPN species resident in a particular soil to prey on *D. abbreviatus*. Laboratory and greenhouse studies report consistently that *S. diaprepesi* or *S. riobrave* kill *D. abbreviatus* and protect citrus seedlings better than equivalent numbers of *H. indica* or *H. zealandica* (Shapiro & McCoy, 2000a, 2000b; Shapiro-Ilan, Duncan, Lacey, & Han, 2005; El-Borai, Brentu, & Duncan, 2007) and that the ability of all of these nematodes to reduce weevil damage to citrus seedlings decreases markedly as soil porosity decreases in a gradient from coarse sands to fine sands to sandy loam soils (El-Borai et al., 2009). An ongoing survey of EPN species diversity and abundance employs real-time PCR to identify and quantify all of the known EPNs endemic in Florida and the introduced species, *S. riobrave* (Table 1).

To date, the survey has revealed no significant differences in average EPN numbers in orchards in the different regions. However the findings support previous observations that endemic species of EPNs have unique spatial patterns related to regional differences in soils (Stuart, El-Borai, & Duncan, 2008). *Heterorhabditis indica* appears generally to be the dominant species across all regions of the state. In orchards on the central ridge, *Steinernema diaprepesi* is virtually ubiquitous and often dominant, but is encountered less frequently in fine sandy soils of the central flatwoods. An unidentified *Steinernema* sp., closely related to *S. glaseri,* is known only from the central flatwoods where it predominates in some orchards. Although *H. zealandica* has been detected in all three regions, it is detected most frequently on the central ridge. The average numbers of species encountered in orchards tend to be highest on the central ridge (2.4), with significantly fewer (1.7) in the central flatwoods. To date, *H. indica* is the only species that has been encountered in significant numbers in the coastal flatwoods. A propensity for *H. indica* to occupy coastal habitats also has been noted in other parts of the Caribbean Basin (Fisher-Le Saux, Mauléon, Constan, Brunel, & Boemare, 1998; Mauléon, Denon, Briand, 2006).

Little is known about the direct effects of soil physical and chemical properties on the population biology of the *Diaprepes* root weevil. However, the various findings reviewed above provide evidence for the hypothesis that patterns of *D. abbreviatus* within and among Florida citrus orchards are partly regulated by the ways in which soil properties affect the natural enemies of *D. abbreviatus*. More explicitly, they suggest that EPNs might help regulate *D. abbreviatus* on the central ridge to the extent that it is often a minor pest, whereas reduced EPN predation in some flatwoods orchards may permit *D. abbreviatus* populations to attain highly damaging levels. If the natural control of *D. abbreviatus* by endemic EPNs varies in different regions of Florida, then understanding how these habitats influence EPN diversity and efficacy could be especially worthwhile by indicating strategies for conservation biological control through the manipulation of habitats to enhance the biocontrol potential of EPNs.

Table 1. The frequency (% of samples) and average dominance (population density as a percentage of all EPN in the sample)[a] of five entomopathogenic nematodes in citrus orchards, in three regions of Florida[b].

Species	*Ridge*		*Central Flatwoods*		*Coastal Flatwoods*	
	F	*D*	*F*	*D*	*F*	*D*
Steinernema diaprepesi	100	36	57	29	0	0
S. riobrave	0	0	5	<1	0	0
Steinernema sp.	0	0	37	8	0	0
Heterorhabditis indica	90	51	84	61	100	99.8
H. zealandica	50	12	5	<1	20	<1

[a] F = frequency; D = dominance.

[b] Data from thirty-two orchards sampled (two samples of 30 soil cores each in a 1 ha area). EPN extracted from 500 cm^3 subsamples from each sample, identified and quantified by real-time PCR.

3. FROM AUGMENTING TO CONSERVING EPNs

To achieve conservation biological control involving EPN, it is necessary to identify environmental states that favor these nematodes. Just as soil may indirectly affect weevil populations through its effects on soil food web components such as EPNs, the resources, competitors and natural enemies of EPNs are also likely to vary in habitats with different physical characteristics. Whereas the direct effects of key soil properties such as water potential, porosity, and temperature on EPNs have been studied in some detail, relatively little attention has been given to understanding the relative importance of biological interactions and how they may vary in different habitats (Kaya & Koppenhöfer, 1996). The profound complexity and variety of soil food webs makes it unlikely that key interactions between EPNs and other organisms can be identified through highly controlled experimentation. However, molecular methods that can be used to identify and quantify organisms at different trophic levels in soil provide cost-effective opportunities to survey natural soil communities in space and time in order to detect relationships that can then be studied for causality under controlled conditions.

3.1. Soil Food Webs and EPN Spatial and Temporal Patterns

Augmentation of EPNs in soil increases populations above an equilibrium density in order to increase the natural level of biological control. However, natural enemies of EPNs respond rapidly to the imbalance, so that EPN numbers and levels of biological control rarely exceed background levels for more than a few weeks (McCoy et al., 2000; Duncan et al., 2003, 2007; El-Borai et al., 2007). Populations of nematophagous mites and collembola have been shown to grow in response to EPN augmentation (Ishibashi, Young, Nakashima, Abiru, & Haraguchi, 1987; Epsky, Walter, & Capinera, 1988; Forschler & Gardner, 1991), and survival of augmented EPNs was inversely related to the final abundance of mites and collembola in field plots (Wilson & Gaugler, 2004). Jaffee and Strong (2005) showed that propagules of some species of nematophagous fungi (NF) increase by 2 orders of magnitude in a localized response to the emergence of thousands of EPN IJs from an insect cadaver. Indeed, the bottom-up trophic cascade that results in more predators following EPN augmentation can apparently be large enough to sometimes reduce EPN populations below background levels for a short period until equilibrium is reestablished (Duncan et al., 2003, 2007). El-Borai et al. (2007) demonstrated that trophic cascades are modulated by the species and amount of EPNs added to the soil.

Duncan et al. (2007) showed that treatments that either increased or decreased the population densities of various NF in the field also affected EPNs. Composted animal manure mulch reduced the abundance of trapping NF while increasing the numbers of sentinel insect larvae infected by EPNs. As noted above, augmenting the EPN community temporarily increased trapping and endoparasitic NF, and population growth of NF was sometimes followed by decreased sentinel infection by EPNs compared to non-augmented plots. The effects of these treatments suggest that NF population dynamics may affect the temporal and spatial patterns of EPN activity. However, the large variety of NF and EPN species, each with different life strategies, means that interactions between NF and EPNs are likely to be very complex. For example, during eight consecutive monthly sampling events, the prevalence of trapping NF in citrus field plots was inversely related to numbers of sentinel weevils infected by *S. diaprepesi* but positively related to those infected by *H. zealandica* in the succeeding month (Duncan et al., 2007). They proposed that these fungi might favor *H. zealandica* by more effectively suppressing its *S. diaprepesi* competitors. Timper and Kaya (1989) demonstrated that the NF *Hirsutella rhossiliensis* killed steinernematids much more effectively than heterorhabditids, and showed that the infection peg of fungal spores did not penetrate beyond the second stage cuticle that is retained as a protective sheath by IJ (3rd stage) heterorhabditids. Steinernematid IJs generally cast the second stage cuticle shortly after emerging into the soil from insect cadavers (Timper & Kaya, 1989, 1992).

A variety of mechanisms other than protective sheaths modulate predation by NF on nematodes. For example, fungi that form adhesive networks trap plant-parasitic root-knot nematodes much more effectively than cyst nematodes (neither of which retain a cast cuticle), whereas fungi producing constricting rings trapped both types of nematodes with equal efficiency (Jaffee, 1998). El-Borai et al. (2007) showed that in sand microcosms the predation rates of five species of endoparasitic and trapping NF on 5 EPN species, all commonly isolated from citrus orchards, were highly species specific. Two endoparasitic NF species that infect via zoospores were highly lethal to all EPN species except *H. indica*, which was unaffected by these fungi in the microcosm assays. Conversely, large endemic steinernematids such as *S. diaprepesi* and *Steinernema* sp. (previously identified as *S. glaseri*) were relatively unaffected by several species of *Arthrobotrys* (trapping NF), compared to significant predation by these fungi on the smaller exotic *S. riobrave* and the heterorhabditids *H. zealandica* and *H. indica*. Thus, the report by El-Borai et al. (2007) did not support causality of the relationships between NF and *S. diaprepesi* or *H. zealandica* reported by Duncan et al. (2007). The lack of predation by zoosporous NF on just *H. indica* is intriguing, however, since these commonly encountered NF require free water for zoospore movement and *H. indica* was the sole EPN species reported from several low lying coastal areas, which tend to have relatively wet soils (Fisher-Le Saux et al., 1998; Mauléon et al., 2006; Table 1).

Organisms other than predators and parasites might influence the abundance of EPNs. *Paenibacillus* is a bacterial genus that is intimately associated with arthropods. Phoretic associations between *Paenibacillus* and EPNs were recently reported and demonstrate remarkable convergence of bacterial species adapting to two paraphyletic nematode genera (Enright & Griffin, 2004, 2005; El-Borai, Duncan, & Preston, 2005). *Paenibacillus* spp. associated with heterorhabditids have spindle shaped spores that adhere exclusively to the nematode sheath (2nd-stage cuticle) whereas those associated with steinernematids have oval spores that adhere only to the 3rd-stage cuticle (Fig. 4). *Paenibacillus nematophilus* spores attached to all tested heterorhabditid species and those in the closely-related order Stongylida (Enright & Griffin, 2004). In contrast, a *Paenibacillus* sp. associated with *S. diaprepesi* appears to be species specific (El-Borai et al., 2005). *Paenibacillus* spp. are frequently observed on all known endemic species of EPNs in Florida but not on the introduced species *S. riobrave*. Like the entomopathogenic bacteria *P. popilliae* and *P. lentimorbus*, the *Paenibacillus* species that are phoretic on EPN complete their life cycle in the insect cadaver but they are not entomopathogenic and they do not appear to affect the reproduction of EPN species. The only known adverse affect to the nematode is impaired motility in proportion to the degree to which a nematode is encumbered with spores. Spore-free steinernematids and heterorhabditids move further and infect more insects that do spore-encumbered nematodes. However, the degree to which *Paenibacillus* spp. can modulate EPN abundance in nature is unknown.

In addition, some opportunistic, free living nematodes are capable of entering insect cadavers and appropriating the nutients produced by the EPN-bacteria symbiosis to the extent that the EPNs fail to reproduce (Duncan et al., 2003; 2007). Some species of EPNs appear to be especially susceptible to this competitive displacement by free living species but, again, the significance of these interactions on EPN population dynamics is unknown. Clearly, much remains to be done to understand how food web components interact to affect EPN patterns in space and time.

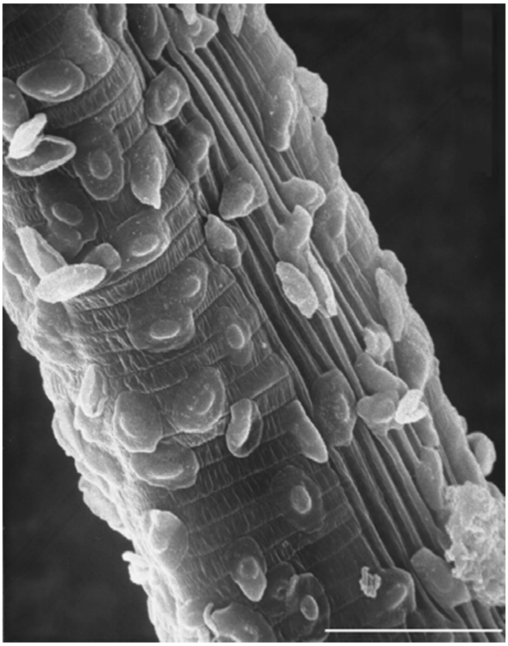


Figure 4. Cuticle of Steinernema diaprepesi *heavily encumbered by spores of* Paenibacillus *sp., a non-pathogenic bacterium that reproduces within insects killed by the nematode and its entomopathogenic symbiont,* Xenorhabdus doucetiae. *Movement through soil by infective juvenile nematodes is impeded by heavy infestations of* Paenibacillus *sp. Scale bar: 10 µm.*

3.2. Identifying Factors that Regulate EPN Abundance and Efficacy

As noted previously, spatial surveys have revealed numerous relationships between EPN prevalence and various soil physical properties such as porosity (texture), water potential, chemistry, as well as cropping and management history. If causality can be demonstrated then some of these relationships have the potential to be exploited to develop cultural practices that enhance biological control. For example, some types of soil mulches have significantly enhanced populations of EPNs (Bednarek & Gaugler, 1997; Duncan et al., 2007) whereas others appear to have no or negative effects on EPNs (Lacey, Arthurs, Unruh, Headrick, & Fritts, 2006). Bednarek and Gaugler

(1997) noted the apparent usefulness of composted animal manure for conservation biocontrol after showing that long-term applications of manure increased EPNs by 3-fold. Animal manure mulches likely increase availability of insect prey and were also shown to decrease the prevalence of some nematophagous fungi that prey on EPNs (Jaffee, Ferris, Stapleton, Norton, & Muldoon, 1994; Duncan et al., 2007). If a number of physical properties can be identified that consistently affect EPN spatial patterns then this information might be used to incrementally improve biological control achieved by either augmented or endemic EPNs.

Understanding the basis of relationships between EPNs and other variables in the field can require a substantial research investment. Whereas EPN associations with some variables have a causative basis, others arise indirectly through the effects of unmeasured hidden variables. Therefore, identifying those variables that are most likely to modulate EPN prevalence or behavior in predictable ways is important. A comparison of the results of surveys using different sample criteria can be helpful in selecting variables of interest.

Campos-Herrera et al. (2007) found that soil moisture was positively associated with EPN recovery frequency in a survey of 100 undisturbed sites (natural areas and field borders) in La Rioja, Spain. However, in a temporal survey of 18 agricultural fields with different management regimes and bordering natural areas, EPNs were unrelated to soil moisture but inversely related to soil disturbance (Campos-Herrera et al., 2008). Because greater irrigation was employed in tilled annual cropping systems with few EPNs than in natural areas or organically grown perennial crops with numerous EPNs, Campos Herrera et al. (2008) speculated that soil disturbance is more important than moisture in regulating EPN spatial patterns. Similarly, Campos-Herrera et al. (2008) reported linear correlations between EPNs and certain soil characteristics that were also studied by Alumai, Grewal, Hoy, and Willoughby, (2006) and Duncan et al. (unpublished). Differences in the survey protocols among these studies suggest the possibility that some of the correlations between soil properties and EPNs are due to the influence of soil texture on both EPNs and certain chemical properties (Fig. 5). Because greater sand content of soil generally favors EPNs, chemical properties associated in some manner with sand content would likely be similarly associated with EPNs. The relationships measured in Ohio and Florida (shown in small font in Fig. 5) are strikingly similar. Organic matter, K^+ and Mg^{++} tended to be negatively associated with both EPNs and percentage sand whereas P was positively associated with both variables. In contrast, these relationships had exactly opposite trends in the Spanish survey. Nevertheless, soil chemicals in each of the three surveys were associated with EPNs in the same manner that they were associated with percentage sand. The Ohio and Florida surveys occurred on several golf courses and within a single citrus orchard, respectively. Thus, cultural practices were similar among sites within each of these surveys, and the effects of porosity and soil colloid surfaces on leaching of some chemicals and the adsorption of others operated on similar levels of nutrient inputs. However, the Spanish survey compared EPN populations in natural areas and in a variety of different annual and perennial cropping systems. The very different levels of inputs between the Spanish sample sites might have caused different relationships between chemicals and percentage sand and, therefore, with EPNs than seen in Ohio or Florida.

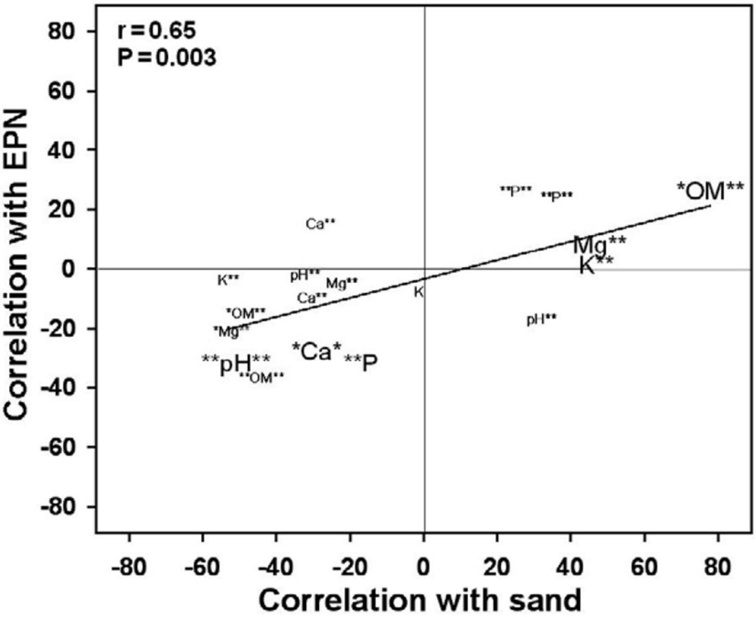


Figure 5. Linear correlations relating soil chemical properties to either the numbers of entomopathogenic nematodes or percentage of sand, in samples taken to study EPN spatial patterns. Symbols *in large font proceed from a survey of different cropping systems in La Rioja, Spain; remaining* symbols *derived from golf courses in Ohio and a citrus orchard in Florida.* Asterisks *on the* left side *of symbols show correlation significance (*, P<0.05; **, P<0.01) with EPNs; those on the* right side *show correlation with percentage of sand* (Ohio data courtesy of Alfred Alumai).

Determining whether specific natural enemies such as NF can significantly regulate EPNs is especially challenging because many NF are saprophytes that are only facultative predators of nematodes and, as noted previously, the predation rate by a given NF species varies for different nematode species (Timper & Kaya, 1989; Jaffee, 1998; El-Borai et al., 2009). Jaffee (2003) found no correlation between predation rate on nematodes and abundance in soil of two NF species that produce traps consisting of adhesive networks whereas abundance and predation rate were weakly correlated for a species producing constricting rings and more highly correlated for two species that produce adhesive knobs. As the fungal dietary requirement for nematodes increases so does the likelihood that the soil population density of these fungi reflects the degree to which nematodes are preyed upon. Species that produce adhesive networks such as *Arthrobotrys oligospora* often behave as saprophytes more than as predators of nematodes whereas *Dactylellina haptotyla* (adhesive knobs) or *D. dactyloides* (constricting rings) appear to rely to a much greater extent on nematodes for their nutrition (Jaffee, 2004). Zoosporoous endoparasitic fungi are obligate parasites of nematodes and some other microscopic metazoans (Kerry & Jaffee, 1997).

Because the predation rate on EPNs cannot be reliably inferred from the abundance of many NF species in soil, more direct methods of assessing predation are needed. Duncan et al. (2007) developed an assay to enumerate NF species recovered directly from nematodes rather than soil, which better reflects levels of predatory rather than saprophytic behavior. Circumscribed soil cylinders were defined and isolated in situ by pounding PVC tubes to a depth of 20 cm in soil beneath the citrus tree canopy. These relatively undisturbed soil cylinders were baited with large numbers of EPNs and then recovered from the field after 3 days. The nematodes extracted from the soil cylinders were placed on water agar to allow growth of NF from nematode cadavers. This method effectively recovered predators and parasites of nematodes, which were invariably all killed within 5 days. However it was necessary to add fresh EPNs to the agar plates after 5 days to induce the formation of fungal fruiting bodies for species identification and the estimation of population abundance based on numbers of EPNs killed. The long period of time during which the fungi competed with one another on the water agar likely skewed the abundance estimates in favor of species best adapted to this artificial habitat.

A more reliable estimate of NF predation rates in soil requires the identification of infected nematodes immediately following extraction from soil. Indeed, direct quantification of target populations rather than estimation from bioassays would facilitate understanding the roles in food webs of many organisms that are currently poorly understood. For example, possible effects of *Paenibacillus* on EPN prevalence can be inferred in bioassays by the degree of spore encumbrance of EPN IJs emerging from sentinel insects (Duncan et al., 2007). However, spore encumbrance increases with the length of time that IJs are in the vicinity of bacteria-infected cadavers in these assays, and the detection of bacteria using sentinel insects depends on EPN abundance in soil. Therefore, methods to directly measure the abundance of these bacteria in soil or on nematodes extracted from soil are needed to accurately assess the degree to which EPNs and *Paenibacillus* interact at different times or in different habitats.

Real-time PCR (or quantitative PCR, qPCR) provides an efficient method of quantifying soilborne organisms such as bacteria and fungi using molecular probes (Atkins, Clark, Pande, Hirsch, & Kerry, 2005; Klob, Knief, Stubner, & Conrad, 2003). The abundance of *S. kraussei* and *S. affine* in fields and meadows was recently compared using qPCR (Torr, Spiridonov, Heritage, & Wilson, 2007). The methods most commonly employed involve the use of fluorescent products that link to double stranded DNA causing increased fluorescence (e.g., SYBR Green®) or the design of specific fluorescent probes (e.g., TaqMan® or hydrolysis probes). In both cases, species-specific primers designed for the target taxon are used. Both systems can function with a high degree of species specificity but, due to the use of a probe that adds an additional level of specificity to the primers, hydolysis probes are generally reported to be more reliable in this regard (Holeva et al., 2006; Leal, Green, Allen, Humble, & Rott, 2007). In both systems, the amount of fluorescence increases during PCR cycling. The quantification cycle (Cq) (also called threshold cycle or Ct) is that at which product amplification enters an exponential phase.

Standard curves of Cq values from known quantities (e.g., numbers of organisms or amounts of DNA) of the species in question can be developed in order to estimate quantities in unknown samples (Fig. 6). Developing the molecular components for a new target species requires several steps, each involving options that affect the cost, accuracy, or reliability of the final method (Fig. 7).

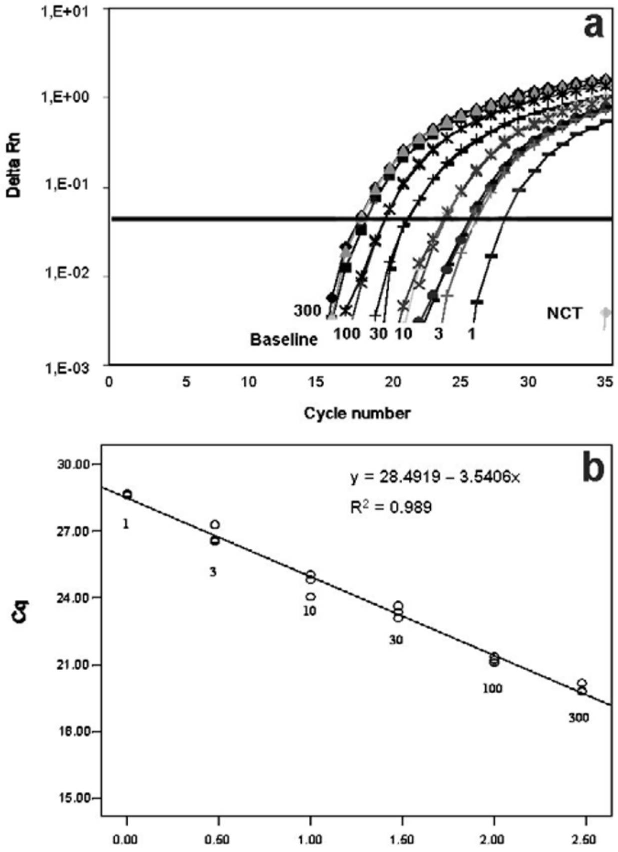


Figure 6. Amplification curves for a qPCR assay with Steinernema diaprepesi *as target species. Fluorescence intensities* (**a**) *produced by different nematode concentrations (1–300 infective juveniles) are shown by the different curves entering the exponential growth at different points (Cq) in the cycling series (NCT = negative control). Linear standard curve of the quantification cycle number (Cq) for the corresponding nematode numbers, expressed as log* (**b**)**.** *All reactions performed in triplicate.*

The relatively few reports using qPCR to identify and quantify cryptic soilborne organisms vary in the adequacy with which experiments are described in order to reproduce the work. Bustin et al. (2009) provide useful guidelines for the accurate presentation of the methods and results obtained using qPCR. Some of methods reported to date are reviewed and compared.

Most species-specific primers (and probes, if required) have been developed to amplify sections of the ITS region. This region of ribosomal DNA is usually well conserved at the species level but provides greater variability between species than more highly conserved regions such as D2–D3. Extensive interspecific variation is necessary to provide enough species-specific primer targets to identify those that will amplify segments of an optimum size for reliable qPCR reactions (i.e., between 80 and 200 base pairs). Compared to other gene regions, the large ITS database in GenBank facilitates validating the species-specificity of ITS primers among organisms characterized to date. In addition to ITS, other regions used to develop primers/probes for use in qPCR include 18S from rDNA (Holeva et al., 2006; MacMillan, Blok, Young, Crawford, & Wilson, 2006), *Msp*I satDNA monomeric unit (François et al., 2007), *Hsp*70 sequence (Leal et al., 2007) and the intragenic spacer (IGS) region of the 5S rRNA gene (Kang, Moon, Lee, Shin, & Lee, 2009).

Figure 7. General protocol to develop real time qPCR primers and probes (from Campos-Herrera, Johnson, El-Borai, Graham, Duncan, 2009).

Once the primers/probes have been validated, sampling and extraction methods can be selected that exploit the advantages and limitations of qPCR. Many protocols for sampling fungi and bacteria in soil extract DNA directly from soil samples that can be as small as a few grams or less. Smith and Jaffee (2009) suggest using a combination of culturing and molecular detection of trapping fungi because primers specific for a major group of trapping fungi (Orbiliales) yielded clones representing just 3 of 8 species that were isolated through culturing, but revealed the presence of 18 species that had not been detected through culturing. It is unclear whether uneven distribution of the species in the small samples (12.5 cm^3 soil) affected the results. In order to optimize sampling and extraction methods, additional studies comparing both species-specific primers and culturing are needed, and they should involve DNA that has been extracted and concentrated from a range of soil sample sizes.

MacMillan et al. (2006) accurately quantified numbers of slug-parasitic nematodes by extracting DNA directly from 10 g soil samples but were not able to do so from 1 g soil samples. However, the samples were artificially infested with nematodes and, in contrast to bacteria or fungal propagules, it is unlikely that the relatively low numbers of nematodes that occur naturally in soil can be reliably detected from such small samples. Studies and diagnostic services that use qPCR to quantify metazoans such as nematodes in soil routinely employ standard methods of sampling and extraction and, therefore, are able to rely on conventional sampling programs designed to optimize cost and accuracy (Hollaway, Ophel-Keller, Taylor, Burns, & McKay, 2004; Stirling et al., 2004; Ophel-Keller, McKay, Hartley, Herdina, & Curran, 2008; Donn, Griffiths, Nielson, & Daniell, 2008). Such programs employ a variety of sampling methods that address the highly aggregated spatial patterns of nematodes, usually by collecting large numbers of samples that are pooled, mixed and subsampled for nematode extraction (Been & Schomaker, 2006).

Subsamples of 500–1000 cm^3 are routinely processed by methods such as sucrose centrifugation to recover nematodes (Jenkins, 1964). There is no reason that such large subsamples cannot be used for molecular diagnostics although a number of studies report the use of smaller subsamples (Table 2), perhaps to reduce the recovery of soil chemicals that can interfere with DNA extraction and PCR reactions (see below). If the objective is estimating predation rates by measuring only those NF intimately associated with nematodes, the nematodes can be further separated from the small residue of soil remaining after sucrose centrifugation by additional centrifugation in a magnesium sulphate density gradient (Duncan et al., 2007). Preliminary experiments (Duncan et al., unpublished data) revealed less fungal DNA and fewer nematodes recovered following the two-step extraction procedure compared to just sucrose centrifugation; and there was no evidence of significant NF DNA in the soil residue. This observation should be confirmed using a variety of soils and NF targets because use of one rather than two extractions reduces time and cost, and the recovery of more nematodes increases the detection efficiency of the system.

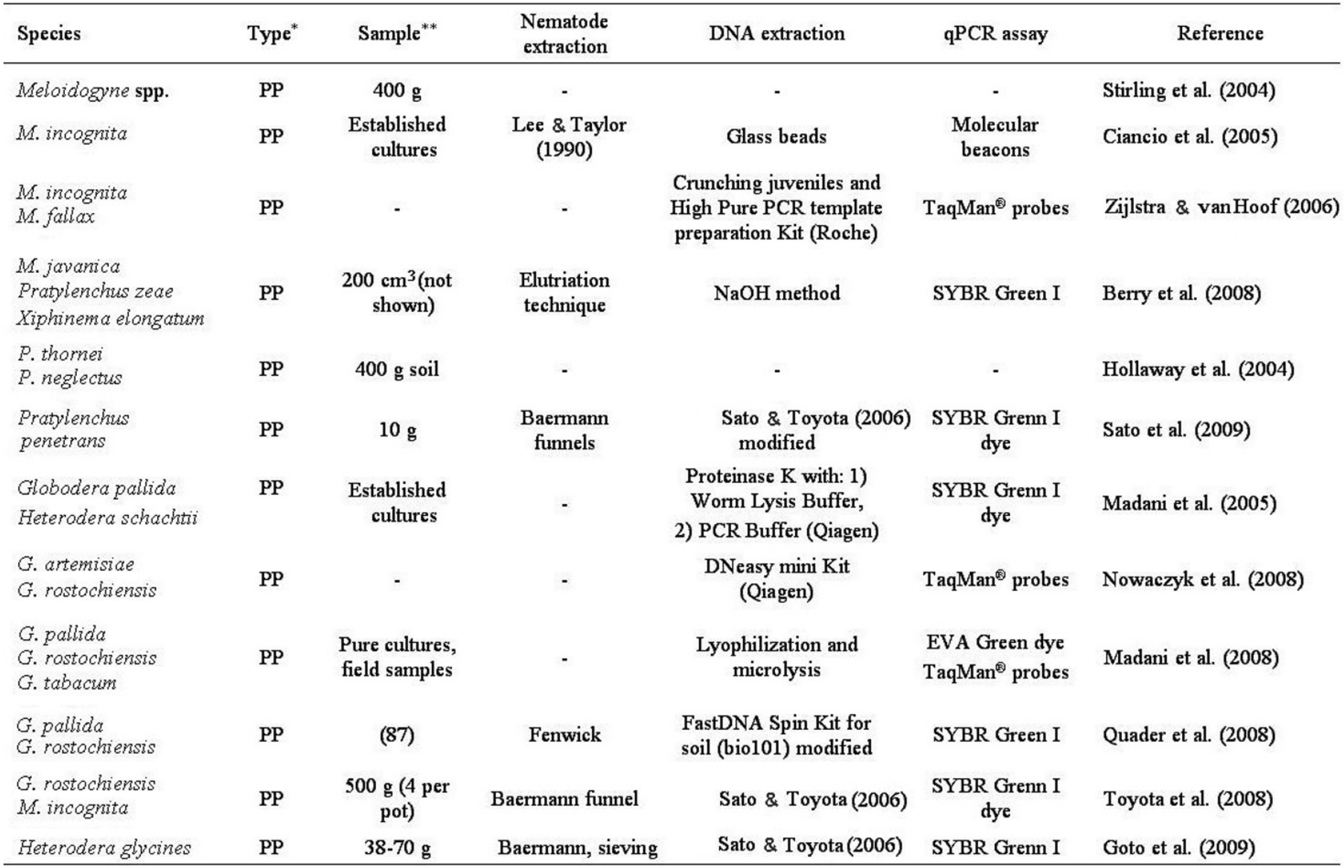
A variety of methods have been used to extract nematode DNA from soil or from nematodes recovered from soil (Table 2). Kits involve higher costs than standard laboratory protocols to purify DNA but are far simpler and faster to use if they perform well with material extracted from soil. When DNA was isolated directly from soil, the PowerMax Soil^{TM} DNA Isolation Kit (MoBio) was more reliable than a standard laboratory method or the Ultra Clean Soil^{TM} DNA Kit (MacMillan et al., 2006). For nematodes extracted from soil, Madami, Subbotin, and Moens, (2005) concluded that the use of proteinase K followed by PCR Buffer yielded more DNA than if it was followed by Worm Lysis Buffer. Donn et al. (2008) found that use of sodium hydroxide extraction, ChargesSwitch PCR CleanUp Kit (Invitrogen), QIAquick PCR Purification System (Invitrogen) and the Wizard PCR Prep DNA Purification System (Promega) all failed to provide either enough DNA or DNA of high enough quality for PCR whereas phenol chloroform extraction or Purelink PCR Purification Columns (Invitrogen) provided high concentrations of DNA acceptable for PCR studies. Campos-Herrera et al. (2009) obtained good quality DNA from nematodes extracted with sucrose-centrifugation using the Unltra Clean Soil^{TM} DNA Kit (Mo Bio).

PCR can be inhibited by chemical contaminants in soil (e.g., humic acid, phenolic compounds) or by use of excessive template. Inhibition can be complete or partial, and so it is important to calibrate qPCR with results obtained from known quantities of the target organism (e.g., nematodes, nematodes infected by NF, etc.) added to populations of nematodes extracted from soil rather than relying on PCR of DNA from pure cultures. Bovine serum albumin has been used to reduce the effects of chemical inhibitors of PCR (MacMillan et al., 2006; Torr et al., 2007; Campos-Herrera et al., 2009). To avoid excess template, dilution of DNA from 4 to 100-fold from samples of unknowns is usually necessary (Madami et al., 2005; Jones, Todd, & Herman, 2006; Kang et al., 2009). Because each soil sample yields a different quantity of DNA that might affect the reaction, the use of a standard DNA quantity is preferable to a standard dilution.

Most authors report very high detection efficiency from the use of qPCR, which is frequently at the level of a single nematode. Several multiplex systems in which single reactions measure more than one nematode species or combinations of nematodes and fungi have been developed that can substantially reduce time and cost (Madami, Ward, & de Boer, 2008; Jones et al., 2006; Berry, Fargette, Spaull, Morand, & Cadet, 2008; Zijlstra & van Hoof, 2006). Unfortunately, the increased likelihood of competition between target DNA or development of interacting primers (primer-dimers) that increase florescence for a false signal can impede the development and performance of multiplex systems. Zijlstra and van Hoof (2006) reported a density-dependent reduction in the efficiency of multiplex qPCR and suggested that individual reactions would be required for precise estimation. Berry et al. (2008) observed similarity of melting temperatures and competition between the amplification of *Meloidogyne javanica* and *Pratylenchus zeae* targets, which made it impossible to distinguish the two species in a multiplex reaction.

At present, studies using molecular techniques to assess EPN population distribution and dynamics remain rare but the development and optimization of these techniques could contribute much to our understanding of these nematodes and the kinds of strategies that might facilitate their enhanced usefulness for biological control in agroecosystems.

Table 2. Methods for study of soil nematodes using real time qPCR.



Species	Type*	Sample**	Nematode extraction	DNA extraction	qPCR assay	Reference
Meloidogyne spp.	PP	400 g	-	-	-	Stirling et al. (2004)
M. incognita	PP	Established cultures	Lee & Taylor (1990)	Glass beads	Molecular beacons	Ciancio et al. (2005)
M. incognita *M. fallax*	PP	-	-	Crunching juveniles and High Pure PCR template preparation Kit (Roche)	TaqMan® probes	Zijlstra & vanHoof (2006)
M. javanica *Pratylenchus zeae* *Xiphinema elongatum*	PP	200 cm^3(not shown)	Elutriation technique	NaOH method	SYBR Green I	Berry et al. (2008)
P. thornei *P. neglectus*	PP	400 g soil	-	-	-	Hollaway et al. (2004)
Pratylenchus penetrans	PP	10 g	Baermann funnels	Sato & Toyota (2006) modified	SYBR Grenn I dye	Sato et al. (2009)
Globodera pallida *Heterodera schachtii*	PP	Established cultures	-	Proteinase K with: 1) Worm Lysis Buffer, 2) PCR Buffer (Qiagen)	SYBR Grenn I dye	Madani et al. (2005)
G. artemisiae *G. rostochiensis*	PP	-	-	DNeasy mini Kit (Qiagen)	TaqMan® probes	Nowaczyk et al. (2008)
G. pallida *G. rostochiensis* *G. tabacum*	PP	Pure cultures, field samples	-	Lyophilization and microlysis	EVA Green dye TaqMan® probes	Madani et al. (2008)
G. pallida *G. rostochiensis*	PP	(87)	Fenwick	FastDNA Spin Kit for soil (bio101) modified	SYBR Green I	Quader et al. (2008)
G. rostochiensis *M. incognita*	PP	500 g (4 per pot)	Baermann funnel	Sato & Toyota (2006)	SYBR Grenn I dye	Toyota et al. (2008)
Heterodera glycines	PP	38-70 g	Baermann, sieving	Sato & Toyota (2006)	SYBR Grenn I	Goto et al. (2009)

Table 2, continued.

Ditylenchus dipsaci	PP	Establised cultures	Filter extraction	Proteinase K and Worm lysis buffer	SYBR Green I dye	Subbotin et al. (2005)
Paratrichodorus pachydermus *Trichodorus similis*	PP VV	200 g soil (8, 6, 3 from different fields)	Decanting and sieving, Baermann funnel	NaOH buffer	TaqMan® probes	Holeva et al. (2006)
Microbial-feeders	FL	100 cm^3 (2 cores × 16 plots)	Centrifugal-flotation	Worm Lysis Buffer	TaqMan® probes	Jones et al. (2006)
Bursaphelenchus xylophilus	PP	Established cultures, wood samples	Baermann funnel	Webster et al. (1990) Zheng et al. (2003)	TaqMan® probes	Cao et al. (2005)
B. xylophilus	PP	Pure cultures, wood samples	Baermann funnel	ChargeSwitch gDNA Plant Kit (Invitrogen)	TaqMan® probes	François et al. (2007)
B. xylophilus	PP	Pure cultures, wood samples	Baermann funnel	Modified method by Burgermeister et al. (2005)		Leal et al. (2007)
B. xylophilus *B. mucronatus*	PP	Pure cultures, wood samples	Modified Baermann	Kang et al. (2004)	SYBR Green I	Kang et al. (2009)
Phasmarhabdites hermaphrodita	SP	0.5 g (10) 1g (10) 10 g (10)	-	1) Lab method 2) UltraClean Soil ™ DNA isolation Kit (MoBio) 3)PowerMax™ Soil Kit (MoBio)	TaqMan® probes	MacMillan et al. (2006)
Steinernena affine *S. kraussei*	EPN	100 mL (5)	Modified sieving method and Baermann funnel	DNeasy mini Kit (Qiagen)	TaqMan® probes	Torr et al. (2007)

* PP = plant parasitic; FL = free living; EPN = entomopathogenic; SP = slug parasitic; VV = virus vector.

** Replications per site/treatment, volume processed and/or number of samples (in parentheses).

CONCLUSIONS

Basic and applied study of the EPN/bacteria symbiosis has increased steadily during the past half-century, coincident with an accelerating awareness of limitations that characterize many conventional pest management practices. An extensive literature on the effectiveness of EPNs as biopesticides is in striking contrast to the paucity of information about the ecology of these cryptic predators of subterranean arthropods. Their population abundance is usually inferred from the numbers of sentinel insects they kill but, unlike plant parasitic and free living nematodes, there are few reports of the actual numbers of these worms in different soil habitats. Consequently, little is known about their population biology – neither the factors that govern population size nor the numbers of worms needed to achieve economic pest suppression in different habitats. The great variety of species with very different life strategies provides tremendous opportunities to study how food webs and soil conditions affect the abundance of nematodes with different traits. Understanding the population biology of EPNs is necessary to discover and exploit new ways to increase their efficacy and, more importantly, their reliability for biological control in managed ecosystems. For example, trophic cascades that result from augmenting EPNs in citrus orchards have the potential to interfere with the effectiveness of this IPM tactic if the non-target effect (i.e., the temporary suppression of EPNs by natural enemies) occurs at a peak time for *D. abbreviatus* egg hatch and larval recruitment into soil. Can better timing of EPN applications reduce this risk? Would the application of EPN species less susceptible to predation by NF modulate the trophic cascade and the potential for non-target effects? What physical characteristics of soils are amenable to change in ways that enhance either the numbers or the effectiveness of EPN? The ready availability of molecular tools to identify and measure EPN and their natural enemies in the soil should facilitate more rapid progress in our understanding of how these organisms co-exist and how we might better manage soils to maximize their biological control potential.

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Section 2

ADVANCED IPM TECHNOLOGIES

INTEGRATION OF INSECT AND MITE MANAGEMENT WITH DISEASE AND WEED CONTROL IN PECAN PRODUCTION

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Abstract. Pecan orchards in the southeastern US are managed to conserve resources, protect the fruit and foliage from injury caused by phytophagous insects, mites, and pecan scab, and remove competition from weeds during the establishment of newly planted trees and in the preparation of the orchard floor as a harvesting surface. Costs associated with pest control are significant each year and the growers use integrated pest management methods to increase the effectiveness of pesticide treatments and reduce control costs. A coordinated research and extension effort over the past 25 years in entomology, plant pathology, weed science and horticulture has reduced the amount of pesticide use by 35%. Four advances have been responsible for the reduction. First, pecan scab sprays are reduced by linking the frequency of applications to the climatic conditions and the cultivar susceptibiltiy. Second, pest-specific insecticides that are toxic to the pests and not toxic to beneficial insects and mites are used to control lepidopterous pests and conserve aphidophagous insects and mite predators. Third, cover crops have been developed to supplement the soil with nitrogen and organic matter and conserve beneficial insects. Fourth, weed studies have led to the elimination of weeds in the first 8 years after planting around young trees, chemical mowing methods in established orchards and selective grass control to increase the growth of clover cover crops. The development and implementation of these and other significant advances in pecan management are reviewed in this chapter.

1. PESTS IMPEDE THE VERSATILITY OF THE PECAN TREE

Pecan [*Carya illinoinensis* (Wangenh.) K. Koch, Fagales: Juglandaceae] is a large tree that is native to the United States and Mexico and is propagated as a shade tree, for timber and for the production of nuts. Although this chapter describes the recent integration of pest control methods in pecans grown for nut production pest problems arise in shade and timber trees as well. As a shade tree in the southern US, pecan has multiple problems and is not recommended for planting (Finch, 2001). However, existing trees are rarely removed and replaced by homeowners or city

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managers. The canopy of a full grown tree, with dimensions of 30.5 m in height and 16.5 m in diameter and covering up to 1/24 ha of land, is often too large for home sites. The main pests of pecan trees planted along the roadside and in the urban landscape, are the gregarious caterpillars – walnut caterpillar [*Datana integerrima* (Grote & Robinson), Lepidoptera: Notodontidae] and fall webworm [*Hyphantria cunea* Drury, Lepidoptera: Arctiidae]. These cause defoliation in the late summer and the fall webworm leaves unsightly webs in the canopy. Biological control with water-miscible formulations *Bacillus thuringiensis* applied as a foliar spray is effective against these two pests. Feeding by the pecan aphids [*Monellia caryella* (Fitch), *Monelliopsis pecanis* (Bissell), *Melanocallis caryaefoliae* (Davis), Hemiptera: Aphididae] also detract from the aesthetic value of pecan as a shade tree. Aphids egest copious amounts of honeydew (Wood, Tedders & Dutcher, 1987). Honeydew deposited on the leaf surface promotes the growth of sooty mold giving the leaves a black appearance. Honeydew deposited on the area beneath the tree canopy often has to be washed off cars and trucks parked beneath the trees. Multicolored Asian ladybeetle [*Harmonia axyridis* (Pallas), Coleoptera: Coccinellidae] was imported for biological control to the US and is an effective control for aphids in pecan trees in areas where the beetle is established. In pecan trees grown for shade, the nut crop is highly depredated by squirrels, birds and insect pests (Worley, 2002).

Pecan lumber is sold as hickory and hickory is used to make tool handles, furniture, cabinetry, ladder rungs, dowels, sporting goods (including baseball bats, skis and archery equipment), flooring, veneer, plywood, fuelwood, and charcoal. Pecan burls and spalted pecan are sold as a specialty wood. The characteristics of hickory and pecan produce a strong, shock-resistant wood that has a white tinge with brown sapwood and red-brown heartwood. Pecan wood is difficult to season and warps during drying. Wood working properties are above average for hickory and slightly less for pecan. Problems arise in splitting while nailing. Machining and glueing the wood are also difficult and the wood is susceptible to bird peck. Pest populations are generally very low in native stands of pecan harvested for timber (Reid & Hunt, 2000).

Yellow-bellied sapsucker, [*Sphyrapicus varius* (L.) Piciformes: Picidae] is a migratory bird that causes bird peck in hardwoods including pecan. The birds eat insects that are attracted to sap flowing from the holes made in the trunk by the bird pecks. Open bird peck holes do not decrease the value of the wood for lumber but they do devalue the wood used for making tool handles. When the bird peck holes pierce the cambium layer of the trunk then the tree produces a callus tissue to repair the hole (aka an occlusion) and the wood looses value. Each yellow-bellied sapsucker returns to the same nesting site each year and repeated annual bird peck damage can extend into the wood and reduce its value. Foresters control the yellow-bellied sapsucker by applying bird Tanglefoot® on the trunk of most of the trees in the orchard leaving a few favored trees untreated to withstand all the injury. The birds nest in decaying trunks of aspen and removal of these nesting sites also reduces injury. Killing the birds is illegal (Ostry & Nicholls, 1978). Pecan are also be used as an alley crop in the southern US. The trees are planted around fields of row crops and the tree roots reduce nitrate leaching from the crop field to the groundwater (Allen et al., 2004).

Pecan nut production in orchards is a viable agribusiness especially in the southern US. Pecan trees will produce a crop of nuts with little or no management. In fact, a large portion of the USA crop is produced on native stands with very low inputs. These stands have much lower average production per hectare than seedling or improved pecan orchards and inputs have to be held to a low cost. Pecans initially were harvested by hand. Harvesters would climb each tree and shake individual limbs or knock nuts off the limbs with bamboo poles, while coworkers would pick them up off the ground. Mechanical trunk and limb shakers are currently used to dislodge the nuts out of the tree and onto the orchard floor. Hand picking off the ground has been replaced by a mechanical process where the fallen limbs are raked over to the edge of the orchard, the nuts and leaves are swept into rows, the leaves are blown off the row and the nuts are picked up from the row by a mechanical harvester. Nuts are sorted and cleaned in the orchard and then transported to the shelling plant where they are sanitized, dried and then typically cracked, shelled and frozen. Harvesting is most efficient when the orchard floor is dry, level, and cleared of weeds and debris. Efficient harvesting is needed to prevent depredation of the nuts by birds and mammals and environmental degradation of the nuts.

Native stands of pecans provided nuts as a source of food for indigenous people of America and early settlers propagated seedling trees through the southern US. Many of the native groves were developed, by European settlers, as a new-world adaptation (Brison, 1974) of the European silvopastoral systems that provided many of the staples of the rural community – milk, meat, hides, wood, and nuts (Auda, 1999). Grafting techniques were developed in the mid-1800s and improved cultivars with desirable characteristics were selected from native and seedling trees or from controlled crosses from amateur or professional plant breeders. Improved cultivars have been propagated across the southeastern US, and in New Mexico, Arizona and California to the extent that the production of nuts from improved cultivars often exceeds the production of native and seedling orchards (Worley, 2002).

Nut production in the US is currently based on ~10 million managed trees planted on ~200,000 ha on 20,000 farms in 24 states producing roughly 146,000 metric tons of in-shell nuts each year. Most orchards are small in area. Sixty-two percent of the farms have less than 6 ha, 32% have 6–40 ha, 5.4% of the farms have 40–200 ha and 0.7% of the farms have more than 200 ha. Orchards with less than 40 ha comprise 56% of the production area in the USA (Wood, 2003). Improved cultivars have contributed to increases in pecan production. Today's new cultivars, however, are only 2–3 generations removed from wild trees (Sparks, 1992) and pesticides – esp. fungicides – have had a more significant impact on increasing USA pecan production than cultivars. In fact, native groves produce approximately 30% of the US nut crop and seedling and improved cultivars planted in commercial orchards produce the remaining crop (Pollack, 2001). Pecan trees have a producing lifespan of at least 80 years and grow to over 30.5 m in height. Production is irregular from season to season. USA production per season ranges from 45.4 to 163.2 million kg of nuts in the shell. The kernel typically comprises ~50% of the mass of the nut in the shell. Growers typically sell pecans in the shell for $1.50–5.00/kg to processors and many growers process, package, and market their own crop as higher value products such as gift boxes, whole kernels, and various candies. Growers in Georgia and Texas produce 60% of the US crop.

2. PEST CONTROL METHODS

Uncontrolled disease, insect and mite, and weedy pest populations, under favorable environmental conditions have the potential of reducing the pecan nut production often to nil. Pests attacking the nuts can take the entire crop in the current season. Pests attacking the foliage can cause premature defoliation in the current year and the trees will not produce flowers and fruit the following year As examples, the primary pest in the humid regions of the southeastern US is pecan scab and control is achieved with preventive treatment of the fruit and foliage with fungicide. Pecan weevil attacks the crop late in the season after the grower has invested considerable resources in the crop and the weevils are most effectively controlled with repeated sprays of insecticide during adult emergence. Black pecan aphid outbreaks defoliate trees quickly and treating after the outbreak does not prevent damage. Black pecan aphids are treated with insecticide when the population exceeds one aphid per leaf. Pecan leaf scorch mite is control with sprays of sulphur or miticide (Dutcher, Hudson & Ellis, 2003). Lack of weed control is directly related reductions in pecan production, esp. in young pecan trees (Smith, Cheary, & Carroll, 2005; Patterson & Goff, 1993; Foshee, Goodman, Patterson, Goff, & Dozier, 1997).

Broad spectrum pesticides and airblast sprayer technology and orchard floor management significantly increase the ability of growers to quickly control these key pests and large portions of the nut crop were conserved by the use the pesticides. Production of nuts increased leading to a period of reliance on chemical control of insects, mites and diseases. Broad spectrum pesticides are crucial to commercial pecan production, offering highly effective control methods that prevent pecan scab and pecan weevil damage, control outbreaks of black pecan aphids, and prepare weed-free herbicided strips along the tree rows.

Pest control in differs in each of the three cultural regimes for pecan nut production (Dutcher et al., 2003). In native stands and groves where nuts are harvested from naturally occurring trees, the orchard floor is cleared at harvest time and the nuts are collected and managers use nitrogen fertilization, mechanical nut harvest rely primarily on natural controls to regulate pest populations with limited use of insecticides (Reid & Mulder, 2003). In seedling orchards where trees are produced from seed and not grafted, and in improved orchards where seedlings are grafted in a nursery to a known cultivar and then planted in an orchard, orchard managers potentially can use all available pest control methods and are limited by the need to balance the costs of control with production potential each season. Each seedling tree produces a unique pecan nut and the harvested product lacks the homogeneity of the nut crop harvested from an improved orchard. However, seedling orchards have greater genetic diversity than improved orchards and pest outbreaks (especially for pecan scab, pecan weevil and black pecan aphid) develop to different levels of severity in each tree. Improved orchards are more susceptible to pest outbreaks over the entire orchard.

Weed control is important and functions to: remove plants that compete with the trees for nutrients and plants that provide food for kernel-feeding hemipterans; and, to the preparation of the orchard floor for mechanical harvesting (Smith & Carroll, 2004). Weed control initially consisted of disking and rolling the soil so that the nuts could be collected from bare soil surface. This method made harvesting easier but the harrow would often open a wound at the base of the trunk of each tree and crown gall [*Agrobacterium tumefaciens* Smith & Townsend, Proteobacteria: Rhizobiales: Rhizobiaceae] would infest the wounded trees and destroy a fair portion of the root system. Growers shifted to a mowed surface between tree rows with an herbicided strip along the tree row to prevent damage to the tree trunks. Through the years the types of herbicides changed but the basic design remained the same. Trees on land that is flooded part of the year along river systems or with flood irrigation have either a bare soil surface maintained with herbicides or the annual weeds are periodically mowed. In the last 30 years, growers have started an integrated approach to weed control by seeding the orchard with various cool season legumes. These are grown as intercrops in the mowed strip or as cover crops over the entire orchard floor. The plants supply nitrogen and increase soil organic matter and also enhance beneficial insects. In the last 5 years, growers have enhanced the growth of the legumes by removing grasses during the winter and spring with, sethoxydim, a selective herbicide.

Agricultural chemical usage in US pecan orchards varies considerably between states and has increased significantly since the domestication of pecan began in the early 1900s. Nitrogen fertilizer, pesticides and new cultivars have increased US production per hectare. Pesticide usage is significant, for example, commercial pecan producers currently use approximately 1/3 million kg of insecticide (active ingredient) each season on 320,000 acres in Georgia and Texas (Smith, Harris, Lee, McEachern, & Ree, 2002; Guillebeau, 2001). The most recent survey results (USDA/NASS, 2000) indicate that approximately 69% of the US pecan acreage is treated each year with supplemental nitrogen fertilizer, 67% is treated with insecticides, 47% is treated with fungicides, and 44% is treated with herbicides.

2.1. Insect and Mite Pest Management in Pecan Orchards

The insect complex associated with pecan in native and improved systems includes 180 species of phytophagous insects and mites (Payne & Johnson, 1979; Harris, 1983) and each is associated by a diverse array of natural enemies (Tedders, 1985). Insect management strategies range from total reliance on natural enemies to intensive integration of chemical and biological controls (Table 1) (Dutcher et al., 2003). The fruit and foliage in pecan orchards are susceptible to insect injury that can result in economic losses for 7 months. Preventive cover sprays of insecticides or miticides are not practical and the control of pecan insect and mite pests has developed into a management scheme. Insects and mites and tree phenology are monitored determine the abundance of the pests and the susceptibility of the tree to injury. Direct control measures are applied when pest abundance exceeds an action threshold and the tree is susceptible to injury. This prevents high costs, resistance, replacement and resurgence problems associated with repeated treatment with insecticides and miticides (Dutcher, Fonsah, & Hudson, 2006).

Table 1. Control methods for the eight major pecan arthropod pests in North American.

