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Successes and failures in the use of parasitic nematodes for pest control

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Abstract

Advances in mass-production and formulation technology of entomopathogenic nematodes, the discovery of numerous isolates/strains and the desirability of reducing pesticide usage have resulted in a surge of scientific and commercial interest in these nematodes. The lessons learned from earlier problems have encouraged scientists and leading commercial companies to increase their efforts toward improving cost efficiency and better product positioning in the market within the confines of product capabilities. The successes or failures of the nematodes against 24 arthropod pest species of agriculture and animals and against a major slug pest in agriculture are discussed in this review. Commercial successes are documented in markets such as citrus (Diaprepes root weevil), greenhouses and glasshouses (black vine weevil, fungus gnats, thrips, and certain borers), turf (white grubs, billbugs, and mole crickets), and mushrooms (sciarid flies). In addition, the successful commercialization of a nematode (*Phasmarhabditis hermaphrodita*) against slugs in agricultural systems is presented. Despite this progress, the reality is that nematode-based products have limited market share. Limited share is attributed to higher product cost compared to standard insecticides, low efficacy under unfavorable conditions, application timing and conditions, limited data and cost benefit in IPM programs, refrigeration requirements and limited room temperature shelf life (product quality), use of suboptimum nematode species, and lack of detail application directions. One or more of these factors affected the market introduction of the nematodes despite promising field efficacy against insects such as black cutworm in turf, sugar beet weevil in sugar beet, sweet potato weevil in sweet potato, and house fly adult in animal-rearing farms. Insects such as cabbage root maggots, carrot root weevil, and Colorado potato beetle are listed on the label of certain commercial products despite low efficacy data, due to insect susceptibility, biology, and/or behavior. To make entomopathogenic nematodes more successful, realistic strategies through genetic engineering, IPM programs, and new delivery systems and/or training programs to overcome their inherent cost, formulation instability, and limited field efficacy toward certain insects are needed.

Keywords: Entomopathogenic nematodes; *Steinernema*; *Heterorhabditis*; *Phasmarhabditis*; Slug nematode; Field efficacy; Commercialization

1. Introduction

The development of large-scale production and ease-of-use formulations created marketing opportunities for entomopathogenic nematodes of the genera *Steinernema*

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and *Heterorhabditis* (Georgis, 2002). However, commercialization of entomopathogenic nematodes has experienced both successes and failures (Shapiro-Ilan et al., 2002). Successes include control of the Diaprepes root weevil *Diaprepes abbreviatus* L. in citrus, the black vine weevil *Otiorhynchus sulcatus* (Fabr.) in cranberries and greenhouses, billbugs *Sphenophorus* spp. in turf, fungus gnats (sciarid flies) *Bradysia* spp. in greenhouses and mushroom flies *Lycoriella* spp. in mushrooms. Yet, these successes often did not lead to capture of a significant share of the pesticide market for these pests. Even where promising efficacy against some insects has been achieved under filed conditions (e.g., artichoke plume moth, *Platyptilia carduidactyla* (Riley), the black vine weevil in mint, and cockroaches in urban industrial environments) under field conditions, commercial sales of nematodes were minimal at best or never realized. Although the host range of entomopathogenic nematodes includes more than 200 insect species, nematodes have only been successfully marketed for a small fraction of these insects. Accordingly, we have selected certain insect pests of animals and crops to address the factors that influence the success or failure of commercial entomopathogenic nematodes.

2. Commercial assessment

The adoption of entomopathogenic nematodes as pest control agents by growers depends upon numerous factors beyond acceptable efficacy. Factors such as cost, shelf life, handling, mixing, coverage, competition, compatibility, and profit margins to manufacturers and distributors contributed to the failure of nematodes to penetrate many markets or to gain significant market share (Tables 1 and 2). Most of the current markets are limited to specific insects such as those of citrus, turf, and ornamentals (Table 1). Unfortunately, due to insect susceptibility, behavior and/or biology, many insects listed on the product labels of certain commercial companies are improper targets (e.g., corn rootworm, cucumber beetles, flea beetles, carrot weevil, root maggots, wireworms, shore flies, and imported fire ants) for nematodes (Georgis, 2004). These insects have a significant market share of the pesticide market (Georgis, 2004).

Georgis and Gaugler (1991) noted that successful market penetration of nematode-based products depends upon providing predictable control. Because of the complex interplay of abiotic and biotic factors, achieving predictability is probably the greatest intellectual challenge facing biological control today. Although nematodes can successfully infect and develop in many different host species, hosts in which optimal infection and development occurs differ with the nematode species or strain. Therefore, screening several different nematode species and strains against a particular target host is essential in development of any control program. The biology and behavior of the nematode and the target host and the environment in which the nematodes are to be applied must also be considered carefully when designing a control strategy.

A large number of field trials are necessary to design and optimize protocols that achieve consistent and satisfactory control. Based on 82 field trials, Georgis and Gaugler (1991) described how factors such as moisture (irrigation frequency and rainfall), thatch depth, soil type, seasonal temperature, nematode strain, and nematode application method could be used to predict failure or successful control of larval scarabaeids.

Recently, Mráček (2002) summarized the results of 70 field tests that were conducted between 1988 and 2002. This summary provides a comprehensive summary of field efficacy of various nematode species against a wide range of insect species in various crops and habitats. Most of these insects live in soil, although some, such as the artichoke plume moth and larval sesiids and cossids, inhabit cryptic environments. Both soil and cryptic habitats protect nematodes from desiccation and UV light, buffer temperature extremes, and promote contact between nematodes and the target insects. Out of the 70 tests, only 12 showed high efficacy. In other tests, the control was inconsistent or ineffective. Those insects that were not controlled successfully usually inhabited an environment hostile to nematodes (e.g., fly maggots in chicken manure, foliar habitats where nematodes desiccate, or sites with high temperatures), were physiologically resistant to nematodes (e.g., the immune response of mosquito larvae), possessed morphological barriers to nematode penetration (e.g., exclusion of nematodes by spiracular plates of certain scarabaeids) or exhibited behavioral traits that allow them to evade or exclude nematodes (e.g., fire ants moving their colonies away from nematodes).

The use of entomopathogenic nematodes against aboveground insects has also been analyzed by Arthurs et al. (2004). They analyzed 136 published greenhouse and field trials that used *Steinernema carpocapsae* (Weiser), and through the use of a general linear model showed that the nematode treatment efficacy depended on the target insect's habitat (bore holes > cryptic foliage > exposed foliage) and trial location (greenhouse > field studies). Relative humidity and temperature during and up to 8 h after application influenced the nematode infection rates, but the addition of spray adjuvants and nematode concentration did not explain a significant amount of variability in the efficacy of *S. carpocapsae*.

3. Effectiveness against nursery and greenhouse insects

The total annual crop sales for the greenhouse and nursery industry in the USA were estimated at over $6.2 billion in 1998 (van Tol and Raupp, 2005). Hardy nursery stock in the Netherlands and the United Kingdom—having the largest production areas in Europe—has an annual crop value of $1.1 billion (van Tol and Raupp, 2006). The nursery industry relies heavily on chemical pesticides. In contrast to greenhouse production, there are only few

Table 1
Major suitable target insects for entomopathogenic nematodes[a]

Market segment	Common name	Scientific name (family, genus, or species)
Apple	Codling moth	*Cydia pomonella* (L.)[b]
	Oriental fruit moth	*Grapholita molesta* (Busck)
	Peach fruit moth	*Carposina niponensis* (Walsingham)
Orchard and nut trees	Navel orangeworm	*Ameylois transitiella* (Walker)
	Pecan weevil	*Curculio caryae* (Horn)
	Litchi longhorn beetle	*Aristobia testudo* (Voet)
	Borers	Sesiidae, Cossidae, Cerambycidae
Citrus	Blue green weevil	*Pachnaeus litus* (Germar)
	Diaprepes root weevil	*Diaprepes abbreviatus* L.
Berries	Black vine weevil	*Otiorhynchus sulcatus* (Fabr.)
	Strawberry root weevil	*O. ovatus* (L.)
	Cranberry girdler	*Chrysoteuchia topiaria* (Zeller)
	Crown borers	Sesiidae
	White grubs	Scarabaeidae
	Common cutworm	*Spodoptera litura* (Fabr.)
Greenhouse and nursery plants	Beet armyworm	*Spodoptera exigua* (Hübner)[c]
	Black vine weevil	*O. sulcatus*
	Strawberry root weevil	*O. ovatus*
	Giant palmetto weevil	*Rhynchophorus cruentatus* Fabr.
	Leafminers	*Liriomyza* spp.[c]
	Sciarid flies	Sciaridae
	Banana moth	*Opogona* sp.
	Crown borers	Sesiidae
	Thrips	Thripidae[c]
	White grubs	Scarabaeidae
Mint	Mint flea beetle	*Longitarsus waterhousei* (Kutschera)
	Mint root borer	*Fumibotys fumalis* (Guenée)
	Root weevils	*Otiorhynchus* spp.
Mushroom	Sciarid fly	*Lycoriella* spp.
Turf	Armyworms	Noctuidae
	Black cutworm	*Agrotis ipsilon* (Hunagel)
	Sod webworms	Pyralidae
	Annual bluegrass weevil	*Listronotus maculicollis* (Kirby)
	Billbugs	*Sphenophorus* spp.
	Mole crickets	*Scapteriscus* spp.
	Cat flea	*Ctenocephalides felis* (Bouché)
	White grubs	Scarabaeidae
Forest	Large pine weevil	*Hylobius abietis* (L.)[d]
	Borers	Sesiidae
Vegetable and field crops	Cutworms	Noctuidae
	Sugar beet weevil	*Temnorhinus mendikus* (Gyllenhal)
	Sweet potato weevil	*Cylas formicarius* (Fabr.)

All soil applications except codling moth, beet armyworm, leafminers, thrips, large pine weevil and borers.

[a] Modified from Georgis (2004).

[b] Drenching fruit bins containing cocooned larvae.

[c] Cryptic or greenhouse conditions.

[d] Stump treatment.

biological control options available for nurserymen. One of the positive exceptions is the biological control of soil-borne pests, using entomopathogenic nematodes.

3.1. Black vine weevil (Coleoptera: Curculionidae)

Otiorhynchus sulcatus is one of the most important pest species of cranberries, strawberries, and nursery ornamentals in USA, Canada and Western Europe. An average of $25–70 million is spent annually in the USA and Canada to control this pest (Shapiro-Ilan et al., 2006) whereas approximately $0.5–2 million is spent yearly to protect against this insect in hardy ornamental production in the Netherlands (van Tol and Raupp, 2006).