Pest	*Control method for pest*[a]	*Relative efficacy*[b]
Pecan weevil *Curculio caryae* (Horn)	Broad spectrum insecticides	High
	Quarantine	High
	Risk rating and spot treatment	Moderate
	Trunk treatment	Moderate
	Entomopathogens, nematodes	Low
	Red imported fire ant as predator	Low
Pecan nut casebearer *Acrobasis nuxvorella* (Neunzig)	Broad spectrum insecticides	High
	Biorational insecticides	High
	Mating disruption	Low
Hickory shuckworm *Cydia caryana* Fitch	Broad spectrum insecticides	High
	Biorational insecticides	High
	Sanitation	Moderate
Black pecan aphid *Melanocallis caryaefoliae* Davis	Organophosphate insecticides	High
	Neonicitinoid insecticides	Moderate
	Insecticidal soap	High
	Harmonia axyridis (Pallas)	Moderate
	Interplanting crape myrtles	Low
Yellow pecan aphid *Monelliopsis pecanis* Bissell	Systemic insecticides	High
	Neonicitinoid insecticides	High
	Insecticidal soap	High
	Harmonia axyridis (Pallas)	Low
	Introduced parasites	Low
Blackmargined aphid *Monellia caryella* Fitch	Systemic insecticides	High
	Neonicitinoid insecticides	High
	Insecticidal soap	High
	Harmonia axyridis (Pallas)	Low
	Introduced parasites	Low
Pecan leaf scorch mite *Eotetranychus hicoriae* (McGregor)	Miticides	High
	Sulphur	High
	Predatory mite release	Moderate
	Dormant oil sprays	Low
Kernel-feeding Hemipterans – Pentatomidae and Coriedae	Broad spectrum insecticides	Moderate
	Trap crops	Low
	Removing alternate host plants	Low

[a] Control methods listed in the table have shown benefits greater than the costs.

[b] Efficacy ratings: total = 100%; high = 91–100%; moderate = 80–90%; low < 80%.

Insecticides offer the single most effective control method for nut-feeding insects. Carbaryl, phosmet, esfenvalerate and cypermethrin are used during the late season for control of pecan weevil, hickory shuckworm and kernel-feeding hemipterans. Pyrethroid insecticides are also used but have a shorter residual activity and lower toxicity against the pecan weevil. Broad spectrum insecticides are not ideal and sprays often destroy beneficial insects leading to resurgence of secondary foliage-feeding pests. Pecan growers with resurgence problems have to apply additional costly and specific aphidicides, systemic insecticides and/or miticides to keep the foliage on the trees.

Systemic insecticides or foliar sprays of insecticides are the most effective controls for outbreaks of foliage feeding insects. Phosmet and chlorpyrifos are used effectively for control of late season outbreaks of the black pecan aphid. These two organophosphate insecticides may become obsolete through reassessment. Imidacloprid sprays and biological control with multicolored Asian ladybeetle are effective against the pecan aphids. Efficacy varies between the three different species and selective control of two aphid species and not the third species opens the feeding niche the third leading to outbreaks. The control of all three species of pecan aphids can leave the foliage open to attack by phytophagous mites and typically leads to an outbreak of pecan leaf scorch mite (Dutcher et al., 2006). Alternative controls for control of nut-feeding pests that do not destroy beneficial insects associated with foliage-feeding pests are possible with the use of selective insecticides.

Growers also adopt insect pest management to reduce the cost of production for pecans. Pecan growers typically minimize the number of spray applications and rely on natural control by predators, parasites and pathogens for many insect pests. One of the most effective biologically-based control techniques for pecan growers is selective insecticides coupled with effective monitoring techniques. to control pecan nut casebearer (Knutson & Ree, 2000) and hickory shuckworm. Replacement of broad spectrum insecticides with biorational insecticides has reduced the incidence of secondary pest resurgence after treatments for these pests. Initially, insect growth regulators were evaluated for insect control against pecan weevil and lepidopterans (Payne & Dutcher, 1985; Tedders, 1977). Efficacy was much lower than the broad-spectrum insecticides and registration was not pursued. Broad-spectrum insecticides became the main control measures for preventing pest damage. Recently, newer and more effective insect growth regulator insecticides, such as, diflubenzuron and tebufenozide, have gained acceptance among growers for control of lepidopteran pests. Broad spectrum insecticides are currently the only effective controls for pecan weevil, kernel-feeding hemipterans and black pecan aphid.

Pecan insects have sufficient reproductive capacities to overcome the mortality caused by control methods and growers are continually battling recurring pest problems each season. The bionomics information for the reproductive capacity and generation time of pecan weevil (Ree, Knutson, & Harris, 2005), pecan nut casebearer (Mulder & Grantham, 2002), yellow pecan aphid, blackmargined aphid, black pecan aphid (Tedders, 1978; Kaakeh & Dutcher, 1992), and pecan leaf scorch mite (Hall, 2001) (Table 2) indicates that only 0.1–70% of the populations need to survive from one season to the next to sustain the pest populations.

Pecan weevil survival of 2.6% per generation is needed to sustain the current population level. Since there is a generation every 2–3 years, an annual mortality of 30.1% per season (for weevils with a 3-year generation time) or 83.4% per season (for weevils with a 2-year generation time) would cause a decrease in weevil from one generation to the next. Multiple generations occur for pecan nut casebearer, aphids and pecan leaf scorch mite and yearly survivals of 0.1–0.2% will ensure a increase in theses pest populations from season to season. Efficacies required to prevent pecan nut casebearer populations from increasing from season to season exceed 99% and two or more control methods often have to be combined to prevent pest outbreaks in an integrated pest management program (cf. Tables 1 and 2). The better chemical control methods typically have efficacies near 95–99%. The impacts of natural controls – e.g. rainfall events (Kaakeh & Dutcher, 1993a) and temperature extremes (Kaakeh & Dutcher, 1993b) on pecan aphids – are important but pests also may increase in the orchard by immigration. Consequently, growers continue to have pest problems from season to season. Pecan weevil problems may be solvable with the integration of several techniques over an extended period.

Table 2. The mortality needed each season to cause a reduction in the season to season abundance estimated from literature values for the reproductive capacity (RC) and generations per year (G), for certain pecan insect and mite pests with the equation: Mortality (%) = 100 · (1 – (1 / (SR · RC)^G)), where the sex ratio (SR) is assumed to be 1.0 for aphids and 0.5 for weevils, casebearers and mites.

Pest[a]	*Reproductive capacity*	*Generations per year*	*Mortality per season (%)*
Pecan weevil	75 eggs/female	0.3– 0.5	30–83
Pecan nut casebearer	50–150 eggs/female	2	99.8–99.9
Black pecan aphid	35 nymphs/female	26	99.9
Yellow pecan aphid	38 nymphs/female	32	99.9
Black margined aphid	125 nymphs/female	16	99.9
Pecan leaf scorch mite	9–36 eggs/female	7-8	99.9

[a] Two pest listed in Table 1 are not listed here. The reproductive capacity of the hickory shuckworm has not been measured and the moths have 2–5 generations per year. Kernel-feeding hemipterans do not reproduce in the pecan trees and increases in abundance by immigrating from alternative host plants.

2.2. Pecan Disease Management

There are multiple diseases of pecan that can impact production by reducing yield or quality of nuts in the present year, or reducing the ability of the trees to produce in subsequent seasons. This is particularly true in the southeastern US where fungal diseases thrive in the warm, humid climate present during the growing season. Production areas of the western US have much drier climates and few problems with fungal diseases. Therefore most of the following comments will pertain to disease control practices in the wetter production areas from east-central Texas through South Carolina. In this region the most damaging disease by far is pecan scab caused by *Fusicladosporium effusum* (G. Winter) Partridge and Morgan-Jones. Most years disease losses are not that high, but growers often spend more money for fungicides than any other production input. In Georgia alone the cost of scab control is estimated to be about $15 million annually, and in a wet year like 2003 the combined cost of lost yield and fungicide sprays was approximately $45 million (Williams-Woodward, 2003). There are other diseases that can cause local damage on some cultivars, but they are generally controlled by fungicides applied for scab. These secondary diseases include Downy Spot (*Mycosphaerella caryigena*), Phytopthora Shuck and Kernel Rot (*Phytophthora cactorum*), Powdery mildew (*Microsphaera penicillata*), Zonate leaf spot (*Cristulariella pyrimidalis*), Anthracnose (*Glomerella cingulata*), Bacterial leaf scorch (*Xylella fastidiosa*), Crown gall (*Agrobacterium tumefaciens*), and a number other minor diseases that rarely cause significant crop loss.

Because losses to disease can be severe under favorable conditions, growers have a high awareness of the need for control programs. This may include practices such as pruning lower tree branches or clearing fence rows around orchards, planting in wide row spacing's, closely mowing the orchard floor, and designing orchards so that prevailing winds flow through it (Latham & Goff, 1991). All these practices are designed to increase air flow, thus reducing leaf wetness periods and therefore levels of infection. General orchard sanitation practices are also utilized, and may include shaking previously harvested trees to dislodge shucks that harbor overwintering pathogen inoculum.

In spite of these practices, growers in areas with frequent rainfall rely heavily on multiple fungicide applications applied with large air-blast sprayers or occasionally airplanes. Fungicides used include triphenyltin hydroxide (TPTH), various sterol demethylation inhibitors (DMI's), dodine, strobilurins, and thiophanate methyl. There have been issues recently with fungicide resistance, and ongoing concerns that it will become an increasing problem (Stevenson, Bertrand, & Brenneman, 2004). These concerns are based on the known risks associated with the fungicides used, the inherent ability of the pathogen to develop resistance, the heavy use of fungicides, and the fact that pecan is a perennial crop and therefore not subject to crop rotation which can greatly reduce the buildup of resistant isolates. However, growers generally still get good disease control if they apply sprays correctly and on a timely basis. Most growers in the southeastern states use a 2–3 weeks spray interval for a total of 7–10 sprays per year, whereas those in more arid areas of the west may make few if any fungicide applications.

Early sprays (i.e. prepollination) are applied to protect the young leaves which are very susceptible to scab infections. Overwintered lesions on stems are a major source of this initial inoculum. Large numbers of conidia are produced from stem lesions in late March and early April, although small numbers are found as late as August (Stevenson, 1995). Therefore one function of the early sprays is to prevent leaf infections that will in turn sporulate and provide secondary inoculum for nut infections that are even more damaging. Scab susceptibility of leaves decreases rapidly with age (Gottwald, 1985), although later season growth flushes can become infected. Late-season fungicide sprays are of questionable benefit when applied after shell hardening according to work by Gottwald and Bertrand (1989). They demonstrated that scab initiated at this time of the season was largely cosmetic since it occurred after the nuts had fully expanded. However, many growers still apply fungicides at this time.

In an effort to reduce the cost of production in areas prone to scab development, several programs have been developed to enable growers to spray only when the environmental conditions are conducive for disease development. The program currently recommended in Georgia is AU-Pecan which is based on the number of rainfall events as well as the predicted chance of rain for the next 5 days. This model has been shown to reduce the total number of sprays in most seasons while maintaining the same level of control, and is available on a site-specific basis for individual orchards using Doppler radar precipitation estimates. A different model based on scab hours, i.e. periods with temperatures > 21.1°C and relative humidity >90%, is being used in Oklahoma (Von Broembsen, Driever, Smith, Duthie, & Carlson, 1999). One limitation to implementation of any advisory is the long time needed for many growers to spray their orchards. This time frame is commonly 1–2 weeks, which necessitates spraying on more of a calendar-based schedule since response to an advisory is too slow. The AU-Pecan model compensates for this to some degree by utilizing the 5-day chance of rain to recommend applications prior to favorable infection periods, rather than waiting until after they occur.

Another consideration in scheduling fungicide sprays based on scab biology alone is the potential development of other diseases. For example, Stuart has been a more scab-resistant cultivar in some areas and often receives fewer sprays than more scab-susceptible cultivars. However, it is very susceptible to downy spot. Downy spot infections occur very early in the season, and prepollination fungicide sprays are essential for control. The spectrum of activity for a given fungicide must also be considered when designing a spray program. A product must have activity on scab, but some excellent scab fungicides do not control other diseases as well. One common example of this is the lack of activity of TPTH and dodine on zonate leaf spot. Both of these fungicides provide excellent control of nut scab, but are weak on zonate leaf spot which also occurs during nut fill. In orchards where zonate can be a problem, other classes of fungicides (particularly DMI's) should be incorporated into post-pollination spray programs to insure control of this potentially damaging disease.

One problem that is exacerbated by fungicide sprays is pecan aphids. As discussed earlier, several species of aphid can be very detrimental to pecan, but there is a beneficial fungus in the Neozygitaceae that can cause high aphid mortality (Ekbom & Pickering, 1990). Unfortunately this fungus is very sensitive to TPTH, and fungal-induced aphid mortality was reduced by 50% in trees sprayed with this commonly used fungicide (Pickering, Dutcher, & Ekbom, 1990). Therefore, reductions in fungicide use not only save money, they also make it less likely that a grower will have to spray insecticides later in the year for aphid control (Pickering, Hargrove, Dutcher, & Ellis, 1990).

Virulence of the pecan scab fungus has been shown to be very cultivar specific (Converse, 1960). Early researcher (Demaree & Cole, 1929) demonstrated that repeated inoculations on the same cultivar increased pathogenicity on that cultivar, and the history of scab-resistant cultivar introductions has consistently been that they become more susceptible to damaging scab epidemics each year (Gottwald, 1989). The basis for this specificity is not fully known, but it occurs sometime later than 4 days after inoculation during the stage of subcuticular growth (Bracewell & Stevenson, 1999). Intercropping a mixture of cultivars has been shown to successfully reduce epidemics of foliar pathogens in other crops (Mundt, 2002), and computer simulations based on apple scab show disease reductions of 65–79% after six generations when planting three cultivars versus a single cultivar (Gessler & Blaise, 1994). While utilizing that model to guide cultivar placement in new orchards has been discussed among pecan growers and researchers, the benefits of planting cultivars in blocks for other management purposes usually outweigh the potential benefits related to scab control.

The race structure of pecan scab populations and their ability to adapt to new cultivars has raised questions regarding the objectives of pecan breeding programs which involve selecting for scab resistance. Breeders have selected for scab resistance for years, and there have also been attempts to introduce new resistance factors from other members of the hickory family via interspecific crosses (Graves & Diehl, 1991). It has generally been recognized that there is a “grace period” after the introduction of a new cultivar before scab becomes adapted to it, and the length of this grace period varies considerably among cultivars. One objective of the Georgia breeding program is to introduce new cultivars with high quality nuts with different resistance genes that can be transitioned into orchards to maintain diversity (Conner, 2003). The current Georgia program is also utilizing DNA markers for resistance genes and examining the physiological basis for scab resistance. Hopefully these efforts will lead to more durable field resistance in new pecan cultivars.

2.3. Weed Management in Pecan Orchards

Weeds are present throughout the world and many interfere with the optimum production of food and fiber. Holm, Doll, Holm, and Pancho (1997) state that approximately 200 plant species world wide are found to cause widespread problems in our crops. Many of these weeds occur in fruit and nut crops. Pecans are grown in the southern US and Mexico, an area that accounts for over 98% of the world’s pecan production (Pena, 2006). Vines, including annual vines such as the morningglories (*Ipomoea* species) and perennial vines such as trumpet creeper (*Campsis radicans*), and both annual and perennial grasses such as crabgrass (*Digitaria* spp.), goosegrass (*Eleusine indica*), bahiagrass (*Paspalum notatum*) and bermudagrass (*Cynodon dactylon*), are some of the most common and troublesome weeds in pecans (Southern Weed Science Society, 2006). Other species commonly found in pecan orchards include nutsedge (*Cyperus* species), horsenettle (*Solanum* species), arrowleaf sida (*Sida rhombifolia*), spurges (*Euphorbia* species), horseweed (*Conyza canadensis*), and pigweed (*Amaranthus* species) Colour pictures and descriptions are found in the *Weed Identification Guide* published by the Southern Weed Science Society (2007).

Numerous weed-competition studies have shown the adverse effects of weeds on agronomic, vegetable, and fruit crops (Zimdahl, 2004). These studies generally document the effects of individual weed species on the growth and/or yield of a crop. Density studies have determined the effect of increasing weed density in season-long competition with the crop. Period of competition studies have determined the length of time the crop can tolerate competition from a specific weed at a specific density without incurring yield loss; or conversely, how long the crop must be maintained free of this weed before it can produce an optimum crop. Different types of information can be obtained with these studies. The critical period of weed control (CPWC) has been defined by Knezevic, Evans, Blankenship, Van Acker, and Lindquist (2002), as the time interval between two measured crop-weed competition components: (i) the maximum amount of time early-season weed competition can be tolerated by the crop before a yield loss occurs, and (ii) the weed-free period required from planting to obtain optimum yield (independent of other confounding factors, i.e. late season drought, insects, diseases, etc.). This period has been shown to vary from zero up to several weeks in work done with annual crops and weeds (Knezevic et al., 2002). In layman terms this answers the questions; how long must I maintain weed control in the crop to obtain optimum growth and yield, or how late can I wait to initiate and maintain weed control without incurring yield loss? Because most of the weed-competition studies were conducted using annual crops grown in narrow spacings (< 1 m) competing with annual weeds, weed control measures were generally applied on a "broadcast" basis. This is generally not the case with weed management in pecan orchards.

Current weed management in pecan orchards involves weed control within a narrow strip centered on the pecan tree row. Also, many of the most troublesome weeds in pecan orchards are perennials. The CPWC has never been determined for pecan and would probably take several years research to determine. Smith (1999) determined that substantial reduction in growth of pecan trees occurred by weed competition from cutleaf eveningprimrose (*Oenothera laciniata*), a cool-season species, implying that the presence of weeds results in reduced growth of pecans even in cool months.

Although several methods of orchard floor management have been used in the past, including mowing and cultivation (Ark, 1954), the use of registered herbicides is currently the most common method used by far (Alabama Cooperative Extension System, 2007; Georgia Cooperative Extension Service, 2007; Smith & Carroll, 2004). Herbicidal active ingredients used to maintain weed control in pecan orchards include oryzalin, pendimethalin, diuron, simazine, norflurazon, fluazifop, sethoxydim, clethodim, flumioxazin, halosulfuron, paraquat, glufosinate, glyphosate, and carfentrazone. These herbicides can be used at different times and for specific weeds in orchard floor management. Some are restricted to non-bearing trees only, and some to trees established at least 2–3 years in the orchard. Most orchard floor management programs use a combination of these products to maintain season-long control in a weed-free strip centered on the tree row. Combinations of registered herbicides used in both preemergence and postemergence programs can provide optimum growth and yield (Faircloth, Patterson, Foshee, Nesbitt, & Goff, 2007).

The area between tree rows is traditionally maintained in grass sod to facilitate movement of spray equipment that is used for fungicide and insecticide applications during the growing season. Consequently, this is known as the "sod-strip" method of orchard floor management. Maintaining a clean (bare ground) strip in the tree row also facilitates harvest in the fall since nuts can be blown away from the trees into the sod strips for pickup by harvesting equipment.

There is also some use of herbicide in subduing unwanted vegetation in the traffic lanes where equipment runs between the tree rows. If there is a sod middle, "chemical mowing" with low rates of glyphosate is a common practice to reduce excess growth and reduce the need to mow (Alabama Cooperative Extension System, 2007; Georgia Cooperative Extension Service, 2007). Low rates of glyphosate are also applied just prior to harvest to reduce regrowth that might interfere with harvest, as mowing once nuts are on the ground is no longer an option. A recent practice has been the use of rolling wiper applicators with glyphosate to remove tall weeds from low-growing desirable plants like "Durana" white clover.

Table 3. Yields of pecans were significantly influenced by weed control in irrigation and nonirrigated, Fairhope, Alabama USA (Patterson & Goff, 1993).

Irrigation and weed control program	*Yield (kg/ha) in indicated year(s)*[a] *1990*	*1991*	*1992*	*Cumulative*
Irrigated				
Total weed control w/ herbicides	254	436	1,628	2,318
Disking	262	454	1,316	2,032
Mowing	11	57	430	496
Grass control w/ selective herbicides	49	137	545	729
No weed control	27	86	308	421
Mean value for irrigated trees	121	233	846	1200
LSD ($P<0.05$) Irrigated	143	129	445	664
Nonirrigated				
Total weed control w/ herbicides	315	286	1,490	2,090
Disking	94	162	762	1,018
Mowing	53	91	584	745
Grass control w/ selective herbicides	116	75	395	587
No weed control	49	41	277	367
Mean value for nonirrigated trees	125	131	701	961
LSD ($P<0.05$) Nonirrigated[b]	NS	56	NS	NS

[a] Yield values were measured in November each year.

[b] NS indicates that the LSD test found no significant differences between yields in the weed control programs in the nonirrrigated trees in 1990, 1992 or cumulative yield.

Research has shown that uncontrolled weeds around newly planted pecan trees can decrease growth and nut yield significantly (Smith et al., 2005; Patterson, Wehtje, & Goff, 1990; Patterson & Goff, 1993). Newly planted "Desirable" pecans without weed control growing in both irrigated and not irrigated situations produced only 18.2 and 17.5% of the yield, respectively, that was produced by trees where total weed control was maintained over the first 8 years of growth (Table 3). This research also shows that mowing around newly planted trees, while controlling annual broadleaf weeds, allowed perennial grasses like bahiagrass to grow, thus robbing the young trees of fertilizer and moisture. Trees in the study that were mowed only as a means of managing the orchard floor produced 35.6 and 21.4% of the yield (irrigated and not irrigated respectively) produced by trees where total weed control was maintained using registered herbicides. This research was conducted using a treated area of 3 m(dia), centered on the tree row. The 3 m treated area was arbitrarily selected for this study. Additional research conducted following this initial study shows that weed-free areas of 3 m(dia) or greater maintained from planting provided optimum growth and yield while areas of 1.8 m (dia) or less reduced growth and eventual yield in the first 2 years of nut bearing (Patterson et al., 1990). Although the significant and detrimental impact of weeds on pecan growth and yield has been documented, weeds can also serve as a host for insect pests (Norris & Kogan, 2005), and negatively influence concentrations of nutrients in young pecan trees (Goff, Patterson, & West, 1991).

3. BENEFITS OF CRIMSON CLOVER AND LEGUMES USE IN PECAN ORCHARDS

In the last 30 years, growers have started an integrated approach to weed control by seeding the orchard with various cool season legumes. These are grown as intercrops in the mowed strip or as cover crops over the entire orchard floor. The intercrop and cover crop plants supply nitrogen, suppress weeds, improve the soil, enhance beneficial insects and benefit wildlife. For centuries, legumes have been used to enhance the fertility and structure of agricultural soils. Historical references as far back as the Roman Empire refer to the benefits these plants can provide. Cato the Elder (234–149 B.C.) suggested improvements to poor vineyard land by inter-planting a legume crop. This crop was then turned under before the plants set seed. The Chinese have also used legumes to maintain soil fertility through centuries of cultivation. Legume use was introduced to pecan orchard management in the early twentieth century. Pecan producers quickly realized the benefits of such plants as blue lupine, vetch, and crimson clover as winter cover crops for the orchard.

Proper orchard management occurs at two separate, yet connected levels. The orchard floor influences the tree crop and its management based on the type of vegetation or lack thereof found in the orchard. An efficient orchard floor cover does not compete heavily with trees for moisture and nutrients and is compatible with orchard insect populations. Weed competition with tree roots is significant throughout the life of the tree. In a newly planted orchard, weed competition can significantly reduce young tree survival and can stunt tree growth (Patterson, 2005a, 2005b). In the case of the mature orchard, vegetative competition can rob the soil of water and nutrients intended for the crop, reducing tree growth and yield, while also promoting alternate bearing. Pecan orchards generally consist of sod culture on the orchard floor with a weed-free herbicide strip approximately 2–4 m wide along the tree row. Row middles are maintained by mechanical mowing or by chemical mowing. The mowed sod middle improves wet weather passage for spraying, harvesting, and other orchard operations.

Pecan trees have an extensive root system, consisting of a tap root which can penetrate as deep as the soil structure and water table permit, and small feeder roots located near the soil surface. While the deeper roots are an aid to survival of the tree in difficult environmental conditions, the feeder roots in the upper 6–18 in. of soil supply the bulk of the pecan's nutritional needs and come into direct competition with vegetation on the orchard floor.

Cool season legumes, such as crimson clover, posses a variety of characteristics that make them compatible with pecan production. Cool season legumes are not especially competitive with trees for soil moisture until mid to late spring. They also serve as an effective source of organically bound nitrogen (N). In addition, cool season legumes stimulate an early increase in beneficial insect populations. The environmental benefits of cool season legumes make their use an especially attractive practice. In addition to the benefits mentioned above, legumes can reduce weed competition, aid in the cycling of nutrients, build soil organic matter, prevent soil erosion and runoff, and serve as an effective source of food and habitat for a variety of wildlife species.

3.1. Pecan Nitrogen Use and Compatibility with Crimson Clover

Prior to World War II, legumes were commonly used as an orchard floor cover due to their N-fixing properties. The arrival of cheap fertilizer sources and mowed sod culture in the orchard led to a decline in the use of legumes as a floor cover in later years (White, Beaty, & Tedders, 1981).

Of all nutrients applied to pecans, N most commonly limits pecan growth and orchard profitability. This element has a dominant influence on vegetative growth and crop production. In recent years, the rising cost of fuel, and its effect upon synthetic fertilizer production, has once again led to a need for alternative sources of N in many orchard operations. Leguminous plants are one very efficient source of organic N.

All legumes, including crimson clover, utilize soil-dwelling bacteria that convert nitrogen from the air into a form that can be used by plants. This is termed "nitrogen fixation". Only particular strains of bacteria provide optimum N production for each group of legumes. When the roots of a leguminous plant come into contact with the appropriate bacteria, the root hairs encircle the bacteria to create a nodule which houses the bacteria. These lumps on the root surface may range in size from a BB to a kernel of corn.

Perennial legumes "fix" N during any time of active growth, usually peaking at flowering. With seed formation, N fixation ceases and the nodules slough from the roots. While they are alive, legumes release little to no nitrogen from the soil. As they die and are decomposed by soil micro-organisms, the N in the roots, stalks, leaves, and seeds are converted to a form available to other plants.

Clover and other legumes are capable of supplying significant amount of N to orchard soils. Studies in Oklahoma suggest that a mixture of legumes including crimson clover, hairy vetch, red clover, and white clover planted to the orchard floor provided over 100 kg of N/ha. In the same study, pecan leaf N concentrations were maintained above a threshold of 2.25% by the above mixture, as well as by crimson clover alone Blue lupine can fix as much as 168 kg of N/ha, while common vetch is capable of fixing 92 kg of N (Smith, Shiferaw, & Rice, 1996).

Pecans are an “irregular bearing” crop, meaning that they tend to bear heavy crop loads for 1–2 years, followed by very light crop loads, depending upon various physiological and environmental factors. A general rule of thumb for the N requirement of pecan trees is 4.5 kg of N for every 45.4 kg of expected crop (Wells, 2007). Depending upon the degree of irregular bearing, mature pecan trees in the Southeastern USs may require from 78.5 to 168.1 kg of N annually for optimum production.

Nitrogen uptake in the pecan tree is driven by demand. There are two critical periods of nitrogen demand during the season the first at early foliage growth and the second at kernel filling. The early spring foliage flush is nourished primarily from reserves held within the tree, while the nitrogen demand during the kernel fill stage is usually satisfied from soil uptake. If N is limited at kernel filling, then the tree will mobilize N from the foliage to the kernels.

Studies have suggested that crimson clover over-winters dependably in the southeastern US and much of the US pecan belt. A vigorous stand of crimson clover will contribute between 78.5 and 168.1 kg of N/ha. When aided by moisture and warm weather to speed up decomposition, up to half the N available from legumes can be released within 7–8 weeks. In the light crop or “off” years, the N supplied by the clover alone, would be adequate for optimum production. Heavy crop or “on” years may require low supplemental N rates in order to bring marketable nuts to maturity and provide a return crop the following year.

3.2. Enhancement of Pest Management

Conserving and encouraging beneficial organisms is key to achieving sustainable pest management. The deep red blossoms of crimson clover attract various species of bees, which feed readily on the abundant nectar. In addition, blooms may harbor beneficial insects such as the minute pirate bug. Pea aphids and blue alfalfa aphids are commonly associated with crimson clover. Although these species are not pests of pecan, they serve as alternative food sources for beneficial predators such as ladybeetles, green lacewings, soldier beetles, predaceous stink bugs, damsel bugs, and hover flies. As the clover declines with the onset of warm weather in June, these beneficial insects move into the trees to feed on pecan aphids and other insect pests, reducing the need for insecticide application.

In addition to reducing insecticide inputs through enhancing beneficial insect populations, the use of legumes in the orchard can also reduce herbicide use. As crimson clover grows, it forms a thick, living mulch. This helps to smother and shade out more troublesome, competitive weed species.

3.3. Soil Building and Sustainability

Clover can improve orchard soils in a number of ways. Protection against erosion is the most obvious benefit, but providing organic matter is an equally important, and more long term goal. Clover can provide habitat and/or food source for important soil organisms, break up compacted soil layers, and help dry out wet soils.

Erosion deprives orchards of topsoil, the most fertile portion of soil with the highest amount of organic matter. When soil particles are dislodged by rainfall, they are more vulnerable to runoff. Cool season legumes can reduce the impact of rainfall on bare ground, slow the action of moving water, increase the soil's ability to absorb and hold water, and help stabilize soil particles. Crimson clover produces more dry matter (6,237–6,683 kg/ha) than many other legumes and is recommended for soil erosion control because of its high early autumn dry matter production. Grass/crimson clover mixtures combine fibrous surface roots with long tap roots and have been observed to reduce herbicide runoff by 94–100%.

As the soil–plant–atmosphere continuum cycles plant nutrients, some are recovered via plant uptake, some are incorporated into organic matter, some are adsorbed to mineral and organic surfaces, and some are precipitated as solid minerals. Excess nutrients are "lost" or removed from the field by runoff or subsurface flow, potentially ending up in water supplies. Nutrients such as N, that are the most water soluble and mobile in the soil, have the greatest potential for the pollution of ground and surface water (Havlin, Beaton, Tisdale, & Nelson, 2005).

Over-fertilization is common in orchard crops because most growers rely on synthetic fertilizers. The amount of nitrogen removed by the crop in proportion to that applied is often far less with tree crops than with more traditional crops. Therefore, the leaching of nitrates into groundwater may be especially serious in orchard crops (Weinbaum, Johnson, & Dejong, 1992). Wiedenfeld, Fenn, Miyamoto, Swietlik, and Marlene (1999) suggested that sod alone on the orchard floor does not sufficiently reduce nitrate leaching. Green manures like cool-season legumes reduce the need for N application and aid in nutrient conservation by utilizing excess fertilizer not assimilated by the pecan trees, preventing undesirable nutrient levels in streams or lakes.

Winter cover crops such as crimson clover grow primarily during a period of tree dormancy, when N uptake by the crop is at a minimum and percolation from rainfall is often the greatest. Although pecan growers have historically had little economic incentive to grow cool season legumes solely to prevent nitrate leaching, it is one of the many benefits they provide.

Orchard floor covers can affect nitrogen uptake by the main tree crop if C:N ratios for the floor cover are high. In such cases, the micro-organisms responsible for decomposition of the highly carbonaceous plant matter require nitrogen to do so, and can lead to the unavailability of N for the main crop (Brady, 1974). Residue from a grass/legume mix has a higher C:N ratio than the legume alone. However, under humid conditions, the C:N ratio of such a mix is less than sod alone. In fact, ratios for the grass/legume mix are such that the release of N is slow, a scenario in which many perennial tree crops perform well. Slow release of nutrients also causes nutrients in the orchard soil to be less vulnerable to loss.

Soil organic matter is composed of partially decayed and partially synthesized plant and animal residues. Although, the organic matter content of a mineral soil is generally only about 3–5%, its influence on soil properties and plant growth are great.

Due to the work of soil micro-organisms, organic matter should be constantly renewed by the addition of plant residues. Legumes such as crimson clover break down quickly; however their root systems remain tough and fibrous, contributing to the accumulation of organic matter. The addition of organic matter to soils improves soil structure, increases water holding capacity, increases cation exchange capacity (the ability of the soil to act as a short term storage bank for positively charged plant nutrients), and provides more efficient storage of nutrients.

Organic matter functions as a "granulator" of soil mineral particles. In most cases, the higher the soil organic matter, the more loose, easily managed, and productive the soil. Organic matter can also serve as a partial source of N, P and S. Through its effect on the physical condition of the soil, organic matter can increase the ability of the soil to hold moisture and make soil water more available for plant growth.

Cation exchange is one of the most common and important of soil reactions. The cation exchange capacity (CEC) of a soil represents the capacity of the soil to hold cation, or positively-charged nutrients such as Ca^{+2}, Mg^{+2}, K^{+}, and NH_4^{+}. The CEC is determined by the amount of clay or organic matter present in the soil. Soils with a higher clay and organic matter content have a higher cation exchange capacity than sandy, low organic matter soils. Hydrogen ions from the root hairs and soil microorganisms replace nutrient cations from the exchange complex. These nutrient cations are then forced into the soil solution, where they can be more readily assimilated by the root surface (Brady, 1974). Due to their effects on improving soil organic matter, legumes can aid in this process.

Legumes help to increase the total number and diversity of soil organisms, which is the key to a healthy, well functioning soil. As organic matter increases, especially if succulent and subject to relatively rapid decay, it encourages microbial action of the heterotrophic organisms responsible for basic decomposition, as well as "free-living" bacteria, such as *Azobacter*, which can also fix N from the atmosphere.

Legumes are closely associated with beneficial fungi, the mycorrhizae, which produce a water-insoluble protein known as glomalin, which binds and glues together particles of organic matter, plant cells, bacteria, and other fungi.

Well aggregated soils are less prone to compaction. Heavy farm implements such as tractors, sprayers, mowers, shakers, and harvesters often make numerous passes over the orchard floor in a given season. Mycorrhizal fungi also have an efficient method of absorbing phosphorous (P) from the soil, which they pass on to their host. Without this relationship, P builds up in the soil. Although it is not leached, it can runoff into streams and rivers through soil erosion. The filaments of the mycorrhizal fungi effectively extend the root system and help the plants tap more P from the soil. Keeping P in an organic form is the most efficient way to keep it cycling in the soil.

The culture of cool-season legume crops has both soil and nutrient conserving properties that are highly advantageous and readily applicable under most humid climatic conditions. In areas of low rainfall, the benefits of legumes will be limited. In such areas decomposition of the crop and its nutrient release may be too slow. Additionally, in such areas, moisture conservation is the primary factor governing soil and crop management. Thus, moisture should be conserved for the main crop. The residual effects of managing legumes as a cool-season cover crop in the orchard are also expected to be reduced on excessively sandy soils.

3.4. Wildlife Benefits

Due to the limited availability of suitable nesting areas in the United States for early-successional songbirds, converting a common attribute of the southern agricultural landscape, such as pecan orchards, to a resource for food and reproductive habitat would provide some of the factors necessary to increase survival and nesting success. Increased abundance of birds and beneficial arthropods and higher species richness in agricultural fields have been linked to habitat heterogeneity (Freemark & Kirk, 2001).

The sod/clover orchard floor mixture along with the pecan overstory allows for an increased landscape heterogeneity. Birds benefit from an increase in prey, an increase in cover for nesting sites and fledgelings, and a reduction in nest loss due to the elimination of mowing during the nesting season (Best, Whitmore, & Booth, 1990; Rodenhouse, Best, O'Connor, & Bollinger, 1993). In addition to enhancing the landscape for non-game songbirds, as well as bobwhite quail, wild turkey, and mourning dove, cool-season legumes provide high quality forage for whitetail deer during the late winter when other food sources have dwindled (Rodenhouse, Best, O'Connor, & Bollinger, 1995).

Clover has been shown to be highly effective at attracting high avian and arthropod densities, increasing wildlife and agronomic benefits compared with conventional management of agricultural systems (Cedarbaum, Carroll, & Cooper, 2004; Warburton & Klimstra, 1984). The reduction of input in the clover system, coupled with its agricultural and environmental benefits, makes this system both a good choice for reducing negative impacts on wildlife and surrounding ecosystems, and for reducing input costs.

3.5. Establishment and Maintenance

In order to establish an adequate stand, crimson clover should be drilled at 16.7–20 kg/ha or broadcast at 22.4–3.6 kg/ha. Establishment should be completed as soon as possible following pecan harvest, preferably in November–December. If clover is seeded prior to harvest, many seeds are removed from the orchard floor with the sweeping and harvesting process. Since legumes require the presence of rhizobial bacteria to effectively fix N, it is important to obtain the correct rhizobial inoculant for the legume being grown. Fresh inoculant and a sticking agent should be mixed with the seed. Otherwise, there will be few nodules and N fixation will be low. Soil pH should be maintained at 6.5 because *Rhizobia* bacteria cannot function properly under highly acidic conditions and will die in soils with pH below 5.0.

Many southeastern orchards have well established populations of annual ryegrass. Where this occurs, the ryegrass often competes with and inhibits growth of clover. This can be prevented by the application of a low rate (1.12 kg/ha) of sethoxydim herbicide in February. The rye grass does not have to be eliminated, only stunted by this application in order to release clover from this competition.

By foregoing mowing of the orchard until clover has gone to seed, producers can take advantage of crimson clover's excellent natural re-seeding ability. This will allow a period of 3–5 years before clover will need to be re-seeded by the grower. Sandy sites will need to be re-seeded sooner than loamy or clay soils, therefore soil type should be taken into consideration when estimating the need for re-establishment.

3.6. Costs and Savings

The approximate cost of crimson clover seed and bacterial inoculum required to produce N-fixing nodules is approximately \$99/ha. At 2005 N prices of \$178/ha, this represents a difference of \$79/ha. Clover can replace from 50 to 100% of synthetic N applied to pecan orchards, depending on the pecan crop load in a given year. This would save growers approximately \$89–178/ha and significantly reduce the grower's reliance on synthetic N. Reduced maintenance and mowing compared to grass covers creates further economic savings and reduces fuel consumption.

Pecan acreage is widely dispersed and hard to track, but reliable estimates indicate that pecans are grown on approximately 550,000 acres in the US, with Georgia making up about ¼ of the total USA acreage. This would indicate a potential savings of approximately \$19,800,000–39,600,000 in N costs, as well as a 41,250 t reduction in the use of synthetic N nationwide with the use of N-fixing legume culture applied to orchard floor management.

One of farming's greatest challenges is to keep N in a stable, storable form until needed by the crop. The use of organic N, such as that produced by legumes, is an ideal way to accomplish this. Combining warm season sod culture and cool season legumes is a practical and effective strategy for conserving and supplying nitrogen for orchard crops, as well as enhancing stewardship of the environment through the enhancement of soil sustainability, beneficial insects, weed suppression, and wildlife.

4. INTEGRATED PEST MANAGEMENT IN PECAN ORCHARDS

Research indicates that biological control is effective with introduced biological control agents against pecan aphids (Tedders, Weaver, & Wehunt, 1973; Tedders, Reilly, Wood, Morrison, & Lofgren, 1990; Tedders & Schaefer, 1994; Mizell, 1984), pecan weevil (Dutcher & Sheppard, 1983; Shapiro-Ilan, 2001; Shapiro-Ilan, Cottrell, & Gardner, 2004)), and pecan leaf scorch mite (Dutcher et al., 2006). Two biological controls that have been readily adopted by pecan growers are the enhancement of aphidophagous insects with orchard floor management and reductions in pecan weevil sprays.

Orchard floor management techniques may improve biological control of pecan aphids. In improved orchards, tree density typically ranges from 12 to 60 trees per hectare. An orchard floor with mowed sod and herbicide strips in tree rows is excellent for harvesting the nuts but has low vegetational diversity (Dutcher, 1993). Natural enemies of aphids and other pecan insect pests are more abundant when the orchard floor is sown with plants that provide alternate prey, nectar, and pollen when pest populations are low. Orchards may require 15–30 trips by heavy machinery each year for all production and harvesting operations, leading to soil compaction (Bugg, Sarrantonio, Dutcher, & Phatak, 1991). Mowing can be reduced substantially by replacing turf grasses with clover (Rice, 1994). Previous studies have shown increased diversity and abundance of native aphid-feeding insects (aphidophaga) with alternative groundcover management in pecan Rice et al. (1998) found clover plus vetch to harbor significantly larger populations of ladybeetles (Family: Coccinellidae) than turf grass cover. Significantly higher populations of ladybeetles were found in various cool (Bugg, Dutcher, & McNeill, 1990) and warm-season (Bugg & Dutcher, 1989) covers. Unfortunately in both cases biological control of pecan aphids did not increase significantly. Multicolored Asian ladybeetle, *Harmonia axyridis* (Pallas), has been successfully introduced and established into the pecan belt since the above experiments were conducted. Multicolored Asian ladybeetle is a much more effective predator on pecan aphids than native ladybeetles. Even though Rice et al. (1998) did not find a significant increase in biological control overall in groundcover-enhanced orchards, they did observe an increase in one isolated orchard where multicolored Asian ladybeetle had recently become established.

Culturing ladybeetles on alternate prey aphids on crapemyrtle plants grown on the orchard floor until pecan aphids become more abundant may be effective (Mizell & Knox, 1993). *Crapemyrtle aphids* is a good candidate as an alternate prey for aphidophaga in the pecan system because they peak on crapemyrtle plants about 2 weeks before pecan aphids. This is ideal timing to build up aphidophaga before the expected peak in pecan aphids. In addition, crapemyrtle aphid and black pecan aphid are taxonomically similar and are attacked by the same natural enemies (Mizell & Schiffhauer, 1987). Intercrops sown away from the herbicide strip and in the mowed sod compete with grasses and not the trees and have additional benefits. Combinations of red and white clovers or clover plus vetch provide adequate nitrogen for pecan (Smith et al., 1996; Diver & Ames, 2000). Supplemental nitrogen fertilizer is effective in maintaining leaf nitrogen concentration at a healthy level when it is applied to the herbicide strip or through the irrigation system (Worley, 1994).

Native and introduced natural enemies of the black pecan aphids do not reduce aphid populations with sufficient speed to prevent serious damage. The population dynamics are well understood (Kaakeh & Dutcher, 1992). Early warning scouting techniques are used to measure abundance, population distribution, and predict outbreaks (Dutcher & Kaakeh, 1992). Black pecan aphid feeding leads quickly to leaflet abscission (Wood, Tedders, & Thompson, 1985; Tedders & Wood, 1985; Tedders, 1978), and control is achieved by quickly resorting to chemical control whenever black pecan aphid abundance exceeds one aphid per compound leaf (Dutcher, 1983; Dutcher & Htay, 1985).

Secondary predators interact with biological controls and these can enhance or hinder control effectiveness. For example, insects are a major source of nutrition for red imported fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). These ants are important predators of pests including southern green stink bug (Krispyn & Todd, 1982), cowpea curculio (Russell, 1981) and pecan weevil (Dutcher & Sheppard, 1983). Pecan weevil larval populations are consistently reduced by 33% after the larvae drop to the soil surface from the pecans and before they burrow into the ground (Dutcher & Sheppard, 1983). Red imported fire ants also interact with aphids and aphidophaga in the pecan trees (Tedders et al., 1990). Ant foraging can be partitioned with insecticide barriers sprayed on the tree trunks so that red imported fire ants will remain on the soil surface to prey on weevils and not interfere with aphidophagous insects in the trees (Dutcher, Estes, & Dutcher, 1999). The effect of these trunk sprays has been effective (Dutcher, 2004; Dutcher et al., 1999) and ineffective in reducing aphid populations (Harris et al., 2003). Red imported fire ants can tunnel under the insecticide barrier without becoming intoxicated and produce a trail to the tree crown, esp. on older trees with heavy bark. Farnesol, an ant repellent that form an impassable odor plume around the trunk successfully prevents Argentine ants from foraging in citrus trees (Shorey, Gaston, Gerber, Sisk, & Phillips, 1996). Current research has found that farnesol was not an effective repellent of ants on pecan trees but certain plant extracts (neem extract, sesbania extract), natural compounds (methyl anthranilate, methyl myristate) and an industrial repellent (methyl carbitol) are effective ant repellents that prevent foraging in pecan trees (Dutcher & Beaver, 2005).

Pecan weevil has a relatively long life cycle and lower reproductive capacity in comparison to other pecan insect and mite pests (Table 2). Growers may achieve control by integrating chemical control for adults biological control with soil application entomopathogens and entomophillic nematodes, and red imported fire ants with and removal of alternate host trees from the woodlots adjacent to the orchard to reduce immigration of adults into the orchard. Risk rating (Mizell, 1984) estimates the relative probability of pest outbreak based on all pertinent information that is known about a particular area and may be useful in integrating control methods for pecan weevil. Pecan cultivars and trees of different ages (sizes) differ considerably to the susceptibility to injury by pecan weevil (Worley & Mullinix, 1997). Certain pecan cultivars have a narrow window of susceptibility to pecan weevil oviposition with either a short kernel development time, or an early or late onset of kernel development (Harris, 1985). Weevil can be controlled in these cultivars with 2–3 applications of carbaryl compared to 4–5 applications for standard cultivars.

Precision applications of carbaryl to tree trunks and spot treatments in highly infested portions of the orchard stems may reduce nut damage in the pecan tree. Nut damage is higher in trees with higher densities of weevils emerging from the soil directly beneath the tree (Dutcher et al., 2003). In early replicated field trials, trunk sprays effectively killed adult weevils on the trunk for up 13 days after application of carbaryl (Cottrell & Wood, 2003). It has been estimated that 70–80% of the adult weevils fly to the trunk first (Raney & Eikenbary, 1968) and could thus be targeted for insecticide application to manage pecan weevils. This would reduce entire canopy sprays, which are known to be detrimental to natural enemies and flare aphid and mite populations (Dutcher & Payne, 1983). The trees in native groves have a unique nut phenology in each tree adding to the variability in pecan weevil distribution (Reid & Mulder, 2003). Pecan weevil distribution in the orchard can be estimated from tree-to-tree measurements of % nut damage, crop load, and known population parameters of the weevil (Harris, 1985) and then validated by extensive trapping of emerging adults with cone emergence traps from a known area of the soil surface (Raney, Eikenbary, & Flora, 1970). Accurate weevil distribution maps would allow the precision application of soil applied biocontrol agents and foliage and trunk sprays of carbaryl.

Significant risk of hemipteran kernel damage is associated with soybean plantings (and other alternate host plants) adjacent to the pecan orchards and the lack of a trap crop for monitoring and control of the hemipterans. Spot treatments of insecticide sprays to the trees in the first two border rows adjacent to the alternate host plants are effective in reducing stink bug damage (kernel spot) throughout the orchard. Legume trap crops between the trees and the alternate host plants are effective and have been used by pecan growers for reducing kernel spot. Even low black aphid populations on less susceptible cultivars cause significant leaf damage and defoliation. However, on less susceptible cultivars the onset of the outbreak is often several weeks later than on susceptible cultivars (Wood & Reilly, 1998).

Kernel spot is caused by several species of true bugs belonging to the families, Pentatomidae and Coreidae. Initially, kernel spot was thought to be caused by disease and treated as such until it was proven (Adair, 1927) that these conditions were caused by several species of kernel feeding hemipterans. The primary kernel feeding hemipteran pests of pecan include the southern green stink bug, *Nezara viridula*; green stink bug, *Acrosternum hilare*; brown stink bug, *Euschistus servus*; Dusky stink bug, *Euschistus tristigmus* and the leaffooted bugs, *Leptoglossus phyllopus* and *L. oppositus*. All of these insects are phytophagus and feed on a wide range of plants (McPherson & McPherson, 2000). Stinkbugs find crops such as cowpeas and soybeans more appealing than pecan trees when plots of these crops are planted near pecan orchards, gravitating toward the trap crop and away from the trees.

The primary challenges pecan producers face in managing these pests include the lack of economic thresholds to make management decisions, the long period of susceptibility to damage (nut set to harvest), the difficulty in scouting for damaging populations and the limitations on insecticide use near harvest. Leguminous trap crops, sown adjacent to seedling and improved pecan orchards, effectively and consistently reduce the incidence of kernel spot by 50% in improved pecan orchards (Smith, 1996, 1999). The trap crops are sprayed as the pods mature with an insecticide to kill the hemipterans before they enter the orchard (Coolman, 2003). Growing trap crops is beneficial for owners of small pecan orchards, as well as people who want to grow their pecan crops organically. Growing a trap crop around the orchard controls stink bugs without spraying the trees. Sunflower, sorghum and millet are also attractive to kernel-feeding hemipterans and produce seed that is attractive to hemipterans in the early fall at the same time as the pecans are susceptible to kernel spot. In native pecan groves, trap crops or broad scale application of pesticides to control stink bugs populations are not practical since livestock and poultry are part of the system. The mid-summer weeds in the groves are attractive to kernel-feeding hemipterans and the weeds provide a place for stink bug populations to develop within the grove itself and mowing of these weeds is an alternative control technique. Trap crops also provide excellent food for quail. Many pecan growers already plant small grains near the orchard border in the fall to feed wildlife and improve hunting, esp. for quail. A pecan grower in Texas achieved significant reductions in the incidence of kernel spot by plant a nontreated trap crop of black-eyed peas (United States Environmental Protection Agency, 2004).

Integrated pest management came to the forefront in pecan pest control after problems arose with pest resurgence, pesticide resistance, pest replacement, and an increase in virulence of the pecan scab fungus. These problems lead to outbreaks of aphids, mites, leafminers and pecan scab. Growers, researchers and extension specialists developed and implemented integrated pest management methods in attempts to solve many of these problems. Current pest management practices for pecan orchards are a genuine integration of various natural, cultural, biological and chemical control techniques. These include: monitoring crop load, orchard floor management, soil amendments, scouting pest populations, enhancement of on natural enemies, decision models, action thresholds, looking for pecan scab resistance in pecan cultivars, inoculative release of introduced insect and mite predators, planting intercrops, selective timing of chemical pesticide sprays and selective pesticides. Reducing broad spectrum insecticide spray frequency with improved monitoring techniques, weather models and assessments damage impact coupled with spot treatments has reduced the incidence of secondary pest resurgence after treatments for pecan weevil and kernel feeding hemipterans (Dutcher & Payne, 1983). Biological controls and biorational or selective insecticides are under development for other insect and mite problems are effectively controlled with (Dutcher et al., 2003).

Integrated pest management research and extension work (Dutcher et al., 2003) in the past 25 years has developed new methods that reduce the amount of insecticide used by 35% (Smith et al., 2002). The future of pecan integrated pest management lays in the development of reduced fungicide spray frequency in the southeastern USA during time periods of low humidity and leaf wetness and reduced soil compaction and herbicide use through orchard floor management. Integration of the tactics enhances aphidophagous insects, improves the soil, and reduces the production costs for the pecan grower. Above and beyond these improvements, integrated pest management in the pecan orchard is the only known strategy for avoidance of secondary pest resurgence, replacement of a primary pests, and pesticide resistance development. These phenomena can double the costs of pest control.

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7

LOCUST HABITAT MONITORING AND RISK ASSESSMENT USING REMOTE SENSING AND GIS TECHNOLOGIES

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Abstract. Locust outbreaks occur on all continents except Antarctica and can affect the livelihoods of one in 10 people on Earth. To prevent economic and environmental losses, locust breeding areas should be periodically monitored, and an early detection-early response strategy should be in place. Traditional, ground survey methods are inefficient to adequately address the large spatial scale of the locust problem. Remote Sensing and the associated geospatial technologies can provide timely data to assess the risk of impending locust outbreaks. This information could be used for targeted preventive management actions in the locust breeding areas. Remotely sensed data are used for monitoring habitats of certain species such as the Desert, Migratory and Australian Plague locusts. However, the vast potential of this technology remains untapped for other locusts. This chapter provides a review of remote sensing and GIS concepts, types of data collected by various remote sensing satellites, and applications of geospatial tools for locust habitat monitoring and risk assessment.

1. INTRODUCTION

Locust outbreaks and subsequent destruction of vegetation result in ecological, environmental and economic problems. Locust swarms can devour green vegetation, including agricultural crops, across large geographic areas thereby upsetting the ecological processes (e.g. carbon and water cycles) of the region or any landscape. Rapid loss in vegetation cover can result in soil erosion and increased run off. Crop damages could result in catastrophic losses to farmers, and this problem could be acute for small, subsistence farmers throughout the world and especially for those in developing countries. Furthermore, locust control efforts, which involve large-scale applications of broad-spectrum insecticides, can produce negative impact on the environment and continue to be very costly, even in the twenty-first century.

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In 2003–2005 a Desert locust outbreak affected 8 million people mostly in Africa, with estimated damage to crops at 80–100% (Brader et al., 2006). To combat the outbreak, 13 million ha were treated with neurotoxins in 26 countries. The cost of the international campaign, including the food aid to affected populations, amounted to half a billion US dollars (Belayneh, 2005).

In order to protect the farmers and the environment from such catastrophes, several national and international agencies and organizations are involved in a host of prevention and control activities. These activities are aimed at either minimizing the large-scale locust plagues or at quickly containing them following an initial outbreak. Since the geographic area involved is often large (across national boundaries), coordination is required in the form of information exchange on the status of locust swarm distribution and damage. In most countries information on locust nymphal development and swarm formation is collected through ground-based surveys. Data collected by field surveys are reported to the national locust control units, which then share them with other national and international agencies. To assess locust risks and develop preventive measures data on land cover habitat condition are required. Vegetation represents the essential component of the locust habitat, providing the insects with nutrition and shelter.

Under the preventive mode, locust control specialists also need information on elevation (or topography), soil moisture, temperature and rainfall, in addition to the vegetation type, status and growth. Specialists use this information to set up effective surveys to assess locust egg-pod or nymphal distribution. During an outbreak, near real-time data on vegetation damage, hopper band and swarm movement will be essential for assessing risks and prioritizing areas for curative treatments. Under either circumstances reliable methods are necessary for collecting information on vegetation status or assessing the damage to the native vegetation or crops resulting from locust outbreaks. Traditional, ground-based survey methods are inadequate to provide accurate and timely information about an ongoing locust outbreak and devise efficient management approaches, since the locust hopper bands move several km and swarms can travel up to 200 km in a day (Uvarov, 1977).

Remote sensing technology can provide necessary data for assessing locust outbreak threats and post-outbreak damage. Remote sensing technology is a means to rapidly collect information on vegetation and earth surface conditions for relatively large geographic areas. These data are routinely used for assessing the status of land and natural resources or for assessing the magnitude of events such as wildfires and hurricanes. Satellite images were used extensively to coordinate the rescue and recovery efforts following the tragic devastation of the South Asian Tsunami in 2004 (Kumar, Chingkhei, & Dolendro, 2007; Wikantika, Sinaga, Hadi, & Darmawan, 2007). Satellites can download these data rapidly to receiving stations on the ground enabling the users to visualize the data in near-real time. Currently, there are remote sensing satellites that collect data for the entire earth every 2 days. In other words, one can monitor the status of any location on the earth's surface every 2 days. Advances in computing hardware and software have enabled sophisticated processing of large volumes of data that was not possible until a few years ago. Through the use of such data, information on earth surface conditions can be updated more frequently, in comparison to the traditional survey methods.

Image data collected by the satellites can be incorporated with other types of information (roads, rivers, villages and administrative boundaries) in a geographic information system (GIS). Using a GIS one could generate a map showing potential areas of interest that are within a specified distance from major roads. This information could be used for targeted locust field surveys or to identify the extent of areas that cannot be easily accessed. Also using the information on administrative boundaries it is possible to generate summary maps showing the extent of infestation and damage, per administrative unit. Such information could be generated in the form of both maps and reports more easily and quickly in comparison to the traditional cartographic methods. In the US, wildfire maps are updated daily using satellite data to enable the firefighters to assess the extent and direction of the wildfires (Keane, Burgan, & Van Wagtendonk, 2001; Hessburg, Reynolds, Keane, James, & Salter, 2007). Also, the Food and Agriculture Organization of the United Nations (FAO) uses GIS extensively to map the land conditions in Africa and generate monthly Desert locust information bulletins (see http://www.fao.org/ag/locusts/en/info/info/index.html).

However, remote sensing technology is not capable of addressing all the information needs of the locust control specialists. Its potential is oversold across several applications resulting in bad reputation among users (Wynne & Carter, 1997). The process of converting data to useful information is rigorous and requires substantial training and knowledge on the part of an image analyst. These skills are critical to successfully extract information off of the images. Nevertheless, several of these risks could be addressed by careful planning and adequate training of the analysts in the use of image processing for information extraction. If implemented correctly, remotely sensed and GIS technologies can provide essential information for managing locust problems worldwide.

2. REMOTE SENSING, GEOGRAPHIC INFORMATION SYSTEMS (GIS), AND GLOBAL POSITIONING SYSTEMS (GPS)

2.1. Remote Sensing

Remote sensing is defined as the science and art of making observations and measurements about objects without coming into physical contact (Campbell, 2006). For example, a human eye remotely senses or “sees” by responding to the radiation emanating from the surrounding objects without any physical contact. All objects above absolute zero (0 K or –373°C) emit electromagnetic radiation and also interact with the incoming solar radiation. When solar radiation comes in contact with an object it can be reflected, transmitted or absorbed to be re-emitted and the pattern of this interaction is unique for each object, which is referred as the spectral signature for that object. For example, green leaves absorb radiation in the blue and red regions but emit in the green region, hence they appear green to human eyes. Changes in the leaf chlorophyll content alter the reflectance pattern which results in different colors of the leaves to human eyes.

Table 1. Spectral ranges in the electromagnetic spectrum (in nm unless otherwise specified).

Spectral range	*Name*
<0.03	Gamma rays
0.03–300	X-rays
300–380	Ultraviolet
400–500	Blue
500–600	Green
600–700	Red
700–1,300	Near infrared
1,300–3,000	Mid infrared
7,000–10,000	Far infrared
1 mm–30 cm	Microwave

Human eyes are sensitive to a narrow region (400–700 nm) of the electromagnetic spectrum (Table 1) referred to as the visible region. All objects interact with electromagnetic radiation in other regions, but human eyes cannot see or sense beyond the visible region. Healthy vegetation, for example, has higher reflectance in the near infrared region than in the green region which can be related to the leaf cell structure (Jensen, 2006). Water content in the leaves influences the amount of reflected radiation in the mid infrared region. Similarly, bare ground, water bodies and other features on the earth surface have their unique reflectance patterns or signatures, outside of the visible region.

In remotely sensed images one would analyze these signatures, relate them to various features on the earth surface and map them. It is possible to distinguish healthy vegetation from plants that are affected by disease, drought or forms of stress based on the amount of infrared reflection. Remotely sensed data can be used to map vegetation distribution and estimate its density. Geologists use remotely sensed data to identify soil types and minerals. Extent and severity of damages to earth surface features following natural catastrophes such as hurricanes, landslides, and wildfires can be also mapped with remotely sensed data.

Chemical (films) or digital (cameras and scanners) sensors are used for recording the interaction of objects or features with electromagnetic radiation in different regions. Chemical-based films or precisely calibrated digital sensors can record values that are proportional to the amount of reflected or emitted radiation by various objects on the earth surface, which can be printed in a photographic film or displayed on a computer monitor for further analyses.

Remote sensing is categorized as passive or active based on the source of the electromagnetic radiation. In passive remote sensing, sensors record the radiation emitted or reflected by earth surface features. For example, when data are recorded in the form of images in bright sunlight (or outdoors) without any other source of radiation it is termed as passive remote sensing. Black & white photos have been in use for more than a century and extensively in World War II to identify and destroy enemy targets. Color infrared photos were acquired since mid-1950s and have found widespread use in vegetation mapping and monitoring (Jenson, 2006). Digital sensors, mounted in satellites, aerial platforms and space shuttles, are used more frequently for collecting remotely sensed data. Digital data are available in ready-to-use formats and can be processed more efficiently than hard copy aerial photographs. Availability of these data in digital format coupled with the advances in computing technology, have resulted in increased use of remote sensing for numerous applications.

Active remote sensing systems use their own source of electromagnetic radiation that is targeted towards various objects and records the interacted information that reaches the sensor. If one uses a flash light in the camera to illuminate indoor objects for recording images, this is active remote sensing. Examples of active remote sensing data include RADAR and LIDAR images that are acquired by targeting electromagnetic radiation in specific regions on various features and recording their interaction pattern. Active remote sensing technology provides opportunities for acquiring images during night time or cloudy days. Both RADAR and LIDAR can penetrate through the top of the vegetation canopy, which is not possible in passive remote sensing, enabling vertical characterization of forests and other features. RADAR can also penetrate soil, hence it is used in archeology for locating and mapping large hidden structures and artifacts.

Utility of remotely sensed data is influenced by its spatial, spectral, temporal and radiometric resolutions among others. *Spatial* resolution determines the size of the smallest feature that can be identified in an image. No standard rules exist to categorize remotely sensed data based on its spatial resolution, however certain guidelines have evolved over time. Images are categorized as high (<5 m), moderate (5 and <120 m), and coarse (>120 m) resolution. Present remote sensing satellites contain sensors capable of obtaining data from 0.64 to 1,000 m. Table 2 lists some of the remote sensing satellites, along with the characteristics of data they collect.

Spectral resolution corresponds to the width of region in the electromagnetic spectrum in which data were acquired. Black & white images record information across the entire visible region (400–600 nm). Hence, B&W images are spectrally coarser in comparison to visible color images where information is recorded in the following three regions of the EMR (Table 1): blue (400 nm), green (500 nm) and red (600 nm). Some satellite sensors capture information in the blue, green, red, near-, mid-, and thermal infrared regions. Hyperspectral images have very high spectral resolution, and are acquired within very narrow ranges (10 nm) of the EMR. For example the red region of the electromagnetic spectrum can be finely divided and the interaction between EMR and features is recorded. It is common to find more than 100 or even 200 spectral bands, in a hyperspectral image.

Table 2. Remote sensing satellites and their data characteristics.

Satellite & country	*Spectral bands*	*Pixel size (m)*	*Swath (km)*
Coarse resolution			
NOAA-GOESS (USA)		1,000	>2,000
SPOT VEG (France)		1,000	
TERRA/MODIS (USA)		250 500 1,000	>2,000
Moderate resolution			
Landsat 5 (USA)	B, G, R, 3 IR	30	185
SPOT-2 (France)	G, R, 2 IR	20	120
IRS 1C (India)	G, R, 2 IR	23	70, 142
IRS 1D	G, R, 2 IR	23	70, 142
SPOT-4	G, R, 2IR	20	120
Landsat 7	B, G, R, 3IR	30	185
TERRA/ASTER (Japan/USA)	G, R, IR 4 IR 3 Thermal IR	15 30 90	60
EO-1 (USA)		30	37
Proba (ESA)		18, 36	14
SPOT-5		10	120
IRS ResourceSat		20	24, 140, 740
IRS-AWiFS (India)		56	350
CBERS-2 (China/Brazil)		20	113
FormaSat (Taiwan)		8	24
ThaiPhat (Thailand)		36	600
MONITOR-E-1 (Russia)		20	94, 160
Beijing-1 (China)		32	600
TopSat (UK)		5	10, 15
ALOS (Japan)		10	35, 70

Temporal resolution is related to the time lapse between successive image acquisitions. For example, Landsat 5 – TM satellite acquires data every 16th day for any given location on the surface of the earth, and therefore its temporal resolution is 16 days. Other satellites (e.g., AVHRR) collect data on daily basis.

Radiometric resolution is a measure of precision of the recorded data and measured in bits. In an 8-bit data recording device, 256 (2^8) levels of brightness values can be recorded, whereas 10-bit device can record 1024 (2^{10}) levels of brightness values. Satellite and aerial based remotely sensed data are available in difference combinations of resolutions (example, 30 m spatial, 6 spectral bands, 16 day temporal and 8-bit radiometric resolutions) and it is up to the analyst to select the correct type of data for matching the informational needs of a task.

2.2. Information Extraction

Remotely sensed data are available in digital format (earlier, these images were printed as hard copy maps and features were manually interpreted). Analysts can display the images in any image processing software (Table 3). Depending on the specific informational needs, enhancements or classification can be carried out on these images. Enhancement refers to a set of image processing steps where information content about one or more features in the image is highlighted. Enhanced products can be used to prepare hard copy maps or further digital processing. Vegetation indices are computed from these images for monitoring vegetation condition. Vegetation indices computed from two different time periods are useful for change assessment studies.

Table 3. Selected examples of remote sensing software.

Software	*Manufacturer*	*Website*
ENVI	ITT VIS	www.ittvis.com
ERDAS imagine	ERDAS Inc.	www.erdas.com
ER mapper	ERDAS Inc	www.erdas.com/ermapper
IDRISI	Clark Labs	www.clarklabs.org
ILWIS	ITC	www.itc.nl/ilwis
Image analyst	Intergraph	www.intergraph.com
PCI	PCI Geomatics	www.pcigeomatics.com

Mapping earth surface features requires a suite of sophisticated image processing tools, such as unsupervised and supervised, neural network and fuzzy logic classifiers. Each algorithm has its own advantage and the analyst decides on the type of classifier for a given mapping project, based on the informational requirements and resource availability along with the time constraints. Most remote sensing textbooks include detailed discussion on image classification algorithms. Products generated from these classification routines can be printed as maps or can be integrated in a GIS for further analyses. Since remotely sensed data are collected on a routine basis, periodic updates can be generated for any area and changes in land cover can be assessed.

2.3. Geographic Information Systems (GIS)

GIS is a computer-based system for storing, displaying, manipulating and analyzing geographic data, that can be tied to a geographic location to be described as geographic data. For example, the number of locust egg-pods (data) collected in each county or district (geographic location) is termed as geographic data. GIS can store, organize and analyze diverse sets of geographic data, such as the number of locust egg-pods, hopper band or swarm density and area, temperature, rainfall received, vegetation at those sites and so on. Users can query a GIS in order to combine information from physical and environmental variables for any application.

As physical and environmental conditions change, one can update the information stored in a GIS and generate new results in a relatively short time. For example, an analyst can select sites for field visits based on distance to roads and vegetation types. Information on roads and vegetation types are stored separately (as spatial data layers) in a GIS and can be combined to generate new or derived information. It is possible to combine other variables (e.g. soil type, elevation values etc.) to narrow down the sampling sites. A thorough discussion on GIS is beyond the scope of this chapter, but numerous textbooks are available. Similarly, GIS software has also grown in number and functionality and some of the commonly used ones are listed in Table 4.

Table 4. Selected examples of geographic information system (GIS) software.

Software	*Manufacturer*	*Website*
ArcGIS	ESRI	www.esri.com
AutoCAD	Autodesk	www.autodesk.com
Cartalink	Clark Labs	www.clarklabs.org
GeoMedia	Intergraph	www.intergraph.com
GRASS	US Army Labs	www.cecer.army.mil
MapInfo	MapInfo	www.mapinfo.com
MicroStation	Bently Systems	www.bently.com
TNTmips	MicroImages Inc.	www.microimages.com
Surfer	Golden Software	www.golden.com
Sage GIS	DLSR	www.dlsr.com.au

GIS technology can be used for analyzing spatial patterns in insect populations. Georeferenced data about insect densities, crop type, and soils for a location can be incorporated in a GIS for producing new map layers (Liebhold, Rossi, & Kemp, 1993). A map layer, generally composed of only one type of data, thus has a theme. Furthermore, themes that represent similar areas can be combined to form a full GIS database. The GIS serves as a tool for analyzing interactions within and between the various spatially referenced data themes. Management and analysis of large spatial databases would be impossible without this type of software.

GIS is being widely used in conjunction with remotely sensed data (satellite imagery) in Africa and Australia for mapping locust habitats (Bryceson, 1989; Cressman, 1997; Voss & Dreiser, 1997). FAO Scientific Advisory Committee considered GIS as the most appropriate technology to aid locust forecasters and researchers (FAO, 1989). GIS is able to improve the specialists' ability to assess and interpret current and historical data on locusts and the environment (Healey, Robertson, Magor, Pender, & Cressman, 1996). Since Desert locust records are among the most complete which exist for an insect pest, a specific GIS "SWARMS" (*Schistocerca* WARning Management System) has been developed offering researchers and decision makers improved information for studying population dynamics and for displaying and testing alternative control strategies (Cressman, 1997; Magor & Pender, 1997).

GIS techniques have also been applied to grasshopper ecology in temperate regions, especially in the US. In Montana, with the use of GIS, regional spatio-temporal grasshopper outbreak characteristics have been examined (Kemp, Kalaris, & Quimby, 1989; Cigliano, Kemp, & Kalaris, 1995). In Wyoming, GIS was implemented to reveal the historic spatial characteristics of grasshopper outbreaks (1960–1993), as well as for spatial analysis of ecological factors related to grasshopper population dynamics (Schell, 1994; Lockwood & Schell, 1995). Schell (1994) studied the spatial properties of grasshopper infestations in Wyoming and found that 72% of grasshopper outbreaks are confined to a particular soil type which has a very limited distribution range (1% of the state). Thus, the GIS analysis revealed that a certain ecological factor was highly correlated with the potential of a habitat to support an outbreak. The results of this study contributed greatly to the optimization of grasshopper survey and management in the state (Schell & Lockwood, 1995, 1997a, 1997b).

2.4. Global Positioning Systems (GPS)

GPS is used, among applications, for determining geographic coordinates while collecting data in the field. GPS consists of a constellation of satellites that transmit signals which are received by hand-held units called GPS Receivers, that can process the signals and determine precise geographic position. Time taken for these signals to travel from each satellite to the GPS receiver is used for computing the distance to each satellite. A minimum of 3 distance measures from 3 different satellites are required to determine a geographic position on the surface of the earth. Sophisticated GPS Receivers can simultaneously receive signals from 8 or more GPS satellites and therefore compute geographic coordinates with higher accuracy. Based on the precision and sophistication GPS Receivers are categorized as recreational, mapping and survey grade GPS. Survey grade GPS Receivers are expensive and provide very accurate measurements, in comparison to the other grades of receivers.

GPS technology is widely used for navigation and surveys. Relatively inexpensive receivers are used in recreational activities while more precise GPS receivers are used for navigating emergency and transportation vehicles and to obtain their whereabouts in real time. GPS technology is used in field data collection for applications such as locating sampling sites or insecticide-treated areas. Previously researchers would mark these locations on a printed map to associate their samples to geographic space. With the help of GPS technology, researchers can record the geographic coordinates while collecting field data and later they can export the coordinates to image processing or GIS software. This process eliminates errors associated with transcribing field notes and annotated coordinates in maps.

Several national and international agencies use GPS technology for collecting periodic information about events on a near-real time basis. Such methods, in addition to reducing the errors associated with transcribing, reduce the time required to generate updated information from field data obtained periodically.

3. REMOTE SENSING AND LOCUST PEST MANAGEMENT

Remotely sensed data collected from airplanes and satellites have been used for mapping locust habitats and also for assessing the damages to vegetation following a locust outbreak. Individual aerial photographs often cover smaller area on the ground in comparison to the area covered by moderate resolution satellite images such as Landsat (Table 2). The geographic area of some coarse resolution satellite images such as AVHRR and MODIS cover several thousand square km. Ability to monitor or map large geographic areas is particularly appealing for assessing locust damages in remote regions of the earth. Showler (2003) categorized the applications as strategic or tactical. Strategic applications include mapping potential or actual locust habitats and using that information to devise suitable prevention measures e.g., chemical treatments. Tactical applications include monitoring ongoing locust plagues from aerial platforms or assessing damages to the vegetation following locust outbreaks. Following sections focus on the habitat requirements and lessons learned from using remotely sensed data and technology for either mapping locust habitats (strategic) or assessing damages (tactical) following locust outbreaks.

In the domain of locust pest management, remote sensing has been used for the detection of changes in vegetation and the measurement of certain meteorological parameters. For example, satellite images were used to detect zones of green vegetation (temporarily mesic habitats activated after sporadic rains) which had the potential for colonization by locust populations in xeric landscapes of Africa (Cherlet, Di Gregorio, & Hielkema, 1990; Cherlet & Di Gregorio, 1993; Voss & Dreiser, 1994, 1997) and Australia (McCulloch & Hunter, 1983; Bryceson & Wright, 1986; Bryceson, 1989). With its broad infrared electromagnetic spectrum, the TM sensor of the Landsat satellite appears to be a useful instrument for detecting different vegetation communities by their reflection in the landscape and mapping of locust habitats at scales up to 1:100,000 (Voss, Drieser, & Popov, 1992, 1993a, 1993b, 1994).

As for the detection of the entomogenic effects on the vegetation, satellite imagery has proved to be useful in forest entomology (Dottavio & Williams, 1983; Rencz & Nemeth, 1985). It also has potential for determining the crop losses due to locusts (Wewetzer, Krall, & Schultz, 1993). An attempt to use satellite imagery in the context of rangeland grasshopper ecology (Schell & Lockwood, 1996) strongly suggested that combinations of thermal and infrared wavelengths can distinguish infested lands from the surrounding areas. Thus, active infestations are apparently revealed by a combination of entomogenic effects, including: (1) more rapid heating of the habitat, perhaps due to increased exposure of soils upon removal of forage by grasshoppers (thermal), (2) decreased plant/soil moisture, perhaps as a consequence of grasshopper feeding (mid-infrared), and (3) changes in plant cell structure, perhaps as a consequence of herbivory-induced stress (near-infrared). A characteristic “halo” effect has been found with active outbreaks, which suggests a gradient of forage loss emanating from a high-density locust band.

Coupled with field observation, remote sensing can be employed to identify soils, vegetation and land use characteristics, and from this initial information, potential and active outbreak areas can be identified. The objective is to discriminate vegetation areas from bare soil and to monitor the changes in vegetation densities and qualities over time. This is achieved using specific indices, such as the Normalized Difference Vegetation Index (NDVI) (Tucker, Hielkema, & Roffey, 1985). The methodology is well developed in Africa. An application of this method allowed identification of potential habitats of the Desert locust from remotely sensed data (Tappan, Moore, & Knausenberger, 1991; Cherlet & Di Gregorio, 1993) and to create corresponding habitat maps (Voss & Dreiser, 1997).

3.1. Desert Locust Biology and Habitat Requirements

The invasion area of the Desert locust (*Schistocerca gregaria*) occupies 29 million km^2 in Africa, S. Europe and SW Asia. During recessions, when population densities are low, the Desert locust inhabits arid and semi-arid lands covering 16 million km^2 from the Atlantic Ocean to NW India (COPR, 1982). Breeding occurs in the areas with 20–25 mm direct rainfall. Preferred oviposition sites are in sandy soils with a mosaic of grasses, herbs and shrubs. Although rain over the area is largely erratic, it tends to fall seasonally. Consequently locust breeding also takes place seasonally in different geographic locations (Fig. 1). The summer breeding zones include the Sahel, West Africa, Sudan, Eritrea, Ethiopia, and the India-Pakistan border. The winter/spring breeding zones include NW Africa, Iran, Pakistan, the Red Sea and the Gulf of Aden coasts, and the interior of Saudi Arabia and Yemen (FAO, 2001).

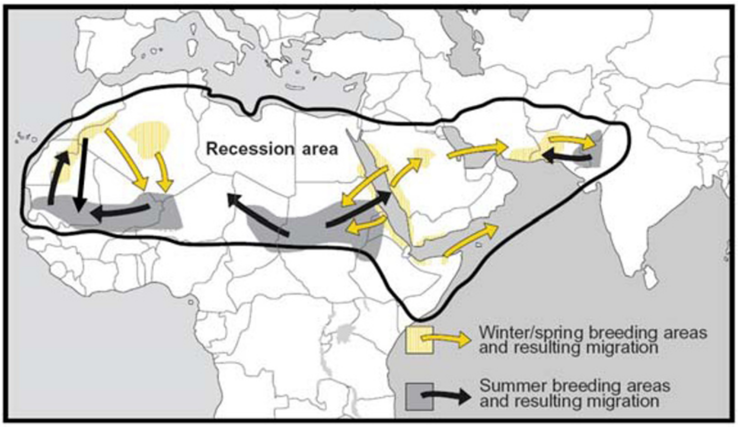

Figure 1. Desert locust seasonal breeding zones and population movements between them (modified from FAO, 2001).

Such complicated spatio-temporal pattern and an extremely large scale of the Desert locust population dynamics make its survey and forecasting extremely difficult. Furthermore, the pest's breeding areas are often concentrated in very remote zones with low resident population or in the zones of the ongoing/imminent military conflicts such as Darfur, Ethiopia-Eritrea or India-Pakistan borders. Ground survey in such zones is difficult or impossible. The international cooperation between bordering countries is crucial, but it is not always in place for the efficient survey of the invasion area of the Desert locust which covers 65 countries.

3.1.1. Habitat Mapping and Post-damage Assessment

Pedgley (1974) was the first to apply satellite data to the Desert locust habitat monitoring. Tucker et al. (1985) recognized the potential of the remotely sensed data for the locust survey and forecasting. Hielkema (1981), Hielkema, Roffey, and Tucker, (1986) and Ghaout (1990) used Landsat imagery to map vegetation in the critical locust's gregarization areas in West Africa. Louveaux, Ghaout, and Gillon (1990) studied the functioning of the winter breeding area of the Desert locust in Mauritania using, among other techniques, the Landsat data. Successful implementation of a preventive Desert locust control strategy requires early and reliable knowledge of areas where vegetation emerges after rainfall, providing suitable conditions for oviposition, egg hatching and hopper development. Cherlet and Di Gregorio (1993) tested the reliability of the NOAA AVHRR satellite data for these purposes. They attempted to calibrate different NOAA vegetation indices including NDVI, using extensive ground-collected field data from Niger. Their proposed calibration included two steps: (1) masking out the areas with no ecological potential for locust breeding; (2) correction of the vegetation index with a factor derived from the soil brightness. This methodology allowed the authors to detect some changes in the low cover vegetation. However, the lack of the background historical database on both, the vegetation and the locusts in certain key Desert locust breeding areas remains a major obstacle precluding the introduction of the remote sensing tools into the survey practice.

Mapping of the Desert locust habitats using satellite imagery was done by the group of the late Prof. Dr. F. Voss in the 1990s. Dreiser (1994) used the Landsat Thematic Mapper data to produce the locust habitat maps for certain areas of Sudan, Mali and Mauritania at the scale of 1:200,000. Voss and Dreiser (1994) used the NDVI to detect the vegetated areas and then classified them with the maximum likelihood technique. The reliability of the resulting maps depended largely on the extensive ground observations and experience of the renowned locust expert Popov (1997) who participated in these studies. Such expertise is often unavailable in other Desert locust breeding areas which hinders the verification of the maps derived from satellite images. The authors concluded that the Landsat data were useful to assess the potential suitability of the Desert locust habitats. However, the actual habitats could be identified using higher temporal resolution imagery like NOAA AVHRR in combination with actual meteorological data from Meteosat and other similar satellites, which involve higher acquisition and processing costs (Voss & Dreiser, 1997). Despland, Rosenberg and Simpson (2004) used the vegetation indices derived from NOAA AVHRR data to connect the Desert locust gregarization and band formation areas in Mauritania and Sudan to landscape structure. The authors concluded that the spatial resolution was insufficient to detect the initial gregarization zones. Similarly, Babah Ebbe (2008) was not able to distinguish the vegetation from bare soil in Mauritania using the NDVI derived from the Landsat TM data. The author concluded that the very low vegetation density in the Saharan and Sub-Saharan zones does not allow for a reliable Desert locust habitat inventory using the Landsat tools. These findings are in line with those of the Desert Locust Information Service (DLIS) of the FAO UN which uses satellite data for forecasting locust outbreaks (http://www.fao.org/ag/locusts/en/activ/DLIS/satel/index.html).

Until recently, DLIS relied on 1 km resolution SPOT-VGT imagery to monitor ecological conditions in a locust breeding areas. Although the sensor was specifically designed for vegetation monitoring, it has become clear that it is difficult to detect the sparse vegetation in the desert – vegetation that appears to be dry to the satellite yet, sufficiently green for Desert locust survival and breeding, resulting in under-prediction of the pest threat. Consequently, DLIS turned to higher resolution imagery, that of 250 m resolution MODIS, consisting of 16-day cumulative images. Analysis of individual channels provides an even more accurate estimation of ecological conditions in Desert locust habitats which are subsequently verified with survey results.

Besides the vegetation, rainfall is another essential parameter necessary for accurate Desert locust forecast and risk assessment. DLIS uses rainfall estimates derived from METEOSAT, mainly infrared and visible channels, to understand better the spatial and quantitative distribution of rainfall in the Desert locust breeding areas. Although images are available every 15 min and estimates every three hrs, DLIS uses daily 24-h cumulative estimates as well as decadal estimates of rainfall processed by Columbia University's International Research Institute for Climate and Society (IRI). DLIS combines satellite-derived estimates with those that originate from meteorological models. Whenever possible, these are verified with ground data.

DLIS collaborates with a variety of universities and other partner institutes such as the IRI, the Italian Institute of Biometeorology (IBIMET), the European Commission Joint Research Centre (JRC), NASA's World Wind Project, and the Catholic University of Louvain (Belgium) in improving the application of remote sensing imagery for Desert locust monitoring and forecasting. SPOT-VGT and MODIS imagery is made available every 10 and 16 days respectively to locust-affected countries. These products are used to help guide national survey teams to potential areas of green vegetation where Desert locust may be present.

Active remote sensing in the form of Vertically Looking RADAR (VLR) was used to observe the Desert locust flights over the Sahara as early as in 1968 (Roffey, 1969). This technique provided novel measurements of aerial density, orientation, direction and speed of flight of solitarious locusts (Schaefer, 1969, 1976). Despite its very promising first results, the use of the RADAR devices for monitoring of the locust swarm migrations was considered impractical, mostly because of the time-consuming nature of the data analysis (Reynolds, 1988; Riley, 1989). Subsequent attempts to use VLR showed its potential to distinguish between the flying Desert locusts and other insects (Smith, Riley, & Gregory, 1993). The obtained data could be a useful complement for the routine locust surveys (Riley & Reynolds, 1997; Chapman, Reynolds, & Smith, 2003).

Finally, it is necessary to point out that currently available satellites are not able to directly detect individual locusts or locust swarms and hopper bands. An attempt of Kibasa (2006) to detect the actual locust groups with the high-resolution Spot 5 data failed, confirming that it was not possible to distinguish the insects from the background soil.

With regard to the GIS applications, the analyses of the enormous amount of geospatial information collected by both, the satellites and field surveys from the vast geographic area of the Desert locust would be impossible without appropriate GIS tools (Hielkema & Snijders, 1994). The GIS SWARMS which was developed by the FAO in collaboration with Natural Resources Institute and the University of Edinburgh (UK) specifically for these purposes contains a number of databases including historical locust data for nearly 100 years, weather data, and background information such as soils and topography (Healey et al., 1996). SWARMS has been used operationally for locust early warning since 1996; it is being constantly revised and updated.

One of the major hurdles in effective Desert locust forecasting is the collection and recording of data in the field and their subsequent transmission to a national locust center in near-real time. FAO DLIS developed, in collaboration with Novacom (France), a handheld device named eLocust2 for field locust officers (http://www.fao.org/ag/locusts/en/activ/DLIS/earlywarning/index.html) to enter and send geo-referenced data in real time. The field officer enters and saves the data into a rugged handheld device which automatically determines the coordinates of the location of the survey or control operation using GPS technology. With a press of a button, the officer sends these data via satellite to the national locust center where they are received as an email attachment, downloaded, decoded and imported into a GIS. This GIS named RAMSES is used for the management and analysis of field results and of locust and environment data at a national level. eLocust2 data from the field are automatically imported into RAMSES and then are exported to FAO DLIS for further analysis, forecasting and early warning.

Furthermore, FAO DLIS utilizes several specialized tools to supplement its analysis and forecasts (Ceccato, Cressman, Giannini, & Trzaska, 2007). A Trajectory Model (developed by Meteo Consult) estimates the source and destination of swarm migrations forward and backward in time. Rainfall estimates and MODIS satellite imagery provided by IRI at Columbia University (New York, USA) are used to try to understand where it has rain and where vegetation is green in the desert. Seasonal predictions of temperature and rainfall 6 months in advance are analyzed. Another model is used that estimates the developmental times of locust eggs and hoppers. The use of these tools in combination with the GIS allows the best possible analysis from which forecasts and early warning can be issued.

Another tool potentially useful for locust forecasting is geostatistics. Woldewahid (2003) used geostatistics to predict the Desert locust densities at distant locations in the Red Sea coastal plains of Sudan, by spatial interpolation through kriging. He found a strong relationship between the Desert locust densities and the millet croplands despite the fact that these croplands occupied only a limited proportion of the study area (5%).

3.2. Migratory Locust Biology and Habitat Requirements

Migratory locust *Locusta migratoria* has the largest distribution area among all grasshoppers and locusts covering almost entirely the temperate and tropical zones of the eastern hemisphere (Fig. 2). Yet the ecological requirements of the species are rather narrow. Within this vast range the locust breeding areas are restricted to grasslands on light soils, often in the wetlands with reed stands along rivers or lakes. There are about 10 subspecies or geographic races of the Migratory locust slightly differing biologically and morphologically (COPR, 1982). The tropical races develop continuously without diapause, while the temperate ones are univoltine.

3.2.1. Habitat Mapping and Post-damage Assessment

To date, remote sensing tools were applied to habitat mapping and damage assessment for two subspecies, *L. m. migratoria* in Central Asia and *L. m. manilensis* in China. In Kazakhstan and Uzbekistan, remotely sensed data collected from satellite platforms were used for mapping potential habitats of the Asian Migratory locust *L. m. migratoria* (AML). Throughout Central Asia, the AML spends most of its life cycle in common reed (*Phragmites australis*) stands, usually, in river deltas.

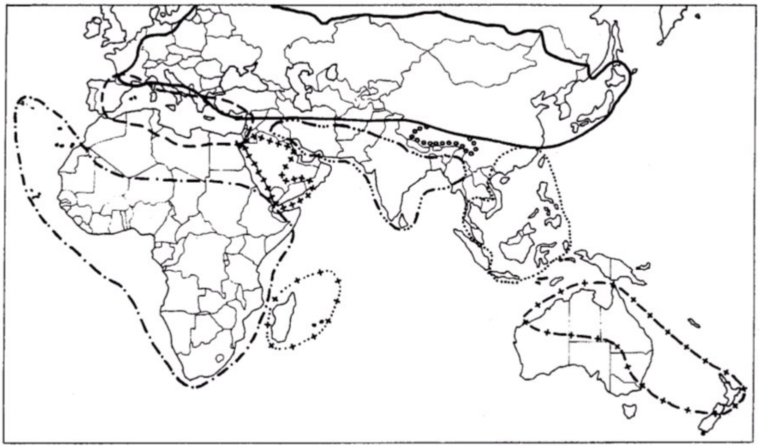

Figure 2. Distribution area of the subspecies of the Migratory locust Locusta migratoria *(modified from COPR, 1982 by Latchininsky et al., 2002).* Locusta migratoria migratoria *and other northern subspecies*___; L. m. cinerescens - - - ; L. m. burmana •••••; *Indian subspecies* ...-; L. m. migratorioides -. - . L. m. capito ...+...; *Arabian subspecies* ++++; L. m. manilensis ....; *Australian subspecies* -+-+-.

To identify the AML habitats, one approach is to map the reed distribution annually or at specific times of the year coinciding with AML developmental stages (e.g., hatching of eggs or oviposition). Sivanpillai, Latchininsky, Driese, and

Kambulin (2006), and Sivanpillai and Latchininsky (2007) demonstrated the utility of information derived from Landsat and MODIS satellites to estimate reed distribution in the River Ili (Kazakhstan) and Amudarya River (Uzbekistan) deltas respectively. Results from these studies showed that satellite data could accurately map the reed stands when they are dominant (>80% classification accuracy). However, satellite data had relatively lower accuracy in detecting reeds growing together with other vegetation like shrubs and sedges (Latchininsky, Sivanpillai, Driese, & Wilps, 2007; Sivanpillai & Latchininsky 2008). Navratil (2007) used the higher resolution multispectral data collected by the SPOT satellite (Table 2) to map the reed distribution for a portion of the Amudarya River delta. Landsat Thematic Mapper 5 data could be used for mapping emerging reeds in the spring (Latchininsky et al., 2007). This time period coincides with the hatching of AML eggs and the satellite-derived information could be used for directing the ground survey towards potential nymphal habitats. Distribution of reeds coinciding with the locust oviposition (late summer) was mapped by Sivanpillai and Latchininsky (2008). Such information could be used for locating locust egg-beds. Satellite-derived information could assist the government pest management agencies to devise treatment plans. Since satellites collect data on a regular basis, reed distribution maps can be updated periodically, providing basis for targeted locust surveys and treatments.

Satellite data acquired prior and after an outbreak have been used for quantifying damages to vegetation. Ji, Xie, Li, Li, and Zhang (2004) used the pre- and post-damage MODIS images for assessing the damages caused by the Oriental Migratory locust *L. m. manilensis* in China's Hebei Province. Using the NDVI values derived from these images, the researchers were able to identify the affected areas. The extent of vegetation damage was grouped into light, moderate, and heavy damage categories. Although MODIS images have relatively coarse spatial resolution (250 m), it was possible to identify 89% of the impacted areas. The authors concluded that satellite-derived information could be more efficient than the traditional ground surveys.

Zha, Gao, Ni, and Shen (2005) assessed the utility of MODIS data acquired over the growing season for monitoring Oriental Migratory locust outbreak in China. Tian, Ji, Xie, Li, and Li (2008) repeated the work conducted by Ji et al. (2004) using Landsat ETM+ data and reported higher classification accuracies compared to MODIS data. Ma et al. (2005) used Landsat ETM+ data to assess damage to vegetation caused by *L. m. manilensis* in China's Dagang region. The ground-based locust monitoring stations established in the 1950s were unable to survey the expanded infested areas. Using the Leaf Area Index values derived from pre- and post-damage images, the authors demonstrated the value of satellite data for routine monitoring of vegetation condition. Tian et al. (2008) repeated the pre- and post-damage assessment work conducted by Ji et al. (2004) but using Landsat ETM+ data, and reported a very high accuracy of 98% for determining the geographic extent of the locust damage. However, the accuracy was slightly lower (92%) when they attempted to categorize the severity of the locust damage from Landsat images. Using soil moisture indices derived from MODIS data, Liu et al. (2008) demonstrated significant differences in soil moistures during severe (2001–2002) and moderate (2003–2004) outbreak years in 3 regions prone to Oriental Migratory locust outbreak in China.

Applications that used satellite data for mapping Migratory locust habitats, assessing risks of infestation and evaluating vegetation damage were comparatively fewer than the number of applications that focused on Desert locust (Section 3.1) or the Australian Plague locust (Section 3.3).

3.3. Australian Plague Locust

Australian Plague locust *Chortoicetes terminifera* occurs throughout Australia (Fig. 3). Its ideal habitats consist of a mosaic of bare ground for basking and egg-laying, short grass cover for feeding and taller sparse tussocks for night shelter (COPR, 1982). The locust can produce three annual generations under favorable weather conditions, primarily sufficient moisture. Hoppers form dense bands which move several 100 m per day. Plagues originate from several recession areas in SW Queensland, Central New South Wales, and NW Victoria where locusts are always present. Usually the onset of the plague is triggered by abnormally heavy rains from late November to January in the recession areas of the Australian dry interior.

From these outbreak centers the huge swarms of adults migrate into agricultural zones covering distances of several 100 km. Rangeland forage is the preferred food for the Australian Plague locust. Migrating swarms, however, inflict severe damage to cereal crops, vineyards, orchards and vegetable gardens.

3.3.1. Habitat Mapping and Post-damage Assessment

Breeding zones of the Australian Plague locust situated in remote and semi-desert locations, the Australian entomologists were among the first to use remotely sensed data for locust habitat mapping. One of the earlier attempts to relate information derived from Landsat data with locust data was conducted by McCulloch and Hunter (1983). This study demonstrated that locust presence was confined to the following three map classes: stony downs, stony plains and high-level flood plains. Further development of the remote sensing tools was done by Bryceson (1984): he was able to detect even small area of vegetative growth after rains with Landsat data. Bryceson and Wright (1986) used several Landsat images modelled the origin and spread of the 1984 Australian Plague locust outbreak. In this study they concluded that it was feasible to use satellite data to monitor changes in vegetation condition which could then be associated with locust breeding areas.

In yet another study, Bryceson (1989) used Landsat MSS data to track the egg-bed areas of the Australian Plague locust in New South Wales. Furthermore, the author showed that satellite data were instrumental in identifying the source areas from which the locust plagues developed (Bryceson, 1990, 1991).

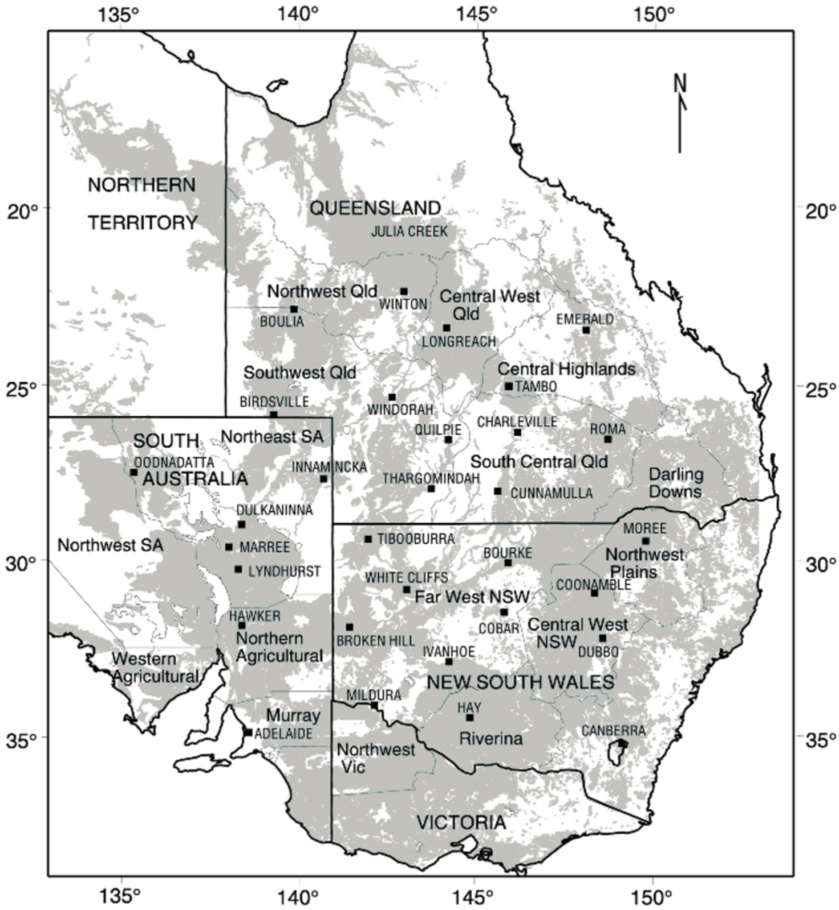

Figure 3. Australian Plague locust habitat (shown as shaded areas*) map (Courtesy: Dr. Hunter and Mr. Deveson, Australian Plague Locust Commission, Canberra, Australia).*

In Australia, locust monitoring and control are executed by a federal agency, the Australian Plague Locust Commission (APLC), specifically created for these purposes. This facilitated the introduction of the meteorological remotely sensed data into the practice of locust forecasting (Bryceson & Cannon, 1990; Bryceson, 1993; Bryceson, Hunter, & Hamilton, 1993; Hamilton & Bryceson, 1993). Multiple information sources, including remotely sensed vegetation and weather data as well as locust infestation data, were integrated into a decision support system developed at APLC (McCulloch, Bie, & Spurgin, 1994; Deveson & Hunter, 2000, 2002; Deveson, 2001).

Locust swarm migrations were tracked in Australia by active remote sensing in the form of vertically-looking RADAR (Drake, Harman, & Hunter, 1998; Drake et al., 2001; Deveson, Drake, Hunter, Walker, & Wang, 2005). The methodology proved useful. However, high costs of the installation and maintenance of the RADAR stations precluded it from practical use.

Hunter, McCulloch, and Spurgin (2008) demonstrated the possibility of detecting the locust nymphal bands from a low-flying aircraft, as a useful survey option. Australia remains an international leader in the operational use of remote sensing and GIS applications in locust management.

3.4. Other Locusts

About a dozen other locust species exist in addition to those described in earlier sections and their distribution ranges often cover vast, sparsely populated and remote areas. These locust species can cause economic and environmental impact similar to any of the locusts discussed above. For example, the Moroccan locust (*Dociostaurus maroccanus*) habitats extend over 10,000 km across N. Africa, Middle East and Central Asia. Moroccan locusts can destroy valuable agricultural crops and adversely impact the livelihood of farmers in these regions. Chemical treatments can be effective only if they are coordinated by all impacted countries. Similarly, the Central American locust (*Schistocerca piceifrons piceifrons*) poses a threat to agricultural operations in Mexico and neighboring countries. Other locusts affect South Africa, South America and Southeast Asia. National Plant Protection Agencies in these regions spend considerable amount of resources for monitoring and managing the locust populations every year.

Applications of the remote sensing and GIS technologies for monitoring and management of the locust species other than the Desert, the Migratory and the Australian plague locusts are very scarce. To our knowledge, such studies were done for only two other locusts. Franc (2007) used high resolution SPOT satellite data to trace habitats of the Red locust *Nomadacris septemfasciata* in Madagascar. Comparing two SPOT images, one dated 1986 and the other dated 2004, he calculated the areas of deforested zones which served as migration pathways for this locust in the basin of the River Sofia. The accuracy of the image classification (77%; Kappa = 0.75) was verified through ground surveys. In 1986, the combined area of such “corridors” was 41,677 ha but as a result of intensive deforestation it increased to 67,607 ha by 2004. The newly cleared areas provided new migration pathways for the Red locust which produced spectacular outbreaks in the early 2000s for the first time ever in Madagascar.

Sivanpillai, Latchininsky, Peveling, and Pankov (2009) used the Indian Remote Sensing (IRS) P6 Satellite –Advanced Wide Field Sensor (AWiFS) data to map the Italian locust *Calliptamus italicus* habitats in a very heterogenic landscape of NE Kazakhstan, consisting of active and fallow croplands, shrub lands, grasslands and riparian zones. This locust species inhabits primarily abandoned agricultural fields (fallows) covered with sagebrush (*Artemisia* spp.) and other broadleaved weeds. Despite its relatively coarse spatial (56 m) and spectral (4 bands) resolutions, the AWiFS data were sufficient to identify most landscape features.

CONCLUSIONS

The idea of using remotely sensed data to identify locust habitats became plausible with the introduction of non-military satellites in the 1970s. Distribution ranges of many locust species cover vast, sparsely populated and remote areas, and the satellite data appeared to be tailor-made to address the spatio-temporal extent of the locust habitats. However, in the last two decades of the twentieth century, the practical use of the satellite data was impeded by inadequate computational hardware capacities and lack of specialized training for locust specialists in remote sensing data acquisition and processing. Hence, after a period of over-enthusiastic claims and views of the remote sensing as a panacea for solving locust problems, the research reports in the beginning of the 2000s sounded more cautious, if not skeptical (Despland et al., 2004; Tratalos & Cheke, 2006). In the present chapter we attempted to summarize the most important achievements and drawbacks of the applications of the remote sensing and GIS technologies to locust monitoring and risk assessment. Out of about a dozen locust species worldwide, this technology has been developed for only three species, the Desert, the Migratory, and the Australian Plague locusts. Numerous advances have been made in the use of satellite remote sensing data for monitoring the Desert locust habitats in Africa. Similarly, the habitats of the Australian Plague locust are being monitored through satellites, aerial photographs and RADAR imaging (active remote sensing) technologies. Satellite data from different platforms were used for habitat monitoring and crop loss assessment for the Migratory locust in Asia. For two other species, the Red and the Italian locusts, only pilot studies were done in this domain.

The use of remotely-sensed information including the data from meteorological satellites became a routine part of the forecasting done by the FAO UN Desert locust Information Service (DLIS). Yet the efficacy of the forecasts is challenged by the vast geographic extent of the monitored territory of the Desert locust recession area (16 million km^2) and, consequently, by the huge amount of information to be collected and processed in near-real time (Van Huis, Cressman, & Magor, 2007).

Habitats of the Migratory locusts, which are confined to reeds around water bodies, can be identified even with satellite platforms of relatively coarse spatial resolution (e.g. MODIS). Continuous reed stands are easily distinguishable spectrally from other land cover classes except when the reeds are mixed with shrubs of other vegetation. For such cases a higher temporal resolution of satellites with a shorter revisiting time is instrumental in following the phenological changes in the vegetation throughout the growing season.

The Australian Plague locust management is implemented by the governmental entity, the APLC, which uses satellite and GIS technologies for locust monitoring and forecasting across a vast area of inner Australia. This country was one of the pioneers in practical introduction of the remote sensing as well as other technologies such as RADAR and aerial photography in locust population management.

Applications of remote sensing and GIS technologies for mapping and monitoring the habitats of most other locusts throughout the world lag behind. Some of these habitats are situated in active agricultural areas of countries such as Kazakhstan, Uzbekistan and Mexico. Locust pest outbreaks in these areas adversely impact the economy and environment. Governments of these countries either independently or jointly (with their neighbors) are engaged in periodic monitoring and treatments to control locust populations and prevent them from damaging agricultural crops. Recently, efforts are underway to form a group of Central Asian and Caucasian countries to coordinate the locust population monitoring and control activities.

Future locust habitat monitoring and surveying activities should incorporate remote sensing and GIS technologies for optimizing the resources available in these countries. Future work should also focus on testing the utility of remotely sensed data for mapping and monitoring the habitat of locust species that are yet to be studied. It is also encouraging that both the number of remote sensing satellites and the countries that launch them have increased since early 1970s. In addition to government agencies several private companies have also launched remote sensing satellites that are collecting data for different parts of the world. With this increased availability of remotely sensed data (Table 2), users have a wide range of choice in terms of spatial, spectral and temporal resolutions while selecting imagery for a particular application. For example, images can be acquired more frequently by obtaining data from different remote sensing satellites with complementary spatial and spectral resolutions (Section 2) providing the required information about locust habitat. Plant protection agencies and international organizations must capitalize on these developments in terms of increased data availability.

With more and more remotely sensed data distributed via the internet the time associated with obtaining the data has reduced tremendously. Previously data were mailed in tapes and discs and it could take several weeks thus reducing its usefulness. It is also important to note that not all countries or regions have access to high-speed internet. However, most remote sensing data can be downloaded directly from the satellites to ground receiving stations and processed in any country or region, thus avoiding the need to transfer large volumes of data through internet. Such activities require collaboration among international agencies (i.e., FAO), impacted countries, and satellite data vendors and agencies.

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8

PLANT DEFENSES AGAINST INSECT HERBIVORY

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Abstract. Herbivory, the act of consumption of plant biomass by specialist animals, regulates the cycling of biotic and abiotic ecosystem components, through a complex process transferring materials among various trophic levels. Herbivores include insects and mammals of varying sizes, the former being most important due to their high diversity. Insects consume the biomass in varying proportions, depending on their size and density. Apparent checks and balances between prey and predators or hosts and parasites are chemically governed functions. Plants and herbivores receive and send signals to each other as well as to organisms in higher trophic levels (predators) through volatile chemicals. Besides several morphological defence mechanisms, plants evolved specific chemical defences against insects. Among herbivores, insects also co-evolved mechanisms to overcome the volatile chemical arsenals of plants. In this review the role of plant defense against insect herbivory is discussed. The plant responses to repel insects and the synthesis of volatile chemicals to attract predatory insects or parasites are reviewed. Plants evolved genes (activated on insect attack) inducing the secretion of volatile chemicals. Such signalling attracts predators or parasites and is absent in plants when they are experimentally injured. Signalling is caused by the reaction with elicitors contained in the oral secretions of herbivorous insect. Through chemically operated keys, plants and insects regulate ecosystem functioning, allowing co-existence in wild and natural ecosystems.

1. INTRODUCTION

Agriculture is one of the major sectors of the Indian economy, since about 70% of human population is dependent on it for livelihood, other than food. The agriculture sector contributes over 40% of the gross national production, but food production has always remained a matter of great concern. During the past few months, the inadequate grain production has added to the global concern. Among other threats to crop productions, herbivorous insects and pests pose a very serious threat to plants in India, as well as all over the world.

Herbivory, the feeding on living plants by animals or insects, is a key ecosystem process whose widely recognized effects on primary production, vegetation structure

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and composition depend on the type and intensity of feeding. Different types of herbivory affect several plant tissues, besides affecting primary production, translocation and accumulation of photosynthates to varying degrees. Herbivory affects a variety of ecosystem properties, primarily through differential changes in survival, productivity and growth of plant species.

In evolutionary terms, the rise of insects represented a major selective force on plants evolution, and led to the selection of plants by their ability to generate defensive adaptations. Insect herbivores are mostly mandibulated that either bite or chew vegetation. The rise of vascular plants led to the co-evolution of sap-sucking feeders, and several other forms of herbivores such as leaf mining, gall forming and nectar feeding insects. The study of plant defense against insect herbivory is not only important from an evolutionary point of view, but is also useful in understanding the extent of its impact on agriculture, human and livestock food sources, as well as on the utility and survival of commodity plants or species of medicinal use. As an example of insects damage due to high rates of herbivory, grasshoppers feed on a wide range of plants and organic material, and voraciously consume green forage approximately one-half of their body weight, every day.

In the present review emphasis is given to herbivory and plant defense mechanisms, as well as to the chemical signalling among plants, herbivorous and predatory insects. In fact, it is worth to determine these mechanisms before either using pesticides or employing other biotechnological means, i.e. genetically manipulated (GM) plants. It is known that the use of pesticides may indeed alter some ecosystem processes or introduce a structural change in density dependent or population regulation mechanisms, apart from the effects related to health hazard implications and costs. Finally, GM plants have several environmental and ecological complications, including the developement of insect resistance in a way similar to resistance to some insecticides. Insect management is in effect a complex and difficult task, as suggested by the insects and plants co-evolution and survival for the past 97 million years (Labandeira, Dilcher, Davis, & Wagner, 1994).

2. PLANT DEFENSE MECHANISMS

Plants curb insect herbivory by synthesizing and releasing complex blends of volatiles. Some of these compounds provide important host-location cues to predator insects or parasites, that are natural enemies of insect herbivores. Synthesis and release of these chemical signals by attacked plants are active physiological processes, triggered by chemical elicitors or substances contained in the oral secretion of attacking herbivores. Certain chemicals contained in the saliva of grazing insect (herbivores) activate the synthesis and release of plant volatiles. The process of attracting predatory insects involves the interaction of specific blends of plant volatiles, with highly sensitive receptor molecules of the predators (De Moraes, Mescher, & Tumlinson, 2001).

Plant volatiles represent a language through which attacked plants send signals to healthy plants in their vicinity and also invite predators or parasites of their herbivores. This adaptation is genetically fixed and controlled by a set of genes present in plants. In particular, a set of five defense genes are induced when a plant is exposed to volatiles proceeding from neraby co-specific leaves infested with insects. All these genes were not induced in healthy plants when exposed to the blend of volatiles from artificially wounded leaves. At least three terpenoids in the infested leaves were responsible for the gene activation process, leading to the release of special blends of volatiles (Arimura et al., 2000).

Volicitin, N-(17-hydroxylinolenoyl)-L-glutamine and β-glucosidase are elicitors of plant volatiles and are released only on insect chewing. Volacitin is a component of the oral secretion of beet worm caterpillars, which induce corn seedlings to synthesize and release volatile chemical signals. β-glucosidase is present in the regurgitant of *Pieris brassicae* caterpillar (Mattiacci, Dicke, & Posthumus, 1995).

Generally, plant defenses can be categorized into two groups, such as constitutive and induced defenses. Constitutive defenses are always present in plants, while induced defenses are synthesized at or mobilized to the site of attack, when a plant is injured. Constitutive defenses range from mechanical defenses to the accumulation of digestibility reducers and toxins. Induced defenses include secondary metabolic products and may induce morphological and/or physiological changes. Both these defenses (inducible and constitutive) are known to increase the defensive ability and effectiveness of attacked plants, against a wide range of insect herbivores.