In general, field tests showed that *Heterorhabditis* species are better than *Steinernema* species in controlling the

Table 2
Major factors affecting market expansion or penetration of entomopathogenic nematodes

Factors	Remarks
Efficacy	Certain product labels with unsuitable target insects Efficacy against certain insects significantly lower than standard insecticides Certain product labels with suboptimum recommended application rates
IPM programs	Limited efficacy and cost benefit field data
Formulation	Refrigeration requirements and limited room temperature shelf life Certain formulations with requirement to prepare nematode spray suspension over a period of time Suboptimum storage by distributors, dealers and growers resulting poor in nematode viability and efficacy
Usage	Certain product labels without proper application directions Application requirements such as temperature, moisture, irrigation, timing, and product coverage impractical in certain crops Improper handling, mixing and application by end-users
Cost	In general, products more expensive than standard chemical insecticides

larvae (van Tol and Raupp, 2006; van Tol et al., 2004). However, not all heterorhabditid species and strains are equally effective in spring applications compared to fall applications (van Tol and Raupp, 2006). Soil temperature is considered the most limiting factor for successful control of this pest (van Tol et al., 2004). An application in summer is not effective against the newly hatched larvae and the application in fall has only limited success because of lower soil temperatures (van Tol and Raupp, 2006). Nematode application in late spring is the optimum time for effective control of the weevil larvae. The first nematode products that became available for growers were giving inconsistent control because of low soil temperature. This has changed in recent years, and new products have became available that are effective at temperatures as low as 12 °C. The increased activity at low temperature has made application in the fall possible. Further selection of nematode strains with lower temperature activity would give growers wider application timing in the fall. van Tol and Raupp (2006) reported lack of continuous efficacy with nematodes applied in summer. It appears that low persistence and lack of nematode recycling in the host reduced their effectiveness against larvae that became available to nematodes several weeks after application. They concluded that nematode species or strains with high persistence ability will provide better results and increase grower acceptance.

3.2. White grubs (Coleoptera: Scarabaeidae)

White grubs are destructive pests of ornamental crops, shrubs and Christmas trees in USA and Canada. Mannion et al. (2001) evaluated *Heterorhabditis bacteriophora* Poinar and *Heterorhabditis marelatus* Liu and Berry and found that both provided poor to moderate control of the Japanese beetle, *Popillia japonica* Newman, infesting various species of potted woody trees at rates of 5×10^9 IJs/ha. Wright et al. (1988) investigated the use of various nematode species applied to potted Japanese yew a few days after inoculation with Japanese beetle and the European chafer, *Rhizotrogus majalis* (Razoumowsky). Control of Japanese beetle grubs with *Heterorhabditis heliothidis* (Khan, Brooks, and Hirschmann) ranged from 60 to 90% and 0 to 58% with *Steinernema glaseri* (Steiner). However, against the European chafer the control with the two nematode species ranged between 0 and 86%. Mannion et al. (2001) and Nielsen and Cowles, 1998 reported poor results with *H. bacteriophora* against Japanese beetle, European chafer and oriental beetle, *Anomala* (*Exomala*) *orientalis* (Waterhouse) in potted cotoneaster. Inability of the nematodes to persist or survive may have been the reason for the unsuccessful control. Application timing is critical for the successful use of nematodes against white grubs in nurseries and greenhouses.

3.3. Fungus gnats (Diptera: Sciaridae)

Fungus gnat larvae damage cuttings of various ornamentals and reduce root weight and vigor of a wide range of ornamentals. Larval feeding is believed to predispose the plants to attack by pathogenic fungi. *Steinernema feltiae* is an efficacious and economical replacement for chemical insecticides in the floriculture industry in The Netherlands, England and Germany (Jagdale et al., 2004). In the USA, the commercial success has been limited probably due to high temperatures in the greenhouses that affect nematode viability and the availability of numerous registered insecticides unlike Western Europe. Moreover, in greenhouse production, certain potting media have reduced the efficacy of the nematodes (Oetting and Latimer, 1991). Apparently, the media affect the survival and infectivity of the nematodes and/or provide ideal conditions for the development of the fungus gnats (Jagdale et al., 2004).

Determining an appropriate concentration, application timing and temperature is crucial in the cost effective control of fungus gnats in greenhouse production. A single application of *S. feltiae* (2.5×10^6 IJs/m^2) against the second, third, and fourth instar larvae and at temperatures below 25 °C produced consistently high level of control (Jagdale et al., 2004). *S. feltiae*, a cold-adapted nematode, has been successfully used to control fungus gnats at temperatures ranging from 12 to 25 °C (Jagdale et al., 2004). However, it is necessary to find effective warm-adapted nematode species to manage fungus gnats in the USA where greenhouse temperatures can exceed 30 °C during the summer. Recently, Jagdale and Grewal (unpublished data) found that *H. bacteriophora* (GPS 11 strain) and *Heterorhabditis indica* Poinar, Karunakar and David were significantly more effective than *S. feltiae* in controlling *Bradysia coprophila* (Lintner) infesting poinsettia and may be more effective at the higher temperature regimes.

Jagdale and Grewal (unpublished data) demonstrated that an early detection of the pest infestation is important to the successful use of nematodes against fungus gnats. They recommended the use of one to three sticky traps per 90 m^2 and to replace the traps on weekly intervals. *S. feltiae* was applied as a soil drench once the adults were observed on the traps. The application was repeated at 7- to 10-day intervals as long as the adults were detected on the traps.

3.4. Leafminers (Diptera: Agromyzidae)

Leafminers, *Liriomyza* spp. are among the major pests of field and glasshouse-grown vegetables and ornamental crops worldwide. Larval mining and adult stippling caused by the leafminers destroy leaf mesophyll, decrease the level of photosynthesis, and allow entry of plant pathogens. The use of various species and strains of steinernematids and heterorhabditids against soil-inhabiting prepupae and pupae stages of leafminers produced variable and inconsistent results (Head and Walters, 2003). In some cases, certain species provided significant control at high rates, but the cost was impractical to the end-users (Tomalak et al., 2005).