Wounding by herbivores activates the systemic expression of defense genes through the octadecanoid signal pathway. The insect saliva, containing chemical elicitors such as volicitin, also triggers the attacked plant to release a bouquet of volatile compounds which attract parasitic or predatory insects to check the attacking herbivore. Volicitin stimulates release of volatiles through the octadecanoid pathway and thereby raises the possibility of cross talk between this molecule and the wound-induced expression of defense genes (Farmer, 1997).

In controlled trials, corn seedlings and cotton plants damaged by caterpillars were observed to release volatiles attracting parasitic wasps, laying their eggs in the caterpillar's body (Turlings, Tumlinson, & Lewis, 1990). The hatching eggs produced wasp larvae feeding on the host, which eventually killed the caterpillar. Over 15 plant species, 10 herbivore and 10 predatory insect species were observed to form a stable, tri-trophic food web (Takabayashi & Dicke, 1996).

Another example of chemical signalling is given by nocturnal moths which lay their eggs on healthy plants, on which newly hatched larvae feed during day light. The plants attacked by the larvae were found to attract parasitic insects. In particular, De Moraes et al. (2001) reported that tobacco (*Nicotiana tabacum*) plants under attack from caterpillars produced two different blends of volatiles during night or day periods. The night time blends of volatiles were small, unsaturated derivatives of fatty acids and discouraged pregnant nocturnal herbivore moths (*Heliothis virscens*) for laying eggs. Night time herbivory food chain was thus discouraged, to avoid further plant damage (De Moraes et al., 2001). At the same time, repelling the night time pregnant moths by a specific blend of volatiles also reduced caterpillars competition with other day time herbivorous insects and also the attacks on their larvae by the predators already invited by diurnal caterpillars. It is important to note that the hatching larvae of nocturnal moths feed during the day time. However, it is not clear whether the night time blends of volatiles are specifically produced or if they are by-products of diurnal volatiles (De Moraes et al., 2001). The dual function of volatiles released on herbivores attack is believed to have evolved simultaneously. The release of night time blends of volatiles of tobacco plant are also constituents of the set of volatiles released during attacks from diurnal insects, and defended tobacco plants from both diurnal and nocturnal herbivore caterpillars (De Moraes et al., 2001).

If the full range of volatile signals used by plant to communicate with each other and with insects could be exploited, and its relevance in ecology also understood (including the identification of genes responsible for volatiles synthesis), plants could then be activated to emit specific blends of defensive volatiles at an appropriate day and night time, well before insects attack. The synthesis of these volatiles even in traces would then contribute to minimize the use of environment detrimental pesticides (Ryan, 2001).

3. INSECT DIVERSITY AND CROP DAMAGE

In spite of the fact that a large number of insect species are yet to be described and reported, about $1.7 \cdot 10^6$ species of insects are already known, representing 56% of all members in the whole animal kingdom. According to another estimate, insects account for 64% of the whole animal biodiversity. Insects are known to have evolved several adaptation mechanisms making them one of the best fitting group in almost all climate and environment conditions (Atwal & Dhaliwal, 2003). The small body size, strong exoskeleton, high mobility through flight, efficient water conservation mechanism, rapid reproduction and resistance allowed their adaptation to diverse climates, and the maintenance of their own biodiversity. Honey bees, silk worm, Lac insect (pigment producers) and pollinators are also economically important species, as also the predators and parasites of herbivorous species. Their life cycle may be small, covering one season only, or last longer, for more seasons.

There are varying reports concerning crop losses due to insect pests attacks. The losses caused by pests, diseases and weeds on a global scale are considered to vary between 35 and 37% (Atwal & Dhaliwal, 2003; Haq, Atif, & Khan, 2004; Brewer, 2001). Crop losses due to various pest categories are very high in both developed and developing countries. In North America, Europe and Japan, estimates of crop losses are 10–30% (Atwal & Dhaliwal, 2003). However, crop losses due to pests are very high in developing regions. In India, estimated crop losses due to insect herbivores until a few decades ago were 18% for cotton, 10% for rice, 5% for oilseed and 5% for pulse crops (Atwal & Dhaliwal, 2003). Older (>50 years) estimates of crop losses due to insects ranged around 13%, whereas other pathogen losses averaged 12%. Arthropod pests, with more than 9,000 species including insects and mites, contributed towards major losses to agriculture crops, not only by direct damages, but also acting as vectors and transmitting various plant diseases (Griswold, 1953). Among all grazers, insect herbivores account today for a 50% of total biomass consumption. Among all herbivores, insects are far most significant grazers as they co-evolved feeding strategies besides the deploy of plants defence mechanisms (Ehrlich & Ehrlich, 1970).

According to a recent report, insects may consume about 10–20% of a crop (Ferry, Edwards, Gatehouse, & Gatehouse, 2004). From previous global estimates, insect pests resulted in crop losses around \$7 billion, while \$3 billion annually were spent in the US on pest control efforts in the early sixties (Janick, Schery, Woods, & Ruttan, 1974). In India, Rangaswami (1983) estimated annual economic crop losses around Rs. 150 thousand million (≈ US\$ 3191 million). Another estimate of total losses of field crops and food grain (pre and post harvest) caused by insects was around Rs. 336.6 billion (approx. US\$ 7.17 billion) per year on a global scale (Atwal & Dhaliwal, 2003). Control of insect pests has hence a high potential in increasing crops production and farmers' revenues, with a potential estimated increase of yields by 13.1–18.7 million tonnes per year. As an example, control of herbivory (flies and pod borers) may reduce total crop damage up to 30%, with an increase of sugarcane yield by 20% or of groundnut production by 15% (Atwal & Dhaliwal, 2003).

The loss to leaf area caused by herbivores varies from 4.8 to 32.5% (Lowman & Box, 1983). Chemical changes in leaf composition are also produced by insect attacks. For example, the insect attack may result into accumulation of phenolics which may reduce in turn grazing intensity by other species (Lowman & Box, 1983). In red alder (*Alnus rubra*), plant memories to insect attacks, in terms of the durability of chemical or physical defense, also varied depending on the feeding insect species (Williams & Myers, 1984).

Further estimates of plant damage due to herbivory may be derived by assays on annual crops or observations on perennial plants. The loss of maize (*Zea mays*) to herbivory by the grasshopper *Choroedocus illustris* follows a density dependent pattern. The damage intensity to the leaf area due to adult grasshoppers varied from 1.64 to 3.55% as 10, 20 and 40 adults attacked, respectively, 32.5, 60.9 and 79% leaves of all plants (Farha-Rehman, 2008). Furthermore, in damaged maize leaves, the insect attack during 15 days led to a proportionate loss of leaf proteins and an increase in proline accumulation (Farha-Rehman, 2008). In the insect herbivory on the Australian woodland eucalypt (*Eucalyptus blakelyi*), Journet (1981) noted that herbivorous insects caused 40% annual foliage damage. The abscission of damaged foliage further enhanced the total annual foliage damage up to 70%.

4. HERBIVORY

4.1. Insect Herbivores

Insects are the primary herbivores in many ecosystems and their size vary from the tiny aphids to very large species. They feed on a vast variety of plants, ranging from algae to angiosperms. The insect grazers form a complex system at the herbivory level in food webs. About 80% of plant material consumed by insects, and their secondary production, can equal or exceed the plant biomass consumed and rebuilt as secondary productivity by vertebrate grazers in grassland.

During the evolution of life on earth, insects have been the most significant herbivores and co-evolved with land plants, since they are dependent on plants for food and shelter. The herbivores co-evolved mechanisms to obtain food from plants despite the set-up of a diverse range of plant defenses. Herbivore adaptations to plant defense have been attributed to the offensive traits linked with promoting increased feeding and use of a suitable host.

Relationship between herbivores and their host plants often resulted in reciprocal evolutionary changes. For example, some herbivores co-evolved ways to hijack plant defenses by sequestering defensive chemicals and using the modified blend to protect themselves from predators.

4.2. Plant Responses to Insect Herbivory

A number of volatile chemicals produced by plants promote or reduce herbivory. The increase of nitrogen, stored as accumulated proline and valine residues, was observed to stimulate grasshopper herbivory during drought stress (Haglund, 1980). Experimental evidence suggested that grasshoppers detect and preferentially feed on grasses treated with the amino acid proline and valine, whose accumulation levels in plants commonly increase under drought. This adversity may lead to insect concentration on drought stressed plants (Haglund, 1980).

Leaf toughness, total phenols and condensed tannins increased in the leaves of five tree species of Australian rain forest, because of insect grazing (Lowman & Box, 1983). In most of these trees leaf toughness and chemical toxicity increased with leaf aging, leading to a corresponding decrease in insect grazing. The herbivory caused leaf area losses between 4.8 and 32.5%, but losses were positively correlated with leaf toughness rather than phenolic contents. Lowman and Box (1983) inferred, from these findings, an interaction of factors including physical and chemical characteristics of leaves, with spatial and temporal factors that induced the variation in insect grazing intensities.

Leaf structure and organization may also protect plants from herbivores, although with complex outcomes. As an example, the tiny hooked trichomes on leaves and stems of *Mentzelia punila* (Family Loasaceae) entrapped and killed not only herbivore aphids (*Macrosiphum mentzeliae*) but also incapacitated a coccinellid beetle (*Hippodamia convergens)* that preys upon the aphid enemy (Eisner, Eisner, & Hoebeke, 1998).

Besides leaf age, the pattern of herbivory may vary with the plant species. Intraspecific variation in the pattern of herbivory on young and mature leaves of *Trichilia cipo* (Meliaceae) and *Cecropia insignis* (Moraceae), two tropical trees, was observed in a lowland rain forest in Panama. Mature leaves of *Cecropia* suffered a five fold greater damage than *Trichilia*, irrespective of the differences in life history, habitat, defensive characteristics and damage levels (Coley, 1983).

4.3. Plant Reaction to Previous Herbivores

The previous herbivore attack of Western tent caterpillar, *Malacosoma californicum pluviale*, on red alder was observed to improve food quality for fall webworm (*Hyphantria cunea*) larvae. Three hypothesis concerning the insect-plant interactions were tested by rearing fall webworm larvae in the laboratory on foliage collected from red alder trees, with different histories of western tent caterpillar herbivory.

Fall webworm larvae grew relatively faster, with heavier pupil weight, when raised on foliage with previous history of herbivory (caused by moderate densities of western tent caterpillar during two previous summers) in comparison to those fed on foliage from unattacked trees. This finding did not fit the hypothesis that previous history of herbivory induced the production of plant defensive chemicals in red alders. Growth patterns of webworms were the same, either fed on foliage from unattacked trees adjacent to those attacked by fall webworm larvae or on the foliage of red alder trees, growing at a longer distance from attacked trees. This study showed, however, that continued insect attacks can deteriorate the food quality of attacked trees (Williams & Myers, 1984).

Seed consuming larvae reduced seed production in turtlehead, *Chelone obliqua*. Seed production was also reduced due to severe herbivory by specialist folivores (Stamp, 1987).

Plant defense signalling can also be induced by biotic attacks. In some interactions, defense was enhanced prior to attack by another organism, whereas in other species, signals were conflicting. Below ground attack may also influence responses to above ground attack and vice versa, due to systemic induction of defense metabolism pathways (Bruce & Pickett, 2007).

4.4. Impact of Herbivory on Ecosystem

In a study on the impact of insect herbivory on eight plant species (including perennial forbs and grasses), the size variability in plant populations influenced insect herbivory, which in turn affected the fitness of individuals under natural successions. The size variability was reduced with herbivores abundance. Insect herbivory reduced size and survival of plants as did competition, which directly affected the size and distribution of plants (Gange & Brown, 1989).

Insect herbivory may also act as a plant population regulatory factor (Williams, 1990). Herbivory limited the distribution of *Eucalyptus pauciflora* in sub-alpine forest in Australia which was replaced by *E. dives* due to increased herbivory preferences. The co-occurrence of *E. dalrympheana* with *E. dives* and *E. pauciflora* was maintained by a density-dependent stabilizing mechanism, caused by leaf parasites. The leaf damage by insect on the ligno-tuberous seedlings of these three subalpine eucalypts was not consistent, but the damage levels in the seedlings of *E. dalrympleana* were lower than those of *E. pauciflora* and *E. dives* (Williams, 1990).

Insect herbivory by grasshoppers accelerated nutrient (nitrogen) cycling, plant production and abundance, and thereby influenced plant species composition over a period of 5 years (Belovsky & Slade, 2000). Enhancement in plant abundance depended on consumption rate of grasshoppers and, under some conditions, grasshoppers decreased nutrient cycling and plant density (Belovsky & Slade, 2000).

The nocturnal plant volatiles induced by caterpillars were observed to repel co-specific females (De Moraes et al., 2001). Tobacco plants released herebivore induced volatiles night and day. The volatile compounds released exclusively at night were highly repellent to female moths *H. virescens*. In fact, tobacco plant released temporarily different volatile blends and lipidopteran herbivores used night time plant volatile signals to select suitable oviposition sites. In night, attacked tobacco plant released volatiles that repelled nocturnal pregnant moths looking for the site of oviposition. In the day, tobacco plants under attack by herbivores released blends attracting parasitic or predatory insects (De Moraes et al., 2001). These responses were beneficial for tobacco plants and the herbivorous insect involved (De Moraes et al., 2001). These findings indicate that host plant on one hand attracted predators of herbivorous caterpillars during day time and female moth co-evolved to avoid such plants for oviposition using some exclusive night time blends to avoid predators of larvae. This co-evolution between tobacco and pregnant female moth maintained ecosystem functioning.

A co-evolutive adaptation was observed as the result of plant-insect interactions (Wittstock et al., 2004). For example, maize rapidly mobilizes the accumulation of a 33-kDa cysteine protease in response to feeding of caterpillars, thereby posing resistance to herbivory. The accumulation of the 33-kDa cystein protease in the maize midwhorl significantly reduced caterpillar growth due to impaired nutrient utilization (Pechan, Cohen, Williams, & Luthe, 2002). The larvae of the specialist insect, *Pieris rapae* (cabbage white butterfly, Lepidoptera) also appear adapted to the glucosinolate-myrosinase system, a defensive chemical arsenal of the host plants.

Trotter, Cobb, and Whitham (2002) studied herbivory, plant resistance and climate in the tree ring records and noted that interactions distorted climatic reconstructions. The resistance or susceptibility of pines to herbivore and climate interaction in the tree ring record were detectable, due to hereditary characteristics. These authors found that herbivory reduced tree rings growth by 25–35% and distorted climate reconstruction on growth rings. Herbivory-induced changes also reduced preference and performance of a variety of insects for a diverse group of plants and ultimately increased their fitness in natural environments, as shown from studies on wild radish (*Raphanus raphanistrum*) (Agrawal, 1999).

4.5. Herbivore Strategies

Several factors affect herbivores feeding, including the type of available food and the biochemical products released during feeding. For example, the lubber grasshopper (*Romalea guttata*) feeds on a wide range of plant species and produces a metathoracic defensive secretion containing primarily phenolics and quinones (Jones, Hess, Whitman, Silk, & Blum, 1987). When reared on onion (*Allium canadense*) and an artificial diet, it secreted volatiles with fewer compounds, in altered proportions as compared to a set of insects reared on diets from a diverse group of 26 plant species, including onion. The diet diversity appeared to have a major impact on the quality and quantity of the autogenous defensive secretions of this generalist herbivore, possibly due to changes in precursors availability, owing to a diverse diet and/or to diet restrictions, leading to a physiological stress caused by partitioning of resources to defensive chemicals (Jones et al., 1987).

Larvae of *Spodoptera eridania* preferred for its diet proteins of *Lotus corniculatus*, as compared to tannins (Briggs, 1990). The larvae were given a choice of *L. corniculatus* plants whose chemical profiles were altered by feeding on plants grown with nutrient fertilization, or with symbiotic nitrogen fixation as their only nitrogen source. The larvae preferred protein rich leaves, rather than leaves with higher tannin contents (Briggs, 1990).

The caterpillar regurgitants were found to amplify the production of the wound induced phytohormone jasmonic acid (JA), but not of nicotine in *Nicotiana sylvestris* (McCloud & Baldwin, 1997). It was noted that herbivory and mechanical damage increased the concentration of the wound-signal molecule (JA) and the defense metabolite (nicotine) in native tobacco plants. However, the larvae of *Manduca sexta* while feeding on damaged leaves modified the plant normal defensive metabolites by reducing systemic JA in roots and, subsequently, the nicotine content in the whole plant (McCloud & Baldwin, 1997).

4.6. Simulation of Herbivory

Several experimental assays attempted to simulate herbivory, in order to gain an insight on the potentials of the biochemical changes and signals induced. Realistic herbivory simulations in terms of plant responses were noted on combining 50% leaf area clipping and JA spraying on *Solidago canadensis*, rather than either clipping or JA spraying alone (Van Kleunen, Ramponi, & Schmid, 2004). Further experimental assays on tomato plants showed that JA is a useful plant elicitor for pest management (Thaler, 1999a).

Pontoppidan, Hopkins, Rask, and Meijer (2005) compared the effect of artificial mechanical wounding with herbivory by diamond-back moth larvae on rapeseed, *Brassica napus*, showing that the changes observed in myrosinase binding protein and myrosinase transcript levels were reflected in protein levels. In a tri-trophic interaction including the bacterial phytopathogen *Pseudomonas syringae*, Cui et al. (2005) reported that the bacterium manipulated the systemic plant defense against pathogens and herbivores. It was noted that virulent strains of *P. syringae* induced systemic susceptibility to a secondary *P. syringae* infection in the host plant *Arabidopsis thaliana* (L.) Heynh. The *P. syringae* infection elicited systemic induced susceptibility caused by the production of coronatine (COR), a pathogen-derived functional and structural mimic of JA, which in turn induced a systemic resistance to attacks by the insect *Trichoplusia ni*.

Recent studies provided evidence for specificity in the elicitation of induced plant responses by different attackers, suggesting that the host suitability for colonizing herbivores may depend on the herbivore species that initially damaged a plant. For example, the existence of a plant-mediated competitive asymmetry between herbivore species on *Solanum dulcamara* showed the dynamic nature of plant resistance, and its potential role in organizing and structuring herbivore communities (Viswanathan, Narwani, & Thaler, 2005).

In simulating/testing of diet components, specific ingredients may be identified which play a significant role in herbivores selections. Konno et al. (2006) found that mulberry (*Morus* spp.) latex, rich in antidiabetic sugar-mimic alkaloids, affected caterpillars dieting. Some ingredients of the latex milky sap exudate present in veins of the mulberry leaves were highly toxic to caterpillars, other than the silkworm *Bombyx mori*, playing a key role in selectivity of insect herbivory. Finally, some ecological factors may also affect plant consumption by herbivores, as shown by

Simonetti, Grez, Celis, and Bustamante (2007) who studied the herbivory and seedling performance in a fragmented temperate forest of Chile. These authors observed that forest fragmentation alters plant-animal interactions, including herbivory. The insects were found to be important herbivores in the Maulino forest and fragmentation had strong indirect effects on plant communities, as mediated through trophic interactions.

5. DEFENSE STRATEGIES

5.1. Plant Defense Strategies

The proteinase inhibitors of tomato plants are known to provide a defense strategy against insect herbivory by the beet armyworm, *Spodoptera exigua* (Broadway, Duffey, Dearce, & Ryan, 1986). The insect herbivory rapidly reduced plant quality and in turn larval growth, as shown by the larval feeding on foliages from insect damaged and undamaged tomato plants. This feedback mechanism involved the induction of tomato proteinase inhibitors resulting from larval feeding, that systemically reduced leaf nutritive value (Broadway et al., 1986). In *Verbascum thapsus*, herbivory was directly related to age dependent leaf pubescence (Woodman & Fernandes, 1991), since less pubescent leaves, of older plants, were more frequently attacked than the more pubescent leaves, of younger plants. The leaf hairs acted both as a barrier against herbivores and also prevent water loss. Similar age-specific mechanical defenses are widespread and effective against generalized chewing insects, such as grasshoppers (Woodman & Fernandes, 1991).

The effect of leaf feeding by the larvae of *Samea multiplicalis*, at densities of 0.8 and 1.6 larvae per plant, severely damaged *Salvinia molesta*, a floating weed, by reducing leaf area, plant weight and ramet numbers (Julien & Bourne, 1988). The root and rhizomes were spared by the moths allowing the plants to continue its growth, but the insect feeding altered the nitrogen balance in above and below ground plant parts (Julien & Bourne, 1988).

Several experimental assays provided evidence for a key role played by JA and jasmonate in plant defense mechanisms. McConn, Creelman, Bell, Mullet, and Browse (1997) noted that jasmonate is essential for insect defense in *Arabidopsis*. The signalling pathways mounting defenses against chewing insects were complex. Mutant plants containing negligible levels of jasmonate showed high mortality (≈ 80%) when attacked by larvae of *Bradysia impatiens*, whereas neighbouring wild type plants were largely unaffected. These experiments precisely defined the role of jasmonate as essential for the induction of biologically effective defense in plant-insect interactions. The herbivory on rape seed plants by cabbage stem flea beetles (*Psylliodes chrysocephala*) induced systemic changes in glucosinolate profile (Bartlet, Kiddle, Williams, & Wallsgrove, 1999). A similar change was noticed when JA was applied to the plants cotyledons, suggesting that JA synthesis was initially induced by herbivory which in turn induced a negative effect on subsequent herbivory (Bartlet et al., 1999). Thaler, Stout, Karban, and Duffey (2001) found that jasmonate-mediated plant resistance may affect a community of herbivores. Several proteins induced in tomato foliage following herbivore damage were linked to reductions in herbivore performance under laboratory condition (Thaler et al., 2001). This induced resistance often suppressed many communities of herbivores. The information on the plant biochemistry, insect preference, performance and abundance may hence be helpful in developing practical tools for insect management, through natural or induced plant defense mechanisms (Thaler et al., 2001).

The amounts of signalling molecule may affect the efficacy of defense metabolites. Quantitative relationships between induced JA levels and volatile emissions in maize during *Spodoptera exigua* herbivory have been reported by Schmelz, Alborn, Banchio, and Tumlinson (2003a). JA induced emission of indole and sesquiterpenes (volatiles) limited the plant ethylene emission and also controlled excessive volatile production. In another study (Schmelz, Alborn, & Tumlinson, 2003b), volicitin from the herbivore oral secretion induced JA levels, and sesquiterpene volatiles were more excessively stimulated in maize than through mechanical damage in absence of volicitin. Relatively more significant increase in ethylene levels were recorded during beet army worm herbivory, than either by wounding or volicitin treatments (Schmelz et al., 2003b).

Engelberth, Alborn, Schmelz, and Tumlinson (2004) reported that airborne signals alert plants against insect herbivore attack. Green leafy volatiles including six carbon aldehydes, alcohols and esters were commonly emitted by plants in response to mechanical damage or herbivory. These volatiles induced intact undamaged neighbouring corn seedlings to rapidly produce JA and emit sesquiterpenes. These green leaf volatiles played a key role in plant – plant signalling and plant-insect interactions. Heil (2004) reported that JA induced defenses in lima bean (*Phaseolus lunatus*) was beneficial under natural condition.

Finally, Howe and Jander (2008) observed that herbivore insects use diverse feeding strategies to obtain nutrients from their host plants. Plants responded to herbivory with the production of toxins and defensive proteins that target physiological processes in the insect. This strategy appears as a highly dynamic form of immunity, initiated by the recognition of insect's oral secretions and emission of signals from injured plant cells.

5.2. Plant Receptor Molecules

Cowpea (*Vigna radiata*) attacked by the fall armyworm *Spodoptera frugiperda* perceived herbivory through inceptins, a proteolytic fragments of chloroplastic ATP synthase γ-subunit regulatory regions (Schmelz et al., 2006). Inceptins (feasibly the receptor molecule) induced volatile, phenylpropanoid and protease inhibitor defenses in cowpea, against fall armyworm. It was also noted that *S. frugiperda* larvae having previously ingested chloroplastic ATP synthase γ-subunit proteins induced cowpea defenses after herbivory.

5.3. Defense Genes in Plants

Intracellular levels of free linoleic and linolenic acids increased in tomato leaves on wounding (Conconi, Miquel, Browse, & Ryan, 1996). The intracellular signalling pathway for activating plant defense genes against attacking herbivores and pathogens is mediated by a lipid based signal transduction cascade. In this pathway, linolenic acid (18:3) is liberated from cell membranes and is converted to cyclopentanones involved in transcriptional regulation of plant defense genes (Conconi et al., 1996).

A wide range of genes in plants are activated on herbivore attacks, and their activation is strongly correlated with the mode of herbivores feeding and degree of tissue damage at the feeding site (Walling, 2000). Phloem feeding whiteflies and aphids producing little injury to plant foliage were perceived as pathogens and activate the salicylic acid (SA)-dependent and JA ethylene-dependent signalling pathways. Volatiles blends provided specific cues to attract specialist parasites and predators to attack infesting herbivores (Walling, 2000).

Herbivory on lima bean leaves induced volatiles elicitor defence genes (Arimura et al., 2000). In uninfested lima bean leaves, five separate sets of defense genes were activated on exposure to volatiles from co-specific leaves infested by *Tetranychus urticae*. All these genes were not activated when uninfested leaves were exposed to volatiles from artificially wounded leaves (Arimura et al., 2000). The expression pattern of these genes was similar to that produced on exposure to JA. At least three terpenoids of the released volatiles were responsible for the gene activation, which were released in response to herbivory but not on artificial wounding. Expression of these genes required calcium influx and protein phosphorylation/dephosphorylation (Arimura et al., 2000).

The herbivore induced volatiles in *Arabidopsis thaliana* attracted the parasitoid *Cotesia robecula* (Van Poecke, Posthumus, & Dicke, 2001). The *A. thaliana* plants infested by *Pieris rapae* emitted volatiles from several major biosynthetic pathways, including terpenoids and green leaf volatiles. Haq et al. (2004) focused on the utility of the protein proteinase inhibitor genes in combating insects, pests, and pathogen as natural and engineered phytoprotection. Devoto et al. (2005) found COII as a key regulator of genes involved in wound. The methyl jasmonate induced secondary metabolism, defence and hormone interactions. COII expressed approximately 84% of 212 genes induced by JA, and approximately 44% of 153 genes induced by wounding. COII displayed a pivotal role in wound and JA signalling (Devoto et al., 2005). The volatile emission on green leaf wounding induced the release of acetylated derivatives and a terpenoid in maize, and these compounds in turn induced JA production in intact plants (Yan & Wang, 2006). The wound-induced green leaf volatiles (GLVs) caused the release of acetylated derivatives and a terpenoid, (E)-4,8-dimethylnona-1, 3, 3-triene (DMNT) in intact maize, which may be a type of plant-plant interaction mediated by airborne GLVs (Yan & Wang, 2006).

Karban and Niiho (1995) worked on plant “memory” to induce resistance and susceptibility to herbivory. Many plants have been found to be highly induced by repeated herbivory, than by a single herbivore bout. The frequency and magnitude of damage were confounded and thus it was not clear if a biochemical “memory” was involved or overall damage amplified the induced response (Karban & Niiho, 1995). In this experiment, the cotton plant attacked by spider mites and mechanical damage of cotyledon induced resistance, but damage of apical buds induced susceptibility, due to differentially affected hormonal regulation in both tissue types (Karban & Niiho, 1995).

Van Poecke, Roosjen, Pumarino, and Dicke (2003) noted that different kind of herbivores induced different signal transduction pathways in *A. thaliana*. The varying blends of volatiles were recognized by specialist parasitoid (*Cotesia rebecula*) of host and non-host herbivores.

5.4. Tri-Trophic Plant Signalling

In many plants, Thaler (1999b) found that defence systems against herbivores were induced through the octadecanoid pathway, which in turn recruited natural enemies (predators or parasites) of herbivores. This pathway was induceable by treating plants with JA or by natural herbivory, as noted in case of tomato plants under insect herbivory. The plants induced with JA increased parasitism of caterpillar pests in an agricultural field (Thaler, 1999b). Either JA or herbivory induced carnivores attraction towards herbivores in lima bean plants (Dicke, Gols, Ludeking, Posthumus, 1999). Lima bean plants herbivory damage by the two spotted spider mite (*Tetranychus urticae*) led to the emission of complex blend of volatiles. These volatiles attracted the carnivorous mite *Phytoseiulus persimilis*, a specialist predator of spider mites which exterminated entire population of spider mites. Dicke et al. (1999) inferred that induction of volatile synthesis in Lima bean plants is almost similar either caused by JA treatment or herbivorous spider mites.

Maize plants (var. LG11) under insect attack released large quantities of volatile compounds and invited parasitic wasps to attack the herbivore (Pare et al., 1998). Volicitin and an elicitor of plant volatile were isolated from beet army worm caterpillars, and were regarded as key components inducing plants to recognize damage caused by herbivory (Pare, Alborn, & Tumlinson, 1998).

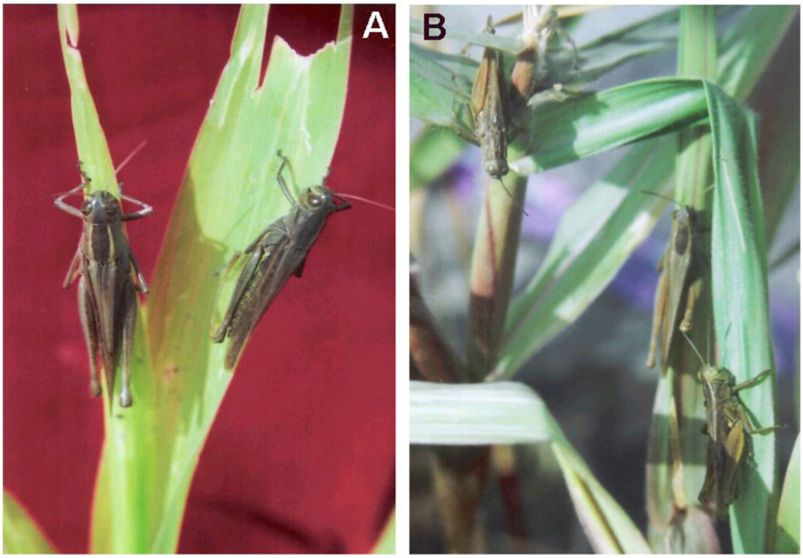

Figure 1. Choroedocus illustris *feeding on young leaves of* Zea mays.

Thaler, Farag, Parepaul, and dicke (2002) found that jasmonate deficient plants reduced direct and indirect defenses against herbivores. Some plant species had negative effects on herbivores whereas others had indirect defense against herbivory, via invitation to the natural enemies of herbivores. It was also noted that damaged wild type plant were more attractive to predator mites compared with undamaged wild plant. In both cases, JA is reported to be an essential regulatory component for the expression of direct and indirect plant defences against herbivory (Thaler et al., 2002). Van Poecke and Dicke (2004) reported that *Arabiodopsis thaliana* defends itself from pathogens, herbivorous insects and mites through induced volatiles emissions, upon herbivory. These volatiles guided predators or parasites to reach their herbivorous prey, with a benefit for both the plants and the carnivores. Similar indirect defense patterns have also been noted in pine, maize and lima bean.

Figure 2. Choroedocus illustris *attacking male inflorescence of* Zea mays.

The proline accumulating leaves are preferred by grasshoppers. Herbivory increased the population of grasshoppers in drought stressed plants (Haglund, 1980). Treatments with low levels of methyl jasmonate resulted in the accumulation of certain vegetative storage proteins in soybean seedlings, within 3 days of exposure. The protein accumulated preferably on shoot tips and primary leaf. Accumulation of some specific protein in response to methyl jasmonate was also noticed in cotyledons. It was inferred that volatile methyl jasmonate acts as gaseous messenger and growth regulator in plants, which alter the nitrogen partitioning (Franceschi & Grimes, 1991).

In a recent study on maize, the grasshopper *Choroedocus illustris* attacked almost every part of the plant. The grasshoppers preferred young leaves mainly in the apical portion (Fig. 1a, b), and also male inflorescence (Fig. 2).

CONCLUSIONS

It is evident from the present review that insects and plant co-evolved and developed well defined mechanisms to regulate the uptake of a limited amount of plant organic matter to higher trophic levels. In this review emphasis has been given to understand the natural mechanism of control over herbivore consumption and the way in which energy is transferred from primary producers to the organisms of higher trophic levels. Plant and insects co-evolution allows the onset of a balanced primary productivity, which is partitioned to the second trophic level and beyond. During their course of evolution, plants not only developed morphological defense mechanisms active against insect herbivory, but also genetic transformations allowing the production of volatile chemicals. Attacked plants use these volatile chemicals as arsenals and signals against attacking pests. These chemicals not only repell herbivores but also control their population, by signalling invitations sent to their predators or parasites. For all these purposes plants synthesize special blends of volatile chemicals only after insect attack. The interaction between herbivores and biotic environment is thus largely based on plant mediated mechanisms, including constitutive traits like modifications in plants anatomy and physiology, or herbivore induced changes in host biochemistry (Elliot, Sabelis, Janssen, Van der Geest, & Berling, 2000; Ode, 2006; Ohgushi, 2005; Price et al., 1980). The plant mediated interactions can furthermore operate among spatially and temporally separated organisms, even at low herbivory levels (Ohgushi, 2005).

The energy cost – benefit ratio between crop losses and pesticides use show that about 50% of losses are due to pests. Under natural conditions, the energy transfer from plants to herbivores has a far lower magnitude (Kormondy, 2003), even without use of pesticides. These compounds have in the ecosystem a far reaching impact, and may eliminate herbivores predators from the ecosystem. Thus, within a single crop cycle, the natural process of controlled biomass transfer from one trophic level to the higher one may be altered, in a structural way. If the process of plant signalling is activated and applied, it may prove to be more effective than pesticides in maintaining stable populations of plants, herbivores and predators.

Plants also evolved direct strategies to repel herbivores, through induced and constitutive defence mechanism. The trichomes constitute a defense feature against a variety of insects. But plants also evolved mechanisms based on volatile substances acting during insect attacks that, on one hand repell an attacking insect directly, whereas on the other hand invite its predators. This mechanism becomes operational only on herbivore attack. The reaction of herbivores oral secretion and plant elicitors activates certain host genes. Thus, plants rely on a system of multiple chemical switches that control the partitioning of biomass to herbivores and predators, at least up to three trophic levels. Moreover, plants have either no or limited "memory" of previous insect attack, with some exceptions. Thus, an equilibrium in the transfer of biomass from hosts to herbivore-predators is maintained naturally, throughout the plants life cycle.

Among pests response to plants arsenals, it is worth to recall how herbivorous insects evolved adaptive mechanism to search suitable healthy plants for oviposition. As shown, nocturnal pests, i.e. *Heliothis veriscence* avoid injured plants during day time for oviposition, to save their offsprings from day time predators (De Moraes et al., 2001).

Herbivory caused by grasshoppers accelerated nutrients cycling and plant production and abundance (Belovsky & Slade, 2000). It is also reported that larvae of *Manduca sexta* modified, before feeding on damage leaves, the host normal defensive metabolites by reducing systemic JA in roots, and subsequently the nicotine content in the whole plant (McCloud & Baldwin, 1997).

Herbivores and predators also rely on receptor molecules, starting from the activation of plants defense genes on an insect attack. Plant defences to herbivory can be simulated by JA, since in some plants the defense mechanism was brought under operation through JA and artificial injury together. However, more experiments are required to induce chemical defense and use this mechanism of chemical signalling for pests control, in a way that may be much more effective and environment friendly than using pesticides. Biological control may prove indeed to be more economic not only for crop losses, but also for the ecosystem maintenance. It must be kept in mind that elimination of one herbivore species may prove to be more detrimental for the ecosystem if it happens to be a keystone species. Any biological control strategy should seek at an equilibrium among plant biomass, herbivores as well as predator populations, to be maintained

Plants adaptive mechanism evolved to ward off herbivores is both structural and functional (constitutive and inducible). Host plants synthesize volatile chemicals as part of their defense strategies against excessive herbivory. Artificial injury induced by leaf clipping in some species did not produce the blend of volatiles which are repulsive for herbivore or provide clues to the predators, since they possess specific genes activating the synthesis of volatiles only on an insect attack and up regulated by the chemicals contained in the herbivores mouth.

The volatiles synthesized in plant tissues after insects attack were useful to ward off insect directly. Also, since the volatiles provide clues to the predator about the preys (herbivores) presence and also signal the neighbouring undamaged plants about the attack of the herbivores, the course of co-evolution acquired a higher level of complexity when certain insect species cleverly adopted to these volatiles. Some herbivores, furthermore, were capable to modify the blends of volatiles and thus used modified plant arsenals (volatiles) to defend themselves from the predator.

Due to co-evolution, most plants had limited memory for insect attack but not in some perennial trees (mainly Australian eucalypts), in which the plant volatiles were synthesized for longer duration and controlled insect population. Similarly, due to the adaptation of herbivores to modify the blends of chemicals, the role of plant volatiles in inviting predators or parasites results in an ecosystem-wide effect, influencing the flow of material and energy along the trophic level, as well as the population equilibrium levels, at each trophic level. Due to co-evolution of synthesis of herbivore repelling volatiles in plants and their modification by herbivores, cyclic population changes, concerning both the herbivores and primary producers, can also be affected. This aspect needs attention and attempts to increase the amount of data through further studies and may prove useful in biological control of crop herbivory.

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9

IPM OF THE RED PALM WEEVIL, *RHYNCHOPHORUS FERRUGINEUS*

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Abstract. The Red Palm Weevil (RPW) *Rhynchophorus ferrugineus* is the major destructive insect pest of a broad range of palm trees. Infestation by RPW varies in relation to the height and age of palm trees and most likely is restricted to 0–1 m height above soil surface and to palms up to 10 years old. The life cycle of the insect in the laboratory may last several months depending on the type of food, temperature as well as the procedure of rearing. A single female may produce more than 400 eggs during its life-time, which may last more than 6 months. Natural enemies recorded for RPW include parasitoids, predators and insect pathogens (bacteria, viruses, fungi and nematodes). However, such recorded natural enemies do not have considerable role against the insect. Chemical control with different insecticides was applied against the insect as spraying, trunk injection as well as fumigation, with phostoxin tablets. Alternative safe methods of control were investigated using entomopathogenic nematodes, fungi or aggregation pheromone traps. The latter proved to be a promising means of control against this insect.

1. INTRODUCTION

The Red Palm Weevil (RPW), *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) is the major destructive insect pest of a broad range of palms including date, coconut, sago, oil palm, toddy and royal palm (Nirula, 1956). It was first recorded in 1889 in India as the most deadly insect pest of the coconut palms (Leefmans, 1920). The insect was later recorded in Ceylon and Philippines in 1906, Indonesia in 1920 (Nirula, 1956) and later detected in the Gulf area in mid 1980s, the United Arab Emirates (UAE) in 1985 (FAO, 1995), Saudi Arabia in 1986, Qatar in 1989 (Abdulla, 1997), the Sultanate of Oman in 1993 (Al-Kaabi, 1993), Kuwait in 1993 (FAO, 1995), Bahrain in 1995 (Hamdi, 1998) and Iran in 1992. The insect was able to cross the Red Sea as it was then found in Egypt in 1992 (Cox, 1993) and in Palestine, Israel and Jordan in 1999 (Kehat, 1999). It was also able to cross the Mediterranean Sea to become a serious pest in Spain, France, Italy and Greece (Manachini, Mansueto, Arizza, & Parrinello, 2008).

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The high rate of spread of this pest is due to the human intervention, by transporting infested young date palm trees and offshoots from infested to healthy areas and countries. For example, the insect invaded Egypt when infested offshoots were imported from UAE in November 1992 (Cox, 1993). Also, as reported by Ferry and Gomez (2002), there was evidence suggesting that the first weevils were introduced into Spain from adult palms imported from Egypt.

Leefmans (1920) published the first original investigation on the pest in Indonesia, and gave a complete description of the pest suggesting various control measures.

2. HABITAT, INFESTATION AND DAMAGE

The crown and trunk of the palm tree represent the natural habitats of all stages of *R. ferrugineus*. In old coconut palms the infestation is restricted to the crown, while in young trees of coconut and date palms it is present in the crown but mainly in the trunk. More often the insect resides in the trunk where it passes many generations, feeding into the soft tissue portions until the trunk is completely hollowed out and the tree falls down. The early symptom of RPW infestation is a brown and bad smelling sap exudated from the point of infestation. However, such a symptom could be invisible, especially when infestations occur at the base of the trunk.

The infestation by RPW varies in relation to the height and age of the date palm trees. In a study in Saudi Arabia 50% of infestations occurred at heights between 0 and 1 m from the trunk base, whereas 38% was within 1–2 m (Anonymous, 2001). As for the age of palms, most of infestation was found in palms 5–10 years old, while prevalence was lower in palms older than 15 years.

In a similar study in UAE, Khalifa et al. (2007) reported percentages of RPW infestation in different date palm plantations as 77.8% at 0–50 cm and 20.4% at 51–100 cm (total prevalence was 98.2% within 0–1 m). No infestation could be detected at heights higher than 3 m. Similarly, prevalence was 12.8% at ages up to 5 years and gradually increased to 64.8% at 6–10 years of age. The infestation was noticed to decrease gradually to reach 17.1, 3.3 and 1.3% at ages of 11–15, 16–20 and >20 years, respectively. However, in Egypt, in old palms some infestations were found at the heights around 6 m, so that the palms were broken at that level (unpublished). This case was attributed to the fact that RPW infestation occurred at the crown of palm and the larvae moved, in tunnels, downward until the tree collapsed at that height.

2.1. Infestation Spreading

Once RPW is introduced into an area or even a country, it has great ability to disperse and expand. For example, *R. ferrugineus* was introduced for the first time in Egypt through imported offshoots transplanted in two close locations in Sharkyia governorate, northeastern of Cairo (Cox, 1993). Since then, great efforts have been done to eradicate the insect using intensive spraying of chemical insecticides in both locations, mass trapping by pheromone traps as well as by cutting, burning and burying the infested palm trees. In 1995, despite these efforts, a RPW infestation was detected in Ismailia governorate (50 km apart from initial focus) and later, in the following years, the insect spread in most governorates in Egypt. This extensive spread is certainly due partly to: *(i)* the exchange and transplanting of offshoots and ornamental adult palms, *(ii)* the partial burning of trunks which did not kill all insects inside such palms and *(iii)* superficial burying of burned palms, that allowed the formed RPW adults to emerge from soil for months.

Another example was reported by Gomez Vives and Ferry (1999) who mentioned that in Spain very soon after RPW killed the first *Phoenix canariensis* in some gardens at Almuñecar, intensive chemical treatments were applied to affected trees. Foliage spraying was conducted with various insecticides and preventive treatment of all palms, even healthy ones, was repeated once a month. Simultaneously, a mass trapping program using aggregation pheromone was initiated. However, despite all such efforts, more than one thousand plants were killed and the area of infestation expanded to villages close to the initial focus.

3. BIOLOGY

3.1. Morphological Characters

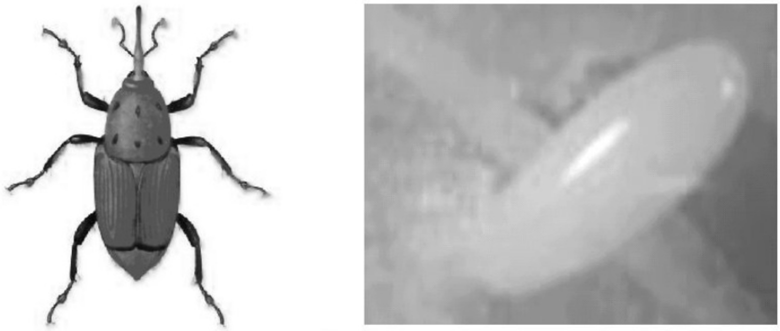
RPW male and female adults are large reddish-brown weevils about 3 cm long with a long curved rostrum. They are capable of flying to long distances. The egg is creamy-white, long oval in shape, smooth and shiny. It is about 2.5 mm long and 1.1 mm wide. The full-grown larva (13 segments) is a conical shaped, plugged in the middle and pointed towards both ends with no legs. The head is light brown with well-developed mouth parts. The average length of the full grown larva is 50 mm with an average of 20 mm in width. The newly formed pupa is creamy in color and turns brown gradually. It is almost 35 mm long and 15 mm wide. The cocoon, constructed from palm tissues, is oval in shape, light brown in color, in average of 60 mm long and 30 mm wide (Fig. 1).

3.2. Life Cycle

The life cycle of RPW was investigated by many authors. In the laboratory, the durations of the immature stages, adult longevity, total number of eggs deposited by a single female and sex ratio varied, as shown in Table 1. Such variations could be attributed to the rearing facilities and the food substrate for the larvae and adults.

3.2.1. Oviposition

The female starts oviposition 2–11 days after emergence from cocoons, in the softer portion of the palm in which they feed (Nirula, 1956). It was believed that RPW attacks only the palms which have been injured by the beetle *Oryctes* *rhinoceros* but it has been proved that the weevil attacks healthy and uninfested palms (personal observations). To oviposit, the female uses the rostrum to bore into the tissues, mainly under the leaf base, to form a hole in which the eggs are laid, then the hole is cemented to protect them from natural enemies.



Adult **Egg**

Pupa **Larva**

Figure 1. Life cycle of the Red Palm weevil, Rhynchophorus ferrugineus.

3.2.2. Feeding

RPWs feeding takes place on the soft portions of the palm. In the field, the weevils select a suitable site with the help of their antennae and thrust in their long rostrum at that spot, to feed on the juice. The feeding scars are quite superficial and can be readily differentiated from the oviposition holes which are deeper (Nirula, 1956). In the laboratory, when weevils feed on pieces of sugar-cane or palms it was noticed that they grind such blocks by their mouth parts, sucking the juice and discarding the remains as ground material (personal observations).

Table 1. Biological parameters of Rhynchophorus ferrugineus *(modified from Murphy & Briscoe, 1999).*

Parameter	Reference					
	Aldafer et al., 1998	Nirula, 1956	Ghosh, 1912	Wattanapongsin, 1966	Viadou & Bigornia, 1949	Faghih, 1996
Incubation period (days)	3.5 (2 – 5)	3 (2 – 5)	3 – 4	3	3	1 – 6
Larval stage (days)	182 (120 – 241)	55 (35 – 71)	35 (25 – 61)	35 – 38	38	41 – 78
Pre-pupal stage (days)	8 (7 – 11)	-	-	2 – 11	-	-
Pupal stage (days)	23 (21 – 26)	16 (12 – 21)	-	11 – 19	-	15 – 27
Total developmental period (days)	♂ 208 (158 – 298) ♀ 223 (145 – 277)	81 (54 – 120)	-	45 – 68 45 - 71	55 (45 – 68) 59 (45 – 68)	57 – 111
Oviposition (days)	96 (54 – 137)	45 (25 – 63)	-	-	-	-
No. eggs / female	227 (55 – 412)	204 (70 – 355)	127 – 276	162 – 350	221 (162 – 350)	3 – 186
Longevity (days)	♂ 161 (76 – 257) ♀ 112 (70 – 335)	81 (48 – 113) 60 (34 – 76)	50 - 90	63 – 109 39 – 72	83 (63 – 109)	39 – 72 20 – 120
Sex ratio	1 ♀ : 0.8 ♂	1 ♀ : 1.3 ♂				

3.2.3. Locomotion

Flight of the weevils is restricted to day time and they have never been observed as attracted by light traps (Nirula, 1956). Al-Khatri and Abd-Allah (2003) studied the daily active periods of *R. ferrugineus* in date palm plantations in Oman, using the pheromone traps. They found that the weevils had two periods of activity, the first between 6 and 9 am and the second between 6 and 9 pm (sunrise in that area during the period of the study was at 6:15 am and sunset was at 5:50 pm). The weevils were noticed to have no activity during the periods between 9 am and 3 pm and between midnight to 3 am. Similarly, Gunawardena and Bandarage (1995) reported that the periods of RPW activity in Sri Lanka were between 6 and 8 am and between 6 and 8 pm.

RPW adults are capable of flying as well as crawling. When they are thrown in the air they fly away in a circle with a buzzing noise and quickly disappear, sometimes landing shortly and boring into the soil. It is believed that the insect is capable of either flying to a long distance or being carried by the winds. It was noticed that when a weevil was attracted to a pheromone trap it landed at less than 1 m apart from the trap, then crawled until reaching it (personal observation). Chinchilla, Oehlschlager, and Gonzaler (1993) reported that the average flight of *R. ferrugineus* adults was around 500 m per day and a small portion of the insect migrated up to 1 km per day. Abbas, Hanounik, Shahdad, and Al-Bogham (2006) found that when marked RPWs were released in date palm plantations, some of them were captured by pheromone traps in other plantations, 1–7 km apart from the release area. Most of such weevils were captured 3–5 days post release.

4. NATURAL ENEMIES

There are few records about the occurrence of natural enemies of *R. ferrugineus*, which might be attributed to the cryptic habitat of the eggs, larvae and pupae which protects them from such natural enemies.

4.1. Parasitoids

Scolia erratica (Hym.: Scoliidae) was reported as an ectoparasitoid of *R. ferrugineus* larvae (Nirula, 1956). However, no biological studies on this parasitoid have been reported. In Brazil, Moura, Mariau, and Delabie (1993) and Moura, Resende, and Vilela (1995) reported the tachinid *Paratheresia menezesi* as a larval-pupal parasitoid of *R. palmarum* and many individuals of the parasitoid adults emerged from a single pupa. However, attempts were carried out to rear this parasitoid on *R. ferrugineus* in the laboratory, but the results were unsuccessful (unpublished). Nirula (1956) mentioned that both RPW pupae and adults were attacked by an unknown species of parasitic mite which killed the pupae and reduced the longevity of adults.

Peter (1989) recorded two mite species, *Hypoaspis* sp. and *Tetrapolypus rhynchophori* (Pymotidae) parasitizing the RPW adults and mentioned that the status of such mites as parasitoids was uncertain.

4.2. Predators

Although the earwigs (Forficulidae: Dermaptera) are considered scavengers, Abraham, Kurian, and Nayer (1973) recorded *Chelisoches morio* as a common predator inhabiting the crown of coconuts in Kerala, India. The authors mentioned that the daily average consumption by nymphs and adults of the predator varied between 5.3 and 8.5 *R. ferrugineus* eggs, or 4.2 and 6.7 larvae, respectively. In Saudi Arabia, the earwig, *Anisolabis maritima* and the anthocorid, *Xylocorus galactinus* were recorded as common predators on RPW eggs, larvae and pupae (Anonymous, 2001).

Anisolabis maritima, showed a higher predatory efficiency. An assay was carried out in Saudi Arabia to evaluate its efficiency as a biocontrol agent against *R. ferrugineus*, on 5 year old date palm offshoots (each caged in a wire-cage, 2 × 2 × 2 m). Three pairs of RPW were introduced in each cage in which *A. maritima* was released after 24 h, at rates of 5, 10, 15 and 20 pairs per offshoot. One month later, 50% of the offshoots were dissected and the numbers of RPW larvae were recorded. The results showed that percentages of infestation in the treated offshoots were 50% (in offshoots with 5 predator couples), 25% (in offshoots with 10, 15 or 20 couples) and 100% (control). A significant reduction in the numbers of RPW larvae was obtained in the offshoots with predators, compared to the control. The larvae found in the five treatments were 12% (in offshoots with 5 or 10 couples), 28% (in offshoots with 15 pairs), 4% (in offshoots with 20 pairs) and 44% (control) (Anonymous, 2001).

The duration of the immature stages of *X. galactinus* were estimated when fed on eggs, 1st instar larvae or pupae of *R. ferrugineus* in the laboratory at 25°C in Saudi Arabia. It was found that the incubation period of the predator egg was 3.6 days (3–4). The nymph (5 instars) lasted 16.5 days (14–19) when fed on 1st instar larvae and 15.5 days (14–17) when feeding on RPW pupae. The average daily consumption of a *X. galactinus* nymph was 1.2–1.8 eggs or 3 larvae. The adult consumed 1.8–2 eggs or 3.6 larvae, daily (Anonymous, 2001).

Xylocorus galactinus was also obtained by M.S.T. Abbas (unpublished) in Egypt from a fallen date palm tree, severely infested. However, this species was found associated with larvae of the house fly, *Musca domestica*, feeding on the fermented decayed tissues of such a tree. In a food preference test, by exposing larvae of *M. domestica* together with eggs and 1st instar larvae of *R. ferrugineus*, the predator attacked and consumed only larvae of *M. domestica*. In agreement with this finding, Tawfik and El-Husseini (1971) recorded *X. galactinus* as a predator of *M. domestica* inhabiting organic manure and animal's dung.

4.3. Pathogens

Banerjee and Dangar (1995) isolated the bacterium *Pseudomonas aeruginosa* from naturally infected adults of *R. ferrugineus* in Kerala, India. The bacterium was found to be pathogenic to adults forced to feed on a suspension of bacterial cells, and mortality occurred 8 days after ingestion.

In India, a highly potent cytoplasmic polyhedrosis virus (CPV) specific to RPW was also found (Gopinadhan, Mohandas, & Nair, 1990). The virus infected all stages of the insect and laboratory infection of late larval stages resulted in the development of malformed adults.

Among fungi, *Beauveria bassiana* was isolated from adults of *R. ferrugineus* in UAE and Saudi Arabia, through a project operated by the Arab Organization for Agriculture Development (AOAD), in the period 1997–2007 (Anonymous, 2008). The fungus was found to be highly pathogenic to both larvae and adults in the laboratory (Hanounik et al., 2000b; El-Safty et al., 2007).

4.4. Nematodes

Praecocilenchus ferruginophorus (Aphelenchida) was recorded parasitizing *R. ferrugineus* adults in India (Rao & Reddy, 1980). The size of the nematodes found in the haemocoel ranged from small intrauterine specimens to larger mature parasitic females, suggesting several simultaneous and unsynchronized life cycles in the weevils. Abbas, Hanounik, Mousa, and Awash (2001b) and Abbas, Hanounik, Mousa, and Mansour (2001c) isolated two entomopathogenic nematodes (EPN), namely *Steinernema abbasi* and *Heterorhabditis indicus* from adult *R. ferrugineus*. They also showed that 20–100% of the RPW adults were found hosting other non pathogenic, unidentified nematodes.

5. CONTROL

5.1. Chemicals

5.1.1. Spraying

Preventative and curative spraying of chemical insecticides have been the most common method applied for RPW control. All groups of insecticides have been tested and applied as foliage and trunk sprayings. For example, Soenardi and Hariadi (1978) reported that application of sevin or carbaryl gave effective field control when applied every 2 months. Also, Abraham, Koya, and Kurian (1975) tested seven insecticides against *R. ferrugineus* in Kerala, India and found that trichlorphon gave the best control, with 92% of the infested palms recovering from weevil infestation. In Egypt, El-Sebaey (2004a), concluded after field trials that offshoots could be protected from RPW infestation for 11–13 weeks, by dipping them for 15 min in a mixture of the insecticides confidor (75 ml/100 l of water), oshin (125 g/100 l) mixed with 250 g/l of mud, before translocation and re-cultivation.

It was observed that many infested trunk bases of date palms harbored reliable numbers of different alive stages of RPW, below the soil surface. Such trunk bases last alive, after removing severely infested palms above the ground level, for a long time (El-Sebaey, 2004a). In this respect, this author carried out an experiment using chemical insecticides to kill all developmental stages of RPW surviving in such trunk bases. Big holes were made in the bases, using a sharp axe, to facilitate penetration of insecticides downward through the base tissues. Six chemical insecticides, dissolved either in water or kerosene, were poured at rates ranging from 5 to 10 l/base. Two weeks later, the treated bases were picked up using a loader machine and cut into parts to allow inspection of alive and/or dead RPW stages, inside the base and root system. The experiment showed that the insecticides Cidial (phenthoate), Basudin (diazinone) and Dursban (chlorpyrifos-ethyl), dissolved in kerosene, caused 100% mortality in larvae and pupae. As for adult weevils they caused 95.6, 95.2 and 97.6% mortalities, respectively. However, the same insecticides, dissolved in water, caused 57, 53 and 49% mortalities in larvae, 37, 55 and 48% mortalities in pupae, and 25, 25 and 26% mortalities in adults. The author related the synergistic action of kerosene to three factors: (i) its function as a good carrier for the chemicals throughout the wood fibers deeply inside the infested roots, (ii) its physical action on the wood as a dehydrated substance which caused wood dryness faster than insect survive, and (iii) its effect on dehydration and toxic action on the insects' cuticle.

5.1.2. Injection

Injection of insecticides into the trunk of the coconut palm was first reported by Rao, Subramaniam, and Abraham (1973), who demonstrated that the direct injection of 0.2% fenthion gave effective control of larvae in the tree. Muthuraman (1984) reported that 10 ml of monocrotophos or dichlorovos injected into a pre–drilled 10 cm deep hole above the infestation site gave 100% recovery of the treated infested trees. In Egypt, El-Sebaey (2004b) tested 15 chemical insecticides against RPW infestation by injecting them at different concentrations in 4–10 holes (10 cm deep), drilled around the infestation site. This author found that all tested chemicals showed 100% recovery of infestation at a concentration of 10,000 ppm, while at 100 ppm dursban, curacron and cidial gave 80% recovery.

An alternative to injecting insecticides into the tree is sealing, inside the tree, a tablet form of a slow release fumigant. Phostoxin tablets (aluminum phosphide) applied at a rate of 0.5–1 tablet per tree was effective in killing larvae, pupae and adults of RPW on coconut (Rao et al., 1973).

Muthuraman (1984) used two 3 g celphos (aluminum phosphide) tablets crushed, placed in holes in date palms and sealed with a paste of cement and copper oxychloride.

Abd-Allah and Al-Khatri (2000a) compared the effectiveness of injecting chemical insecticides and using fumigation tablets (aluminum phosphide) against RPW infestation. In this experiment, three holes (30 cm deep and 1.9 cm wide) were drilled into the tree: one at the point of infestation (where the brown bad smell sap was oozing), the second 20 cm above and the third 20 cm below the first hole. Into each hole 50 ml of the insecticide formothion 33% was poured using a plastic tube (45 cm long and 1.3 cm diameter). The holes were sealed with moistened clay. In case of fumigation tablets, the part of the trunk where oozing occurred was cleaned and the decaying tissues and grubs were removed as much as possible. One aluminum phosphide tablet (3 g) was placed in the formed cavity then sealed with moistened clay.

The treated palm trees were inspected at 2-week intervals for 10 weeks. The experiment showed that injecting formothion in the infested date palms was much more effective compared to the fumigation tablets. The authors concluded that aluminum phosphide was not effective in controlling RPW in infested trees. They attributed this failure to (i) the escape of gas through many crevices in the tree and (ii) the feces and frass which, forming a thick paste, block the larval tunnels so that it is difficult for the gas to diffuse to reach the larvae.

5.2. Aggregation Pheromone Traps

Trapping of palm weevils started with utilizing insecticide – treated palm stems (Mariau, 1968; Griffith, 1969) followed by utilizing treated fruits or sugar-cane in plastic buckets hung on the trunk at heights of 1–1.5 m (Delgado & Orellana Moreno, 1986). A male–produced aggregation pheromone was first identified for *R. palmarum* by Rochat, Gonzales, Mariau, Villanueva, and Zagatti (1991a) and Rochat et al. (1991b). Chinchilla et al. (1993) reported that the most efficient and convenient traps for *R. palmarum* infesting oil palm consisted of 19 l plastic buckets, containing such a pheromone and carbofuran-treated sugarcane.

Hallett et al. (1993) demonstrated that 4-methyl-5-nonanol (ferrugineol) is an aggregation pheromone to *R. ferrugineus* and *R. vulneratus*. While another compound, 4-methyl-5-nonanone (ferrugineone) was found to have bioactivity only for *R. ferrugineus*. In field trials, these authors found that *R. ferrugineus* was captured in traps with ferrugineol alone or in a 10:1 ratio with ferrugineone. Increasing amounts of ferrugineone significantly decreased attraction of *R. ferrugineus*. Interestingly, they also found that using 10 alive males of *R. ferrugineus* in trap instead of ferrugineol and ferrugineone (in 10:1 ratio), was as attractive as the latter for both *R. ferrugineus* and *R. vulneratus*.

5.2.1. Trap Design and Components

Abd-Allah and Al-Khatri (2000b) evaluated nine different designs of pheromone traps (20 l plastic bucket hung on tha palm trunk at a height of 1.7 m), for their rate of capturing *R. ferrugineus* adults. They found that the most efficient pheromone trap was the open one (without a lid) followed by the trap with a plastic lid. The latter had six lateral holes (3 cm in diameter) and four similar holes on the lid. Both traps contained a pack of commercial aggregation pheromone attached to one side of the bucket. The traps were provided also with 1 kg of dates, 5 g of yeast and 5 l of water.

A modification in the trap with a lid was made through a project for RPW biological control adopted by AOAD, carried out in Gulf countries in 1997–2007

(Anonymous, 2000). A pack of kairomone (synthetic volatile material induced by palm tissues which attracts RPW) is attached beside the pack of the aggregation pheromone on the inner surface of the lid, to enhance the rate of capture. Recently, some companies are producing a pack of a mixture of aggregation pheromone and kairomone.

A field trial was carried out to evaluate the rate of catch of the pheromone plus kairomone traps, compared to those with pheromone only. The trial revealed that the pheromone plus kairomone traps captured more weevils than the traps without kairomone. However, the difference in capture rates between traps was insignificant. Pieces of palm tissues, date fruits or sugar-cane were considered to have a role in pheromone traps. The catch of *R. ferrugineus* in pheromone traps containing the commercial aggregation pheromone and dates was hence compared to the catch in traps containing either the pheromone alone or dates alone. The numbers of RPW captured in 16 traps during a year were 1,752, 181 and 54 insects for the three treatments, respectively (Al-Saoud, 2007). Similarly, Chinchilla et al. (1993) reported that pheromone traps of *R. palmarum* captured 6–30 times more weevils than traps containing palm tissues or sugar-cane alone.

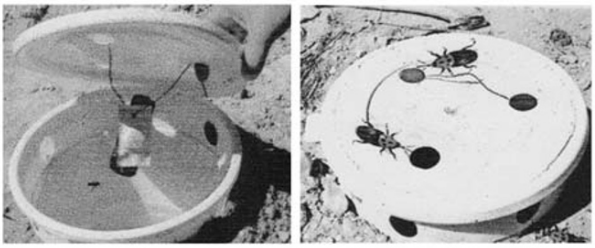


Figure 2. Pheromone terrestrial traps.

5.2.2. Trap Installation

Pheromone traps were reported to be attached or hung close to palm trees at 1–1.7 m height (Oehlschlager, Chinchilla, & Gonzales, 1992; Chinchilla et al., 1993; Abd-Allah & Al-Khatri, 2000b). In field trials, rate of catch of RPW was evaluated in pheromone traps with plastic lids in three date palm plantations installed at a rate of 1 trap per ha (Anonymous, 2000). In each plantation, 6 traps were buried in the soil up to the lateral holes below the lid of the trap (terrestrial traps, Fig. 2) and another 6 were hung on the palms trunks at a height of 1.5 m (aerial traps). The weevils from both traps in the three plantations were collected weekly, from April to November, and transferred to laboratory where their numbers were recorded. It was found that the monthly average capture of terrestrial traps was 2–3 folds the aerial traps (Fig. 3).

5.2.3. Density of Traps

A field trial was carried out in UAE to compare the catch of pheromone traps installed at rates of 1, 2 or 4 traps per ha, in three date palm plantations

(Anonymous, 2001). Such plantations contained close total numbers of date palm trees of the same age, as well as similar levels of infestation. The trial revealed that the total number of RPW captured within 5 months at the 4 traps per ha density was 2.4 and 2 fold the 1 trap per ha and the 2 traps per ha, respectively. However, the calculated annual costs and labor for the 4 traps per ha was almost 4-fold the 1 trap per ha density. Chinchilla et al. (1993), in contrast, reported that trap density of 1 per ha was just as effective as 6 traps per ha, in capturing *R. palmarum*.

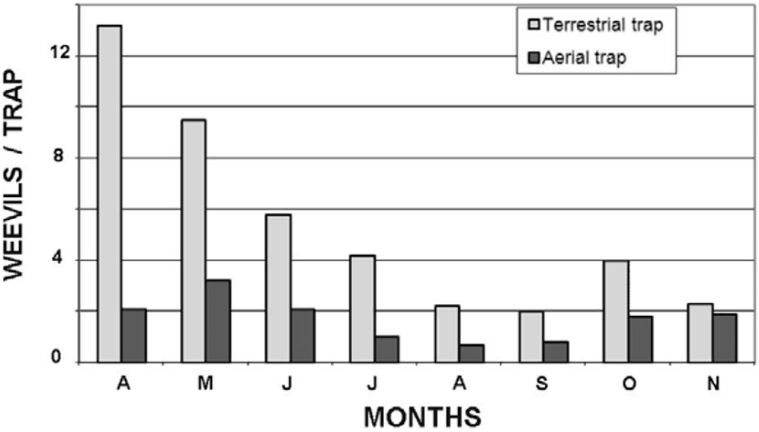


Figure 3. Average monthly numbers of Red Palm Weevils captured by terrestrial and aerial pheromone traps.

5.2.4. Utilization of Pheromone Traps

Pheromone traps could be utilized for different purposes, including reducing the population of *R. ferrugineus*, or estimating the insect population fluctuation and its sex ratio in nature.

5.2.4.1. Traps Efficiency

Performance of the pheromone traps could be evaluated by estimating percentages of RPW capture in date palm plantations. An evaluation was carried out by Abbas et al. (2006) in Ras Al-Khaima, UAE during 2000 and 2001 by releasing marked RPW in three date palm plantations and estimating the rate of capture of the released insects by pheromone traps, installed at a density of 1 trap per ha. Adults RPW were marked using small pieces of thin colored plastic, glued onto the thorax of the insect. Different colors and different shapes were used, representing different date palm plantations (one color per plantation) and different dates of release (one shape per release date). Releases were carried out at rates of 10–40 individuals per release and plantation. During the period from April to December 2000, only males were released in three date palm plantations, representing three locations 10–20 km apart from each other (each plantation contained 12 pheromone traps). Such pheromone traps were inspected 3–4 days post release, and the captured weevils were collected and transferred to laboratory where the marked specimens were counted.

During January–December, 2001 both males and females of RPW were marked and released in only two plantations (n. 1 and 2). In addition, groups of marked males and females were released, periodically, in other four date palm plantations. Such plantations were among 130 date palm plantations in Ras Al-Khaima provided with pheromone traps at a rate of 1 trap per ha.

This trapping system was carried out by the Ministry of Agriculture in UAE as a method for controlling RPW. All captured weevils were collected weekly and transferred to the laboratory, thus allowing the monitoring of the released marked weevils. Percentages of capture by pheromone traps did not include those marked weevils which were captured in the successive weeks after the first capture (3–4 days post release). Also, they did not include the marked weevils captured in date palm plantations other than those where they were released. The study revealed that monthly percentage of marked RPW captured by pheromone traps, 3–4 days post release, from April to December 2000 ranged from 0 to 7% in plantation 1, from 6 to 27.5% in plantation 2 and from 12 to 33% in plantation 3 (Fig. 4). The respective averages in 2001 ranged from 0 to 12.5% in plantation 1 and from 3.6 to 31.7% in plantation 2.

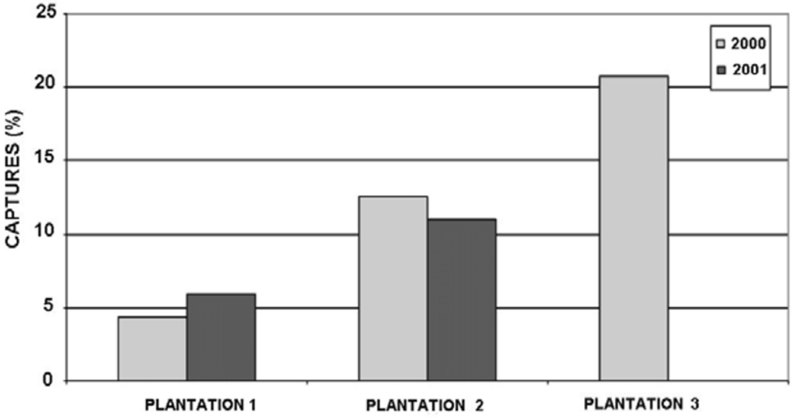


Figure 4. Average captures (%) of marked Red Palm Weevils released in three date palm plantations in 2000 and 2001.

The captures of the released weevils did not occur in all releases as no marked weevils could be captured from 15 releases in plantation 1, 10 releases in plantation 2 and three releases in plantation 3, out of the 32 releases in each plantation performed during the year 2000. Similarly, no marked weevils could be captured from 19 releases in plantation 1 and 11 releases in plantation 2, out of 41 releases in 2001. However, the rate of capture reached 80% when 16 marked weevils were captured out of 20 released in plantation 2 in the last week of December, 2000. The percentage of capture of marked females did not differ significantly from that of marked males in the six plantations in 2001. Averages of 12.2% of marked males were captured compared to 11.9% marked females. This study showed that pheromone traps capture both sexes of RPW almost equally.

The low capture rate in plantation 1 could be attributed to the high rate of infestation by RPW as well as the high incidence of weeds in this plantation. The high rate of infestation by RPW led to the production of high rate of natural aggregation pheromone (secreted by males) and kairomones (volatilized from infested palms), which were much more attractive to released males than synthetic pheromone and kairomone. Kalshoven (1981) reported that volatiles from infested palms and from fermenting palm sap (as a result of infestation) were well known to attract palm weevils. The high incidence of weeds, in turn, attracted the weevils as a habitat providing shade and shelter. In contrast, the high rate of capture in plantation 3 could be attributed to the very low rate of infestation by RPW, as well as to the old age of the palm trees (more than 20 years old) in this plantation. Thus, the pheromone and kairomone in the traps were more attractive to the released marked weevils.

5.2.4.2. Estimating RPW Population Fluctuations

Population fluctuation of *R. ferrugineus* was studied using pheromone traps at a rate of one trap per ha, to assess the variations in numbers of resident weevils from 1 plantation to another, to provide epidemiological data about the infestation levels. Also the study determined the peak(s) of RPW populations which can help in identifying the best timing of application of protective chemicals. Abbas et al. (2006) studied the population fluctuation of *R. ferrugineus* in three date palm plantations (1, 2 and 3) in Ras Al-Khaima, UAE during 2000 and 2001, using the terrestrial pheromone traps. The study revealed that the populations of the weevils increased gradually to reach a peak in March or April, and then gradually decreased til the end of the year.

The study showed also that the RPW population was much less prevalent in plantation 3 compared to plantations 1 and 2 (Fig. 5). The results showed that protective chemical insecticidal sprays against RPW in this province should be started in March, followed by a further spray in April.

5.2.4.3. Estimating Reduction of RPW Populations

Continuous use of pheromone traps, changing the pheromone pack and the food substrate (dates or palm tissues) in the proper time, should result in a progressive decrease of the *R. ferrugineus* population in the treated areas. The total numbers of weevils captured by pheromone traps in the above mentioned three plantations (1, 2 and 3), for example, were much lower in 2001 compared to 2000. A total of 65,000 weevils were captured within 18 months by pheromone traps installed in more than 130 date palm plantations in Ras Al-Khaima, site of the three tested plantations. In agreement with such observations, Oehlschlager et al. (1993) reported that mass trapping with aggregation pheromone traps reduced *R. palmarum* populations and the incidence of the associated red ring disease in oil palm plantations. As for *R. ferrugineus*, Oehlschlager (2007) mentioned that there was a strong evidence that trapping, in combination with chemical spraying, decreased infestation by 64%, while smaller scale experiments indicated that trapping alone reduced infestation by 71%. Muralidharan, Vaghasia, and Sodagar (1999) obtained similar results and reported that trapping reduced the capture rate of *R. ferrugineus* by 75%, within 3 years.

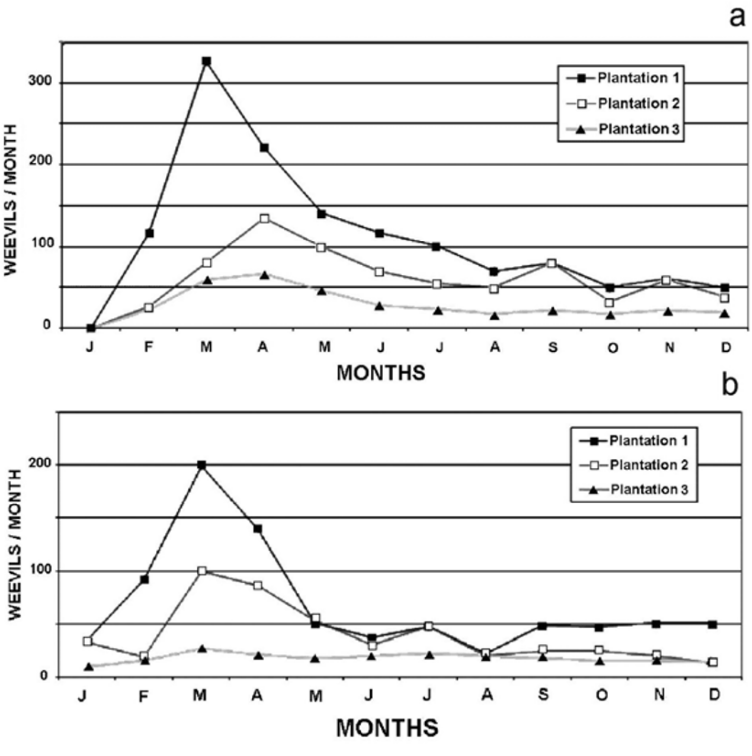


Figure 5. Population fluctuations of Red Palm Weevilsl in three date palm plantations in the years 2000 **(a)** *and 2001* **(b).**

5.2.4.4. Estimating Sex Ratios

In field trials no significant differences were observed between response patterns of female and male *R. ferrugineus* or *R. vulneratus* to the aggregation pheromone (Hallett et al., 1993). In agreement with this report, Abbas et al. (2006) found that when marked RPW males and females were released in date palm plantations the frequencies of captured females did not differ significantly from that of males. Abraham, Faleiro, Shuaibi, and Alabdan (2001), however, reported that *R. ferrugineus* captured by pheromone traps were most likely female dominated, as the sex ratio in captured weevils was 1 male:2.7 females.

El-Garhy (1996) found this ratio to be 1 male:2 females (in Ismaelyia, Egypt) while Abbas et al. (2006) found that out of 18,047 RPW captured from five date palm plantations (Ras Al-Khaima, UAE) during 2000 and 2001, the females represented 60.2% of the total catch, while males represented 39.8% (almost 1 male:1.51 females). Finally, Abd-Allah and Al-Khatri (2005) compared the catch of different colours of pheromone traps hung on the palm trees at 1 m height in Sultanate of Oman. These authors found that the orange or red traps captured almost 2-fold RPW compared to the blue ones. However, sex ratio in the captured weevils was almost 1 male:1.1 females, in the 3 traps.

5.3. Entomopathogenic Nematodes

EPN from the families Steinernematidae and Heterorhabditidae are widely regarded as being excellent biological control agents for a number of insect pests in soil and cryptic habitats (Gaugler & Kaya, 1990; Kaya & Gaugler, 1993). They possess many positive attributes including their wide range hosts, safety to vertebrates, plants as well as non target organisms, exemption from registration in many countries, ease of in vitro production and application using standard spray equipments. The two families bear mutualistic bacteria in the intestine, belonging to the genera *Xenorhabdus* (in Steinernematidae) and *Photorhabdus* (in Heterorhabditidae).

The free-living, non-feeding 3rd instars (infective juveniles) of these nematodes possess attributes of both insect parasitoids or predators and entomopathogens. Like parasitoids and predators, they have chemo-receptors and are motile; like pathogens, they are highly virulent, killing their host victims within 24–48 h.

5.3.1. Pathogenicity to RPW

Several laboratory studies were carried out to evaluate the efficiency of EPNs against larvae and adults of *R. ferrugineus*. Abbas and Hanounik (1999) tested the virulence of *Steinernema riobravis*, *S. carpocapsae* (All strain) and *Heterorhabditis* sp. (Egyptian isolate) against larvae and adults of RPW. They found that the three nematode species caused 10–100% mortality in the larvae, at concentrations ranging from 30 to 240 infective juveniles (IJs) per larva. The trial was carried out in Petri-dishes lined with filter paper. The LC_{50} values were 51, 61 and 56.6 IJs per larva for *S. riobravis*, *S. carpocapsae* and *Heterorhabditis* sp., respectively. Adults RPW were less susceptible to the nematode infection, as the corresponding LC_{50} values for the three species were 900, 1,100 and 1,416 IJs per adult. However, such infected adults produced 2,000–242,000 IJs per weevil, but no correlation was found between dose and IJs production.

Abbas et al. (2001b, 2001c) were able to isolate *S. abbasi* and *H. indicus* from RPW adults collected from date palm plantations at Al-Hamranyia, UAE. They tested the pathogenicity of these two species to larvae and adults in Petri dishes and found that 5th larval instar was less susceptible than the 3rd instar to both species, at concentrations of 100 and 200 IJs per larva. The LC_{50} values for 3rd and 5th instars were 69.2 and 97.7 IJs per larva, respectively, for *S. abbasi* while for *H. indicus* they were 123 and 128.8 IJs per larva. However, only 11.5 and 8.6% of dead RPW larvae infected with *S. abbasi* and *H. indicus*, respectively, produced IJs. The average number of IJs produced per larva was 33,000 (2,000–113,000) for *S. abbasi* and 35,000 (5,000–85,000) for *H. indicus*.

Slight differences in virulence towards adult weevils were found between *S. abbasi* and *H. indicus*, at concentrations of 12.5 and 25 IJs/cm^2 of sand surface, in a trial carried out in plastic cups lined with moistened sterilized sand. No difference was noticed at concentrations of 50 and 100 IJs/cm^2. The calculated LC_{50} values were 23.2 and 25.1 IJs/cm^2 of sand for *S. abbasi* and *H. indicus*, respectively. Of the dead adults infected with *S. abbasi* and *H. indicus*, 93 and 89%, respectively, produced IJs with respective averages of 983,000 (93,000–3,055,000) and 776,000 (145,000–2,820,000) IJs per weevil at the applied concentration of 100 IJs/cm^2 of sand. It should be noted that the recommended commercial application of EPNs as biocontrol agents was reported to be $2.5– 5\cdot 10^9$ IJs/ha (Georgis & Hague, 1991). This rate is equivalent to 25–50 IJs/cm^2 of soil surface.

Saleh and Alheji (2003) compared virulence of four species of EPN to the 3rd and 8th larval instars, as well as adults of RPW in laboratory. Such tested nematodes were *H. indicus* (from Saudi Arabia), *H. bacteriophora* HP88 (from USA), *S. abbasi* (from Sultanate of Oman) and *S. carpocapsae* (from Germany). A concentration of 100 IJs per larva (in 9 cm Petri dishes lined with filter paper) was used for the assay with larvae, whereas concentrations of 10–100 IJs/cm^2 of sand surface were used for adults (in 9 cm Petri-dishes lined with 50 g fine sand). The results indicated that 3rd instar larvae were highly susceptible as all tested nematode species caused 100% mortality within 2–3 days. The 8th instar larvae were found to be less susceptible, as percentages of mortality were 60% by *S. abbasi*, 70% by *H. indicus* and *H. bacteriophora*, and 80% by *S. carpocapsae*. RPW adults were also less susceptible to nematode infection than larvae. Mortality at the tested concentrations, 10–100 IJs/cm^2 of sand surface, ranged from 17 to 75% for *H. indicus*, from 25 to 83% for *H. bacteriophora*, from 33 to 75% for *S. abbasi* and from 33 to 92% by *S. carpocapsae*. The respective LC_{50} values were 49.9, 40.2, 32.4 and 6.4 IJs/cm^2 of sand. Such LC_{50} values correspond to 3,172, 2,555, 2,060 and 406 IJs per adult RPW, respectively.

Shamseldean (2002), however, reported that adult *R. ferrugineus* was the most susceptible stage to nematode infection when testing the efficiency of 13 species and/or isolates of the *Heterorhabditis* and 2 isolates of *Steinernema* against larvae, pupae and adults. He stated also that the last instar larva was less susceptible than the pupa. Shamseldean and Atwa (2004) reported that three Egyptian isolates of *Steinernema* were highly pathogenic to RPW larvae and adults. High mortality rates (100%) were recorded when adults were treated with those 3 isolates, while mortalities in last larval instar ranged between 78 and 90%.

5.3.2. Field trials

5.3.2.1. Injection of Nematodes

Nematodes injection is carried out by making 3–4 artificial tunnels (15–20 cm deep) using an electrical hammer drill with a 20–40 cm long screw, above and around the infested spot, in the palm trunk , where creamy to dark brown sap is noticed (a symptom of RPW infestation). Nematode suspensions are injected in such tunnels through perforated plastic tubes inserted into such tunnels. After injection, the opening of the tunnels are covered with damp soil to avoid reinfestation.

Shamseldean (2002) carried out field applications of EPN against RPW infestation (during 1998–2001) by injecting the trees with 120 ml of the nematode suspension at a concentration of 3,000 IJs/ml, injected in each artificial tunnel with a total of 1,440,000 IJs per tree. The treated date palms were checked 1 month later and during the following 5 months to check the recovery from infestation. The numbers of treated palms were 36 (in 1998), 45 (in 1999), 30 (in 2000), 27 (in 2001). Egyptian isolates of EPN were used: *H. bacteriophora* (strain EKB20), *H. indicus* (strain EGBB) and *Steinernema* sp. (strain EBNUE). The author mentioned that the treated palm trees, in all applications, were found healthy with no symptoms of old or new infestation. Shamseldean and Atwa (2004) stated that injection of three Egyptian isolates of *Steinernema* in infested date palm trees resulted in 88.9 and 91.9% recoveries when the isolate EGG4 was used, compared to 77.7 and 77.1% when the isolate EBNE was used during 2001 and 2002, respectively. In 2003, percentages of recoveries obtained were 83.3 and 72.2%, by EGG4 and EIKE isolates, respectively.

Abbas, Saleh, and Okil (2001a) applied the same technique of nematodes injection to seven infested date palm trees, but used another method to evaluate the efficiency of the injected nematodes. Two weeks after injection the leaf-axils in the treated area was removed until reaching the open of the natural tunnel made by the insect larva. It was interesting that no dead larvae could be obtained in the treated trees and all larvae found were alive and healthy. The nematodes used in this trial were *S. abbasi*, *S. riobravis*, *S. feltiae*, *S. carpocapsae* and *H. bacteriophora.* El-Bishry, El-Sebaey, and Al-Elimi (2000) obtained almost similar results.

Another method of injecting nematodes in the infested palm trees was tested by Abbas, Hanounik, Mousa, and Al-Bagham (2000). The leaf-axils were removed from the infested spot on the trunk (showing symptoms of infestation) until reaching the entrance of the larval tunnel. The tunnels were then injected with the nematode suspension containing 5,000 IJs/ml after which the entrances were plugged with damp soil. The area where the leaf-axils were removed was also covered with a thick layer of damp soil, to prevent new infestations. Thirty one tunnels in 25 trees were injected, each with 50–100 ml. of the *S. riobravis* suspension. Two weeks later, the injected tunnels were checked for dead and/or alive larvae. Out of the 31 injected tunnels, only four contained nematode-infected dead larvae, while 12 tunnels contained alive healthy larvae, with a total 25% mortality. However, no dead or alive larvae could be found in the other 15 tunnels. The absence of larvae in the latter tunnels was interpreted as the larvae probably migrated through sub-tunnels inside the trunk, before or after the nematode injection.

Saleh and Alheji (2003) used a third method for injecting a nematode suspension in the infested date palm trees, treating 30 active tunnels, in 20 trees, with fresh exudates and frass, by making few small holes with an electric drill at the site of infestation, to reach the tunnel network in the trunk. A suspension of *H. indicus* at the rate of 10,000 IJs/ml was injected through the holes at the site of infestation, then blocking the holes with soil. Two weeks later, the tunnels were inspected and numbers of dead and alive larvae or adults were recorded. The results indicated that percentages of mortality were 58.8% in larvae and 43.5% in adults.

In conclusion, the recovery of treated palm trees expressed by "no symptoms of old or new infestation" as mentioned by Shamseldean (2002) and Shamseldean and Atwa (2004) appears controversial. Injecting nematodes in artificial tunnels around the spot of infestation does not warrant that the infective juveniles reach RPW larvae inside their tunnels. The walls of the artificial tunnels form barriers as the IJs can not penetrate such walls and/or move through wood tissues.

El-Bishry et al. (2000) attributed the poor results of injecting nematodes to the deleterious effect of frass in larval tunnels on the injected IJs. These authors mentioned that juveniles of five species of EPN were killed within 24 h when placed on the frass of infested and decomposed tissues of date palm. However, our studies (unpublished) revealed that the feces of RPW larvae and frass obtained from larval tunnels did not affect viability or pathogenicity of infective juveniles of *S. riobravis* or *H. indicus* when placed on such material for 15 days. The findings of El-Bishry et al. (2000) could be related to the fact that the frass and decomposed tissues of infested palm are subjected to fermentation by microorganisms which produce alcohols and other toxic materials toxic to the juveniles.

Compared to nematode injection, the chemical insecticides injected in artificial tunnels can penetrate such tunnels, are absorbed by the wood tissues and may reach the larvae inside their tunnels.

5.3.2.2. Spraying Nematodes

Trunk Spraying

A semi-field trial was conducted by Abbas et al. (2000) to estimate the efficiency of nematodes sprayed against adults of RPW. Sixteen young trees (3–5 years old) were individually caged by 2 × 2 × 2 m cages made of wooden frame and wire screen. The trunks of 12 trees representing three treatments were sprayed with one l of nematode suspension containing 2 × 10^6 IJs per tree. The amount of suspension was enough to wet the whole short trunk and was sprayed carefully so that it did not reach the soil. Two commercial antidesiccants, Liqua-Gel (Miller Chemicals and Fertilizer Corporation, USA) at a rate of 100 ml/l and Leaf-Shield (Aquatrols Corporation of America) at a rate of 2.5 g/l, were added to the nematode suspension in the first and second treatments, respectively. The last four trees were sprayed with water as control. Ten females and five males of RPW were released in each cage immediately after treatment.

The palm trees were inspected daily for 10 days and the dead weevils were transferred to laboratory and kept individually in White-traps for extracting the infective juveniles (IJs) produced by the infected insects. Dead weevils which did not give rise to infective juveniles were dissected to check infection. The results indicated that mortality in RPW adults was 8.9% when the anti-desiccant Leaf-Shield was used with nematode suspension and 13.3% when Liqua-Gel was used. However, nematode suspension without anti-desiccant gave 11.7% mortality.

In general anti-desiccants are utilized with the commercial formulations of EPN to enhance their persistence and performance in the field (Georgis, 1990; Kaya & Gaugler, 1993). The poor efficacy of *S. riobravis* sprayed on palm trees despite the addition of anti-desiccants could be attributed to the adverse effect of sun heat and UV radiation on the IJs. The leaf-axils of palm trees do not provide enough shade or shelter to IJs. In addition, soil, not leaf-axils, is the natural habitat for the nematodes.

Hanounik et al. (2000a) carried out a similar trial using a *Heterorhabditis* sp. isolated from Saudi Arabia, alone or with Leaf-Shield (100 ml/l of water) or Liqua-Gel (2.5 g/l of water). The caged date palm trees were first artificially infested by releasing 10 RPW adults on the trunk of each tree and 1 h later the trees were sprayed by the nematode suspension. Each tree received an average of 3.75 × 10^6 IJs in 2 l of water. The trees were inspected daily starting from the 3rd day until the 7th day post-treatment to record weevil mortality. The results showed that mortalities in *R. ferrugineus* adults were 65% (by using the nematode alone or with Liqua-Gel) and 87.5% (by using the nematode with Leaf Shield). Such results, differ from those obtained by Abbas et al. (2000) and may depend on the experimental procedure applied. The RPW adults in procedure of Hanounik et al. (2000a) were released on the trunks of the palm trees before spraying the EPN suspension, which means that the weevils were sprayed directly with the infective nematodes.

5.4. Soil Treatments

According to field studies and observations (Abbas et al., 2000), it was concluded that RPW adults sometimes inhabit soil, probably seeking shade and shelter. This conclusion was based on the following reasons: (i) up to 20–100% of RPW adults collected monthly by pheromone traps were found to be parasitized with unidentified non pathogenic nematodes, (ii) both *S. abbasi* and *H. indicus* were isolated from RPW adults (Abbas et al., 2001b, 2001c), (iii) young date palm trees (3–10 years old) were found to undergo severe infestation by RPW at or below soil surface, (iv) terrestrial pheromone traps were found to capture 2–3 fold RPW, compared to aerial traps (hung at 1–1.5 m height). Abraham, Shuabi, Faleira, Abuzuhairah, and Vidyasagar (1998) mentioned that on young growing date palms, the weevils take shelter under the splitting bark and lay eggs within the newly emerging roots.

Ferry and Gomez (2002) reported that larvae of *R. ferrugineus* could be found in any place within the palm even in the very base of the trunk where the roots emerge. The emerged adults in this case emerge in the soil. Also, El-Sebaey (2004a) found that many infested trunk bases of date palm harbored reliable numbers of RPW surviving in these parts, just under the ground level. Such weevils were found alive after removing the infested collapsed palms for a long time and infested the growing roots in the soil.

Table 2. Mortality (%) of Rhynchophorus ferrugineus *adults released in cages with soil treated with* Steinernema riobravis *at a rate of* 8×10^6 *IJs per cage.*

Mortality	*Treated cages*						*Untreated cages*	
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Total	86.7	46.7	80.0	86.7	73.0	100	20.0	13.8
Due to nematode infection	86.7	33.3	60.0	66.7	60.0	86.7	–	–

5.5. Semi-Field Trials

As reported by Abbas et al. (2000), eight 3–5 years old date palm trees were caged individually by 2 × 2 × 2 m cages made of wooden frame and wire screen. In six cages, the soil was sprayed with 10 l of EPN suspension with 8×10^6 IJs of *S. riobravis*, while in the other 2 cages the soil was sprayed with 10 l of water as control. Ten females and five males of RPW were released in each cage 3 h post treatment. The cages were inspected daily and the dead weevils were transferred to laboratory and kept in White-traps to extract the IJs produced by infected weevils. Dead weevils not giving rise to IJs were dissected to check infection. The results of this trial (Table 2) showed that *S. riobravis* caused considerable mortalities among RPW adults in the treated cages. Average mortality due to nematode infection was 65.6% (range 33.3–86.7%). Interestingly, 40% of the dead weevils were found on the trunk at leaf-axils.

Saleh and Alheji (2003) conducted similar trial by using *S. carpocapsae* and *H. bacteriophora* at a rate of 2 million IJs in 3 l of water per tree. The trial was carried out in Saudi Arabia in February, with daily mean temperature between 8 and 20°C. *Steinernema carpocapsae* caused 77.5% mortality in RPW adults while *H. bacteriophora* caused only 17.5 % mortality. The authors mentioned that the low effect of *H. bacteriophora* could be interpreted as this species was isolated from a tropical area, therefore it was not adapted to low prevailing temperatures. Such findings confirm the importance of the biological and ecological characteristics of the EPN applied, since soil is the natural habitat of EPN and most of the successful control with EPNs was achieved against soil-inhabiting insects (Georgis, 1990).

In conclusion, soil application of EPNs could be recommended as a biological tool for RPW control, within a durable IPM strategy. Persistance after release is a fundamental property of any biocontrol agent, in view of its practical exploitation. *Steinernema abbasi* and *H. indicus*, sprayed in a field study around date palms, survived in the treated soil for at least 1 year (Abbas & Mousa, 2003).

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10

CONTROL OF URTICATING LEPIDOPTERA OUTBREAKS WITH *BACILLUS THURINGIENSIS* AERIAL TREATMENTS

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Abstract. The main species of urticating Lepidoptera are presented, together with the environment-friendly methods based on biological control agents. These include the bacterium *Bacillus thuringiensis* and the technologies developed to treat large park and forest areas, in wild as well as urban environments. Advances in the aerial control methods developed and the effects of formulations on the treatments efficacy and persistence are briefly described.

1. INTRODUCTION

A limited number of Lepidoptera is considered as seriously harmful in forest and urban parks and this characteristic is linked to the possibility that massive population explosions may occur (Myers, 1988). From the ecological point of view, dramatic density changes or "outbreaks" are phenomena common to various animal species, including insects, and occur through cycles of increasing population density changes (Berryman, 1987). The capability to increase enormously in number and to attack trees in normal-growing conditions, acting as a prime debilitating or "early damage-causing biotic factor" makes the infestation of some species of defoliating Lepidoptera particularly dangerous. Furthermore, their attacks can predispose trees to infections by pathogenic agents, that exploit the reduced capability of the host to react.

Among Lepidoptera there are species noted not only for the direct damage caused by defoliating activities, but also for their capability to interact with agro-

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forestry productions and other human activities, including aspects related to environmental hygiene. To this instance are referred those species whose larvae, during specific developmental stages, bear urticating hairs, i.e. tegumentary appendages, connected to special glands, capable of eliciting adverse reactions in humans rangin from dermatitis and conjunctivitis to Ig-E mediated anaphylactic reactions (Wirtz, 1984; Arlian, 2002).

In this chapter recent advance on these issues are discussed, with particular attention to environment-friendly methods based on biological control agents, including the G+ bacterium *Bacillus thuringiensis* and the technologies developed to treat large park and forest areas in wild as well as urban environments.

2. URTICATING LEPIDOPTERA

The larval hairs, which act as defence mechanisms, are very small (about one tenth of mm) and are generally harpoon-shaped. Their dissemination in the environment raises serious implications for the public hygiene. When hairs penetrate the skin and mucosal tissues of man or other warm-blooded organisms, they cause severe irritations due to both the physical action of penetration (favoured by their shape) and the chemical activity of a soluble protein released by the accidental breaking of the hairs. These structures are set up on the larval body in particular areas called “mirrors”, whose morphology and distribution are characteristic for each species. The “mirrors” are produced at every moult, reaching their maximum breadth in last-age larvae that can present from hundred–thousand to, sometimes, million urticating hairs (Fig. 1b, c).

The effects produced on man by urticating hairs present a remarkable interest in health-care, either for epidermic reactions, more or less persistent, aroused on the majority of victims or for other consequences, sometimes observed in individuals particularly responsive or sensitized, owing to reiterated contacts. Some worth-mentioning effects are those related to mucous tissues and sensory organs, mainly eyes. Inflammatory reactions can be particularly dangerous for main airways when massive inhalation occurs, due to unsafe operations performed during pest control by workers not adequately protected (Lamy, Novak, Duboscq, Ducombs, & Maleville, 1988).

Thanks to their tiny size, urticating hairs are easily carried away by air currents. For this reason, during intense and widespread outbreaks, remarkable drawbacks may occur not only close or within areas stricken by the disease, but also in locations considerably far. In recent years, when monitoring the diffusion of this and other structures or arthropods parts, these phenomena were referred as “Animal Atmospheric Pollution”. At the same time, monitoring operations of pollen and other allergenic structures transported by air are performed on this kind of organic materials (Lamy, 1990; Werno & Lamy, 1990; Gottschling & Meyer, 2006).

Lepidopteran larvae bearing urticating hairs with a defence function are known among many species of temperate and tropical regions within the following families: Thaumetopoeidae, Lymantriidae, Saturniidae, Nymphalidae, Lasiocampidae, Bombycidae, Arctiidae, Noctuidae, Anthelidae, Eupterotidae, Limacodidae and Megalopygidae.

The main species of urticating Lepidoptera, responsible of severe infestations among countries around the Mediterranean see, belong to the genus *Thaumetopoea* (Fam. Thaumetopoeidae) and *Euproctis* (Fam. Lymantridae) (Roversi, 2006). In the Palaearctic region, the members of the Thaumetopoeidae family form a small group of species that represents a formidable menace in wide areas even because of the progressive expansion of their distribution range. The increase in their distribution area is the consequence of both general climatic trends, characterized by milder winter temperatures, and anomalous climatic events allowing colonization of new territories, either at higher altitudes as well as in areas northern than those reached until a few years ago.

The two main species known for the negative effects of their infestations are the Pine Processionary Moth (PPM), *Thaumetopoea pityocampa* (Den. et Schiff.) and the Oak Processionary Moth (OPM), *T. processioneae* (L.). Proceeding towards northern territories other species are worth mention, such as *T. pinivora* (Treitschke) whose colonies are reported to have increased their extension in areas of North Europe completely undamaged until recent times.

The individuals of the three species exhibit a sub-social behaviour, their larvae living in groups throughout their development. Moreover, the first two species sometimes build nests of remarkable dimensions, so that a huge amount of urticating hairs can be present inside the nests for a long time.

PPM is a lepidopteran with a wide distribution range in the Mediterranean area and Middle-East Europe. It is considered the most important insect pest of pines in southern Europe and North Africa, affecting many indigenous and exotic pines, as well as other Pinaceae (in order of preference, *Pinus nigra austriaca*, *P. sylvestris*, *P. laricio*, *P. pinea*, *P. halepensis*, *P. pinaster*, *P. canariensis*, *Cedrus* spp. and *Larix decidua*) (OEPP/EPPO, 2004). Within its range of distribution, *T. pityocampa* is common in extremely diversified habitats, varying from coastal formations to mountain woods at altitudes higher than 2,000 m. Particularly severe problems are reported at the end of winter, when long processions of mature larvae abandon pines in a line, as they come down from trees to deepen themselves into the ground, where pupation will take place (Fig. 1a). In this last phase, contacts with people and animals are very frequent.

OPM is an univoltine forest defoliator of deciduous oaks, widely distributed in central, western and southern Europe (Agenjo, 1941). The larvae are equipped, from the third instar, with urticating hairs and are active in spring–summer. They exhibit a gregarious behaviour throughout their life, constructing sack-shaped nests on the stem or axis of large branches, in which they pupate in a papery cartoon. This pest can completely defoliate oak stands but it is also found on isolated trees in avenues and parks. Since the 1990s, heavy attacks by OPM have become increasingly frequent throughout very large areas in various European countries (Flemming, 1997; Tomiczek & Krehan, 2003; Lövgren & Dalsved, 2005; Wulf & Pehl, 2005), including Holland, where outbreaks had not been reported for a century (Bosma & Jans, 1998; Stigter & Romeijn, 1992; Stigter, Geraedts, & Spikers, 1996).

In recent years, OPM became a problem even in United Kingdom (Townsend, 2007). In southern Europe heavy and extensive infestations occurred both in Middle European oak environments and forests, and in sub-Mediterranean inhabited mainly by the Turkey oak *Quercus cerris* (Camerini, Caronni, & Roversi, 2002; Roversi, 2002). Nest construction on oak stems not only enhances risk of accidental contacts in green areas but, mostly represents a considerable hazard for forest-workers committed to carry out coppice cut or to collect firewood.

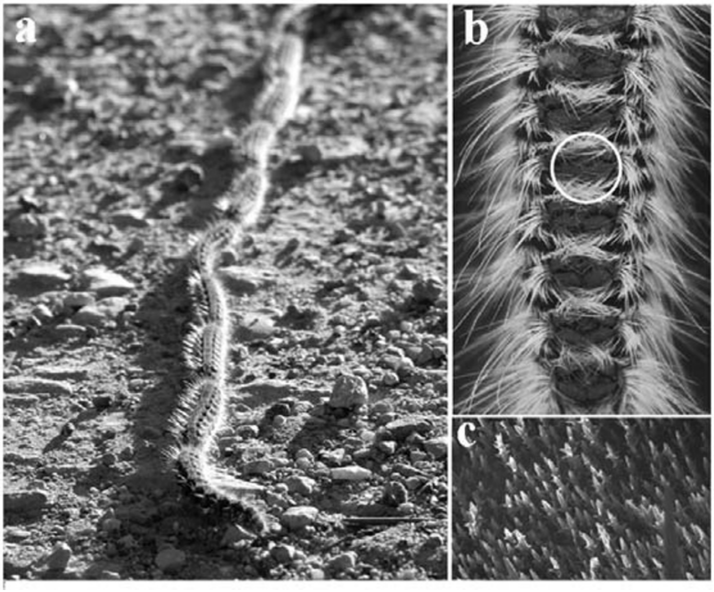

Figure 1. Thaumetopoea pityocampa *larval procession* (**a**)*, dorsum detail of a mature larva with a "mirror" of urticating hairs* (**b***,* white circle) *and SEM magnified densely thickened urticating hairs of a "mirror"* (**c**)

Euproctis chrysorrea (L.), a moth widespread in North Africa and Europe including Italy, has been introduced also to North America. This species is common from the sea level up to the beech mountain altitude, and is also frequent in coastal environments on *Arbutus unedo* and in inland regions on oaks and various Rosaceae shrubs, particularly *Crataegus* spp. On the latter it forms permanent hotbeds even in urban and suburban parks. During heavy infestations it can defoliate entire woods, making them unfit to deploy. Since the second instar, *Euproctis* larvae bear, on their back, dense strands of tiny urticating hairs, 0.1 mm long, with tri-cuspidate tip.

Equally important cases are reported in other geographic areas. A clear example is the leaf skeletonizer *Uraba lugens* Walker (Lepidoptera Nolidae), a serious pest of forestry and *Eucalyptus* stands, particularly in risky areas (e.g., urban zones or near waterways). *Uraba lugens* is widespread in the majority of Australian territory where periodic outbreaks occur which can lead to extensive defoliations. The species is well established also in new Zealand (Farr, 2002; Mansfield et al., 2006). The larvae of *U. lugens* bear, on their back, hairs that after being thrust into man skin inject istamine, causing irritation, sometimes severe, and wide itching rash (Southcott, 1978).

Lepidoptera families including urticating species are present and spread in almost every continent except the most cold regions. Among them the Limantriidae is the most important one, due to the genus *Euproctis.* In addition to the two species *E. chrysorrhoea* (Fig. 2) and *E. similis* (Fuessly), worth mention are also *E. edwardsii* (Newman) in Australia on *Eucalyptus* spp., *E. lunata* Walker on *Acacia* trees in the Indian region, *E. scintillans* (Walker) on *Robinia pseudoacacia* L., *E. bipunctapex* Hampson in Singapore and *E. pseuconspersa* (Strand) in Japan (Ooi, Goh, Loe, & Goh, 1991; Dunlop & Freeman, 1997; Ohtaki & Takino, 1998; Balit, Ptolemy, Geary, Russel, & Isbistes, 2001; Subramanian & Krishnamurthy, 2002; Kalia & Pandey, 2004).

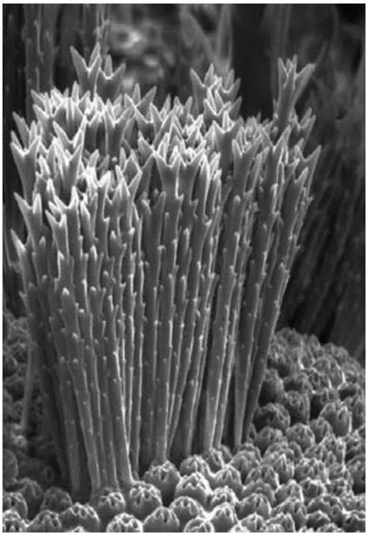

Figure 2. Euproctis chrysorrhoea*: detail of a tuft of urticating hairs with tri-cuspidate tips.*

Many authors reported reactions of “tussockosis” to irritating setae of the Douglas Fir Tussock Moth larvae, *Orgyia pesudotugata* (McDunnough) in the US Northwest (Perlman, Press, Googins, Malley, & Poareo, 1976; Press et al., 1977). Moreover, in some species, adult females rather than larvae exhibit urticating hairs such as in African and South American species belonging to the genus *Anaphe* (Family *Notodontidae*) and to the genus *Hylesia* (Family Saturniidae), like *A. panda* (Boisduval), *H. urticans* Floch & Abonnenc, *H. iola* Dyar and *H. lineata* (Druce) (Lamy, Pastureaud, Novak, & Ducombs, 1984). The reactions caused by adult moths or butterflies are called “Lepidopterism”. Damages to people and animals are caused in this case by barbed setae in the anal tuft (Fig. 2), the tips of which show glands secreting a toxin, normally released by female on egg clusters as an effective deterrent against egg predators and parasitoids, like i.e. the Lycenidae *Eumaeus atala florida* Rueber (Rotschild, Reichstein, Von Euw, Aplin, & Harman, 1970) and the Thaumetopoeidae *Ochrogaster lunifer* Herrich-Schäffer (Floater, 1998).

3. AERIAL CONTROL

In the last decades, the increasing need to carry out direct monitoring interventions as a consequence of diffused infestations of urticating species, showed the limit of control means deployed only after damages was already assessed. It is important to underline that the use of biocides to reduce large phytophagous populations cannot solve, in the short term, public health and hygiene problems. In fact, the mass of urticating hairs can remain for a long time in the environment rendering the areas hit by infestations unfit for any use, even for many years.

From this overall picture of serious issues raised by lepidopteran infestations and given that, in general, the interventions are set up in forest ecosystems in which wide range pesticides are not allowed because of their negative environmental fallout, new adequate strategies must be promoted. Among them, it is necessary to combine the set up and maintaining of efficient monitoring task forces, to forecast the beginning of new attacks, with the improvement of methods and means for the timely deploy of biopesticides with a low environment impact.

Formulations of *B. thuringiensis* var. *kurstaki* (Btk), a naturally sporulating soil bacteria, have been used for years in North America and Europe against lepidopteran defoliators, in coniferous and broad-leaved woods (Martin & Bonneau, 2006; Van Frankenhuyzen & Payne, 1993; Van Frankenhuyzen, 2000; Roversi, 2008). The formulations are chosen on account of their effectiveness and specificity, as well as of the rapidity with which the spores are killed by the UV radiation (Wilson & Benoit, 1993; Leong, Cabo, & Kubinski, 1980).

In Canada and USA, most of the treatments are applied with airplanes. In Italy, as well in other European countries like France and Germany, helicopters are preferred for control of defoliator lepidopterans, because of their small size and the more or less irregular borders of the areas to be treated. Further reasons are the close association of the treated surfaces with cultivated areas or the general morphology of their environments, which rarely present uniform landscapes over large surfaces (Lentini & Luciano, 1995; Luciano & Lentini, 1999; Martin & Bonneau, 2006).

For aerial spreading of Btk formulations, helicopters have proved more useful when wind speed is less than 16 kmh, to reduce drift. The best equipment is the electrically operated rotary nozzle, mounted on bars to wet at ultra-low swath intervals of about 30 m at each flight. GPS equipments proved also useful to record both the flight and the complete treatment coverage.

Btk spraying experiments carried out in New Zealand against the Tussock Moth *Uraba lugens* Walker, proved the reliability of an ULVA-8 spinning disc operating at 12,500 rpm and mounted above a track conveyor belt to obtain very small droplets, with a median volume diameter of 150 μm, varying the dosis applied by changing the belt speed and the flow rates (Mansfield et al., 2006). In Spain, Pascual, Robredo, and Galante (1990) showed that aerial treatments using a plane distributing soluble powders of Btk at the dose of 5 l/ha^1 (1,500 cc of commercial product with 8,500 u.i./mg^1 and 3.5 l of water), resulted in high mortality of OPM larvae, in colonies artificially transferred to areas that were then experimentally treated.

Unlike standard protocols today available for other harmful defoliators of mesophilous forests, i.e. *Lymantria dispar* (L.), noxious also to cork oak in North

Africa, or *Choristoneura fumiferana* (Clem.), which is often very dangerous to many nearctic conifer stands (Lentini & Luciano, 1995; Bauce, Carisey, Dupont, & Van Frankenhuyzen, 2004), no standard protocols are available thus far in Italy for Btk products aimed to control main urticating lepidopteran defoliators. Data are now available only on *T. pityocampa*, based on experiments proceeding from other countries. In Italy, observations began in the 1960s (De Bellis & Cavalcaselle, 1969; Triggiani & Sidor, 1982; Currado & Brussino, 1985; Niccoli & Tiberi, 1985; Ambrosi, Salvatori, & Zanotelli, 1993; Battisti, Longo, Tiberi, & Triggiani, 1998).

Further investigations on the capability of aerial treatments by means of biopesticides against PPM were carried out also in other European countries, especially in France where this species is often noxious mostly in tourist areas, i.e. in the Maritime Alps (Demolin, Martin, & Lavanceau, 1993; Demolin & Martin, 1998; Martin & Bonneau, 2006).