Greenhouse tests have demonstrated the potential of using nematodes, especially *S. feltiae*, as foliar treatments against the larval stages of various leafminers (Hara et al., 1993; Head and Walters, 2003; Williams and MacDonald, 1995; Williams and Walters, 2000). In general, to achieve reliable control, optimum spray volume is essential to allow the nematodes to come in contact with the larval stages. Maintaining high relative humidity (above 90%) in the greenhouse and/or moisture on the plants for at least 6–8 h after nematode applications is critical for successful control (Arthurs et al., 2004; Williams and Walters, 2000). The best control of *Liriomyza trifolii* Burgress was achieved with 2–4 weekly applications of *S. carpocapsae* or *S. feltiae* at 1×10^6 IJs/m^2 against the second and the third instars larvae (LeBeck et al., 1993; Williams and Walters, 2000). The second instar larva is the most nematode-susceptible stage of *Liriomyza* spp. (LeBeck et al., 1993). In addition, the use of adjuvants with the spray enhanced the efficacy and the persistence of the nematodes (Williams and MacDonald, 1995).

3.5. Western flower thrips (Thysanoptera: Thripidae)

Western flower thrips, *Frankliniella occidentalis* (Pergande) is the most important pest of ornamental plants worldwide. In addition to the risk of transmission of viruses, they feed on rapidly growing tissues of plants, leading to excessive aesthetic damage upon bud break. The use of various species and strains of steinernematids and heterorhabditids against soil-inhabiting prepupae and pupae produced low and inconsistence results (Piggott et al., 2000; Wardlow et al., 2001). As a result in recent years foliar applications of the nematodes targeting the larvae and the adult stages of the thrips were attempted with encouraging results (Bennison et al., 1998; Piggott et al., 2000; Wardlow et al., 2001). Although there is no published experimental data, a new formulation of *S. feltiae* is now commercially available against the western flower thrips (Shapiro-Ilan et al., 2006).

Based on Piggott et al. (2000) and Wardlow et al. (2001), an optimum spray volume and the use of a wetting agent adjuvant are essential to allow the nematodes to penetrate the growing points of the plants, the place in which the western flower thrips females lay there eggs. Wardlow et al. (2001) emphasized that 5–9 weekly applications of *S. feltiae* at 3.2×10 IJs/m^2 is needed to suppress thrips populations.

4. Effectiveness against sciarid and phorid flies in mushroom houses

Cultivation of edible mushrooms [*Agaricus bisporus* (Lange)] is an economically valuable industry with an annual production valued at $759 million in the USA and $305 million in the UK (Long et al., 2000). Amongst the species of sciarid flies that are found associated with mushrooms throughout the world, *Lycoriella auripila* (Fitch), *Lycoriella mali* (Fitch) and *Lycoriella solani* Winnertz, are the most significant species adversely affecting mushroom cultivation (Grewal, 2000). *L. mali* is responsible for about 20% annual crop loss in the USA (Cantwell and Cantelo, 1984). Although adult flies are found in the mushroom houses throughout the year, they generally infest them when freshly pasteurized or spawned compost is brought in. Larvae feed on the compost and destroy its structure and water retention capacity, which in turn inhibits mycelial colonization causing a significant reduction in mushroom yield. In addition, the larvae make mushrooms unsaleable by tunneling and feeding on the growing mycelial front, fruiting primordial, and stripe tissues (Grewal and Richardson, 1993). Larvae are most destructive to the casing, which is a 4–5 cm thick layer of peat-moss and chalk spread on the surface of spawned compost.

The use of appropriate nematode rates, application site, and application timing for the cost effective management of mushroom flies is economically important for mushroom growers. Several concentrations ranging from 1.5 to 3.0×10^6 *S. feltiae*/m^2 have been tested to achieve satisfactory control of sciarid infestations (Fenton et al., 2002; Grewal and Smith, 1995), with consistent efficacy at 3.0×10^6 IJs/m^2 applied 4–7 days after casing (single application) or half rate at casing and the other half 4–7 days after casing (split application). In most cases, the efficacy was comparable to the standard insecticide, diflubenzuron.

Currently in the UK, the application of *S. feltiae*-based product is recommended at 3.0×10^6 IJs/m^2 as a standard rate to manage sciarid flies. A genetically selected strain of *S. feltiae* produced similar levels of control of *L. solani* (92–99%) when applied at the recommended rate of 3.0×10^6 or at 1.0×0^6 IJs/m^2 (Tomalak, 1994).

Application of entomopathogenic nematodes has a major advantage over chemical pesticides due to their capacity to recycle and persist in the mushroom substrates and over time suppress the sciarid flies in the mushroom houses. Grewal et al. (1993) found that ScP strain of *S. feltiae* persisted better than the unselected strain in the casing layers infested with *L. mali*, and Tomalak (1994) observed the persistence and recycling of the same strain of *S. feltiae* for over 51 days in casings infested with *L. solani*. Jess and Kilpatrick (2000) found a correlation between increased total yield of mushrooms and nematode persistence. Recently, Fenton et al. (2002) reported a very high level of recycling and persistence of *S. feltiae* in mushroom houses and this correlated with the continuous suppression of sciarid flies throughout the experiment.