Very few data are available for the Brown Tail Moth (BTM) *E. chrysorrhoea* as well as for OPM. The latter species, has spread recently northwards in Europe convincing some EU Member States to introduce national or regional control programmes for public health reasons (EFSA, 2009). However, only in 2008 an experimental protocol has been issued by means of aerial spreading of Btk over large areas. Employ doses were stated at 2.5 l/ha[1] of 12.7 BIU (Billion International Units)/l of commercial formulation and were distributed at ultra-low volume at the time of bud opening, against 1st and 2nd instar larvae (Roversi, 2008).

Before aerial Btk treatments are deployed, it is also useful to know the exact time of presence of larvae, which varies normally according to the different urticating. For instance, OPM and BTM have different larval presence. In Italy, for the former species it is necessary to treat by the end of April or at the beginning of May, when buds are opening and leaves growing. However, it is important to treat larvae before they reach the third instar, when they become urticating. Early treatments in spring are also capable to better wet the crowns inside. In fact, to reach a good biopesticide spraying all over the new foliage it is necessary to avoid some larval groups to escape.

For BTM, the different life cycle patterns lead to treatments in the late summer or autumn, just before the larvae hide themselves inside winter nests. However, in mild Mediterranean climates with mild winters where BTM can feed and survive on mixed scrubs, treatments can be delayed to the end of autumn. As to PPM, treatments can be performed by the end of summer, when the newly hatched larvae start to feed on needles. The winter control of PPM is possible by means of Btk formulations applied on third instar larvae or more (L4–L5) (Martin & Mazet, 2001). Furthermore, it is worth to remark that PPM can show different life cycle patterns, according to the different seasonal conditions and climates. In fact, PPM eggs can be laid sometimes also at the beginning of autumn (Battisti, 1989).

Numbers of researches have been carried out in the recent years also on the Btk impact on non-target species, as well as on the persistence of the microbial products in the treated environment (i.e., spores-crystal complex), or the host plant effects (Cooke & Régnière, 1999; Appel & Schultz, 1994; Kouassi, Lorenzetti, Guertin, Cabana, & Mauffette, 2001; Bauce, Bidon, & Berthiaume, 2002; Carisey, Bauce, Dupont, & Miron, 2004), so that the Btk residual activity is now a key-factor in operational forestry and urban programs (Gindin, Navon, Protasov, Saphis, & Mendel, 2007).

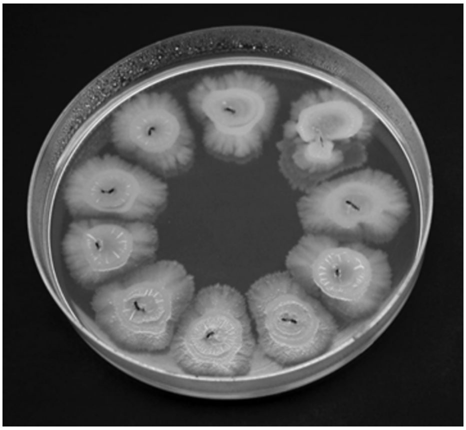

Figure 3. Petri dish with Btk *colonies developing from already died 2nd instar larvae collected in the field after an aerial treatment.*

In experimental assays carried out in spring 2004 on Turkey oak woods in Tuscany (Central Italy), coinciding with the early phase of a new *T. processionea* outbreak, a wide aerial Btk treatment was carried out, followed by a 1-month evaluation of its effect on non-target lepidopterans. The control area was checked daily with traps placed underneath the crowns (Roversi, Rumine, & Barzanti, 2006). These trials showed a low effect of the treatment against OPM, with a small number of dead larvae of other species (Roversi et al., 2006).

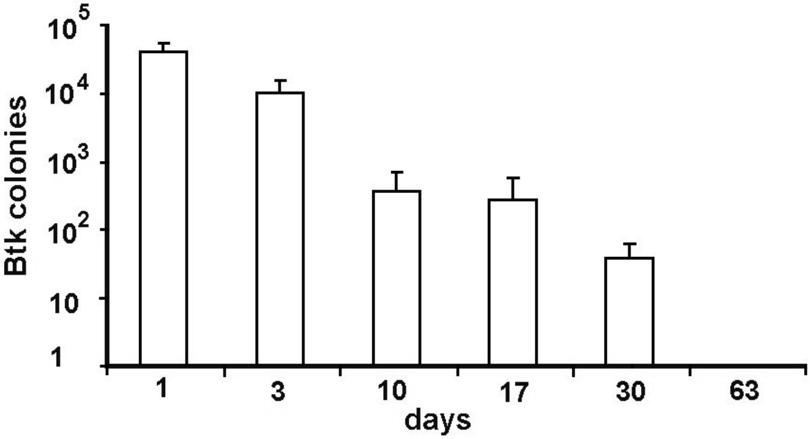

*Figure 4. Numbers of Btk colonies developed from 1 ml water washings of Turkey Oak leaves collected from the upper part of the tree crowns, after treatment with 31.75 Btk BIU/ha, at Berignone Forest (Tuscany, Central Italy). Leaves were collected in a period of about months (*Bars *show standard deviation)* (Roversi et al., unpublished).

Further laboratory analyses of both the insects midgut and leaves revealed the presence of bacterial spores inside the whole crown, together with the development of colonies from 100% of the OPM larval cultures. These were obtained from larvae died within the first 9 days after the treatment (Fig. 3). Further controls of Btk persistence using agar cultures of water washings from leaves, collected at both high and medium crown levels, revealed a marked reduction of the living spores 10 days after the treatments (Fig. 4). In some cases, on the contrary, colonies of Btk spore were obtained from leaves collected 2 months or more, after treatments (Roversi et al., unpublished data).

Other field experiments confirmed the persistance of Btk applications based on milk formulations, used successfully against *T. wilkinsonii* Tams in Israel. Data showed that the formulation has a rain-fasting effect, resulting in the retaining of more than half of the Btk activity after 8 days (Gindin et al., 2007).

In conclusion, the exploitation of biopesticides based on Btk is likely to be strongly increased in the future, owing to both the scientific evolution and the government policies, encouraging the use of alternative pest control products.

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Section 3

MITES BIOLOGICAL CONTROL AND IPM

11

IPM POTENTIALS OF MICROBIAL PATHOGENS AND DISEASES OF MITES

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Abstract. An overview is given of diseases in mites, caused by infectious microorganisms. Many pathogens play an important role in the regulation of natural populations of mite populations and are for this reason subject of research on the feasibility to develop such pathogens to biological control agents. Several examples are given of successful application of pathogens for the control of mite pests, but also failures are discussed. Most studies concern fungal pathogens of tetranychids and eriophyids; some of these fungi are possible candidates for biological control agent of species of noxious mites. An interesting group of pathogens form the intracellular symbionts: bacteria that may cause unusual effects in their hosts, such as parthenogenesis, feminization, male killing and incompatibilities. This group of bacteria is present in many invertebrates species and are presently widely studied as new molecular techniques have become available that make detection of such symbionts possible. Attention is also given to quality control of beneficial mites that are being used in integrated control programs. Beneficial mites, as e.g. predatory mites, may also be infected by microorganisms (bacteria, viruses, microsporidia), resulting in poor performance of the predator. Prospects for the application of pathogens in IPM systems are discussed.

1. INTRODUCTION

As other organisms, Acari may also be subject to disease, usually caused by pathogenic microorganisms. Our knowledge on pathogens of mites, however, is still fragmentary, in contrast to what we know about pathogens of insects. The rapid development of invertebrate pathology in the second half of the twentieth century has largely been due to the study of insect pathogens. Relatively few mite pathogens are known, despite the large number of mite species. Approximately 48,000 species have hitherto been described and it is estimated that this number represents only 10% of the total number of mite species.

That mite pathogens have been studied less comprehensively is not surprising: their frequently small size renders disease diagnosis often difficult and pathophysiogical studies almost impossible. From the other hand, the often large

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reproductive potential of mites makes many acarine species ideal model organisms for detailed epidemiological investigations.

Pathogens in populations of mite species often play a major role in the regulation of population size and population density in natural habitats. In several instances, we see that populations of pest species in agricultural systems are kept below the economic threshold level by the occurrence of a disease. Such observations often provide impetus for further studies on the pathogen involved that may eventually lead to the successful application of a pathogen for the biological control of a mite pest. In addition, the occurrence of disease may be undesirable, e.g. in the case of cultures of beneficial mites: several cases have been reported on the occurrence of disease in mass cultures of predatory mites. In many instances, such observations have led to comprehensive studies on the mite pathogen involved.

Interest in pathogens of mites is increasing. This is apparent from literature reviews that have been published in recent years. We refer to reviews by Van der Geest (1985), McCoy (1996), Poinar and Poinar (1998), Samish and Řeháček (1999), Chandler et al. (2000) and Van der Geest, Elliot, Breeuwer, and Beerling (2000). A survey of pathogenic fungi infecting plant-inhabiting mites can be found in Van der Geest (2004). The student is referred to these reviews if more detailed information is required than is given in the underlying chapter.

Mites obtain their food in general by inserting their mouth parts into their vertebrate host or plant tissue. It is therefore unlikely that mites would contract disease through the alimentary tract unless the vertebrate host or food plant is infected by a pathogen. As penetration through the mouth parts is hampered, the pathogen should follow other infection routes. A plausible infection route is through the integument. Fungi are in general capable to penetrate a mite (or other arthropod species) through the integument. It is therefore not surprising that most mite pathogens are found among the fungi.

In this chapter, the different groups of microorganisms that may cause diseases in Acari will be treated.

2. VIRUS DISEASES

Relatively few viruses are known from mites, in strong contrast to what is observed in insects, from which more than 1,600 viruses have been described in about 1,100 species. Insect viruses are often embedded in larger inclusion bodies, as is the case for nuclear polyhedrosis viruses. The rod-shaped virus particles are situated in proteinaceous bodies that protect the virus against unfavorable conditions when released from the insect cadaver. Such structures are not found for viruses that are known from mites. Well-studied viruses in mites are found in two spider mite species: the citrus red mite, *Panonychus citri*, and the European red mite, *P. ulmi.*

The first record on a virus disease in a spider mite is by Muma (1955), who noted diseased mites in a natural population of the citrus red mite (CRM) in Florida, USA. Affected mites showed signs of diarrhea and the cadavers were adhered to the leaf surface by a black resinous material that was excreted from the anus. The disease has later also been reported in California by Smith, Hill, Munger, and

Gilmore (1959). They observed spherical particles inside diseased mites and assumed that these were virus particles. Later, it could be demonstrated that a rod-shaped, non-inclusion virus is the cause of the disease (Reed & Hall, 1972). The virus particles are approximately 194 × 58 nm in size and enclosed in an envelope of circa. 266 × 111 nm. The virus is formed inside the nuclei of epithelial cells of the midgut, but later it moves out of the nucleus, into the cytoplasm. The pathogen is transmitted when healthy mites ingest the feces of infected mites.

The spherical particles were studied in more detail by Reed and Desjardins (1978). These authors found spherical particles of three different sizes, but only in laboratory reared mites. The particles were apparently acquired by the mites from the green lemons on which they were grown. No detrimental effects of the particles on the mites were observed, although the spheres did multiply inside the mites. In diseased mites, birefringent bodies of irregular shape were found. The size of these bodies may vary from a few micrometers up to 50 µm in diameter (Smith & Cressman, 1962). The function of these bodies is not known, but they seem to be associated with the formation of the so-called fecal pellets, that probably contain guanine as excretion product. Similar bodies have been found in mites showing poor physiological condition (see Section 6.1).

The virus disease is common in citrus groves in California and Arizona and causes a considerable reduction in the population density of the CRM (Reed, 1981). In the 1960s, efforts were made to use the virus as a biological acaricide in order to control the CRM. A drawback in the use of viruses for biological control is that multiplication is only possible in living cells. Control experiments were carried out by spraying aqueous suspensions of macerated diseased mites (Shaw, Chambers, & Tashiro, 1968). For this purpose, mites were grown on green lemons and harvested after infection. This is a very laborious method and can only be done for small field experiments. Another more efficient method was the collection of infected mites in the field by means of a vacuum-suction devise. The mites were subsequently kept on lemons for an additional 6–7 days in order to increase the level of infection. Application of field collected material usually rendered better results than virus suspensions obtained from laboratory cultured mites. The results in small field trials were promising, but large field applications were less satisfactory. Reasons are that the virus is easily inactivated by sunlight when applied as an aqueous suspension and that high temperatures have a negative effect on the virulence of the pathogen. An advantage of using viruses as biological control agent is that they have a narrow host spectrum: they are able to infect only a few species of spider mite and show no effect on other organisms, e.g. phytoseiids (Shaw, Moffitt, & Sciven, 1967).

Steinhaus (1959) studied a virus disease of the European red mite (ERM) in California. He observed spherical particles with a size of 40–60 nm inside affected mites and assumed that these were virus particles. A virus disease was also observed in *P. ulmi* in fruit orchards in Ontario (Putman & Herne, 1966). This disease was caused by a rod-shaped virus that developed inside the nuclei of fat body cells. There is no conclusive evidence that we are dealing with the same virus as the one that described from California. Affected mites can be recognized by a darker color of the immature stages, but diseased adults show no difference in color. The most conspicuous symptoms, however, is the presence of birefringent bodies inside the midgut of the mites. These crystal-like structures probably contain guanine. The virus may cause epizootics in natural populations, but only at high densities of the spider mite. Field experiments were carried out by Putman (1970) by introducing infected immature mites into a peach orchard. In this way, a considerable reduction of the mite population could be obtained. Sprays of aqueous suspensions of the virus were less successful. It was assumed that the leaves release virus inhibitors that inactivate the virus. Recent studies on virus diseases in tetranychids have not been conducted.

Transmission electron microscopy (TEM) has revealed virus-like particles in diseased females of the predatory mite *Metaseiulus occidentalis* (Poinar & Poinar, 1998). Diseased females in laboratory colonies showed a reduced oviposition and often died suddenly with a paralyzed appearance (Hoy & Jeyaprakash, 2008). Three types of icosahedral virus particles were detected by TEM analysis. One type was situated in epithelial cells, 47 nm in diameter with an electron dense core of 35 nm. Poinar and Poinar (1998) assumed that these particles were similar to those reported earlier in the citrus red mite and also similar to those found in epithelial cells of diseased and healthy twospotted spider mites (see above). It might be possible that the predatory mites obtained these particles from their prey, *T. urticae.* A second type of virus particles in *M. occidentalis* was approximately 38 nm in diameter with an electron dense core of 20 nm. These particles were located in large numbers in the nuclei of midgut cells, but free virions were also observed in the cytoplasm and lumen of the midgut. A third type of virus particles was 45 nm in diameter with a 35 nm dense core. These particles were only observed in tissue of the alimentary tract. We know little about the etiology of the disease and whether these observed particles are the cause of the disease and whether the predator becomes infected by feeding on infected prey. The predatory mites studied belonged to crowded laboratory colonies, no field material had been included in the study.

Virus-like particles have been observed in the yolk of eggs developing inside gravid *Neoseiulus cucumeris* by Steiner (1993) and inside *Phytoseiulus persimilis* females by Bjørnson, Steiner, & Keddie (1997). In adults of these species, also birefringent crystals were observed, but there may be no relation between the presence of the virus-like particles and these crystals. Virus infections in cultures of predatory mites are undesirable as such conditions may affect the efficacy of the predator as biological control agent.

Several phytophagous species are known to transmit plant viruses. For example, the dry bulb mite *Aceria tulipae* has been reported as vector of two viruses of *Allium* sp. (Van Dijk & Van der Vlugt, 1994) and *Brevipalpus* spp. are known as vector of, among others, citrus leprosis virus in citrus in Latin America (Rodrigues, Kitajima, Childers, & Chagas, 2003), of orchid fleck virus in many orchid species world-wide (Kondo, Maeda, & Tamada, 2003) and of coffee ringspot virus in coffee in Brazil and Costa Rica (Chagas, Kitajima, & Rodrigues, 2003). It is doubtful whether these vector mites suffer from the presence of viruses inside their body, although the *high plain virus* and *Pigeon Pea Mosaic* virus seem to replicate in their respective vectors (Kumar, Duncan, Robert, Jones, & Reddy, 2002). The *Brome Mosaic Virus*, usually transmitted by a beetle, is capable to multiply in the eriophyid *Aceria tulipae.* It may cause severe cytopathological effects in the midgut cells of the mite (Paliwal, 1972). Similarly, mites e.g. Dermanyssoidea play a role in the transmission of viruses to vertebrates (e.g. equine encephalitis viruses, West Nile virus, fowl pox virus) (Valiente Moro, Chauve, &

Zenner, 2005). It is not within the scope of this article to describe these viruses comprehensively, as they are considered to be plant, resp. vertebrate viruses.

Several viruses are known from the honeybee (*Apis mellifera*), some of which are associated with the varroa mite *Varroa jacobsoni* and the honeybee tracheal mite (HBTM) *Acarapis woodi* (Sammataro, Gerson, & Needham 2000). These viruses may always be present in the bee, either in a latent or in an unapparent form. Wounds inflicted by the mites may activate the viruses in the bee. These viruses are probably not capable to infect the varroa mite or the HBTM, but the presence of these mites affects the incidence of virus disease in honey bees. The varroa mite may also play a role in the transmission of Bee Kashmir Virus (KBV), although the virus was already known from bee colonies before *Apis mellifera* colonies were infested by *V. destructor*. The presence of the virus in the mite has been demonstrated by several authors (e.g. Chen, Pettis, Evans, Kramer, & Feldlaufer, 2004). Virus-free mites may become infected by coinhabiting in the same cell as virus-infected mites. Whether transmission to honey bees occurs mechanically or biologically is a question that has not yet been conclusively solved. Shen, Yang, Cox-Foster, and Cui (2005) suggested that varroa mites cause suppression of the immune system of the honey been which leads to activation of latent virus infections.

Liu (1991) found virus-like particles in a sample of HBTM that originated from Scotland, but no such particles were found in HBTM samples from California. Tissues of affected mites showed extensive lysis, while most cells were tightly packed with virus-like particles. The virions are 27–30 nm in size and are arranged in paracrystalline arrays forming hexagonal patterns. The ultrastructural morphology of the particles indicates that we may be dealing with a picorna-like virus. Such viruses resemble picornaviruses that are found in vertebrates. Picorna-like viruses are also known from honeybees, but on the basis of histopathological studies it was assumed that the virus found in HBTM is not derived from honeybees, but actually multiplies inside the mite.

Kleespies, Radtke, and Bienefield (2000) performed a search for diseases in varroa mites in parasitized bee colonies. They found mites with characteristic internal black-colored changes of the gut and the fat body. On living adult bees, 3.6% of the mites showed this anomaly, in brood cells, even 8% of the juvenile mites were affected. Disease incidence and intensity of the symptoms can be enhanced by changes in environmental conditions, such as deficiency of bee brood, deficiency of pollen, abnormal brood temperature and death of the host. The authors found that longevity of black-colored mites was reduced by 43%. Cytopathological studies showed the presence of a large number of spherical virus-like particles, especially in the nuclei of fatbody and muscle tissue. The particles measure approximately 27–60 nm in diameter and were very similar to the particles found by Liu (1991) in HBTM. *Per os* infection experiments with extracts of fatbody tissue derived from symptomatic mites were unsuccessful.

Ongus et al. (2004) detected virus-like particles in varroa mites that were collected in bee hives in The Netherlands. The virions were mainly present in the cytoplasm of mite tissue and resemble the virus-like particles found by Kleespies et al. (2000). Immunochemical studies revealed that the virus was localized in the abdominal part of the alimentary tract and in the gastric caeca, but not in the salivary glands (Ongus, 2006). It was possible to isolate the virus and to determine the base sequence of the virus genome. The virus (*Varroa destructor* virus 1) is a single stranded RNA genome and, based on the base sequence, it was decided that it belongs to the genus *Iflavirus* (Ongus et al., 2004). Viruses in this genus belong also to the picorna-like viruses. The virus is closely related to *deformed wing virus*, known from honey bees. The latter virus causes morphological anomalies in wings of bees.

3. DISEASES CAUSED BY BACTERIA

The most widely studied bacterium in invertebrate pathology is *Bacillus thuringiensis.* It was first described in 1915 by Berliner, who isolated it from soil samples in the Thuringian Forest in Germany. Simultaneously with spore formation, a crystalline body is formed in the bacterium. Upon ingestion by an insect, this crystal (δ-endotoxin) falls apart into toxic subunits that may cause paralysis of the alimentary tract, resulting in the death of the insect. Most varieties (serotypes) of *B. thuringiensis* show an effect on larvae of Lepidoptera, but some also on other groups of insects, e.g. Coleoptera and Diptera.

Very comprehensive research has been carried out on *B. thuringiensis* that has resulted in the development of several commercial preparations that are mainly used against lepidopterous pests. Also, the gene encoding for the crystalline toxic body has been isolated and transferred into crop plants, e.g. corn and cotton, making these crops resistant towards a number of lepidopterous pests. Several serotypes of *B. thuringiensis* produce in addition an exotoxin, the β-exotoxin, named thuringiensin. This exotoxin is excreted by the bacterium into the culture medium. It has a nucleotide-like structure and inhibits DNA-dependent RNA polymerase. This results in a blockage of mitosis. When thuringiensin is applied to young holometabolous insects, morphological deformations may occur in the adult stage.

Field applications of thuringiensin were successful against the citrus red mite *P. citri* (Hall, Hunter, & Arakawa, 1971) and *Tetranychus pacificus* (Hoy & Ouyang, 1987). Later, Royalty, Hall, and Taylor (1990) conducted experiments by testing two different formulations of thuringiensin against the twospotted spider mite *T. urticae.* The results indicated that thuringiensin might be a potential acaricide. In particular young instars are susceptible, since these have a high growth rate. Various physiological processes in young organisms require higher RNA synthesis than in the older slower growing stages. A major drawback is that thuringiensin is toxic for a wide range of organisms. Not only are spider mites affected, but also beneficial mites, such as *Phytoseiulus persimilis:* oviposition starts to decline after 2 days and ceases completely after 3–4 days in both predator and spider mite *T. urticae* (Guo, Zuo, Zhao, Wang, & Jiang, 1993). The chemical is apparently a nonselective acaricide that should not be used in combination with predatory mites.

The spore-crystal complex of *B. thuringiensis* has been tested on spider mites by Krieg (1972), but no mortality was observed. However, Chapman and Hoy (1991) conducted experiments in which *T. urticae* and *Metaseiulus occidentalis* were treated with a commercial preparation of *B. thuringiensis* var. *tenebrionis.* This variety of *B. thuringiensis* shows an effect on beetles and is recommended for use against the Colorado Potato Beetle, *Leptinotarsa decemlineata.* No effect was noted on the twospotted spider mite, but the preparation did show a toxic effect on *M. occidentalis*. Eggs were not affected, but if juveniles were treated, only 65% reached the adult stage. This toxic effect could be enhanced by starving the mites: the authors assumed that starvation may lead to a higher uptake of the material, or that the mites were more exposed to the preparation as starving mites tend to move faster. It is also possible that starvation acts as a stress factor. The authors have no explanation for the toxic effect on the predatory mite: the preparation did not contain the β-exotoxin (thuringiensin) known to be toxic for mites.

In more recent years, isolates of *B. thuringiensis* have been found that do show toxicity towards spider mites and house dust mites (Payne, Cannon, & Bagley, 1993; Payne, Cannon, & Ralph, 1994). It has been suggested to isolate the δ-endotoxin of these isolates and to formulate it as an acaricide. One may also transfer the gene, encoding for this specific δ-endotoxin into a crop plant in order to protect the crop against spider mite infestations.

An interesting discovery is the isolation of a *B. thuringiensis* strain from dead twospotted spider mites, *T. urticae* (Jung, Mizuki, Akao, & Côte, 2007). In sporulating cultures of the bacterium, roughly spherical parasporal inclusion bodies are formed. This crystalline body is composed of at least two polypeptides of 86 and 79 KDa. The crystal is not toxic to the twospotted spider mite, but after cleavage with trypsin, it is cytocidal to some human cancer cells.

In Table 1, a list is given of bacteria that have been isolated from mites. We realize that this list may not be complete, but we have tried to limit the list to bacteria that show pathogenicity towards mites. Many species of bacteria may be isolated from mites (and other organisms): in many instances, these may not appear to be pathogenic.

Aksoy, Ozman-Sullival, Ocal, Celik, and Sullivan (2008) studied the effect of *Pseudomonas putida* biotype B on the twospotted spider mite *T. urticae*. The bacterium had been isolated from greenhouse soil in Turkey and was tested on newly emerged, copulated females. The authors observed a strongly reduced egg production and no hatching of the eggs was noted. The results showed that the bacterium may be very effective in causing mortality in *T. urticae* populations. Further research is required to find out whether this organism may be developed to a microbial miticide.

The predatory mite *Phytoseiulus persimilis* has been mass cultured for several decades for the biological control of spider mites in various field and glasshouse crops. This predator species is very important in integrated pest control programs and has stimulated research on predator-prey interactions and foraging behavior. It has been shown in several instances that adult female predatory mites are attracted to volatiles that are emitted by plants infested by prey. This emission of volatiles by the plant after herbivore attack is apparently a defense mechanism against herbivorous mites. Schütte (2006) noticed a change in response to prey-induced plant volatiles in a laboratory colony of *P. persimilis*. This population showed a lower attraction to these volatiles than other populations of the predator. It could be demonstrated that the change in behavior is caused by the involvement of a bacterium. Transmission of the bacterium occurs through feces and debris. There is no evidence that vertical transmission (from one generation to the next) occurs. The bacterium could be isolated and was described as *Acaricomes phytoseiuli* (Pukall, Schumann, Schütte, Gols, & Dicke, 2006). Comparative analysis of the 16S rDNA sequence revealed that it belongs to the Micrococcaceae, and that it is related to the soil bacterium *Arthrobacter globiformis*, to *A. rissicus*, a bacterium originally isolated from air in the Russian space laboratory and to *Renibacterium salmoninarum*, the causal agent of kidney disease in salmon. The bacterium clearly affects the effectiveness of *P. persimilis* as biological control agent as infected mites show a lower response to the volatiles emitted by the plant after herbivorous attack. At this moment, there are no signs that the disease is present in commercial populations of *P. persimilis*, nor is it widespread in laboratory populations of this predator.

Table 1. Bacterial infections in mites.

Species	*Mite host*	*Mite family*	*References*
Bacillus thuringiensis	Spider mites House dust mites	Tetranychidae Pyroglyphidae	Payne et al. (1993, 1994)
B. thuringiensis	*Tetranychus urticae*	Tetranychidae	Jung et al. (2007)
B. thuringiensis var. *israelensis*	*Dermatophagoides pteronyssinus*	Pyroglyphidae	Saleh et al. (1991)
B. thuringiensis var. *tenebrionis*	*Metaseiulus occidentalis*	Phytoseiidae	Chapman and Hoy (1991)
B. sphaericus	*Dermatophagoides pteronyssinus*	Pyroglyphidae	Saleh, Kelada and Shader (1991)
Bacteroidetes	*Metaseiulus occidentalis*	Phytoseiidae	Hoy and Jeyaprakash (2008)
Enterobacter	*Metaseiulus occidentalis*	Phytoseiidae	Hoy and Jeyaprakash (2008)
Pseudomonas putida	*Tetranychus urticae*	Tetranychidae	Aksoy et al. (2008)
Acaricomes phytoseiuli	*Phytoseiulus persimilis*	Phytoseiidae	Pukall et al. (2006).
Rickettsia sp.	*Metaseiulus occidentalis*	Phytoseiidae	Hess and Hoy (1982)
	Vatacarus ipoides	Trombiculidae	Thomas and Poinar (1973)
Rickettsiella phytoseiuli	*Phytoseiulus persimilis*	Phytoseiidae	Šut'áková (1988)
Wolbachia	*Bryobia* sp.	Tetranychidae	Weeks (personal communication)
	Eutetranychus orientalis	Tetranychidae	Breeuwer and Jacobs (1996)
	Oligonychus biharensis	Tetranychidae	Breeuwer and Jacobs (1996)
	Tetranychus yusti	Tetranychidae	Breeuwer and Jacobs (1996)
	Tetranychus kanzawai	Tetranychidae	Breeuwer and Jacobs (1996) Gomi, Gotoh, and Noda (1997)

Table 1 Continued

	Tetranychus neocaledonicus	Tetranychidae	Breeuwer and Jacobs (1996)
	Tetranychus turkestani	Tetranychidae	Breeuwer and Jacobs (1996)
	Tetranychus quercivorus	Tetranychidae	Gotoh, Abe, Kurihara, and Suzuki (1995)
	Tetranychus urticae	Tetranychidae	Breeuwer and Jacobs (1996) Tsagkarakou, Guillemaud, Rousset, and Navajas (1996)
	Metaseiulus occidentalis	Phytoseiidae	Johanowicz and Hoy (1966) Breeuwer and Jacobs (1996)
	Neoseiulus barkeri	Phytoseiidae	Breeuwer and Jacobs (1996)
	Neoseiulus bibens	Phytoseiidae	Breeuwer and Jacobs (1996)
	Phytoseiulus persimilis	Phytoseiidae	Steiner (1993) and Breeuwer and Jacobs (1996)
Cardinium sp.	*Brevipalpus phoenici*	Tenuipalpidae	Groot and Breeuwer (2006)
	Brevipalpus californicus	Tenuipalpidae	Chigira and Miura (2005)
	Euseius finlandicus	Phytoseiidae	Enigi and Schausberger (2007) Hoy and Jeyaprakash (2008)
	Neoseiulus californicus		
	Metaseiulus occidentalis		
Spiroplasma	*Tetranychus urticae*	Tetranychidae	Enigi and Schausberger (2007), De Luna, Valente Moro, Guy, Zenner, and Sparagano (2009)
	Dermanyssus gallinae	Dermanyssidae	
Serratia marcescens	*Metaseiulus occidentalis*	Phytoseiidae	Lighthart, Sewall, and Thomas (1988)

Mite and also tick species are frequently infected with intra-cellular microorganisms (Šut'áková, 1988, 1994; Steiner, 1993; Munderloh & Kurtti, 1995; Bjørnson et al., 1997) These microorganisms have been placed into the family Rickettsiaceae within the class α-Proteobacteria. The family Rickettsiaceae (or Rickettsia-like organisms) consists of three tribes: Rickettsieae, Ehrlichieae and Wolbachieae (Weiss & Moulder, 1984). Representatives of these tribes have a number of features in common: they are obligate intracellular gram-negative bacteria naturally found in arthropod hosts, they multiply inside eukaryotic cells and are often surrounded by multiple membranes. Some are also capable of infecting humans and other vertebrates and are frequently pathogenic in these secondary hosts They often cause severe diseases such as spotted fever, typhus and scrub typhus (Hayes & Burgdorfer, 1989). A good classification of these organisms has been hampered by the fact that classical microbiological identification tools cannot be used because of their obligate intracellular lifestyle. Since a number of years, tools have become available to sequence DNA of organisms. Sequence information on 16S rDNA genes has revealed interesting aspects of the order Rickettsiales, including their phylogeny. For additional information see Van der Geest et al. (2000).

The best studied group of rickettsia are those that are vectored by ticks, as they often cause disease in vertebrates and are therefore of great medical and veterinary importance. In plant-inhabiting mites, rickettsia-like infections have mainly been observed in microscopic surveys (Šut'áková, 1988, 1994; Hess & Hoy, 1982; Steiner, 1993; Munderloh & Kurtti, 1995; Bjørnson et al., 1997). They are described as intracellular microbes often localized in the reproductive tissue of their host, but no data are available on their possible effect on mites.

Hess and Hoy (1982) studied diseased laboratory colonies of the predatory mite *Metaseiulus occidentalis* more comprehensively, as these colonies produced only few eggs and often died out. Two different pathologies could be discerned. Some adult females were plump in appearance and had cream- to pink-colored plugs excreting from their rectum. Such females rarely produced eggs, although they appeared gravid. Immatures and males seldom showed this plug. The second condition affected both females and juveniles. These females failed to lay eggs and immatures often died, in particular during molting. Two forms of *Rickettsia*-like organisms were described, based on structure of the cell wall and the presence of cytoplasmic inclusions. Type A was observed in all mites examined, while type B was present in approximately two-thirds of the mites investigated. The Type A organisms were small, ovoid and 0.75 μm in length and 0.5 μm in width, with a trilaminar membrane of 7 nm thick, while Type B was rod-shaped, and measured 0.5 μm in width and 2 μm in length. The Type A organisms were located intracellularly, singly or in groups of two or three in all tissues except ovarian and nervous tissue. The Type B organisms were both inter-and extracellular within membrane-bound vacuoles of cells that are similar in structure to mycetomes in insects. For a more detailed description is referred to Hoy and Jeyaprakash (2008).

Since the mid-1990s, molecular screening for intracellular microorganisms is possible. This has resulted in the discovery of *Wolbachia* in tetranychid and phytoseiid mites (e.g. Gotoh et al. 1995; Gotoh, Gomi, & Nagata, 1999; Gotoh, Sugasawa, & Nagata, 1999; Breeuwer & Jacobs, 1996; Johanowicz & Hoy, 1996; Tsagkarakou et al., 1996). Probably all *Rickettsia*-like organisms that have been reported in the early microscopic surveys are members of the genus *Wolbachia* belonging to the family Rickettsiaceae. These bacteria are parasites of the reproductive system of mites and insects and cause many unusual effects in their hosts, such as parthenogenesis (infected females produce only females), male killing (male embryos die while female embryos develop into adults), feminization (infected male embryo's develop into females) and cytoplasmic incompatibility.

Wolbachia is a bacterium that is vertically transmitted to the next generation through the females. It modifies host reproduction in several ways in order to enhance its own spread (Weeks & Breeuwer, 2001). It induces parthenogenesis in the spider mite *Bryobia praetiosa,* causing males not to be produced. This is to the advantage of the bacterium, as only females are able to transmit the pathogen to the next generation. Probably over 1 million species of insects are infected, and infection rates in other taxa of arthropods may be similar.

Van Opijnen and Breeuwer (1999) showed that *Wolbachia* can induce cytoplasmic incompatibility in the arrhenotokous[1] twospotted spider mite *Tetranychus urticae* between uninfected females and infected males. Cytoplasmic incompatibility is expressed through a male-biased sex ratio and low hatchability of eggs. It could be suppressed by removing *Wolbachia* from spider mites reared on a diet with antibiotics. The authors also demonstrated that heat treatment (32° C.) can eliminate *Wolbachia* from infected mites. It was shown that 71% of the mites were "cured" after being reared for four generations at 32°C. The infection could be completely eliminated by keeping the mites for 6 generations at 32°C.

In a recent study Hoy and Jeyaprakash (2008) studied *M. occidentalis* females with large anal plugs. These females were collected from crowded laboratory colonies. The authors used 16S PCR primers to amplify the bacterial DNA and subsequently cloned and sequenced the products. It was remarkable that only bacteria were found that could also be detected in "healthy" females, *viz. Wolbachia*, *Cardinium*, an undescribed species of *Bacteroidetes* and an *Enterobacter* species. The authors assumed that stress could increase the titer of one or more endosymbionts which may lead to "disease". The authors cite the statement of Dale and Moran (2006) that "as more cases of chronic bacterial infection are characterized, the distinction between pathogenesis and mutualism has become increasingly blurred. Infection by a particular bacterium may be beneficial to a host under circumstances but harmful in other hosts or environments" Another interpretation for the occurrence of the disease in *M. occidentalis* is that the predator obtains *Rickettsia*-like organisms from its food. *Rickettsia*-like organisms have been detected in twospotted spider mites. However, there is no conclusive evidence that the predator obtains the endosymbionts from its prey (Hoy & Jeyaprakash, 2008).

Groot and Breeuwer (2006) showed the presence of the symbiont *Cardinium* in the thelytokous[2] mite species *Brevipalpus phoenici. Cardinium* spp. belong to the Flexibacteraceae (Phylum: Bacteroidetes; Class: Sphingobacteria). It was demonstrated that *Cardinium* induces thelytoky by feminizing unfertilized haploid eggs. Isofemale lines were set up and in some of these lines, males were produced by only very young daughters, while older females produce daughters exclusively. That a bacterium was involved was apparent from the fact that a treatment with antibiotics resulted in an increased number of male progeny. The presence of *Cardinium* was also shown in *B. californicus* (Chigira & Miura, 2005). Tetracycline-treated females produced many male progeny while untreated females produced only female progeny. This is an indication that *B. californicus* is feminized by *Cardinium*. Comparison of infected females with uninfected males and other closely related species, revealed that *Cardinium* does not have a negative effect on the fitness of *B. californicus*. The bacterium has also been found in a population of

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[1] Arrhenotokous: capable of producing male offspring only.

[2] Thelytokous: capable of producing female offspring only.

Eotetranychus suginamensis in Japan (Gotoh, Noda, & Ito, 2006). One species of spider mite, *Eotetranychus pueraricola* harbored both *Cardinium* and *Wolbachia,* but these symbionts seemed to have no effect on the reproduction of the host. *Cardinium* has also been found to play a role in insect species, e.g. *Encarsia* sp. *Cardinium* is also present in field populations of the predatory mites *Euseius finlandicus* and *Neoseiulus californicus* and the spider mite *Eotetranychus uncatus* (Enigi & Schausberger, 2007). The endosymbionts has also been detected in the poultry red mite *Dermanyssus gallinae* (De Luna et al., 2009).

Another genus of endosymbionts is the genus *Spiroplasma.* This genus belongs to the family Spiroplasmataceae, class Mollicutes, phylum Firmicutes. These bacteria are fastidious organisms which means that they can only been grown on rich nutrient media. *Spiroplasma* has been shown to kill males of *Danaus chrysippus* (Lepidoptera) and also of other insect species. Interspecific transmission of *Spiroplasma poulsonii*, a male-killing symbiont has been demonstrated between *Drosophila* sp. and *Macrocheles* sp., an ectoparasitic mite that feeds on the hemolymph of *Drosophila* (Jaeniki, Polak, Fiskin, Helou, & Minhas, 2007). Enigi and Schausberger (2007) showed the presence of *Spiroplasma* in the twospotted spider mite, but its effect on reproduction and other biological parameters is not yet known. *Spiroplasma* has also been detected in the poultry red mite *Dermanyssus gallinae* (De Luna et al., 2009).

The ecological implications of modification of reproduction in herbivorous and predatory mites by intracellular symbionts is an interesting field of study. It will be clear that such modifications will have a great impact on the development of mite populations.

4. DISEASES CAUSED BY FUNGI

The Kingdom Fungi is a monophyletic assemblage which comprises at present four phyla: Chytridiomycota, Zygomycota, Basidiomycota and Ascomycota. In addition, there is a group called the Deuteromycetes (or Fungi Imperfecti), fungi of which no sexual stage is known. In the past, many parasitic fungi were classified in this group, but were reclassified after the sexual stage had been discovered. Most of these "reclassified" fungi belong to the Ascomycota. Nucleotide sequence analysis also suggests that the majority of these fungi belong to the Ascomycota. A large number of fungi capable to infect arthropods are found in the Deuteromycetes.

Chytridiomycota and Zygomycota share common features, in particular the absence of cross walls (septa) in their hyphae. The Chytridiomycota (chytrids) represent a primitive group of aquatic fungi. Chytrids have gametes that are mobile by means of flagella. No chytrids are known that infect mites.

Zygomycota are characterized by the formation of zygospores. These sexual spores are not contained within a specialized fruiting body or sac, but are unenclosed (or naked) between their parental hyphae. The haploid nuclei at the end of two hyphae fuse to a diploid zygote. This zygote undergoes immediately meiosis to form haploid cells that develop into zygospores.

Ascomycota are also called "sac fungi" as their sexual spores (ascospores) are enclosed in tube-like sacs, known as asci. The formation of ascospores is similar to that of zygospores, but the spores are not naked but contained within asci. Ascomycota have cross walls in their hyphae.

Basidiomycota are also known as the “club fungi”. Their sexual spores are formed on often very complex fruiting bodies called basidia. This group of fungi possesses also septa inside their hyphae.

4.1. Zygomycota

In this phylum, only the order Entomomophthorales (meaning insect destroyers) (class Zygomycetes) contains species that are pathogenic for Arthropoda. These fungi are mainly obligatory pathogens of insects and other arthropods, often with a restricted host spectrum. However, some species are parasitic on desmids (green algae) or fern prothalli while other species may be saprophytic on plant debris (Moore-Landecker, 1996). Hosts that are infected by fungi of this group are usually not killed before all available nutrients have been utilized, in contrast to, e.g. Deuteromycetes infections where mycotoxins often cause the death of the host prior to the complete utilization of food reserves. Hajek (1997) assumed that the complete utilization of the live host by the fungus seems to be consistent with the obligate nature of this group of fungi.

As mentioned above, Zygomycetes are characterized by their sexual reproduction, the so-called zygospore formation, usually inside the parasitized host. Zygospores bud laterally from conjugation bridges between gametangia[3] (hyphal bodies). These thick-walled, dark-colored spores are also called resting spores and may germinate to produce conidiophores with conidia attached. Other types of resting spores are chlamydospores, thick-walled, nonsexual spores originating from transformed hyphal cells, and azygospores that may arise from parthenogenetic development of hyphal bodies (short segments of mycelium inside the host). Azygospores may also be the result of budding of chlamydospores, or they may be formed at the tip of hyphae that arise from chlamydospores or hyphal bodies. Resting spores (Fig. 1) seem to aid the fungus in surviving unfavorable conditions, e.g. periods of drought. Conditions under which resting spores germinate are not well-understood.

More common is the formation of sporangiospores or ballistospores. These asexual spores are nonmotile and are formed within a sac-like structure, the sporangiole. Under nearly saturated conditions, these spores (by most mycologists called: primary conidia) are forcibly discharged from the spore bearing structure (sporangiophore, conidiophore). Primary conidia are often sticky due to the presence of a mucous substance causing them to adhere to a substrate after they have been released. The conidia may either form hyphae directly that may infect a new host or form secondary conidia.

Six families are recognized within the Entomomophthorales; pathogens of arthropods are mainly found in the Entomophthoraceae, Neozygitaceae and Ancylistaceae. These families are recognized on the basis of nuclear characters (size,

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[3] A single-celled structure producing gametes (sex cells) or gametic nuclei.

number, stainability), mode of formation of resting spores, and mechanism of discharge of primary conidia.

Figure 1. Resting spores of Neozygites tanajoae *in the cassava green mite* Mononychellus tanajoa. *Photograph courtesy of Dr. Fabien C.C. Hountondji.*

Entomophthoraceae are obligate pathogens of arthropods. In this family, primary conidia are released in a cannon-like manner when the conidiophore ruptures at the apex. The conidium is actually a bud at the apex of the conidiophore that is filled with protoplasm from the conidiophore. When the conidium is fully developed, both conidium and conidiophore absorb water quickly under humid conditions. The osmotic pressure is larger in the conidium than in the conidiophore and this fact results in the forcible discharge of the conidium. When the spore lands on a suitable host, it may infect the host after germination. These primary conidia may form secondary conidia if landed on a non suitable substrate. A well-known example is *Entomophthora muscae,* a species capable to infect flies. Mites infecting species are known in the genus *Tarichium*. Of species of this genus, only resting spores are known and species determination is largely based on the shape and size of the resting spores. *Tarichium* spp. have been isolated in soil samples from several species of mites, in many cases Oribatidae (e.g. Bałazy & Wiśniewski, 1982, 1984).

Neozygitaceae are obligate pathogens of insects (mainly Homoptera) and mites. They release primary conidia that germinate to form smaller secondary conidia with an adhesive haptor on long, thin conidiophores. The resting spores are often dark-colored and ornamented on the outside wall. They possess two pores and are formed inside the hosts.

Ancylistaceae contains a number of representatives that may not only infect arthropods, but also vertebrates. For example, *Conidiobolus coronatus* causes serious infectious of mucous membranes of humans and other vertebrates (Maiti et al., 2004). Other species in this family are found in the soil and are often saprophytic. Primary conidia are forcibly discharged towards a light source and germinate to form secondary conidia that usually have the same shape as the primary conidia. The secondary conidia are either forcibly discharged or discharged passively from long slender conidiophores.

4.1.1. Course of Infection

Entomophthoralean fungi usually penetrate their host through the cuticle by means of a germ tube that is formed by the conidium. Mite infecting species are mainly found in the Neozygitaceae. The primary conidium is released from the conidiophore and forms on top of a slender tube a much smaller secondary conidium, called capilliconidium. The capilliconidium is considered to be the infectious propagule. When landed on a suitable host, it penetrates through the cuticle by means of a newly formed germ tube (Fig. 2). After penetration, mycelium is formed within the body cavity of the host that fragments into a number of smaller segments, the hyphal bodies. Hyphal bodies are the propagative units and multiply by budding and by undergoing fission. In addition, rhizoids may be formed: sterile hyphae, often branched, that perforate the ventral side of the host and attach the host to the substrate. The infected host becomes in this way fixed to the substrate, also after its death. Favorable conditions (high relative humidity) allow the formation of conidiophores that grow through the cuticle of the host. On top of these structures, conidia are formed that form a halo around the dead host after discharge from the conidiophores. The spores have a mucous substance on the outside and stick to the substrate on which they have landed. They may be picked up by new, uninfected hosts. A high relative humidity (near saturation point) is required at two points in the course of the disease: germination of the spores needs a high relative humidity, but also sporulation requires humid conditions.

Figure 2. Capilliconidium of a Neozygites *sp. on a leg of a spider mite Photograph: Leo P.S. van der Geest.*

The presence of rhizoids is an important property of the fungus for a successful transmission of the disease to a new host: the dead host (mummy) remains fixed to the plant surface and does not fall on the soil. In this way, chances for a successful transmission are increased.

The formation of resting spores is an interesting phenomenon. Sometimes, resting spores are present in large numbers. It is assumed that they may assist the fungus to overcome poor favorable conditions, such as extreme periods of drought. However, we still know very little about the role of resting spores and the conditions under which they germinate are poorly understood. The fungus may also survive unfavorable conditions in other manners. Klingen, Wærsted, and Westrum (2008) showed that *Neozygites floridana* may survive cold winter periods in the form of hyphal bodies inside hibernating females of *T.urticae.*

Most entomophthoralean fungi are difficult to culture in artificial culture media. For mass production, it is often necessary to use live host. Species that can be cultured in artificial media have usually a broad host spectrum. On the other hand, species with a restricted host range can hardly be grown outside their host, although some progress have been made with very complex culture media. Entomophthorales observed in mites are given in Table 2.

Table 2. Entomophthoralean fungi infecting mites and their hosts.

Fungus species	*Mite species*	*Mite family*	*References*
Not identified Entomophthoralean species	*Arctoseius* sp.	Arctoseiinae	Bałazy and Wiśniewski (1989)
	Macrocheles peregrinus	Macrochelidae	Milner (1985)
	Pergamasus crassipes	Parasitidae	Milner (1985)
	Tetranychus desertorum	Tetranychidae	Walter (1999)
Resting spores of Entomophtoralean species	*Agistemus*	Stigmaeidae	Van der Geest, De Moraes, Navia, and Tanzini (2002)
	Asca sp.	Ascidae	
	Amblyseius igarassuensis	Phytoseiidae	
Basidiobolus sp.	*Tetranychus urticae*	Tetranychidae	See: Chandler et al. (2000)
Conidiobolus sp.	*Bryobia* sp.	Tetranychidae	Bałazy, Mietkiewski, Tkaczuk, Wegensteiner, and Wrzosek (2008)
Conidiobolus brefeldionis	*Tyrophagus perniciosus*	Acaridae	See: Chandler et al. (2000)
Conidiobolus chapowski	*Pergamasus*	Parasitidae	See: Chandler et al. (2000)

Table 2 Continued

Conidiobolus coronatus	*Dendrolaelaps* sp.	Digamasellidae	Bałazy et al. (2008)
Conidiobolus obscurus	*Phytoseiulus persimilis*	Phytoseiidae	See: Chandler et al. (2000)
	Mononychellus tanajoa	Tetranychidae	Nyiira (1982)
	Tetranychus bimaculatus		See: Chandler et al. (2000)
	Tetranychus urticae		
Conidiobolus thromboides	*Tetranychus urticae*	Tetranychidae	See: Chandler et al. (2000)
	Eotetranychus sexmaculaturs		Selhime and Muma (1966)
	Mononychellus sp.		Bartkowski Odindo, and Otieno (1988)
	Mononychellus tanajoa		Delalibera, Sosa Gomez, De Moraes, De Alencar, and Farias Araujo (1992)
	Panonychus citri		Fisher (1951)
	Tetranychus evansi		Humber, De Moraes, and Dos Santos (1981)
	Tetranychus pacificus		Steinhaus and Marsh (1962)
	Tetranychus tumidus		See: Chandler et al. (2000)
	Tetranychus turkestani		Carner and Canerday (1968)
	Vatacarus sp.	Trombiculidae	See: Chandler et al. (2000)
Erynia phalangicidae	*Pergamasus* sp.	Parasitidae	Bałazy and Wiśniewski (1984)
Zoophthora radicans	*Tetranychus urtcae*	Tetranychidae	See Chandler et al. (2000)
Neozygites sp.	*Euseius citrifolius*	Phytoseiidae	Furtado, De Moraes, and Keller (1996)
	Alaskozetes antarcticus	Ameronothridae	Bridge and Worland (2004, 2008)
Neozygites abacaridis	*Abacarus hystrix*	Eriophyidae	Miętkiewski and Bałazy (2003)
	Aculodes mckenziei		Bałazy, Mietkiewski, Tkaczuk, Wegensteiner, and Wrzosek (2008)
	Aculodes sp.		
	Aculus fockeui		

Table 2 Continued

Neozygites acaricida	*Halotydeus destructor* *Penthaleus major*	Eupodidae Penthaleidae	See Chandler et al. (2000)
Neozygites acaridis	*Halotydeus destructor* *Penthaleus major*	Eupodidae Penthaleidae	James (1994)
Neozygites floridana	*Bryobia* sp. *Eotetranychus banksi* *Oligonychus gossypii* *Oligonychus hondoensis* *Oligonychus pratensis* *Panonychus citri* *Tetranychus ludeni* *Tetranychus tumidus* *Tetranychus urticae*	Tetranychidae	Miętkiewski, Balazy, and Van der Geest (1993) Weiser and Muma (1966) Yaninek, Saizonou, Onzo, Zannou, and Gnanvossou (1996) Nemoto and Aoki (1974) Dick, Buschman, and Ramoska (1992) Fisher (1951) Rameseshiah (1971) Saba (1971) Smith and Furr (1975)
Neozygites tanajoae	*Mononychellus tanajoa* *Oligonychus gossypii*	Tetranychidae	Delalibera, Hajek, and Humber (2004)
Neozygites tetranychid	*Tetranychus urticae*	Tetranychidae	Weiser (1968)
Tarichium acaricolum	*Pergamasus* sp.	Parasitidae	Bałazy and Wiśniewski (1984)
Tarichium azygosporicum	*Pergamasus* sp.	Parasitidae	Bałazy, Wiśniewski, and Kaczmarek (1987)
Tarichium distinctum	*Pergamasus* sp.	Parasitidae	Bałazy et al. (1987)
Tarichium hyalinum	*Tectocepheus velatus*	Parasitidae	Bałazy et al. (1987)
Tarichium monokaryoticum	*Tricouropoda szczecinensis*	Uropodidae	Bałazy et al. (1987)

Table 2 Continued

Tarichium svalbardense	*Dinychus carinatus*	Prodinychidae	Bałazy et al. (1987)
	Pergamasus sp.	Parasitidae	Bałazy and Wiśniewski (1984)
	Veigaia sp.	Veigaiidae	Bałazy et al., 1987
Tarichium obtusoangulatum	*Uropoda minima*	Uropodidae	Bałazy and Wiśniewski (1984)
Tarichium pusillum	*Pergamasus* sp.	Parasitidae	Bałazy and Wiśniewski (1984)
Tarichium sphaericum	*Trachyuropoda coccinea*	Trachyuropodidae	Bałazy and Wiśniewski (1984)
Tarichium subglobosum	*Pergamasus* sp.	Parasitidae	Bałazy and Wiśniewski (1984)
	Uropoda minima	Uropodidae	Bałazy and Wiśniewski (1984)
Tarichium tenuisculpturatum	*Pergamasus* sp.	Parasitidae	Bałazy and Wiśniewski (1984)
Tarichium uropodinis	*Trachyuropoda coccinea*	Trachyuropodidae	Bałazy and Wiśniewski (1982)
Tarichium verruculosum	*Celaenopsis* sp.	Celaenopsidae	Bałazy et al. (1987)
	Unidentified	Galumnidae	

4.1.2. Natural Entomophthoraceous Infections

The first record of an entomophthoralean fungus infection in spider mites was observed by Fisher (1951) who noted adult mortality from 32 to 95% in populations of the citrus red mite *Panonychus citri.* The fungus was in particular prevalent during late summer and early autumn throughout the Florida peninsula. Weiser and Muma (1966) isolated the fungus later from the Texas citrus mite *Eutetranychus banksi* and described it as *Entomophthora floridana.* The fungus has since been reported from several other spider mites species: For example, it was observed in *Tetranychus tumidis* on cotton in the humid subtropical regions of Florida (Saba, 1971), in *T. evansi* on tomato crops in Brazil (Humber et al., 1981), in *T. ludeni* on bean in India (Ramaseshiah, 1971), in *Oligonychus hondoensis* on cedar in Japan (Nemoto & Aoki, 1975) and in *T. urticae* on field corn in North Carolina, USA (Brandenburg & Kennedy, 1982). The fungus has also been reported in Poland on the twospotted spider mite *T. urticae* and on *Bryobia* sp. (Miețkiewski et al., 1993).

Keller and Wuest (1983) noted infections by *Neozygites adjarica* in *T. urticae* on bean in Switzerland, but later investigations showed that this fungus is identical to *N. floridana*; *N. tetranychi*, described by Weiser (1968) as a pathogen of the twospotted spider mite from a fruit orchard in Southern Bohemia, Czech Republic, is also considered to be a synonym of *N. floridana* (Keller, 1997).

Bridge and Worland (2008) observed a *Neozygites* infection in the cryprostigmatic mite *Alaskozetes antarcticus* (Ameronothridae). This mite was collected at Rip Point, Nelson Island off the West coast of the Antarctic peninsula. A few specimens appeared to be infected by a fungus that was provisionally described as *Neozygites* cf. *acaridis*. The mite survives the harsh climatic conditions by lowering the freezing point by the accumulation of low molecular weight cryoprotective compounds such as glycerol, together with the removal or masking of ice-nucleating substances from its body.

The introduction of the cassava green mite *Mononychellus tanajoa* into the cassava growing regions on the African continent has led to a comprehensive search for natural enemies of this pest. This has resulted in the isolation of a *Neozygites* sp. that is very specific for the cassava green mite in Venezuela by Agudela-Silva (1986) and later also in Brazil by Delalibera et al. (1992). The fungus is also known from the African continent. Originally, the fungus was considered to be identical to *N. floridana*, although it did show differences in, among others, its very restricted host spectrum and in the size of the conidia. Recently, the fungus has been described as *N. tanajoae* (Delalibera et al., 2004). It can be distinguished from *N. floridana* on the basis of 18S ribosomal DNA (rDNA) sequences, host range, nutritional requirement for growth in vitro, tolerances to low temperatures (4° C) and ability to withstand specific cryopreservation techniques. *N. tanajoae* isolates from Brazil and Africa have identical 18S rDNA sequences but they presented 5.7 and 9.94% pair wise distance from *N. floridana* isolates. *N. tanajoae* proved to differ sufficiently from other mite-pathogenic fungi referred to as *N. floridana* to justify the description as a new species.

4.1.3. Role of Neozygites floridana *in the Field*

The role of *Neozygites* spp. in the field has been studied in a number of instances. Numerous examples show that *Neozygites* sp. may have a major impact on populations of spider mites in agricultural crops. Saba (1971) observed *Neozygites floridana* in populations of *T. tumidus* in cotton field in the humid subtropical part of Florida. A reduction of 220 spider mites per cotton leaf down to less than 1 spider mite was noted during a period of warm humid weather with heavy rainfall. This reduction could be mainly attributed to the presence of the fungus disease. Similar observations were made by others, e.g. Smith and Furr (1975) who established that *Neozygites* in the main factor that limits late-season population increases of *T. urticae* on cotton in the delta of the Mississippi and Carner (1976) who found the fungus throughout the states Georgia, South Carolina and Alabama. He considered *Neozygites* a very important factor in regulating spider mite populations in cotton.

Treatment of crops with fungicides may affect the incidence of fungus disease in populations of spider mite: Brandenburg and Kennedy (1983) observed a lower proportion of spider mites infected by *Neozygites* in lima beans when the fungicide benomyl was applied. The fungicide did not affect sporulation of the pathogen, but seemed to have an effect on conidial germination and growth of the fungus.

Immature stages of *T. urticae* are more susceptible to *N. floridana* than adult mites, while adult females are more susceptible to infection than adult males (Susilo Nordin, & Brown, 1994). The authors suggest than the thinner cuticle of the immature mites could be the cause that immature stages are more vulnerable to the fungus disease. That males are less susceptible to infection is probably due to the stronger cuticle of this sex compared to that of females. Males have to compete with other males for females which may have resulted in a tougher cuticle. On the other hand, females have a more elastic opisthosomal cuticle, needed for ovarial development and oviposition. The authors speculate that the cuticle of females might be thinner, or somewhat different in chemical composition.

Infection by entomogenous fungi results from penetration of the fungus through the cuticle of the host by a combination of enzymatic and mechanical processes. Successful infection by a fungus depends largely on the effectiveness of various antifungal substances present in the host cuticle. The authors claim that differential susceptibility of different stages and sexes of mites can be attributed partially to the presence or absence of antifungal substances. This finding is in contrast with observations by Elliot (1998) on an epizootic of *Neozygites tanajoae* in the cassava green mite *M. tanajoa* in Brazil. Far fewer capilliconidia of the fungus were found on field-collected juveniles than on adult females, while the vast majority of killed mycosed mites were adult females. Elliot (1998) assumed that the limited movement of immature stages makes them less likely to pick up capilliconidia from the leaf surface. For additional information on the role of *N. floridana* in natural populations of spider mites is referred to Van der Geest et al. (2000).

4.1.4. The Cassava Green Mite and Neozygites tanajoae

The unfortunate introduction of the cassava green mite (GCM), *M. tanajoa*, from Latin America into East Africa during the 1970s led to a comprehensive search for natural enemies of this pest. The mite dispersed within 10 years across the cassava belt in Africa, threatening cassava production in vast areas of the continent (Yaninek, 1988). It was already clear from the beginning that chemical control of the CGM would be no option. Cassava is a marginal crop in Africa and the farmers would not be able to afford the cost of chemical control. Moreover, chemical control would result in an unacceptable environmental pollution in large parts of the continent. It was clearly understood that classical biological control would be the most desirable solution for the problem. Several predatory mites of the family Phytoseiidae were collected in various locations in Latin America and introduced into Africa.

During the search for natural enemies, also a species of *Neozygites* was found, first in Venezuela (Agudela-Silva, 1986), later also in Brazil by Delalibera et al. (1992). The fungus has a very narrow host spectrum and does not infect other spider mites or predatory mites. The fungus, initially considered to be *N. floridana,* but later described by Delalibera et al., (2004) as *Neozygites tanajoae,* is considered a good candidate as biological control agent for the CGM in Africa, because of its high specificity (De Moraes & Delalibera, 1992). A drawback, however, is the fact that the fungus cannot successfully be grown on artificial media (Leite, Smith, De Moraes, & Roberts, 2000), which means that it should be released in the field in the form of fungus infected dead mites. A detailed study was therefore performed in order to gain information on the suitability of the fungus as biocontrol agent. In a series of experiments, Oduor, De Moraes, Yaninek, and Van der Geest (1995a), Oduor, Yaninek, Van der Geest, and De Moraes (1995b), Oduor (1995c), Oduor, De Moraes, Van der Geest, and Yaninek (1996a), Oduor, Yaninek, Van der Geest, and De Moraes (1996b), Oduor, De Moraes, Van der Geest, and Yaninek (1997a) and Oduor, Sabelis, Lingeman, De Moraes, and Yaninek (1997b) studied the biology of *N. tanajoae* and its effect on the CGM. They demonstrated that the production of primary conidia is affected by ambient humidity, temperature and photoperiod: production of conidia increased with higher temperatures between 13°C and 23°C, but no conidia production was obtained above 28°C. Also, germination of capilliconidia on cassava green mites is affected by exposure time, temperatures, ambient humidity and photoperiod.

Infectivity of capilliconidia is lost after several days: only 3.5% of the spores germinate after a storage period of 10 days. It was also found that germination occurred at all temperatures studied (13–33°C), even at 13°C, more than 15% of the capilliconidia germinated. This is an indication that the minimum temperature for germination may still be lower. An interesting observation is also that more capilliconidia germinate at 18°C than at higher temperatures. Germination in the dark is considerably higher than in light, while this process is also greatly affected by ambient humidity. The fungus apparently sporulates under natural conditions early in the morning before sunrise when the temperature is still low and the relative humidity high. Primary conidia can then disperse and produce capilliconidia that wait on the leaf surface to be picked up by a suitable host.

N. tanajoae cannot be mass produced in artificial culture media. Propagation needs to be performed in live mites, which is a laborious, and also expensive process. Storage of dead, non-sporulating infected mites (also called mummies) appears to be possible over prolonged periods of time in well-sealed plastic containers with cotton wool partially soaked in glycerol to establish a low humidity. In this manner, the fungus remains viable for periods of 6–7 months.

The fungus causes significant reductions in cassava green mite populations in cassava fields in the State of Bahia, Brazil (Delalibera, De Moraes, & Sosa Gomez, 1999), reaching infection levels up to 75% during the wet season. Elliot et al. (2000) conducted a 4-year study in cassava fields in a semi-arid region in northeastern Brazil. The authors found that the fungus had a significant effect on CGM populations, but results were not very consistent for the different years. The fungus was not found in live mites during dry periods and for that reason it was assumed that resting spores were the most feasible mechanism to survive these periods of drought. Resting spores were found in great numbers during an epizootic in 1995, but they were not found in epizootics in early and mid 1998. Elliot (1998) considered perennial survival in the field of the fungus in mummies highly improbable as experiments had demonstrated that no viable fungus could be detected in dead mites after 2 month of hot and dry storage, both in the field and in the laboratory. In a later report, Elliot, De Moraes and Mumford (2008) discussed results of an experiment in which they monitored a population of the CGM and its natural enemies in central Bahia. Despite the presence of very high levels of the predatory mite *Neoseiulus idaeus,* there was an overexploitation of the cassava plants by the cassava green mite, which led to a total leaf loss. A simple regression model predicted a potential epizootic by *N. tanajoae,* but at that time, no inoculum of the fungus was present. There could have been an epizootic after the CGM population crashed, but then there were no hosts present to infect. The study showed the ineffectiveness of the predator and the importance of a correct timing of the pathogen. The authors concluded that the fungus may be a useful addition to biological control in this system with another predator than *N. idaeus.*

Yaninek et al. (1996) carried out a survey of fungi occurring in mites in the Republic of Benin, West Africa and found *N. tanajoae* in CGM and in *Oligonychus gossypii.* The fungus was present during the entire year, although the incidence of the disease was considerably greater in the wet season. Infection levels reached by the Benin strain were never as high as those of the Brazilian isolates where infection levels close to 100% may sometimes be reached. However, disease incidences in *O. gossypii* were about twice as high as in CGM. This species of mite is indigenous to Africa, in contrast to the cassava green mite. The *N. tanajoae* strains native to Africa are thought to be weakly pathogenic towards the CGM as they cause only low levels of mortality in CGM populations.

Based on these studies, *N. tanajoae* pathotypes from Latin America were being considered as a biological control agent of the CGM in Africa. An international collaboration between the International Institute for Tropical Agriculture (IITA, Cotonou, Benin), Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA, Brazil) and the University of Amsterdam (The Netherlands) was set up in order to guide the introduction and release of the fungus into Africa. An in vivo release procedure has been developed at the (IITA) as it is not yet possible to culture the fungus in artificial culture media. Field experimental releases were performed in order to test the possibility to induce the development of epizootics in the field (Hountondji, 2005). The in vivo release method consisted of the release of 1-day old infected mites on young leaves of with CGM infested cassava fields Two different Brazilian strains were released in Southeastern Benin, resulting in disease incidence levels of 34 and 26.5% respectively, while the maximum prevalence for a Beninese isolate was only 4.5%. The fungus is in the first site endemically present, but had never been found in northeastern Benin. Post-release monitoring in Southeastern Benin showed after 10 months the presence of the fungus in 3 out of 20 cassava field where the fungus had been released. In Northeastern Benin, epizootics were also observed with infection levels between 15 and 70%. An interesting observation is that infection levels were consistently higher in fields inoculated with Brazilian isolates compared to those inoculated with the Beninese isolates.

The epizootics were especially observed during periods of hot days and cool nights with high relative humidity. The establishment of the Brazilian isolates at the release site could be confirmed. However, resting spores, believed to be essential for the survival of the fungus during dry periods were hardly found. Further studies will be needed to determine the suitability of the fungus as biocontrol agent of the CGM in Africa.

Hountondji (2008) discussed the interactions within the cassava green mite-*Neozygites tanajoae* system. He states that most fungal pathogens lack the capacity to search for their hosts but that they have rather developed a sit-and-wait strategy. Field applications of *N. tanajoae* against the CRM has resulted in only limited success and for that reason, studies were conducted on the tritrophic interactions in the system. An interesting finding is that herbivores trigger the release of volatile chemicals (HIPV) that promotes sporulation of the fungal pathogen, whereas the host mites avoid the haloes of conidia. However, the mite does not avoid the pathogen when the fungus is inside the mummified cadaver. Mechanically damaged leaves produce also volatile, but these “green leaf volatiles” seem to delay sporulation. Hountondji (2005) speculates that a delay in the formation of conidia until the HIPV’s are produced is to the advantage of the fungus. The production of HIPV’s is a signal for the fungus that herbivores (hosts for the fungus) are present.

4.2. Deuteromycetes

The Deuteromycetes, also mitosporic or anamorphic fungi, and formerly called the Fungi Imperfecti, is a group of fungi of which no sexual stage is known. It is difficult to place these fungi in the fungus classification system, since this classification is mainly based on the mode of sexual reproduction. The majority of Deuteromycetes are probably the asexual stages of Ascomycota and Basidiomycota, with the largest number belonging to the Ascomycota. The approximately 20,000 species (2,600 genera) of Deuteromycetes (or Deuteromycota) lack any common phylogenetic origin or relationship and have only been grouped together for the sake of convenience. Classification of Deuteromycetes is mainly based on morphological similarities. The criteria typically used are color, shape, size and septation of the conidia (whether the spores are unicellular, or made up of multiple cells). The effect of this classification is that unrelated fungi have been “lumped” together while possibly related fungi have been classified into different form-classes because of differences in the visual appearance of the spores. When the sexual stage (teleomorph) is discovered, the correct name of the fungus will be that of the teleomorph.

A group in the Deuteromycetes is the form-class Hyphomycetes. These fungi have a mycelium but lack a sporocarp (structure in which spores are formed). The spores are borne on separate or aggregated conidiophores (stroma). We speak of a synnema when the conidiophores are united at the base and of a sporodochium when the hyphae are aggregated to a cushion-line structure on which the conidiophores are formed. Many of the spores of the Deuteromycetes have morphologically distinct features so that the spores of this group are some of the most easily identified. These fungi are also easily cultured so that more research has been conducted on this group of fungi than on many others.

4.2.1. Hirsutella *Infections in Mites*

A well-known genus in the Hyphomycetes is *Hirsutella.* Approximately 80 species have been described in this genus; most species are pathogenic to tropical invertebrates, some are pathogens of Acari, in particular of eriophyids. Teleomorphs of *Hirsutella* have been reported as *Cordyceps* and *Torrubiella* spp. (*cf.* Chandler et al., 2000). *Hirsutella* species observed in mites are given in Table 3.

The first published report of a *Hirsutella* infection in an eriophyid goes back to 1924: Speare and Yothers (1924) observed a sudden decimation of a large population of the citrus red mite (CRM) (*Phyllocoptruta oleivora*) on grapefruit in Florida. The population density of CRM was in June sometimes extremely high with 5,000 mites on a single grapefruit, but soon after the maximum density had been reached, populations went down to almost zero. The authors were able to discern fungal hyphae in mite cadavers and also noted that disease incidence was much lower after application of a fungicidal copper spray. Fisher (1950) described this fungus later as *Hirsutella thompsonii* (Fig. 3).

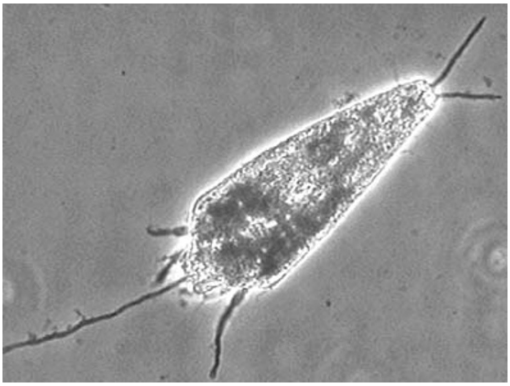

Figure 3. Epitremerus goniathrix *(Eriophyidae), infected by* Hirsutella *sp. Photograph: Leo P.S. van der Geest.*

The conidia are the infective propagules of the fungus. They are formed on phialides (flask-like conidiophores) that taper into a long narrow neck, and produce usually only 1–3 conidia in a dense terminal sphere of slime. This gives the impression that only one spore on each phialide is produced (Fig. 4). The mucous coat facilitates adhesion of the spores to the host cuticle. Penetration of the fungus is usually through all parts of the host's body, but in case of spider mites, it is usually through the legs. The fungi enter the host through the cuticle which is composed of about 30% chitin embedded in a protein matrix. Entomogenous fungi often produce proteases that may play a role in the early stages of penetration by exposing the chitin fibrils. Subsequently, chitinolytic enzymes are excreted that causes degradation of the fibrils into chains of glucose-N-acetyl of variable length.

Table 3. Hirsutella *species infecting mites and their hosts.*

Fungus species	*Mite species*	*Mite family*	*Reference*
Hirsutella sp.	*Amrineus cocofolius* *Epitremerus goniathrix*	Eriophyoidea	Van der Geest et al. (2002)
	Mononychellus sp.	Tetranychidae	Bartkowski et al. (1988)
	Notostrix attenuata	Eriophyoidea	Van der Geest et al. (2002)
	Pronematus sp.	Tydeidae	Cabrera and McCoy (1984)
	Propilus syagris *Retracus jonhstoni*	Eriophyoidea	Van der Geest et al. (2002)
	Tarsonemus sp.	Tarsonemidae	Van der Geest et al. (2002)
H. brownorum	Mites in soil		Humber (1992)
H. gregis	*Abacarus hystrix*	Eriophyoidea	Minter, Brady, and Hall (1983)
	Unidentified sp.	Acaridae	Miętkiewski, Bałazy, and Tkaczuk (2000)
H. danubiensis	*Tetranychus urticae*	Tetranychidae	Bałazy et al. (2008)
H. haptospora	*Uropodina* sp.	Uropodoidea	Humber (1992)
	Urobovella sp.	Uropodoidea	Miętkiewski et al. (2000)
	Unidentified species	Parasitiformes	Miętkiewski et al. (2000)
H. kirchneri	*Abacarus hystrix*	Eriophyoidea	Minter et al. (1983)
	Eutetranychus orientalis	Tetranychidae	Sztejnberg, Doron-Shloush, and Gerson (1997)
	Hemisarcoptes coccophagus	Hemisarcoptidae	Sztejnberg et al. (1997)
	Panonychus citri	Tetranychidae	Sztejnberg et al. (1997)
	Phyllocoptruta oleivora	Eriophyoidea	Cabrera and Dominguez (1987a) and Sztejnberg et al. (1997)
H. necatrix	*Abacarus hystrix*	Eriophyoidea	Minter et al. (1983)
	Tetranychus cinnabarinus	Tetranychidae	Sztejnberg et al. (1997)

Table 3 continued

	Dendrolaelaps cornutus	Digamasellidae	Miętkiewski et al. (2000)
H. nodulosa	*Aceria guerreronis*	Eriophyoidea	Cabrera and Dominguez (1987b)
	Phyllocoptruta oleivora	Eriophyoidea	Cabrera and Dominguez (1987a)
	Polyphagotarsonemus latus	Tarsonemidae	Peña, Osborne, and Duncan (1996)
	Steneotarsonemus fragariae = *Phytonemus pallidus*	Tarsonemidae	Miętkiewski et al. (2000)
H. rostrata	*Dendrolaelaps tetraspinosus*	Digamasellidae	Bałazy and Wiśniewski (1989)
	Proctolaelaps sp.	Ascidae	Bałazy and Wiśniewski (1989)
H. thompsonii	*Abacarus hystrix*	Eriophyoidea	Lewis, Heard, Brady, and Minter (1981)
	Acalitus vaccinii	Eriophyoidea	Baker and Neunzig (1968)
	Aceria cynodoniensis	Eriophyoidea	McCoy (1996)
	Aceria guerreronis	Eriophyoidea	Humber (1992)
	Aceria sheldonii	Eriophyoidea	McCoy (1996) and Sosa Gomez and Moscardi (1991)
	Aceria sp.	Eriophyoidea	McCoy and Selhime (1977)
	Aculops lycopersici	Eriophyoidea	In: Chandler et al. (2000)
	Calacarus heveae	Eriophyoidea	Tanzini, Alves, Tamai, De Moraes, and Ferla (2000)
	Colomerus novahebridensis	Eriophyoidea	Hall, Hussey, and Mariau (1980)
	Epitremerus goniathrix	Eriophyoidea	Van der Geest et al. (2002)
	Notostrix formosae	Eriophyoidea	Van der Geest et al. (2002)
	Phyllocoptruta oleivora	Eriophyoidea	Fisher (1950)
	Retracus elaeis	Eriophyoidea	Urueta (1980)
	Rhynacus sp.	Eriophyoidea	Cabrera, Caceras, and Dominguez (1987)
	Vasates destructor	Eriophyoidea	McCoy (1996)

Table 3 Continued

	Polyphagotarsonemus latus	Tarsonemidae	Peña et al. (1996)
	Vasates mckenzie	Tarsonemidae	Miętkiewski et al. (2000)
	Dolichotetranychus floridanus	Tenuipalpidae	Humber (1992)
	Eutetranychus banksi	Tetranychidae	McCoy and Selhime (1977)
	Eotetranychus sexmaculatus	Tetranychidae	McCoy and Selhime (1977)
	Eutetranychus orientalis	Tetranychidae	Gerson, Kenneth, and Muttath (1979)
	Mononychellus tanajoa	Tetranychidae	Yaninek et al. (1996)
	Panonychus citri	Tetranychidae	McCoy and Selhime (1977)
	Oligonychus gossypii	Tetranychidae	Yaninek et al. (1996)
	Tetranychus cinnabarinus	Tetranychidae	Cehrnin, Gafni, Mozes-Koch, Gerson, and Sztejnberg (1997)
	Oligonychus ilicis	Tetranychidae	Gardner, Oetting, and Storey (1982)
	Tetranychus urticae	Tetranychidae	Gardner et al. (1982)
	Tetranychus turkestani	Tetranychidae	In: Chandler et al. (2000)
	Trachyuropoda coccinea	Trachyuropodidae	Bałazy and Wiśniewski (1982)
H. tydeicola	*Lorryia formosa*	Tydeidae	Cabrera, see: Samson and McCoy (1982)
	Tydeus californicus	Tydeidae	Cabrera, see: Samson and McCoy (1982)
	Tydeus gloveri	Tydeidae	Samson and McCoy (1982)
H. vandergeesti	*Amblyseius* sp.	Phytoseiidae	Bałazy et al. (2008)
	Neoseiulus sp.		
	Seiulus sp.		
	Typhlodromus sp.		
	Tarsonemus lacustris	Tarsonemidae	

When grown in artificial cultures, a toxic protein is produced with a molecular weight of 15–16 kDa (Vey, Quiot, Mazet, & McCoy, 1993). This protein, hirsutellin A (HtA) is a heat stable, non-glycosylated protein with properties similar to ribosome-inhibiting proteins. It is a single chain polypeptide that is composed of 130 amino acids (Herrero-Galán et al., 2008). HtA shows ribotoxin activity and interacts with phospholipid membranes. When administered orally or by injection, it causes strong cytotoxic effects against several insect species (pycnosis of the nucleus and lesions in the midgut, malpighian tubules, hypodermis, fatbody, hemocytes, muscles and silk glands of larvae). The toxin affects a wide range of arthropods, including mites. It was tested against the citrus rust mite *Phyllocoptruta oleivora*, the natural host of the fungus, where it caused considerable mortality among adult mites, while oviposition of females was lowered prior to their death (Omoto & McCoy, 1998). Maimala, Tarter, Bouciar, and Chandrapatya (2002) studied 162 strains of *H. thompsonii* and found that more than half of these strains produced the toxin. They were able to characterize the gene encoding for the toxin, but the presence of the gene could not be associated with enhanced insecticidal properties of the fungus. Other toxins have also been reported to be produced by *Hirsutella* sp., such as hirsutellin B and phomalactone. It is not within the scope of this manuscript to discuss these products further.

H. thompsonii regularly causes epizootics in populations of *P. oleivora* under natural conditions in Florida and affects both nymph and adult stages. The asexual spores are produced outside the host body on the plant surface. Conidia germinate under favorable conditions (high relative humidity) and enter the body by means of a germ tube. After penetration, a ramifying growth of the fungus can be discerned. The fungus erupts after

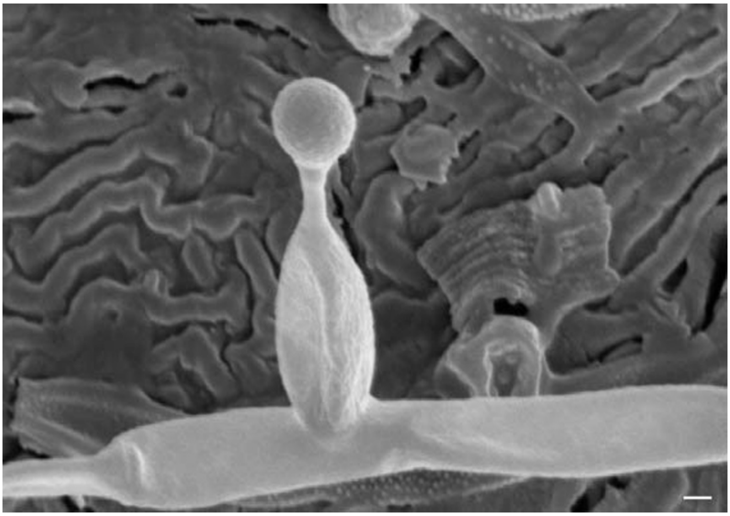

Figure 4. Phialid of Hirsutella thompsonii *with conidia. The globular structure on top of the phialid may hold several conidia which are contained in a slimy sheath. Photograph courtesy of Dr. Marcel R. Tanzini.*

death of the mite through the cuticle and forms new spores. It takes about 4 h for a spore to penetrate the cuticle of the host, while time from infection to formation of new spores is around 4 days at 25–30°C (Fig. 4).

The fungus can easily be grown on artificial media and this property together with its high infective potential towards the citrus rust mite (CRM) has led to research aimed at the development of the fungus as biological control agent. Large-scale laboratory and industrial production methods have been developed for the production of mycelial and conidial preparations (McCoy, Hills, & Kanavel, 1975; McCoy, 1981). It was planned to introduce the fungus early in the season as a prophylactic to reduce outbreaks of the CRM. Commercial production of conidial preparation in the USA was developed in 1975–1976 by Abbott Laboratories and full registration was received for a mycoacaricide for the control of eriophyids on citrus under the name Mycar^{TM}. Several hundreds of kilograms were sold of the product but commercial production was discontinued in 1985 as too many factors affected the stability and reliability of the acaricide.

Interest in the production of *H. thompsonii* based acaricides for use against eriophyids continued in other countries even after the production of Mycar^{TM} in the USA was discontinued. Experiments have been conducted in several countries, e.g. Brazil, Argentina, Mexico and Sri Lanka, but these studies have yet not led to a commercial biological control product. More successful were studies in India where the coconut mite, *Aceria (Eriophyes) guerreronis,* is a major constraint to coconut farming in India, the third largest producer of coconuts in the world. Biological control has been considered for a long time, despite the availability of other control methods, including chemical and botanical pesticides and nutrition-based cultural methods (Sreerama Kumar & Singh, 2001; Sreerama Kumar, 2006). Research has resulted in the identification of the most infective mite pathogen, *H. thompsonii*, as important regulator of the coconut mite (Fig. 5). Further research had led to the production of a biological acaricide, Mycohit^{TM}, with a mixture of mycelium

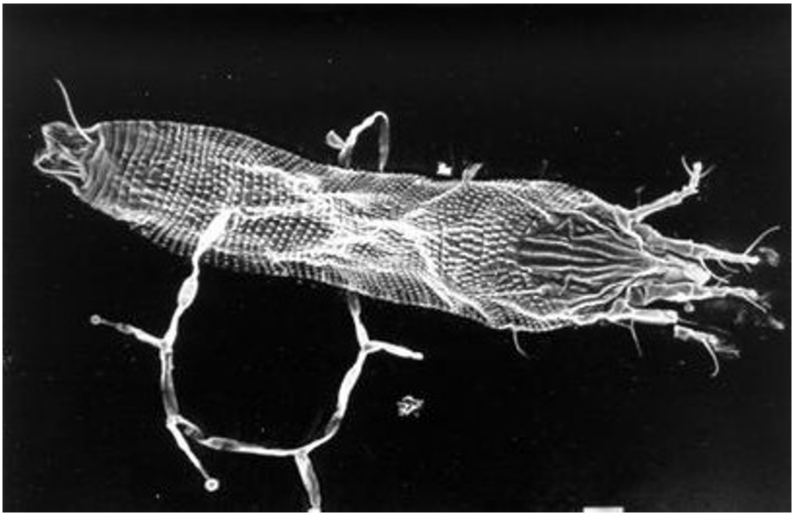

Figure 5. Aceria (Eriophyes) guerreronis *infected by* Hirsutella thompsonii. *Photograph courtesy of Dr. P. Sreerama Kumar.*

and conidia as active ingredients. In a series of field experiments, it was shown that Mycohit^{TM} is a very effective acaricide that brings down the population to over 90%. For the application of the product, youngsters are hired that climb into the trees in order to carry out the applications (Figs. 6 and 7).

Further research has led to the development of Mycohit-M^{TM}, a preparation that contains only mycelium (Sreerama Kumar & Singh, 2008). The authors also studied the effect of several adjuvants (glycerol, yeast extract powder and dehydrated malt extract) on the growth and spore forming of the latter preparation. In the presence of these adjuvants *H. thompsonii* biomass produced under laboratory conditions more colonies and a higher number of conidia. These products also showed protection against sun irradiation under field conditions. Studies are presently also conducted in Brazil on the control of the coconut mite by use of *H. thompsonii* (cf. Van der Geest et. al., 2000). Various isolates of the fungus were introduced from Mexico into Brazil and were mass produced on rice and corn grids, but the mortality due to the fungus was too low (about 35%). However, it was possible to increase the virulence of the fungus by passage through the host. The fungus has been established in the field, although damage due to *A. guerreronis* is still too high.

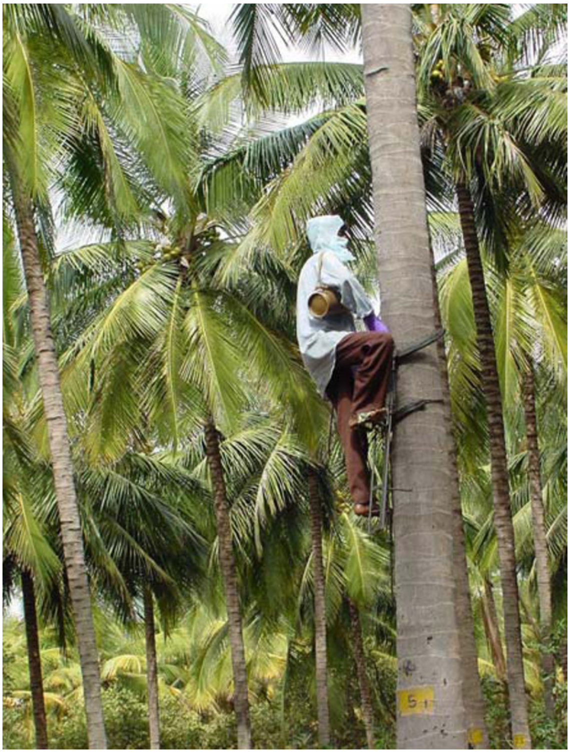

Figure 6. Spraying of coconut trees with Mycohit. Photograph courtesy of Dr. P. Sreerama Kumar.

Several attempts have been made to use the fungus also for the control of other mite pests. However, most of these attempts were unsuccessful, as e.g. the control of spider mites (*T. cinnabarinus*, *Eotetranychus orientalis* and *T. urticae* in greenhouses (Rombach & Gillespie, 1988). *Hirsutella kirchneri* has also been considered a good candidate for the control of plant-inhabiting mites. Its pathogenicity was tested by Sztejnberg et al. (1997) against a number of different mites. It was shown to be effective against the eriophyid *P. oleivora*, and against the spider mites *Eutetranychus orientalis*, *Panonychus citri*, *T. cinnabarinus* and in some degree against *Hemisarcoptes coccophagus* (Hemisarcoptidae). However, no infectivity was found towards the broad mite *Polyphagotarsonemus latus* (Tarsonemidae), *Rhizoglyphus robini*, *Tyrophagus putrescentiae* (Acaridae) and *Typhlodromus athiasae* (Phytoseiidae). No infectivity was noted towards a number of beneficial arthropods.

Figure 7. Spraying of coconut trees with Mycohit. Photograph courtesy of Dr. P. Sreerama Kumar.

A few reports deal with the occurrence of the *H. thompsonii* on the cassava green mite *M. tanajoa* in the cassava belt in Africa. Yaninek et al. (1996) observed the fungus on CGM and on *Oligonychus gossypii* during an inventory in Benin, and Odongo, Odindo, Brownbridge, and Kumar (1998) carried out experiments in which CGM infected plants were treated with suspensions of *H. thompsonii.* In the treated plots, 76.6% disease incidence was obtained, but rainfall caused a reduction in disease prevalence in the mites. The authors concluded that the application of the fungus has promise for controlling the CGM, but that the fungus should be applied in harmony with other natural mortality factors such as rainfall.

The fungus has also been recorded in *Calacarus heveae* (Eriophyidae) an important pest in rubber plantations in Brazil (Tanzini et al., 2000). This mite is a major pest in the Michelin rubber plantation in Itiquira, Mato Grosso, where it causes considerable economic damage. *Calacarus heveae* feeds on the upper side of the leaves, in contract to most other rust mites (Figs. 8 and 9). Populations of several hundreds of mites per rubber tree leave are no exception. However, the mite populations are usually decimated after a few weeks by the action of *Hirsutella thompsonii* (Figs. 9 and 10). These epidemics are usually too late to avoid damage to the plantation (defoliation of the trees).

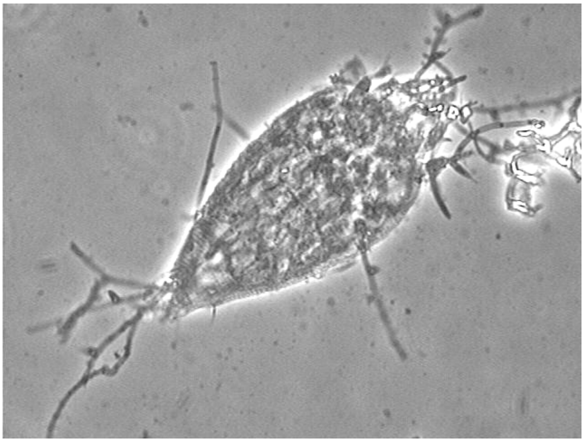

Figure 8. Calacarus heveae*, killed by* Hirsutella thompsonii. *Photograph: Leo P.S. van der Geest.*

Hirsutella thompsonii is normally associated with prostigmatic mites (Eriophyidae and Tetranychidae), although it has also been observed to infect mesostigmatic mites (McCoy & Selhime, 1977; Bałazy & Wiśniewski, 1982).

Gerson, Gafni, Paz, and Sztejnberg (2008) tested *Hirsutella thompsonii, H. kirchneri* and *H. necatrix* with respect to their effect on several mite species: spider mites, rust mites, broad mites, but also scavengers and predatory mites were included in the study. The results of these experiments led to the conclusion that none of these fungus species gave satisfactory control of mite pests, neither in greenhouses, nor outdoors.

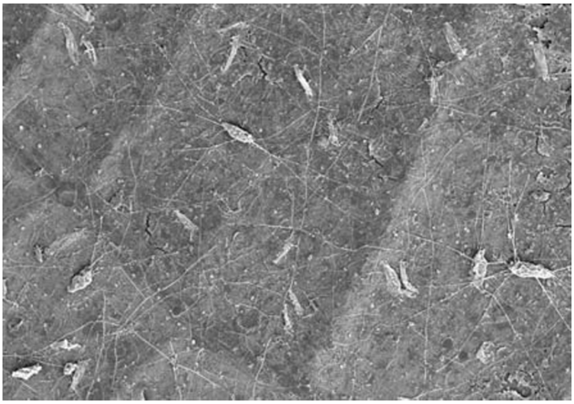

Figure 9. Rubber tree leaves with numerous cadavers of Calacarus heveae. *The mites have been killed by the fungus* Hirsutella thompsonii. *However, the disease cannot prevent substantial economic damage to the rubber plantations in Itiquira, MG, Brazil, as the epidemics come too late in the season. Photograph courtesy of Dr. Marcel R. Tanzini.*

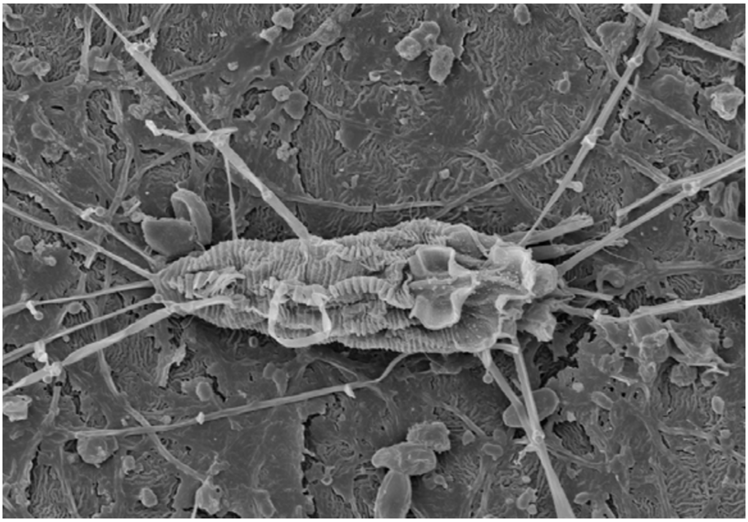

Figure 10. Calacarus heveae *(Eriophyidae), killed by* Hirsutella thompsonii. *Photograph courtesy of Dr. Marcel R. Tanzini.*

4.2.2. Other Deuteromycetes Infecting Mites

Some of the other Deuteromycetes are probably the most comprehensively studied entomopathogenic fungi. However, of these fungi, only few records have been made of natural infections in mites. Leatherdale (1965) observed a mycosis of the blackcurrant mite *Cecidophyopsis ribis* by *Paecilomyces eriophytis,* later also found in Italy on *Phytoptus avellanae,* a mite causing big bud disease in ornamental and fruiting hazel (*Corylus avellana*). Experiments were carried out with the fungus isolate from *C. ribis* to infect the European red mite *Panonychus ulmi* and the eriophyids *Aceria hippocastani* (from horse chestnut) and *Cecidophyes galii* (from goosegrass). The fungus proved to be pathogenic for the first two species, but no infection was obtained in *C. galii.* The fungus has also been reported by Baker and Neunzig (1968): high infection rates were noted in the blueberry bud mite *Aceria vaccinii* (Eriophyidae) in North Carolina, USA, when high temperatures coincided with heavy rainfall and high relative humidity. Disease incidence declined from August onwards, when conditions were less favorable for the fungus. The population density of the blueberry mites then increased.

One of the earliest experiments in which a fungus was tested against a phytophagous mite was a field application of *B. bassiana* spores for the control of the twospotted spider mite *T. urticae* (Dresner, 1949). Mites were treated with a dust containing 0.5% spores of the fungus, resulting in a mortality of 71%. However, these experiments did not lead to the development of a microbial acaricide.

Deuteromycetes have widely been studied for the control of insect (and in a few instances mite) pests. A number of these fungi (e.g. *Metarhizium*, *Beauveria*) have a broad host spectrum and can easily be mass produced on relatively simple culture media. In Brazil, research is being conducted to evaluate several Deuteromycetes as possible control agent of the twospotted spider mite (Tamai, Alves, Lopes & Neves 1998). They tested 152 different isolates for the fungi *B. bassiana*, *B. brongniartii*, *Beauveria* sp., *Metarhizium* sp., *Paecilomyces lilacinus* and *P. farinosus.* Only isolates of *Beauveria* spp. caused mortality between 35 and 95%. The pathogenicity of some isolates was further tested: one isolate gave even better control than obtained with chemical pesticides. The fungus was also effective against other pests in chrysanthemum, such as thrips and aphids (Alves, Tamai, & Lopes, 1998).

Three different fungus species were investigated by Peña et al. (1996) with respect to their potential as biological control agent of the broad mite *Polyphagotarsonemus latus* (Tarsonemidae). This very polyphagous pest species thrives under warm and humid conditions, and this seemed to be a good reason to study the feasibility of using fungi as control agents. Three fungi were tested: *B. bassiana*, *H. thompsonii* and *Paecilomyces fumosoroseus* under controlled temperature and humidity conditions in the laboratory and in the greenhouse. All fungi were capable to infect the mites: higher doses resulted in a faster death of the mites, while density of the mites also affected disease incidence. The authors concluded from their experiments that the fungus selected should cause epizootics within 2–3 days following application. Promising results for the control of the broad mite on mulberry have been obtained with *M. anisopliae* by Maketon, Orosz-Coghlan, and Sinprasert (2008). The fungus is effective against larvae and adults, but no ovicidal effect was noted. The broad mite has also been found in association with *Hirsutella nodulosa* (Peña et al., 1996). No other associations of this mite with fungi have been reported.

Shi, Feng, and Liu (2008) noted an ovicidal effect of sprays of an emulsifiable *B. bassiana* formulation against the twospotted spider mite *T. urticae.* The fungal isolate was obtained from mycosed aphids and has been formulated for the control of whiteflies in greenhouses. The dried conidial powder was suspended in a mixture of 95% industrial paraffin as oil carrier and 5% fatty alcohol polyethylene glycol ether as emulsifier. The authors are of the opinion that the formulation has greatly enhanced egg mortality, also at lower relative humidities. Oily formulations may give a better attachment of the conidia to the target pest and a better protection of the conidia against desiccation. The formulation has also provided significant control of the citrus rust mite in orchards in East China and of *T. truncates* and *T. turkestani* in cotton in the Tarim Basin of northwest China.

Isolates of fungi may show large differences in virulence to their hosts. For example, Bugeme, Maniania, Knapp, and Boga (2008) studied 23 isolates of *Metarhizium anisopliae* and 3 isolates of *B. bassiana* with respect to their pathogenicity versus the spider mite *T. evansi.* Temperature greatly affected observed mortality of the spider mites, but also germination of the spores and growth were affected and varied with isolate.

The fungi *M. anisopliae* and *H. thompsonii* are being investigated as control agents of *Varroa jacobsoni* (Kanga, James, & Boucias, 2002). They are pathogenic for the varroa mite and the conditions under which they can infect mites are similar to those found in bee colonies. Meikle, Mercadier, Holst, and Girod (2008) studied the impact of formulations of *B. bassiana* on varroa mites and on the honey bees. They formulated conidial preparations of two strains of the fungus with either carnauba or candelilla wax powder and studied the effect on colony health and mite fall (mortality). Bees were nof affected by the fungal preparations, but mite fall was consistently higher than the control. Results were encouraging, but more knowledge should be acquired on conidia dosage, number of applications and conditions within the bee hives.

Eken and Hayat (2008) conducted an inventory for natural enemies of the twospotted spider mite *Tetranychus urticae* in Turkey. They found several isolates of *Cladosporium cladosporioides* (Moniliaceae), that were subsequently tested in the laboratory with respect to their pathogenicity towards *T. urticae*. The fungus caused mortality levels of 51–75% when sprayed on leaflets infested with twospotted spider mites. These results are encouraging and additional experiments should be conducted. *Cladosporium* infections were also found by Van der Geest et al. (2002) in *Retracus johnstoni,* an eriophyid feeding on the palm tree *Syagrus romanzoffiana* in Brazil. The fungus has been isolated on potato-dextrose agar, but no further experiments have been carried out with this isolate.

Interesting experiments were performed by Sanassi and Amirthavalli (1970) with the velvet mite *Trombidium gigas* (Trombidiidae). Mite were infected with spores of the fungus *Aspergillus flavus*, a fungus also capable of infecting humans and problematic in foods as it produces the very toxic aflatoxin. Three different methods were applied: injection of spore suspensions into the body cavity, spraying of spore's suspensions on the integument of the mite and dusting of spores on the integument. In all cases infection of the mites was obtained. Changes in the structure of the integument were noted (Sanassi & Oliver, 1971). The first sign of the disease is the loss of the scarlet-red plumose cuticular setae of the mites. Each seta consists of a main central stem from where minor secondary branches arise. The base of the central stem is normally slightly enlarged and is buried in sockets present in the epi- and procuticle.

The fungus apparently digests the lipoproteinaceous epicuticular layer that attaches the base to the cuticular sockets. Subsequently, the process is followed by additional chemical changes in the composition of the cuticle. Epidermal cells display interesting pathological symptoms. In uninfected mites, epidermal cells form a syncytium, but after infection a remarkable reaction occur in these cells. The cells line up in almost a single row just below the outer limiting border of the epidermis. Vacuoles appear in the cytoplasm of the epidermal cells, while organelles such as mitochondria and Golgi apparatus are not anymore discernable.

In a search for control agents of the citrus red mites (CRM) in Israel, field collected mite cadavers were examined for the presence of pathogens. This has resulted in the finding of three species of fungi that were found to be associated with mites. The fungi were described by Boekhout et al. (2003) as novel species belonging to the Ustilagomycetes, a class that is also called the smut fungi. It is a large taxon with over 1,400 species in 70 genera that are almost exclusively plant pathogens. The three fungi are anamorphic (they have no sexual state) and belong therefore to the Deuterymycetes. However, morphologically they are similar to yeast-like fungi which have been classified in the Ustilaginales. Based on molecular properties, they should be considered to belong to two different lineages within the Exobasidiomycetidae of the Ustilaginomycetes (Basidiomycota). These fungi, described as *Meira geulakonigii*, *M. argovae* and *Acaromyces ingoldii*, are hard to isolate from field-collected material: they are slow-growing fungi and for their identification physiological and molecular methods are needed. This probably explains the fact that they have only recently been discovered.

The three fungi were further studied with respect to their potential as biological control agents of phytophagous mites. Laboratory investigations showed that all three fungi affected mites, although *M. argovae* showed no effect towards *T. urticae.* (Gerson et al., 2008). *Meira geulakonigii* caused considerable mortality of spider mites and citrus red mite. An interesting observation was that none of the fungi invaded the mites, although the fungi did grow on the mite's cadavers. It is assumed that mortality among the mites was caused by the action of fungal toxins.

Further tests showed that the fungus was endophytically present within the sealed grapefruit flowers and in the flavedo (the tough outer skin) of grapefruit. There was no evidence that the fungus caused any damage to the plants, which led the authors to the assumption that *M. geulakonigii* serves as a "body guard" of grapefruits. Also, *M. geulakonigii* had only minimal fungicidal effect on some predatory mites. This property, together with its tolerance to many insecticides and acaricides, suggests that this fungus has possibilities for integrated control programs.

4.3. Ascomycota

The Ascomycota is a large taxon of fungi with approximately 2,000 genera and over 30,000 species. Members of the Ascomycota bear the sexual spores within an ascus, originally a cell that at first contains a diploid nucleus resulting from karyogamy (the fusion of nuclei or nuclear material that occurs during sexual reproduction). The nucleus undergoes subsequently meiosis, resulting in the formation of haploid ascospores inside the asci. These asci are often borne in or on top of a sporocarp. Very few Ascomycota have been isolated from mites. These isolations concern mainly Laboulbeniales infections in a number of mite species. Laboulbeniales is an order of fungi with more than 2,000 species. They are small, often minute fungi that have an obligate association with arthropods, mainly insects, and they lack mycelium. On their host, they appear as scattered or densely crowded bristles or bushy hairs which may form furry or velvety patches on certain part of the host' integument. These plume-like structures and triggers help in ascospore release when the arthropod comes into contact with a mature thallus.

The site of attachment is usually limited to definite regions on the integument of each host. The entire thallus (body) is derived from enlargement and subsequent cell division of the two-celled ascospore. Below the surface of the arthropod cuticle absorption through a peg- or root-like haustorium provides a nutrition source for the fungus; however, the fungal parasites of this group do not appear to cause much damage to the host. A few species have been found on mites, including a *Rickia* sp. and an unknown species on *Hirstionyssus* (Demanyssidae) (Steinhaus & Marsh, 1962).

5. DISEASES CAUSED BY EUKARYOTIC MICROPARASITES

Traditionally, the animal kingdom has been divided into two subkingdoms: the Protozoa (unicellular eukaryotes) and the Metazoa (multicellular animals). In modern taxonomy, a kingdom Protista is considered of which the members are either unicellular, colonial or multicellualar. All these organisms are eukaryotes,

The Protista are divided into three major groups: the Protozoa, the Algae and the Fungus-like protists. Protozoa are defined as single-celled eukaryotic organisms, that feed heterotrophically (feeding both on both organic and inorganic raw materials) and exhibit diverse motility mechanisms. Traditionally, four divisions are discerned, based on their mode of mobility:

- Flagellata (Mastigophora) are protozoa that move by means of flagellar action. Some flagellates have their flagella attached in a structure called an undulating membrane. They often have symbiotic relationships with multicellular organisms.
- Rhizopoda (Amoebozoa) Rhizopoda are protozoa that move by employing pseudopodia, which are covered by membranes. These pseudopodia are cytoplasmic extensions that are not only used for locomotion but also to engulf food. Amoebae live in moist terrestrial and aquatic environment.
- Apicomplexa (or Sporozoa). The Sporozoa are parasitic spore formers that do not move by their own power. *Plasmodium vivax*, the cause of malaria, is a sporozoan.
- Ciliata (Ciliophora) Ciliata are protozoa that move by means of cilia action. Cilia are small and numerous; flagella are large and few. Because of the tremendous variety in ciliary arrangements and functions, ciliates are among the most diverse cells in the biological world.

Table 4. Protozoan infections in mites.

Species	*Mite host*	*Mite family*	*References*
Apicomplexa			
Acarogregarina corolla	*Scutovertex minutus*	Scutoverticidae	Erhardová (1955)
Asterophora caloglyphi	*Caloglyphus moniezi*	Acaridae	Geus (1969)
Erhardovina bisphaera	*Damaeus clavipes*	Damaeidae	Purrini and Ormieres (1981)
	Damaeus onustus	Damaeidae	Purrini and Ormieres (1981)
	Eupelops hirtus	Eupelopidae	Purrini and Ormieres (1981)
E. carabodesi	*Carabodes coriaceus*	Carabodidae	Purrini and Ormieres (1981)
E. euzeti	*Euzetes seminulum*	Euzetidae	Lipa (1982)
E. fuscozetesi	*Fuscozetes setosus*	Ceratozetidae	Purrini, Bukva, and Bäumler (1979)
E. fuscozetesi	*Euzetes globulus*	Euzetidae	Purrini and Ormieres (1981)
E. oribatarum	Mite		see Lipa (1971)
E. phtiracari	*Phthiracarus globosus*	Phthiracaroidea	Purrini and Ormieres (1981)
	Phthiracarus piger	Phthiracaroidea	Purrini and Ormieres (1981)
E. platynothri	*Platynothrus peltifer*	Nothroidea	Purrini and Ormieres (1981)
E. postneri	*Hermannia gibba*	Hermannioidea	Purrini et al. (1979)
E. scutovertexi	*Scutovertex minutus*	Scutoverticidae	Erhardová (1955)
Erhardovina sp.	*Limnochares aquatica*	Hydrachnellae	Issi and Lipa (1968)
Gregarina sp.	*Eupelops subuliger*	Eupelopidae	Purrini et al. (1979)
	Eupelops torulosus	Eupelopidae	Purrini et al. (1979)
	Rhysotritia ardua	Phthiracaroidea	Purrini et al. (1979)
Erhardovina sp.	*Damaeus geniculatus*	Damaeidae	see Lipa (1971)

Table 4 Continued

Erhardovina sp.	*Damaeus oblongus*	Damaeidae	see Lipa (1971)
Gurleya sokolovii	*Limnochares aquatica*	Hydrachnellae	Issi and Lipa (1968)
Unidentified gregarine	*Tyrophagus putrescentiae*	Acaridae	Steiner (1993)
Adelina acarinae	*Nothrus silvestris*	Nothroidea	Purrini (1984)
Microspora			
Intexta acarivora	*Tyrophagus putrescentiae*	Tyroglyphidae	Larsson, Steiner, and Bjørnson, (1997)
Microsporidium phytoseiuli	*Phytoseiulus persimilis*	Phytoseiidae	Bjørnson, Steiner, and Keddie (1996)
Microsporidium sp.	*Amblyseius barkeri*	Phytoseiidae	Beerling and Van der Geest (1991a, 1991b)
Neoseiulus cucumeris		Phytoseiidae	Beerling and Van der Geest (1991a; 1991b)
Napamichum aequifolium	*Limnochares aquatica*	Hydrachnellae	Larsson (1990)
Nosema acari	*Damaeus onustus*	Damaeidae	Purrini and Weiser (1981)
	Damaeus clavipes	Damaeidae	Purrini and Weiser (1981)
N. euzeti	*Euzetes seminulum*	Euzetidae	Lipa (1982)
N. führeri	*Phthiracarus globosus*	Phthiracaroidea	Purrini & Weiser (1981)
N. helminthorum	*Ceratoppia* sp.	Liacaroidea	Moniez (1887)
	Ceratoppia bipilis	Liacaroidea	Dissanaike (1958)
	Xenillus tegeocranus	Liacaroidea	Dissanaike (1958)
N. hermanniae	*Hermannia gibba*	Hermannioidea	Purrini et al. (1979)
N. ptyctimae	*Rhysotritia ardua*	Phthiracaroidea	Purrini and Bäumler (1976)
N. sperchoni	*Sperchon* sp.	Sperchontidae	Lipa (1962)
N. steganacari	*Steganacarus striculus*	Phthiracaroidea	Purrini and Weiser (1981)

Table 4 Continued

N. steinhausi	*Tyrophagus putrescentiae*	Tyroglyphidae	Weiser (1956)
Oligosporidium occidentalis	*Metaseiulus occidentalis*	*Phytoseiidae*	Becnel, Jeyaprakash, Hoy, and Shapiro (2002)
Pleistophora cephei	*Cepheus dentatus*	Cepheoidea	Purrini and Weiser (1981)
P. dindali	*Carabodes coriaceus*	Carabodidae	Purrini and Weiser (1981)
P. platynothri	*Platynothrus peltifer*	Nothroidea	Purrini and Weiser (1981)
P. oribatei	*Carabodes femoralis*	Carabodidae	Purrini and Weiser (1981)
	Damaeus clavipes	Damaeidae	Purrini and Weiser (1981)
	Microtritia minima	Euphthiracaroidea	Purrini and Weiser (1981)
	Phthiracarus piger	Phthiracaroidea	Purrini and Weiser (1981)
	Phthiracarus sp.	Phthiracaroidea	Purrini and Weiser (1981)
	Physotritia duplicata	Phthiracaroidea	Purrini and Weiser (1981)
Thelohania microtritiae	*Microtritia minima*	Euphthiracaroidea	Purrini and Weiser (1981)

The above classification is still being used, but we know now on the basis of ribosomal RNA gene sequencing that various groups in the Flagellata and Rhizopoda are not closely related. There may be greater genetic difference between two groups of Flagellata than between flowering plants and vertebrates. In modern classification, the following seven phyla are distinguished: Apicomplexa, Sarcomastigophora, Microspora, Ciliophora, Acetosphora, Myxospora and Labyrinthomorpha. It is not within the scope of this chapter to go into details about this classification.