The phorid fly, *Megaselia halterata* (wood) is of secondary importance but it still causes considerable problems for mushrooms. Adults are very strongly attracted to compost or casing in which spawn is running. Gravid females lay eggs close to the growing mycelia and close to the surface of the compost. Larvae feed on mycelia, generally in lower layers of compost. Adult flies become most problematic when they enter the crop soon after spawn run. This results in a new generation.

Chemical insecticides including diazinon, malathion, and diflubenzuron are currently recommended as surface sprays to control phorid flies in the mushroom houses. However, recent withdrawal of these chemicals from the market prompted a need for alternative methods. Researchers have demonstrated that heterorhabditids and steinernematids, including *S. feltiae* can infect phorid fly larvae in mushroom production houses, but the level of control has been significantly lower compared to sciarid flies (Scheepmaker et al., 1998). Recently, Long et al. (2000) demonstrated that three isolates of *Steinernema* spp. caused approximate 75% reduction in phorid fly populations compared to controls. Further investigations may lead to selection of an effective species that is cost effective against phorid flies.

5. Effectiveness against vegetable insects

Numerous insect pests of economic importance are encountered in vegetable crop production. Being high value crops, the introduction of biological pest control agents such as entomopathogenic nematodes has stimulated great interest worldwide on both above- and below-ground pests. However, despite promising laboratory and field trials against soil pests, the use of commercial nematodes has been insignificant (Bélair et al., 2003).

5.1. Carrot root weevil (Coleoptera: Curculionidae)

The carrot weevil, *Listronotus oregonensis* (LeConte), is an important pest of carrot, celery, and parsley in northeastern North America. Adults overwinter on or near the soil surface, associated with plant material and debris. In the spring, they crawl over the soil surface, and later females deposit eggs on the plant petioles. The young larvae bore into plant crowns and roots or feed at the surface of larger roots. The spring migration of the adults from their overwintering sites into carrot fields provides the opportunity to infect them either through application of nematodes by spraying or use of baits. Application timing can have a marked effect on nematode efficacy. For example, early season application of *H. bacteriophora* provides greater plant protection for carrot and parsley (Miklasiewicz et al., 2002). In laboratory studies the larvae were more susceptible than adults and overwintered adults were substantially less susceptible than newly emerged and 2-month-old adults. Infected females still alive after 2 days stopped ovipositing (Boivin and Bélair, 1989). This last effect was especially interesting as most control approaches aim to prevent oviposition by females in the spring.

In Quebec, Canada, field application of *S. carpocapsae* (Weiser) as a drench or as a bait in muck-grown carrots at the rate of 4.4×10^9 IJs/ha reduced carrot weevil damage by 59% (Bélair and Boivin, 1995). In Ohio, USA, soil spray application of *S. carpocapsae* and *H. bacteriophora* in muck-grown carrot and parsley at the rate of 3.3×10^9 IJs/ha had no effect on yield, but *H. bacteriophora* treatments persisted longer and resulted in greater insect mortality and plant survival (Miklasiewicz et al., 2002).

Although the nematodes show some promise for controlling carrot weevil, they cannot compete against conventional chemical pesticides. Because in carrot production the economic threshold is less than 2% infestation the use of nematodes should only be considered under light insect pressure. The demand for organic vegetables has increased significantly in recent years, providing opportunities for nematode-based products.

5.2. Cabbage maggot (Diptera: Antomyiidae)

The cabbage maggot, *Delia radicum* (L.), is a cosmopolitan pest of radish, rutabaga and other cole crops. Eggs of the economically important first generation are deposited around and on the stems of early-season (April and May) plants. The larvae hatch and tunnel into root tissue and can reduce yield through plant stunting or death.

The cabbage maggot has been one of the most extensively studied targets for nematodes. Despite this, the level of control has remained variable (Shapiro-Ilan et al., 1996). *S. carpocapsae* and *S. feltiae* have been the most commonly used species in field evaluations. Control levels have generally been lower than the insecticide treatments (Vänninen et al., 1999), but in some cases were comparable (Bracken, 1990). Timing and conditions for nematode applications have to optimal because *D. radicum* larvae are only in the soil for a brief period. Organic root brassica production is a potential niche market for nematodes.

5.3. Sugar beet weevil (Coleoptera: Curculionidae)

The sugar beet weevil, *Temnorhinus* (=*Conorhynchus*) *mendicus* (Gyllenhal), is the major insect pest in all western Mediterranean countries, especially in southern France, Italy, Spain, and northern Africa. This species completes one generation per year and overwinters as adults in the soil. Chemical insecticides are effective only against the adults. All larval instars, pupae and newly emerged adults of *T. mendicus* are susceptible to nematodes (Curto et al., 1999) with the greatest efficacy being achieved by a spray against newly hatched larvae directly on the crop following irrigation or rainfall. Field application at 25 IJs/cm^2 gave 90–95% weevil mortality and was significantly better than the chemical insecticide treatments. Nematode-infected weevils were observed 1 year after application, and nematode persistence was greater in clay and loamy soils compared to peat soil. However, effective application with existing farm equipment and the availability of large amounts of nematodes at a low price are required before the nematodes can be used economically.

5.4. Colorado potato beetle (Coleoptera: Chrysomelidae)

Colorado potato beetle, *Lepinotarsa decemlineata* (Say), the key pest of potatoes, has four larval instars, the last of which drops from the plant and burrows into the soil for pupation. The beetle completes 1–3 generations per year, depending on latitude. Most studies have investigated soil treatments with various nematodes species against fourth instars (Wright et al., 1987). Foliar applications led to rapid desiccation of the nematodes, although anti-desiccants have been shown to increase the effectiveness of *S. carpocapsae* (MacVean et al., 1982). The field use of nematodes has been simulated in cages filled with soil against spring and summer generations of the beetle. Nematodes were sprayed on the soil surface 1 day before adding fourth instar larvae (Stewart et al., 1998; Wright et al., 1987). In these tests larval mortality was generally lower than in laboratory tests (79% with *S. carpocapsae* Mexican strain at 93 IJs/cm^2, and 67% with *H. bacteriophora* (=*heliothidis*) at 155 IJs/cm^2).

In a greenhouse test, *S. carpocapsae* formulated as a pesta formulation was applied against the prepupae (Nickle et al., 1994). Nematodes emerged successfully from the pellets and killed 94% of the prepupae at 82 IJs/cm^2. Berry et al. (1997) showed that *H. marelatus* was very effective against the beetle when applied at 50 IJs/cm^2. However, nematode persistence in the soil and reproduction in the spring generation of the larvae were not evident (Berry et al., 1997). In further studies, Armer et al. (2004a) also used 50 *H. marelatus*/cm^2 and reported a 50% reduction in adult beetle populations. Dissection of dead beetles from the field showed that nematodes did kill the prepupae and pupae, but no nematode reproduction occurred.

The effectiveness of nematodes in potato fields appeared to be reduced by various factors, such as the depth of beetle pupation (1–15 cm) and the migration of beetles from neighboring plants and fields (MacVean et al., 1982). In addition, Armer et al. (2004b) demonstrated that *H. marelatus* and its symbiotic bacterium, *Photorhabdus luminescens* (Thomas and Poinar), did not reproduce in beetle larvae and prepupae because of an inhibitory compound(s) in the beetle's hemolymph, thus excluding long-term control of the beetle.

5.5. Sweet potato weevil (Coleoptera: Curculionidae)

The sweet potato weevil, *Cylas formicarius* (Summers), is the most important insect pest of sweet potato. It can cause damage both in the field and in storage because its entire life cycle takes place within the stems and tubers and all three larval instars are present at the same time. Larval feeding induces terpenoid production in plants, so even slightly damaged tubers become unpalatable and are not marketable. The weevil completes 5–8 generations per year. Adults emerge from the pupal chambers or remain in the stems or tubers. Since the late 1980s, a number of research projects have evaluated the virulence, effectiveness, and persistence of steinernematids and heterorhabditids against this insect. Field tests have demonstrated that nematodes seek out and kill weevil larvae and pupae and reproduce in their cadavers. Experiments have demonstrated that a well-timed single application of nematodes may provide better control than multiple applications (Jansson et al., 1991). Nematodes are more effective than chemicals at reducing weevil densities, and heterorhabditids appear to be more effective and more persistent than steinernematids against both larvae and pupae. *H. bacteriophora* (HP88), *Heterorhabditis* sp. (Jansson et al., 1993), and *Heterorhabditis megidis* Poinar, Jackson, and Klein are particularly effective (Ekanayake et al., 2001). Research has demonstrated that nematodes have the potential for managing the sweet potato weevil in the field and on stored roots, but cost remains a limiting factor.

6. Effectiveness against the Diaprepes root weevil (Coleoptera: Curculionidae) in citrus

Several curculionid species feed on the leaves and roots of citrus trees. While few are of economic importance, the polyphagous Diaprepes root weevil, *D. abbreviatus*, is a major pest of citrus and numerous other crops in Florida and the Caribbean Basin (McCoy, 1999). Data regarding the geographical distribution and economic effect of the weevil are scarce, but more than 20,000 ha of citrus in all production regions of Florida were known to be infested in 1995 (Hall, 1995). The blue green weevil, *Pachnaeus litus* (Germar), is also pest of citrus and widely distributed in Florida.

Adults of Diaprepes root weevil feed on young citrus leaves and eggs are oviposited between two leaf surfaces, which are then glued together for protection. Eggs are generally produced from early summer into winter. Upon hatching, neonate larvae drop to and enter the soil where they feed on the fibrous and major roots over the next several months. Adults emerge from the soil throughout the year with peak emergence often occurring during spring (Duncan et al., 2001). Feeding by late instar larvae can severely damage roots and reduce yields. Moreover, wounding of the root cortex also favors infection by *Phytophthora* spp. The resulting pest–disease complex can kill trees and debilitate orchards to the point of unprofitability (Graham et al., 2003). Soil conditions such as poor drainage and flooding that are conducive to *Phytophthora* infection and root damage by anoxia also seem to favor increased population growth of this weevil.

Two aspects of the weevil's life cycle make the insect difficult to manage. (1) All life stages are active in orchards during all but the winter months and (2) adults and larvae are spatially separated and must be targeted individually either in the tree canopy or in the soil. Because adults emerge continuously from soil to produce offspring, which re-enter the soil, non-persistent control methods targeted at only adults or larvae can only briefly reduce the pest population density. Because persistent pesticides (e.g., deldrin and chlordane) are unavailable, combination of non-persistent tactics timed to kill both phases of the population is a strategy often used by growers. Because no chemical pesticides are registered in Florida to manage the soil-borne stages of the weevil, growers have widely adopted the use of commercially formulated entomopathogenic nematodes since they became available in 1990.