Two phyla of the subkingdom Protozoa, the Apicomplexa and the Microspora, have members that are found in arthropods. Some of these are also pathogens of vertebrates, while others are commensals or weak pathogens; sometimes, they are highly virulent and pathogenic organisms (*cf.* Tanada & Kaya, 1993).

Protozoa associated with mites are mainly found in the Apicomplexa, with the classes Gregarina and Coccidia, and in the phylum Microspora. The class Gregarina is divided into the orders Eugregarinida and Neogregarinida. Several members of the former order have been detected in mites.

The phylum Microspora also contains several pathogens of mites. Table 4 lists protozoan species that have hitherto been isolated from mites. There is little information available on the population dynamical effects of these organisms on natural mite populations.

5.1. Apicomplexa Infections in Mites

Apicomplexans have very complex life cycles, with much variation among the different apicomplexan groups. Both asexual and sexual reproduction is involved, although some Apicomplexa skip one or the other stage. The life cycle starts when an infective stage, the sporozoite, enters a host cell, and then divides repeatedly to form numerous merozoites. Some of the merozoites transform into sexually reproductive cells, or gamonts. These gamonts join together in pairs and form a gamontocyst. Within the gamontocyst, the gamonts divide to form numerous gametes. Pairs of gametes then fuse to form zygotes, which give rise by meiosis to new sporozoites, and the cycle starts all over again.

Apicomplexa are transmitted to new hosts in various ways; some, like the malaria parasite, are transmitted by infected mosquitoes, while others may be transmitted in the feces of an infected host, or when a predator eats infected prey.

Gregarinia, or gregarines have mature gamonts (trophozoites) that are large and extracellular. The gamonts are found in the digestive tract and body cavities of invertebrates and they possess organelles (see Tanada & Kaya, 1993). They have usually similar gametes (isogametes) and undergo syzygy: mature gamonts detach themselves from the midgut and line themselves end to end in pairs or in large numbers to form a prenuptial association. The zygotes form oocysts within gametocysts. Eugregarines have a life cycle that consists only of gametogony and sporogony, but the neogregarines, considered more primitive, have an additional schizogony (a multiple fission process). This schizogony (or merogony) occurs intra- or extracellularly and causes the presence of larger numbers of the pathogen than in case of the eugregarines. These higher numbers result in a more virulent infection.

As early as 1885, the eugregarine *Gregarina oribataram* was reported in an unidentified mite (see Lipa, 1971). Unidentified gregarine infections were observed in the oribatids *Damaeus oblongus* and *D. geniculatus* (Damaeidae) by Michael in 1884 and Wellmer in 1911 (see Lipa, 1971). *Gregarina scutovertexi* was described by Erhardová in 1955 from *Scutovertex minutus*, an oribatid mite that serves as vector of the tape worm *Monieza expansa*. Several other eugregarine species were described by Purrini et al. (1979) and Purrini and Ormiers (1981). For more details is referred to Table 4.

An interesting eugregarine infection was also noted in the intestinal wall of the water mite *Limnochares aquatica* (Hydrachnellidae) (see Issi & Lipa, 1968), while *Gregarina euzeti* was detected in the oribatid *Euzetes seminulum* (Lipa, 1982). Despite all these reports on gregarines, no data are available on the impact of these infections on the host. It is generally believed that gregarines are of low virulence.

Coccidia differ from the gregarines in their gamogony: female gamonts of gregarines give rise to a number of gamonts whereas those of Coccidea only to single gamonts. One coccidian species has been described from an oribatid (Purrini, 1984).

5.2. Microspora Infections in Mites

Microspora (or Microsporidia) are obligate intracellular parasites with a broad host range including all animal groups. They rank among the smallest eukaryotes (1–40 µm) with the shortest eukaryotic genome and are generally considered to be a separate phylum within the subkingdom Protozoa. However, recent molecular studies indicate that they may be considered to be extremely reduced fungi. Replication takes place within the host's cells, which are infected by means of unicellular spores.

Microsporidia are unusual in lacking mitochondria and in having mitosomes.[4] They also lack motile structures such as flagella. The spores are protected by a layered wall made of proteins and chitin. Their interior is dominated by a unique coiled structure called the polar filament. Spores, the infective stage, are ingested by the host and, in the midgut, their polar filament is instantaneously emitted as turning a garden hose inside out. The polar tube penetrates the host cell and the contents of the spore are pumped through it without destruction of the host cell. Within the host cell, an increase in numbers followed by the development into new spores takes place. In this way, the infection spreads throughout the host. Many different tissues may be affected. The spores may be released into the environment during the life of the host or after its death. The spores are the only stages that can survive outside the host. Many microsporidia have very complex life cycles with several spore types and host involved. Microsporidiosis is often transmitted vertically (from mother to offspring), usually without spores as intermediary. Vertical transmission may be transovarial, by passage though the ovary, or transovum, a form of transovarial transmission in which the pathogen enters the egg while it is still in the ovary.

Taxonomy was in the past mainly based on spore size and shape. These characters are, however, unsatisfactory and for that reason, ultrastructural characteristics have been used. This has caused a considerable revision in this group of pathogenic organisms (Sprague, Becnel, & Hazard, 1992). Recently, several research groups have started to integrate molecular techniques for phylogenetic studies of Microsporidia. This will undoubtedly result in a new revision of these taxa.

The first microsporidium described in mites was *Nosema steinhausi* (Weiser, 1956) from the stored product mite *Tyrophagus putrescentiae* (Tyroglyphidae). The disease affects both adults and nymphs. Experiments in a colony of the mite showed that the disease progressed from about 10% infection at the start of the experiment to

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[4] A mitosome is an organelle found in some unicellular eukaryotic organisms. The mitosome has only recently been found and named, but its function has not yet been well characterized. It is sometimes termed a crypton.

75% after 2 months. The colony was completely eradicated after a further 2 weeks. Several other authors have described microporidia from various species of mites. For more details refer to Table 4.

In the late 1980s poor performance was noted of phytoseiids used as biological control agent for thrips and spider mites in greenhouse crops and this observation has led to studies on the possible involvement of pathogens in predator colonies. Beerling and Van der Geest (1991a, 1991b) studied a microsporidosis in mass cultures of the predators *Amblyseius barkeri* and *Neoseiulus cucumeris* (Phytoseiidae) that are being used for the control of the thrips pests *Frankliniella occidentalis* and *Thrips tabaci* (Thripidae) on vegetable and ornamental crops in greenhouses. Diseased predators show a low reproduction and predation capacity of the mites unsatisfactory. Predatory mites were sluggish in their movement and had a swollen and whitish appearance (Beerling & Van der Geest, 1991a).

The presence of numerous spores could be detected in squash preparations of the mites and it was assumed, that the pathogen involved belonged to the Pleistophoridae (Microspora). Also infected stored product mites were observed. Further work showed that three different spore types are found in the predator cultures. It is difficult to say that three species of Microsporidia are involved, since some species have several spore types during their life cycle. Recently, a new species of a microsporidium parasite, *Intexta acarivora*, was observed in the gut epithelium of the forage mite *T. putrescentiae* (Larsson et al., 1997), obtained from a commercial culture in The Netherlands. Mites of this culture are used as prey for a commercial rearing of *N. cucumeris*.

Beerling and Van der Geest (1991a, 1991b) also studied infected mite strains from a commercial rearing in The Netherlands, but it is not known whether this has the same origin as the samples studied by Larsson et al. (1997). The spore size values of this microsporidium do not correspond with any of the values of the spores in Beerling, Rouppe van der Voort, and Kwakman (1993).

Bjørnson et al. (1996) studied colonies of *P. persimilis* that were obtained from suppliers of biological control agents. On the basis of spore morphology, three distinct microsporidia could be observed in strains of *P. persimilis,* obtained from three different suppliers. The ultrastructure of the pathogen and the course of the disease of a colony obtained from Europe were studied in more detail. Schizonts were observed inside the nuclei of the digestive cells of the ventriculus and within the protoplasm of cells that line the caecal wall and the muscle tissue underlying it. The properties of the pathogen made it difficult to assign it to an existing genus. For that reason, it was placed in the collective group *Microsporidium*. Vertical transmission for this microsporidium was proven, as mature spores were observed in developing eggs inside gravid females (Fig. 11). The performance of an infected colony was greatly affected (Bjørnson & Keddie, 1999): mean fecundity and prey consumption of infected mites were significantly reduced. Short-term survivability was variable and was not a good measure of predator quality. However, uninfected females lived longer than infected females.

Figure 11. Cross section of a microsporidian spore and a crystal of Phytoseiulus persimilis. *The polar filament of the spore is not visible. Photograph by Dr. Susan Bjørnson.*

Poor performance of the predators, due to the presence of pathogens in the mass cultures is a threat to integrated pest management in especially glasshouse crops (e.g. Steiner, 1993). It is clear that more attention should be paid to a good monitoring system. The presence of pathogens should be established early in the manufacturing process, which requires a fast and reliable detection method. In the past, visual inspection with the aid of a binocular or compound microscope was the only way to establish the presence of these pathogens. A polarizing filter is needed to avoid confusion with the birefringent crystals that are commonly present inside adult phytoseiids (see Section 6.1).

The availability of a method to detect the disease at an early stage is of great importance for the commercial production of natural enemies. Beerling et al. (1993) developed an ELISA to detect the presence of microsporidiosis in predator mass-rearings. Monoclonal antibodies were produced against one spore type (oblong), that was present in both prey and predator species. A next step would be the use of more sensitive molecular techniques which makes the detection of microsporidiosis possible regardless of spore type, and even before spores are formed (Malone & McIvor, 1996).

Recently, a new species of microsporidia was described by Becnel, Jeyaprakash, Hoy, and Shapiro (2002) from the predatory mite *Metaseiulus occidentalis.* Mites infected with *Oligosporidium occidentalis* do not show any external or gross signs of infection, but electronmicroscopical analysis reveales that the pathogen develops in eggs, larvae, nymphs and adults of the predator. Mature cells of *O. occidentalis* can be found in cecal cells, lyrate organ cells, ganglia, epithelial cells, muscle, inside the ovary and in developing and mature eggs. Female predators have in general a shorter life span, a low oviposition rate and fewer female progeny. No effect could be shown on longevity of males or on male progeny survival to larval and adult stages.

The infection may affect the performance of the predator. Heat treatments of infected colonies had some success: eggs placed for 7 days in a chamber at 33°C and subsequently at 27°C showed a reduced infection rate, but the disease was still not completely eliminated. Mites emerging from these eggs still showed spores inside their body, but it was assumed that most of these spore were dead as the majority of the mites survived. After a number of weeks, infection rates were again high, indicating that the heat treatment was only partially effective. However, when eggs were placed at 33°C. and when their progeny was also kept at this temperature, disease-free mites were obtained. It was also shown that the disease may be horizontally transmitted, probably by cannibalism.

At this moment, no other cure exists for microsporidiosis in predatory mite mass rearings. Anti-microsporidial compounds, such as albendazole, fumagillin, metronidazole and nifedipine were not successful in eliminating microsporidiosis in mass cultures of *P. persimilis* (Bjørnson, 1998). Therefore, efforts should be made to keep the starting cultures of predatory mites disease-free. Heat treatment of infected eggs, or rearing infected individuals at elevated temperatures, has been shown to reduce disease prevalence in some cases; however, the most effective and practical means for rearing microsporidian free predatory mites is to start a new rearing with progeny from uninfected females.

6. OTHER DISEASES

6.1. Symptoms Ascribed to Poor Condition

In this chapter, we discuss a condition in mites that cannot be ascribed to the action of a pathogen. In a number of instances, rectal plugs, usually in combination with abdominal discoloration have been observed in phytoseiids (Tanigoshi, Fagerlund, & Nishio-Wong, 1981; Bjørnson et al., 1997). This discoloration is usually manifested as two white stripes along the dorsal sides of the body within the Malpighian tubules. The condition is frequently found in laboratory colonies of predatory mites and it may be a sign of poor condition of the predator. Tanigoshi (1982), for example, considered the condition a sign of senescence. Affected mites are often lethargic and have frequently numerous densely packed, birefringent dumbbell-shaped bodies, mainly in the Malpighian tubules, rectum and anal atrium.

In *Cheyletus eruditus*, abdominal discoloration is also associated with the occurrence of birefringent crystals in the excretory organs, especially when reared under crowded conditions. These crystals are considered to be the normal excretory products (Hughes, 1950) and probably consist of guanine and uric acid (McEnroe, 1961). These compounds are insoluble and are probably stored in the malpighian tubes prior to excretion. The crystals observed in *P. persimilis*, however, contain high levels of potassium, low levels of phosphorous and sulphur and traces of chlorine, very unlike the common waste products mentioned above (Bjørnson, 1998). Large numbers of crystals in mites are especially found in laboratory mites, reared under crowded condition (Fig. 12), and it is assumed that they are an indication of a poor condition of the mites.

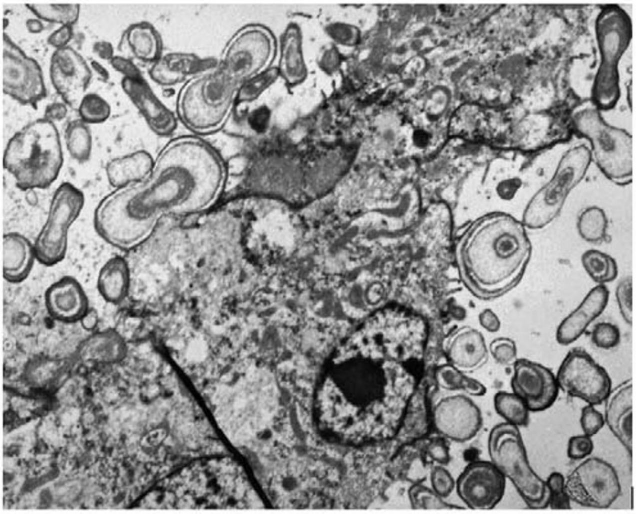

Figure 12. Crystals in tissue of Phytoseiulus persimilis. *Such crystals are often an indication of a poor condition of the mite. Photograph of Dr. Susan Bjørnson.*

6.2. Identification of Pathogens

Recognition of an organism causing disease in noxious mites will aid in the process of implementing control strategies against such mites, while it will also be of great importance to know disease causing organisms in mites that are mass reared for biological control purposes. However, identification of pathogens is not in all instances easy: for example, virus recognition in diseased mites may require very comprehensive research before one may decide that a virus is the disease causing agent. In contrast to insects, very few viruses have been isolated from mites and the viruses known to cause disease in mites are not as readily identifiable as some of the insect viruses. A large number of insect viruses belong to the baculoviruses, rod-shaped viruses that may form large inclusion bodies (polyhedra) in the cell nuclei (nuclear polyhedrosis viruses), or smaller so-called granula (granulosis viruses). Other viruses, the cytoplasmic polyhedrosis viruses are icosahedra and are also included in large inclusion bodies of irregular form. Identification of such viruses can partially be done by light-microscopic means, in combination with more sophisticated molecular techniques. Such viruses are not known from Acari. Hitherto, only few viruses have been identified as pathogen of mites.

Bacteria causing disease are often obligate intracellular organisms. Classical identification based on characters such as nutrient requirement and structure of colonies on artificial media is not possible as these bacteria cannot be grown outside it host's cell. However, the availability of molecular techniques has made identification of such intracellular bacteria possible by, among others, analysis of 16S rDNA gene sequences. The best-known species of such intracellular bacteria belong to the genus *Wolbachia*, probably the most widely-spread parasitic bacterium known. Abnormal sex ratios, incompatibilities between strains of mites and absence of male offspring may point to the presence of *Wolbachia* sp. or other symbionts. Feeding infected mites with antibiotics may remove these parasitic bacteria and will result in "normal" offspring. Molecular analysis of 16S rDNA will finally prove the presence of *Wolbachia* or other intracellular bacteria.

The largest number of pathogens of mites is found in the fungi. Pathogens of mites are mainly found in the Zygomycota and Deuteromycota (or Fungi Imperfecti). For a proper identification, it is usually necessary to study sporulating fungi. This can be accomplished by incubating the infected mites under conditions of a high relative humidity.

Zygomycota are characterized by the absence of cross walls (septa) in their hyphae and the presence of tick-walled, sexual spores (zygospores). However, the primary taxonomic emphasis is on asexual reproductive structures. The asexual spores (conidia) are released from the conidiophores by force and form often a halo around the host's cadaver. Species infecting mites form secondary conidia upon germination of these conidia. The secondary conidium is much smaller in size and is called capilliconidium. Identification to the species is often difficult and requires the aid of specialists. Of some species, only zygospores (or resting spores) are known. Taxonomy of these species is mainly based on the properties of these spores. These species have been lumped together in the genus *Tarichium.*

Several Deuteromycetes have been isolated from Acari, belonging to the following genera: *Aspergillus*, *Beauveria*, *Cephalosporium*, *Hirsutella*, *Paecilomyces*, *Sporothrix*, *Tolypocladium* and *Lecanicillium* (*Verticillium*). A key to the genera of Deuteromycetes infecting insects and mites can be found in Samson (1981). Identification requires examination of conidium ontogeny which is the primary character for typifying the different genera. There are two modes of blastic conidiogenesis: phialidic and sympodal. A succession of conidia is produced by a phialidic conidiogenous cell. The shape of the phialide is dependent on the genus: flask-like in *Paecilomyces* and *Hirsutella*, awl-like in *Lecanicillium* and cylindrical in *Metarhizium.* The conidia are produced in chains (*Paecilomyces*, *Metarhizium*), or they are contained in slimy heads or droplets (*Lecanicillium*, *Fusarium*). Phialides of *Hirsutella* form conidia that are held together in a slimy sheath. This gives the impression that only one conidium is being produced. Sympodial development is observed in species of the genera *Beauveria* and *Sporothrix.* Conidia are formed singly on a laterally proliferating conidiogenous cell that often shows a geniculate or zigzag type of elongation. For more details is referred to Samson (1981).

7. PROSPECTS OF ACAROPATHOGENS FOR INTEGRATED PEST MANAGEMENT

Comprehensive research has been conducted to study the possibilities to use pathogens for the control of insects and other invertebrate pests. There are several cases known how pathogens may decimate populations of phytophagous mites under natural conditions. An early example is a virus disease of the citrus red mite in citrus groves throughout California and Arizona (Reed, 1981). However, successful application of the virus failed for a variety of reasons. Mass production of the virus is difficult as the virus can only be grown in living mites. This fact makes mass production very laborious and expensive. Furthermore, the virus becomes rapidly inactivated by sunlight when applied in aqueous formulations, while high temperatures, common in citrus orchards in California and Arizona, also have a negative effect on the virulence of the virus. Inactivation of the virus by sunlight may be overcome by the addition of ultraviolet protecting substances. The main reason that this virus, although very host specific, has never been successful are the difficulties encountered during mass production. Biological acaricides with viruses as active ingredient are not foreseen for the near future, as few viruses of phytophagous mites are known. The situation with respect to the varroa mite *Varroa jacobsoni* may be completely different. Several viruses have been isolated from this parasitic mite and more research may lead to the discovery of viruses that could be used for the control of the varroa mite. A point to worry about is that the varroa mite may act as vector of honey bee viruses. Host specificity experiments are for that reason of great importance.

Many fungal pathogens show a high pathogenicity towards phytophagous mites. There are several examples showing how fungi are able to cause large epidemics in natural populations of tetranychids and eriophyids. One of the main obstacles for a successful application of fungal pathogens for the control of invertebrate pests in agricultural crops is the ambient condition within the vegetation. Almost all fungi require a relative humidity near the saturation point for both spore germination and spore formation. Entomophthorales epidemics have frequently been observed in insect and mite populations: *Neozygites floridana* is a fungal pathogen that may cause large reductions in population sizes of several species of spider mite. Such epidemics usually occur later in the season, when population sizes of spider mites are large and when relative humidity is near the saturation point. Damage to the crop has then already been inflicted.

Experiments have been conducted to advance epidemics by inundative releases of the pathogen. The high virulence against certain target pests and their high specificity make these fungi attractive for inundative releases. However, entomophthoralean species are very fastidious: culturing and sporulation in artificial media is hardly possible which makes mass production of these fungi very expensive and laborious, as they should be grown in living mites. In addition, the infective stages of these fungi are rather short-lived and this characteristic makes their application difficult. It has been suggested that the use of these fungi in greenhouses may show good prospects (Maniania, Bugeme, Wekesa, Delalibera, & Knapp, 2008).

In many greenhouses, high value horticultural crops are grown where environmental coniditions that normally favor the efficacy of these fungi can easier be manipulated than in outdoor crops. In addition, horizontal transmission of the fungus may be more efficient when spider mites densities are sufficiently high, making repeated inundative releases unnecessary. However, low spider mite densities would be an disadvantage for successful control of the pest concerned.

From the other hand, classical biological control with these fungi seems to be attractive in certain circumstances. Efforts have been made to use *Neozygites tanajoae* as a control agent for the cassava green mite in Africa by releasing the fungus in cassava fields in Benin as a classical biological control agent. The fungus has later been isolated from the release areas where it caused a higher mortality among cassava mites than the local strains of *N. tanajoae* did. However, more knowledge is required. We know too little how the fungus overcomes unfavorable periods, about the role of resting spores and under which conditions may resting spores sporulates. Interesting observations were made by Elliot et al. (2008) in a cassava field in the state on Bahia, Brazil. Epidemics of the fungus were virtually absent when cassava green mite populations reached high densities, as not sufficient fungus inoculum was present. Later in the season, sufficient fungus inoculum was observed, but at that time no mites were present due to defoliation of the cassava plants. It is very hard to manipulate such a system, as mass production, followed by inundative releases is impossible at a large scale.

The fungus does not seem to be the solution for the cassava green mite problem in Africa, although it is promising that the fungus seems to become established in the release areas. The fungus may be an important factor in the control of the cassava mite, when applied in combination with predatory mites. A point of concern is also the use of chemical pesticides: it is known that, in particular fungicides may have a detrimental effect on the fungal pathogen. Careful selection of chemicals used in a crop system is of utmost importance.

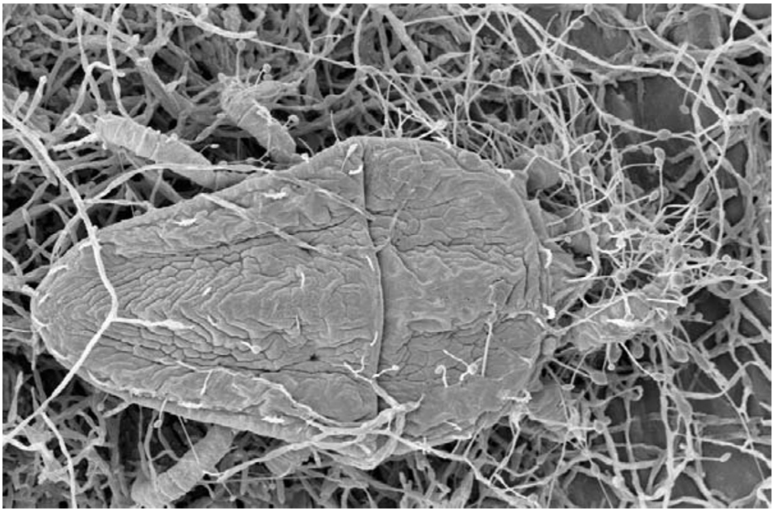

Figure 13. Brevipalpus phoenicis *infected by* Lecanicillium (Verticillium) lecanii. *Photograph courtesy of Dr. Marcel Tanzini.*

Several Deuteromycota are known with a high virulence towards mites and other invertebrates. This group of fungi can in general be grown in artificial media and mass production is therefore no problem. The first attempt to control an invertebrate pest with a fungus of this group was already conducted in Russia in 1888, when Krassilstschik sprayed a suspension of spores of *Metarhizium anisopliae* in the field for the control of the sugar beet curculio *Cleonis punctiventris* (*cf.* Steinhaus, 1949). Since then, many attempts have been made to formulate mycopesticides, mainly for the control of insect pests (De Faria & Wraight, 2007).

Until now, 171 products have been developed worldwide with entomo- and acaropathogens as active ingredients. Of these, 129 products are still available. The number of products that have been developed for use against mites is small: only 17 products are recommended for use against Acari. The main part of these pesticides contains *B. bassiana* as active ingredient, but four preparations are based on *Lecanicillium* sp. (formerly *Verticillium lecanii*) (Fig. 13). Many of these species have a broad host spectrum, like *Beauveria bassiana* and *Metarhizium anisopliae.* The genus *Hirsutella* contains several members with a high specificity towards mites. The best studied species is *H. thompsonii,* originally described from the citrus rust mite *Phyllocoptruta oleivora*. Acaricidal preparations with *H. thompsonii* as active ingredient were developed in the 1980s for the control of the citrus rust mite, but the production was discontinued because of instability of the product. It is hopeful to notice that the interest in the production of *H. thompsonii* based acaricides has recently been renewed in India, and in some Latin American countries, but now for the control of rust mites in coconut. This renewed interest has led in India to the production of a successful bio-acaricide.

In Colombia, biopesticides have been developed that contain a mixture of invertebrate pathogens, e.g. Microbiol Completo contains *B. bassiana*, *M. anisopliae*, *Nomuraea rileyi*, *Isaria fumosorosea* and *B. thuringiensis.* It is recommended for the control of a variety of insect species and Acari. It is remarkable that in particular in Latin American countries, many bioinsecticides are being developed with fungal pathogens as active ingredients. The climatic conditions may be more suitable for the applications of such pesticides than, e.g. in Europe. As mentioned earlier, a major drawback in the use of fungi is their dependence on a high relative humidity during spores germination. Oily formulations seem to be a solution to this problem. Oily substances protect the spores against desiccation and allow sporulation at a somewhat lower relative humidity.

Hirsutella thompsonii var. *synnematosa* has been introduced from Zimbabwe and *H. thompsonii* var. *vinacea* from North Carolina as classical biological control agents for the control of *Eriophyes sheldoni* and *Phyllocoptruta oleivora* in Argentina. Infection levels after release were high, but no information is available about their persistence. The project has been discontinued (*cf.* Maniania et al., 2008).

A point of major concern in integrated control programs is the quality of biological control agents. Predatory mites have been used for several decades for the control of spider mites in horticultural and agricultural crops. Low performance of predators has revealed the presence of pathogens. For reviews is referred to Schütte and Dicke (2008), Bjørnson (2008) and Hoy and Jeyaprakash (2008). Microspora species have been causing problems in mass cultures of several species of Phytoseiidae, in particular *Amblyseius barkeri* and *A. cucumeris*, but they are also known to occur in *Phytoseiulus persimilis* and *Metaseiulus occidentalis.* Sanitary measures and careful selection of uninfected lines of predatory mites are still the only solution to these problems. *Acaricomes phytoseiuli* is a bacterium that affects the behavior and predacious capacity of *P. persimilis.* This bacterium has not yet been encountered in mass rearings for the predator, but it will be clear that screening for such pathogens is necessary. Another group of bacteria that can cause considerable damage in mass cultures of beneficial mites and insects are *Wolbachia* sp., *Cardinium* sp., *Spiroplasma* sp. and other bacteria that affect sex ratios and that may cause incompatibilities between strains of the same species. This type of bacteria is widespread in mites and other vertebrates

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12

IPM STRATEGIES THROUGH SPECIALIST AND GENERALIST PHYTOSEIIDS (ACARI, MESOSTIGMATA)

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Abstract. The exploitation of natural enemies, especially the predaceous mites phytoseiids, has become a fundamental factor for IPM in integrated crop production in Europe and worldwide. In controlling harmful insect and mite species, various species/strains from this group of predators are commonly used and marketed. Intrinsic biological traits of phytoseiids allow their ranking in different life style types. These factors, together with their quick adaptability to face new emergencies (i.e. phytophagous and exotic pests outbreaks and new environment or climatic conditions) confirm their role as a significant and sustainable tool in biological control. In this chapter the foundations for a more intensive adoption of phytoseiids are underlined, including the enhancement and introduction of new strategies aiming at a long term and efficient pest control.

1. INTRODUCTION

The need to increase food availability hardly couples with better chances to limit plant pests in modern agriculture, and several actions aim worldwide at increasing crop production and facilitating food and commodities trade. These efforts include lengthening of the plant/crops seasonal life or strategies increasing the consistent uniformity of varieties and cultivars used which represent, in fact, useful factors for business at small time scale. However, these actions are not always suitable to prevent a multitude of pest infestations on a regional scale. Since pests are also persistently poised for plant invasion, preventive and early remedial pest management strategies and treatments are needed at regular time intervals, in almost any cropping system (Sparks, 1999; Jarvis, Mar., & Sears, 2006; Gerson & Weintraub, 2007).

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Given the worldwide trend towards crops with high genetic uniformity, much of the biodiversity found in the context of agroecosystems is sustained and maintained by means of the marginal environments surrounding productive areas. Any loss of such diversity due to crop management choices reduces farmers capacities to cope with plant stress factors. These include biotic factors like extreme temperatures, drought and salinity (Wang, Vinocur, & Altman, 2003). Consequently, due to the synergies occurring among biotic and abiotic stress factors, the problems that much of the world population practicing subsistence agriculture has to face are also emphasized. Furthermore, and typically in plants that regularly shut down, periodic inspections by specialists involved in integrated pest management (IPM) are needed for monitoring (with related costs). On the other hand, in more intensive agricultural systems, crop production in field or under protected conditions, whether in climate-controlled greenhouses and glasshouses or tunnels with little or no climate control, is increasing worldwide, due to market demand, demographic pressure and/or unavailability of suitable environments. Although these last cropping systems are closed and relatively protected, they still remain vulnerable to known or invasive pests.

The general and pressing consumers demand for healthy products, the increasing legal restrictions on pesticides, along with the increase in resistance to pesticides, is driving the greenhouse industry to apply as many non-chemical solutions as possible, so that different kinds of control strategies have to interact (Hussey & Scopes, 1985; Tanigoshi, Martin, Osborne, & Peña, 2004; Gillespie & Raworth, 2004). In addition, during the last decade there has been a consistent and widespread increase in market of foodstuffs and other "biological" products. These are associated with natural and healthy processes even if this association is frequently far from to be ascertained. Many countries, i.e. United Kingdom, have adopted strategies to reduce reliance on pesticides and encourage, or in some instances force, growers to consider other strategies in managing pests. This aim was pursued in 2006 by i.e. lowering registration fees associated with the development of biopesticides, in an effort to increase availability of reduced-risk pesticides to growers (ESRC, 2008).

Campaigns against the use of pesticides in agriculture in the EU led to an increase of the production area and market size of the greenhouse industry, introducing biological control in many agricultural systems. Frequently and realistically, in spite of the great emphasis on the use of "natural" techniques concerning control and production requiring the exclusion of artificial products, biological/organic agriculture do not appear so different from most modern "conventional" regimes: these processes can be indeed largely based on the use of active ingredients, natural but still toxic that, on a different scale, could yield problems similar to those already known for pesticides (i.e. residues on foodstuffs, side effects on non-target organisms).

The global situation of pests control and the increasing efforts, both in biological and IPM agriculture, related to the adoptions of sustainable ways can be affected by the lack of whole efficacy of these products, more than by a loss of strategic trust in pesticides. Given this context and perspectives, the control of pests matches frequently and unavoidably with the release and/or enforcing of natural enemies and antagonists already present on a crop (Bale, Van Lenteren, & Bigler, 2008).

Among natural enemies of arthropod pests, predaceous mites Phytoseiids (Acari: Mesostigmata), became in the recent years a fundamental tool for IPM in integrated crop production in Europe as well as worldwide. Various species or strains belonging to this group of predators are commonly used and marketed to control harmful insect and mite species. The phytoseiids ranking in different life style types, together with their quick adaptability to face new emergencies confirm their role as a significant and sustainable tool in biological control. In this chapter we review the basic concepts related to and the possible use of phytoseiids, including the enhancement and introduction of new strategies for efficient pest control, durable in time.

2. CONCEPTS ON NATURAL ENEMIES AND/OR ANTAGONISTS IN IPM

The role of natural enemies and/or antagonists in IPM and the degree at which biological control agents (BCAs) can be exploited vary from crop to crop and from area to area. Some questions need consideration and precise answers, before deciding on effectiveness of natural enemies against major pests on a crop system and defining the guidelines for their application or safeguard. One the most important issues concerns the occurrence of effective natural enemy on the considered pest and if, from an economic and ecologic point of view, it is more convenient to introduce mass reared enemies or to facilitate the colonization of the crop by local populations, from the surrounding areas. The crops or vegetation types adjacent to the targeted crop are important, since some plants can act as possible refuge for natural enemies, while others can harbour unwanted pests.

Crops grown under "organic" or "conventional" regimes obviously need different approaches for pest management. If the crop is "organic" it can provide added incentives to the use of biocontrol methods. However, a crop does not have to be grown organically to benefit by the use of biocontrol methods. "Soft" chemical options must be found for use in conjunction with natural enemies. Furthermore, crop life span and environment have to be suitable enough for harboring natural enemies. Generally, it can be difficult to establish BCAs in short lived crops. Also, the knowledge about plant life stages suitable for natural enemies is important, since BCAs may be most appropriate at a particular stage of the growing cycle.

In addition to these questions, there are those related to the cost, practicality and degree of difficulty encountered when controlling key pests with chemical means alone and/or natural enemies, and when assuming which strategy is more demanding in terms of cost, efficiency or healthy status returns. Furthermore, difficulties are encountered when switching from a conventional towards an IPM or organic system. Practices and routines need to be modified continuously as new information must be gained during this process. Regular monitoring is necessary to identify pest outbreaks and their location within a crop. Also, "soft" controls methods should be checked and tested, since they may be available for some pests, but not for others.

When an antagonists-based strategy is adopted, some damage induced by pests must be tolerated, since a minimum number of individuals may be required to support a useful population of its natural enemy. Deciding whether or not spraying (and when) can represent a further difficulty. However, if "soft" options are available for the pest in question, this is not such an issue. The identification of the most appropriate release timing is necessary when introducing natural enemies and to get them established quickly and maintained along. The introduction of appropriate numbers of mass reared BCAs is another condition necessary to facilitate quick establishment.

Another aspect to be considered is providing a suitable environment. Very hot dry conditions are not conducive to some BCA's and research programs were recently developed (Palevsky et al., 2006). Adjustments may need to make to favour BCA's, e.g. shade, windbreaks, overhead watering. Having an expectation that one cannot spray chemicals at all could be incorrect and may result in failure of the IPM system. BCA's usually recover from occasional sprays of moderately toxic products and can remain at useful levels.

Actually, by referring to the state of knowledge on animal organisms and to the complex represented by phytophagous-natural and/or commercial enemies, consolidated strategies (and not only theoretical approaches) are available for farmers. They aim, among the several factors involved, to the reconstitution of the populations balance and to the improvement of the action against enemies. This objective is pursued by: (i) the diffusion of antagonists, (ii) the elimination of sources of perturbance and (iii) the adoption of environmental and cultural management measures.

This chapter deals with the phytoseiid mites, which have a significant role on control of crop pests in greenhouse and field conditions, worldwide. By focusing on the definition of DeBach (1964), biological control is: *the study and uses of parasites, predators and pathogens for the regulation of host (pest) densities*. Two main principles are included in this review: (1) most organisms are consumed by other organisms and this can be exploited by the man as “natural control”, (2) this natural/biological control reduces, rather than eradicates, the pest. As a consequence, a number of important pests can be kept at a low population density by biological control agents over long time periods or, differently, populations of pests are reduced but further releases, or additional methods are needed to achieve an adequate level of control. Among BCAs, phytoseiids are, for their traits, highly capable to efficiently interface with rapidly changing contexts, as crop and environment turn over. At the same time, the great versatility of some species seems to guarantee long lasting control expectancy of pests and a presidium of the area with an acceptable level of related costs.

3. WHY PHYTOSEIIDS?

3.1. Mass Rearing

The breeding of auxiliaries is a very complex task, requiring the development of refined and very reliable techniques. The high cost involved, together with the need to be supported by a sure market, has sometimes considerably limited the use of natural enemies. However, the environments to be protected usually hosts intensive or expensive cultures and, on the other hand, do not facilitate the dispersion of the antagonists. For this reason, processes optimising their efficacy and persistence are needed.

A combination of control efficiency with low production costs and receptive markets is needed to support the development of effective mass rearing technologies for selected antagonists, including importation and cultures of appropriate natural enemies on alternative food, automated productions, testing of technologies for production and quality of predators, processing, storage, packaging, distribution and release of natural enemies of insect and/or weed pests. Recently, more and more effective augmentative and classical biological control strategies for key pests have been developed.

Phytoseiids satisfactorily match all the needs of the production pathway and their production and marketing sensibly increased in the last years (Van Lenteren, 2003). Some species i.e. *Phytoseiulus persimilis* and *Neoseiulus californicus* (Fig. 1) are worldwide employed in control strategies. They have been widely and successfully used in the biological control of tetranychids phytophagous mites, for over half a century and more recently for thrips and aleurodids (Gerson & Weintraub, 2007; Messelink, Van Steenpaal, & Ramakers, 2006; Messelink, Maanen, Van Steenpaal, & Janssen, 2008). For many phytoseiids species the rearing can be conducted on a number of alternative preys, which lowered considerably the costs.

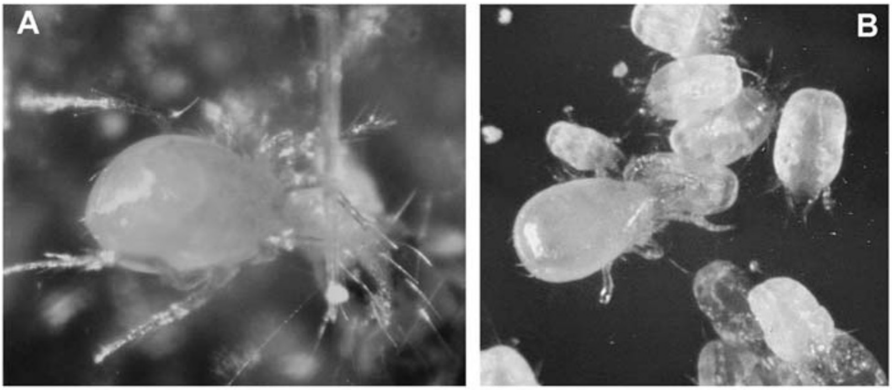

Figure 1. Phytoseiulus persimilis *preying on* Tetranychus urticae *on strawberry leaf* (**A**)*, and* Neoseiulus californicus *preying astigmatid mite on rearing unit* (**B**).

The large number of studies on biological traits of phytoseiids allowed producers to determine and/or automate the best mass rearing techniques and to establish their potential for control. At the same time research from bioindustries contributed to increase the bulk of knowledge, not only in terms of production standards and/or protocols to evaluate the quality and efficiency of reared predators, but also in terms of benefits for the theoretical study of predator-prey interactions.

3.2. Fitness and Adaptation Characters

Phytoseiids have a short life span and are relatively easy to rear in the laboratory. For these reasons they are frequently chosen as a subject for basic studies on the mechanisms regulating the herbivores-predators-plants relationships. Some effective phytoseiids proved to retain high developmental and population increase rates on prey (Table 1), as well as good numerical and functional responses on prey density (Nachman, 1981; Sabelis, 1986; Castagnoli & Simoni, 1999). Data are available also on population dynamics and density changes in both seasonal and spatial distribution studies (Sabelis & Bakker, 1992; Sabelis & Janssen, 1994), as well as on mechanism and cues which determine the search of prey and infested plants (Sabelis & Van der Baan, 1983; Takabayashi, Dicke, & Posthumus, 1991). Data on the characteristics and occurrence of diapause and on the abiotic factors involved in its induction, maintenance and termination (i.e. photoperiod, temperature and food availability), are available for phytoseiid mites, as well as knowledge about the physiological mechanisms and related applied aspects (Veerman, 1992).

Table 1. Intrinsic rate of increase (r_m) and population doubling time at about 25°C of phytoseiid species, largely utilized in biological control.

Phytoseiid species	*Prey*	r_m (day^{-1})	*Doubling time (days)*	*References*
Phytoseiulus persimilis	Tetranychids	0.317	2.19	Takafuji and Chant (1976)
Neoseiulus californicus	Tetranychids	0.259	2.68	Castagnoli and Simoni (1991)
Neoseiulus cucumeris	Tetranychids Thrips	0.174 0.178	3.98 3.89	Castagnoli and Simoni (1990)
Amblyseius swirkii	Whitefly	0.213	3.25	Nomikou, Janssen, Schraag, and Sabelis (2001)
Galendromus occidentalis	Tetranychids	0.190	3.65	Tanigoshi, Hoyt, Browne, and Logan (1975)

However, the phytoseiids reproductive strategy is the trait which perhaps makes these predators quite peculiar. In arrhenotokous arthropods males arise from unfertilized eggs and, by controlling the fertilization process, mothers can adjust the sex ratio in their offspring. In pseudo-arrhenotokous phytoseiid mites, males are haploid, even if arising from fertilized eggs. The haploid state is achieved through elimination of a chromosome set during the embryonic development. Phytoseiid females can control the sex ratio in their offspring and this control seems extremely flexible (Nagelkerke & Sabelis, 1998). As predicted by current evolutionary theory of sex allocation, sex ratios approached half males, half females under random mating, whereas a female bias was observed under sib-mating. It is suggested that arrhenotoky is selected for when there is a substantial risk of high portion of unmated females in the population: pseudo-arrhenotoky may evolve by external/environmental pressure, since it retains the possibility to reinstall lost genetic information in the maternally derived chromosome, by using the paternal chromosome as a template for DNA-repair. This precise control of sex allocation in phytoseiids is probably the significant mechanism by which these predators can regulate their density, depending on both phytoseiid female and prey densities. They can in fact adjust offspring sex ratio in response to the presence of conspecifics or their cues and also to synchronize their population with that of prey (Nagelkerke & Sabelis, 1996). The prediction of optimal sex ratio by means of modeling is selectively advantageous when local mating groups vary in size and are usually small, as in the case of experimental laboratory studies on phytoseiids. At a larger spatial scale than the local mating group, the prediction appears to be less precise, may be due to operating and interfering selection levels (Nagelkerke & Sabelis, 1998).

3.3. Life Style Types

The characterization and ranking of phytoseiid mites is a helpful and significant tool (McMurtry & Croft, 1997), allowing a rating of species based on some morphological, reproductive and developmental aspects. Furthermore, traits like feeding and diet needs (McMurtry & Rodriguez, 1987; Schausberger & Croft, 1999) and adaptation to certain foods (Castagnoli, Simoni, & Liguori, 2003), were considered to ascribe the predators to the different life styles. McMurtry and Croft (1997) considered that a four-types classification may be efficient to rank the different species of phytoseiids and gave emphasis to the biological control of spider mite pests (Table 2). However, new life style types might be identified.

Recent studies aimed at estimating/identifying the more significant traits involved in phytoseiids rating and to possibly generate more stable classifications (Croft, Blackwood, & McMurtry, 2004). The mainly and first exploited traits/factors to rate phytoseiids were: (i) *Feeding*: the ability to prey and to feed on various prey and other food types (McMurtry & Rodriguez, 1987; McMurtry, 1992), a primary criterion considered for classification; (ii) *External morphology*: apparently, no strong correlation was found between body size and generalist–specialist phytoseiids (Schuster & Pritchard, 1963; Chant & Hansell, 1971) and more evidence is needed to establish the association between body size and different life styles (Croft et al., 2004); more correspondence was found in the evaluation of the adult dorsal shield setal length, in association with the feeding specialization (Sabelis & Bakker, 1992). Also, the mouthpart apparatus may be different between specialists and generalists (Flechtmann & McMurtry, 1992), but it is not clear to what extent this could work for a clear attribution to a life style; (iii) *Biological parameters*: research focused on reproduction, development and mortality; the specialist phytoseiids generally show intrinsic rates (Sabelis & Janssen, 1994) and sex ratio values higher than generalist (Nagelkerke & Sabelis, 1996) as well as shorter developmental times (Luh & Croft, 1999, 2001). Concerning mortality, studies characterized more exactly the dependence on prey density and the rate by which this changes. Through mortality, species differently adapt to varying prey or food levels, in the different life style types: the specialist species shows a tendence towards fast response to higher prey densities than a generalist, whereas a generalist tends to persist for a longer time, at limited or scarce prey density (Walzer & Schausberger, 2005; Simoni, Castagnoli, & Liguori, 2005).

Table 2. Categorization of phytoseiids life style types by McMurtry and Croft (1997).

Type	Strategy [a]	Crop [b]
Type I spp.		
Phytoseiulus persimilis	1, 2, 3	L, G
P. macropilis	2	L
P. longipes	2	L, G
Type II spp.		
Galendromus annectes	1, 2	T
G. helveolus	1, 2	T
G. occidentalis	1, 2, 3	T, L, V
Typhlodromus (T.) rickeri		T
Neoseiulus bibens	1	L, Sh
N. californicus	1, 2	T, L, V
N. fallacis	1, 2, 3	T, Sh, L
N. idaeus	1, 3	Sh, L
N. longispinosus	1, 2	T, Sh, L
N. tiki	1	Sh, L
Type III spp.		
Typhlodromus (T.) pyri	1, 2	T, V, Sh
T. (T.) exhilaratus	1	T
T. (Anthoseius) caudiglans	1	T
T. (A.) doreenae	1	V
Metaseiulus arboreus	1	T
M. citri	1	T
M. pomi	1	T
Paraseiulus soleiger	1	T

Table 2 continued

Type III spp. *(continued)*		
Phytoseius macropilis	1	T
P. spoofi	1	T
Amblyseius andersoni	1, 2	T
A. eharai	1	T
A. swirskii	1	T
Neoseiulus barkeri	2	G
N. cucumeris	1, 2	L, G
N. umbraticus		L, T
Kampimodromus aberrans	1,2	T, V
Typhlodromalus aripo	1, 3	Sh
T. limonicus	1	T, Sh
T. manihoti	1, 3	Sh
Typhlodromips sessor		T
T. newsami	1, 2	T
Iphiseius degenerans	1, 2	T, Sh, G
Type IV spp.		
Euseius addoensis	1	T
E. elinae	1	T
E. finlandicus	1	T
E. fructicolus	1	T
E. sojaensis	1	T
E. tularensis	1	T
E. stipulatus	1,3	T
E. victoriensis	1	T

[a] Strategies: 1 = conservation; 2 = augmentation; 3 = importation and establishment.
[b] Crops: L = low-growing; G = greenhouse; T = tree; Sh = shrub; V = grapevine.

A considerable amount of studies has been recently added concerning the evaluation of the different degree of responses by generalists and specialists predators, as a consequence of their different physiology and behaviour. Even if a build up in basic physiological studies is still necessary, physiological and behavioural responses were analysed frequently and in different contexts. Preliminary studies indicate appreciable differences among life styles. One of the most remarkable concerns the response to odours produced by preys, other foods, competitors, host plants and other habitat-related elements (Blackwood, Luh, &

Croft 2004; Gnanvossou, Hanna, & Dicke, 2003; Schausberger & Croft, 2001). In particular, host–plant relationships seem to be especially affected by the intrinsic physiological traits, and generalists have closer associations with host plants than specialist phytoseiids do (Kreiter, Tixier, Croft, Auger, & Barret, 2002; Tixier, Kreiter, Croft, & Auger, 2002). Specialists also tend to aggregate mostly in proximity of preys, by suffering sometimes higher mortalities (McMurtry & Croft, 1997; Faraji, Janssen, & Sabelis, 2002), whereas the perception of the prey cues can lead to different inter and/or within plant dispersal.

It should be considered that the phytoseiids life style categorization is not a dogma, although it can be represent a dynamic underlying support in setting control strategies of different pests. To determine the value of each definition, a sort of holistic approach would be advisable, by considering more factors and criteria in the grouping. Future studies are needed to estimate both the optimal numbers of traits and numbers of life styles type, simultaneously. Such fitting problems, or more robust classifications, probably will require the use of a multi-sample classification methods. By this point of view, significant trials were performed by Luh and Croft (1999, 2001) by a computer-based genetic algorithm, and by Blackwood et al. (2004), by a discriminant analysis model. The results obtained by Luh and Croft (2001) were in full agreement with the ranking of McMurtry and Croft (1997): just one, *Neoseiulus longispinosus*, out the 20 species considered in the two categorizations shifted by one style type level. The selection of subsets of variables optimizing the classification and reducing the chance of misclassification of traits included for best fit, shows to be effective as identifying potential useful indicators of life style type.

3.4. Single or Multiple Antagonists Release

Several herbivores can be usually attacked by numerous predator species but, historically, studies concerning single prey-single predator interactions were mainly performed (Holling, 1966; Hassell, 1978; Kareiva, 1994). A controversial issue was the use of a natural enemy complex, as opposed to a single enemy strategy, to achieve the best biological control (Ehler, 1990; Riechert & Lawrence, 1997; Losey & Denno, 1998). As concerns mite pests management, control in different agricultural systems was generally performed by adopting the release of a single phytoseiid species. More sporadic were evaluations about the release of two or more species. The interaction and dynamics of different phytoseiids spp. with, evenly, different life styles, may return different responses in comparison with the expected outcome (Helle & Sabelis, 1985; McMurtry & Croft, 1997; Schausberger & Walzer, 2001; Castagnoli, Simoni, & Nachman, 2001).

Walzer and Schausberger (2005) evidenced that the combination of a specialist predator as *Phytoseiulus persimilis* and a diet-generalist as *Neoseiulus californicus* in a sustainable way, might control spider mite, with higher efficiency in perennial crops. Control on eggplant and pepper was achieved by single *N. californicus* release (Castagnoli, Liguori, & Simoni, 2005). Simoni et al. (2005) evaluated the same phytoseiids and *P. persimilis* alone, by means of single and combined releases, on the same infested solanaceae as well as on tomato, in order to evaluate if their effect was additive, multiplicative or detrimental in the action of predators. The not-overlapping degree of specialization, narrow in *P. persimilis*, wider in *N. californicus*, and the consequent asymmetry in the response guaranteed, for some weeks, fast (mainly due to *P. persimilis*) and longer (mainly due to *N. californicus*) term equilibrium of the prey-predator system and of biological control, especially on eggplant and pepper.

As concerns the ascertained intrinsic traits and versatility of some phytoseiid, *N. californicus* appears able to adapt to different food and climatic conditions (Castagnoli & Simoni, 2004), and it may be considered feasible and convenient if combined to or in light shifts of phytoseiid releases, or in application with some other kind of biocontrol agents (i.e. other mites, insects or the fungus *Beauveria bassiana*). Obviously, the adoption of such a tactic needs an intensive monitoring, a full evaluation of the possible effect of the microrganism on the predator and an assessment concerning the possibility that the phytoseiid is able to recognize the treated substrate (Simoni, Guidi, & Tarchi, 2009).

3.5. Cannibalism and Intraguild Predation

The coexistence and interaction of two different phytoseiids species sharing a common prey resource can undoubtedly determine new functional-trophic relations (Rudolf, 2008). Intraguild predation and cannibalism are, in cases of combined release of predators, variables to be included in current models aiming at overcoming the discrepancy between theory and empirical data.

Although cannibalism may often represent a weak, unuseful interaction in nature, it may have significant consequences at the population level (McCann, Hastings, & Huxel, 1998). Cannibalism can be a crucial factor contributing to population structure, dynamics and control in a given habitat (McCann et al., 1998), affecting the quantity and quality of food for the remaining individuals. Predator-predator interactions such as competition, intraguild predation (IGP), and cannibalism affect the development and coexistence of predator populations, and can have significance in the biological control of commonly exploited pest organisms (Castagnoli, Liguori, & Simoni, 2002; Schausberger & Croft, 2000). Furthermore, phytoseiids are suitable models for studying cannibalism and related phenomena, due to their diversity and variability, small size and ease of rearing in the laboratory. In future research and in the frame of tactics and strategies of control, most fields are to be exploited, i.e. kin the link of cannibalism with discrimination and disease transmission, the interplay between cannibalism and dispersal, and the effects of cannibalism on population dynamics and species communities.

4. CONCLUSIONS

Given the number and complexity of factors involved in the wide pattern of natural enemies and antagonists, phytoseiid mites appear able to significantly interact in the dynamic equilibria of different prey-crop contexts. The modality of agricultural pests control by these predators can range from classical biological control, with introduction and establishment of foreign species, to safeguard and augmentation of indigenous species. Perspectively, it appears convenient to stress further the search for appropriate control strategies and tactics, on which phytoseiids have a dominant role. Phytoseiids proved to be able to quickly adapt to new environmental conditions (Helle & Sabelis, 1985). Laboratory selection of phytoseiids produced several strains with different traits as no-diapausing strains, strains adapted to live on unsuitable hosts or more resistant to adverse climate or pesticides (Castagnoli, Liguori, Simoni, & Guidi, 1998; Castagnoli, Liguori, & Simoni, 1999; Castagnoli et al., 2003; Drukker, Janssen, Ravensberg, & Sabelis, 1997; Fournier, Pralavorio, Berge, & Cuany, 1985). Frequently, these adapted strains have been mass reared and released for pest management programs in glasshouses and agricultural cropping systems. The majority of researches on the argument show that the release of phytoseiids for augmentation or classical biological control programs involves minimal risk for the environment and indigenous predators, with great benefits (Pilkington, Messelink, Van Lenteren, & Le Mottee, 2009).

The development of recombinant DNA techniques for the genetic manipulation of crops and microorganisms may be successfully applied also to phytoseiids, and much debated opinions concern risks of transgenic phytoseiids release. Great attention and punctual researches still need to address the real impact in the environment of phytoseiids that have been manipulated with genetic techniques. These studies, further to answer to the question, could again provide opportunities to expand our understanding of the ecological impact of phytoseiids in agricultural and natural environments, and lead to improved pest management tactics (Hoy, 1992).

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