Steinernema glaseri, *S. carpocapsae*, and *H. bacteriophora* were the first species evaluated for control of the weevil (Bullock and Miller, 1994; Downing et al., 1991; Schroeder, 1992). Use of the latter two species, at rates ranging from 100 to 600 IJs/cm^2, suppressed the emergence of adult weevils in the field by as much as 60–80% for up to 1 year. Despite their widespread use, the efficacy of products containing *S. carpocapsae* and *H. bacteriophora* was less apparent in subsequent field trials (Duncan et al., 1996). In contrast, commercially formulated *Steinernema riobrave* Cabanillas, Poinar, and Raulston at rates of 100 or more IJs/cm^2 effectively reduced numbers of weevil larvae and adults (Bullock et al., 1999; Duncan and McCoy, 1996; Duncan et al., 1996). Of several species evaluated in bioassay and greenhouse trails, *S. riobrave* and a Florida isolate of *H. indica* were the most effective against the Diaprepes root weevil, and reproduction by *H. indica* in the weevil exceeded that of other species (Shapiro-Ilan and McCoy, 2000a,b). *S. riobrave* and *H. indica* are currently the only two nematode species marketed in the Florida citrus industry. *H. indica* is formulated as a paste and *S. riobrave* can be obtained in liquid or water dispersible granular formulations. In 1999, approximately 20% of the hectares infested with this weevil were treated with nematodes (Shapiro-Ilan et al., 2002). Populations of the blue green weevil are also reduced by application of nematodes (Duncan et al., 2002). Suppression of *Phytophthora nicotianae* (Waterhouse) in soil occurs simultaneously with a reduction of weevil larvae, presumably due to reduced insect damage to the root system (Duncan et al., 2002).

Field trials to evaluate efficacy of *S. riobrave* on sandy soils in which young trees were treated by hand at rates of 100–250 IJs/cm^2 resulted in 77–93% reduction of Diaprepes root weevil larvae 1 month after treatment (Duncan and McCoy, 1996; Duncan et al., 1996) and 48–100% suppression of adult weevils for more than 1 year (Bullock et al., 1999). Efficacy has been less apparent and more variable when *S. riobrave* and *H. indica* were evaluated on mature trees, ranging from 0 to 66% suppression of larvae or adults (Duncan et al., 2003b; McCoy et al., 2002). Mature trees are treated with lower rates of nematodes than young trees (typically 11–25 IJs/cm^2) due to the added cost of treating the larger area beneath the canopy. The reduced efficacy from lower rates likely accounts for some variation in the estimates of nematode efficacy, but additional factors are undoubtedly important. Quality control of formulated product has been an occasional problem (Duncan et al., 2003b). Application methods and spray volumes before, during and after treatment varied among experiments. Nematodes are applied to mature trees through various low volume irrigation systems or with various types of herbicide applicators, and there have been no comparative studies of the efficiency with which these systems deliver viable nematodes. Soil type and texture vary from deep, well-drained sands on Florida's central ridge to shallow, sometimes poorly drained soils of various textures in the coastal and inland 'flatwoods' regions. The many physical characteristics of soils interact in a wide variety of ways to influence nematodes behavior; however, coarse soil texture and high porosity generally favor the movement, persistence, and efficacy of nematodes (McCoy et al., 2002).

At least six species of nematodes have been identified in Florida citrus orchards, but information about natural regulation of Diaprepes root weevil by endemic nematodes is scarce (McCoy et al., 2000). A 4 year study of the profitability of nematodes augmentation to manage this weevil showed that endemic nematodes killed sentinel larvae in the citrus rhizosphere at rates as high as 50% per week, but that repeated application of *S. riobrave* reduced the prevalence of endemic nematodes (Duncan et al., 2003b). Most of the endemic nematodes species maintained higher prevalence than *S. riobrave* in the field, and some of them persisted and maintained infectivity much longer than *S. riobrave* in the absence of a host in soil microcosms (Duncan, unpublished data). Although *S. riobrave* increased the rate of biological control for a short time following its application in the field, competition between the exotic and endemic nematodes appears to have reduced the prevalence of nematodes during the long intervals between applications of *S. riobrave*, possibly reducing the net efficacy achieved by nematode augmentation. The results suggest a potential benefit from augmenting the nematodes community with species or strains that are not only effective in the short-term, but which are also capable of long persistence either because of inherent longevity or because they are better adapted to survive in Florida soils (Curran, 1993).

As nematode populations grow in response to the introduction and population growth of the weevil in an orchard, other components of the food web constrain the level of natural control achieved. The importance of endemic nematodes to the net biological control in a system augmented with exotic nematodes (Duncan et al., 2003b) underscores a need to better understand the forces that regulate endemic species as well as the post-application biology of exotic strains or species (Curran, 1993). Although some of the biotic agents and abiotic factors that regulate nematode population dynamics are known, their effects on nematode communities in nature are poorly understood (Kaya, 2002; Kaya and Koppenhöfer, 1996). In addition to exotic nematodes, free-living bactivorous nematodes can compete with the entomopathogenic nematodes in the insect host cadaver and may be significant regulators of nematode densities in Florida citrus groves (Duncan et al., 2003a).

Paenibacillus sp. appears to be widespread in phoretic association with heterorhabditid species (Enright and Griffin, 2005; Enright et al., 2003). Spore attachment by *Paenibacillus* sp. to the nematode cuticle impaired the nematode's motility and thereby reduced the population growth rate of *Steinernema diaprepesi* Nguyen and Duncan, a species prevalent on Florida's central ridge (El-Borai et al., 2006). Density dependent regulation of nematodes by some types of nematophagous fungi has been demonstrated, but the relative involvement of these antagonists in food webs involving insect pests and nematodes remains unknown (Jaffee et al., 1996). Further study of temporal and spatial patterns of known food web components (abundance of prey, nematode species, and their competitors and antagonists) is needed to help reveal key environmental factors and density dependent processes that regulate both insect and nematode numbers and to provide a basis to conserve and effectively augment the natural pest control by nematodes.

After more than a decade using nematodes to help manage Diaprepes root weevil, some Florida citrus growers are convinced that nematode augmentation is a valuable IPM tactic, whereas others consider augmentation to be ineffective. Research suggests that, depending on circumstances, both outlooks are valid. One of the few certainties is that growers apply nematodes in their orchards because they lack alternative pest control tactics. New IPM methods will be developed at the expense of nematode usage unless methods to increase nematode effectiveness are found. Although documented cases of outstanding control of this weevil by nematodes (e.g., Bullock et al., 1999; Duncan et al., 1996) are the exception, they demonstrate the high potential of nematodes for biological control. Advances in nematode production methods to permit the economical delivery of increased rates would greatly enhance the effectiveness of augmentation. Continued research to improve application methods and timing, discover or develop species and strains with superior efficacy and persistence, and identify habitats favorable to nematode augmentation are additional strategies likely to produce incremental improvements in the profitability of this tactic.

7. Effectiveness against turfgrass insects

A variety of wear-tolerant grass species are grown to provide permanent or semi-permanent managed ground cover for recreational spaces in the urban environment. Such turfgrass areas include sod farms, parks, cemeteries, lawns, golf courses, and athletic fields. Between golf courses and professional and homeowner lawn care, turf maintenance is a $45 billion industry in the USA alone. Large variations exist among the different turf maintenance systems in value, input, demands, damage thresholds, and, consequently, tolerances for pests. Because the damage thresholds are generally low, numerous insects are considered pests. Several important insect turf pests are amenable to control by nematodes. Those pest species that have received the most attention as targets for nematodes include white grubs (Coleoptera: Scarabaeidae), mole crickets (*Scapteriscus* spp.), billbugs (*Sphenophorus* spp.), and the black cutworm [*Agrotis ipsilon* (Hufnagel)]. Other pests that have been controlled experimentally with nematodes include annual bluegrass weevil [*Listronotus maculicollis* (Kirby)], cutworms and armyworms (Noctuidae), sod webworms (Pyralidae), and crane flies (*Tipula* spp.)

7.1. White grubs (Coleoptera: Scarabaeidae)

White grubs, the root-feeding larvae of scarab beetles, are serious turfgrass pests throughout the world. Some of the most serious pests are introduced species such as *P. japonica*, *A. orientalis*, and *R. majalis*, in the eastern USA. Most important species have an annual life cycle. The adults emerge in late spring or summer, and eggs are laid in the soil below the turf. By late summer, most larvae have developed into the third and final instar. After overwintering, the larvae may feed for a few more weeks before pupating in the soil. The extensive feeding activity of the larger larvae can kill large areas of grass especially under warm, dry conditions. In addition, vertebrate predators can tear up the turf to feed on the grubs even at relatively low larval densities.

White grubs are primarily managed with chemical insecticides. Organophosphate and carbamate insecticides have a relatively short residual in the soil and are more effective when applied against young larvae, i.e., first and second instars, at which time they can provide control in excess of 70% (Potter, 1998; unpublished data). The later the applications are made, the higher the variability in results and the lower the efficacy. Due to their toxicity many of these 'harder' chemicals are being phased out by regulatory agencies. In the USA, only the organophosphate trichlorfon and the carbamate carbaryl are still registered for white grub control but are likely to retain registration for a while. Over the last decade new types of insecticides have become available that have a much lower toxicity, i.e., the neonicotinoids imidacloprid and clothianidin and the insect growth regulator halofenozide. Because these compounds are less effective against older larvae (Potter, 1998) they are applied on a preventative basis, involving the treatment of large turf areas that may need only partial or no treatment. The preventative use makes these chemicals more expensive, but they are very effective (>80% control) and relatively safe, and therefore, an attractive management option, especially where cost is not a major issue (e.g., many golf courses). In many countries, these 'preventatives' are not registered for most turfgrass uses (e.g., Germany).

Attempts to use nematodes for inundative white grub control were triggered by the commercialization of entomopathogenic nematodes in the early 1980s. Generally, *Heterorhabditis* spp. and *S. glaseri* were more effective than *S. feltiae* and *S. carpocapsae* (Klein, 1993). However, most field tests in the USA concentrated on *S. carpocapsae* and *H. bacteriophora* because these species were available in large numbers from commercial companies. Georgis and Gaugler (1991) analyzed 82 field trials conducted against *P. japonica* between 1984 and 1988 and concluded that *H. bacteriophora* strains (at 2.5×10^9 IJs/ha) used under the right conditions were as effective as standard chemical insecticides, whereas *S. carpocapsae* was ill-adapted for white grub control. Since that time, much of the work has focused on discovery and evaluation of new species and strains, elucidation of factors affecting nematode efficacy, and determination of the interactions between nematodes and other control agents. At the same time, advances in production technology, particularly the development of liquid culture for *Heterorhabditis* spp., increased production efficiency, and making the use of nematodes for white grub control more feasible.

Recent studies have clearly shown that white grub species differ in their susceptibility against entomopathogenic nematodes and that the relative virulence of different nematode species also varies among white grub species (Grewal et al., 2002; Koppenhöfer and Fuzy, 2003a; Koppenhöfer et al., 2004; Koppenhöfer et al., unpublished data). Among white grub species that are important pest of turfgrass in the USA, *P. japonica* appears to be the most nematode-susceptible species, whereas larvae of other white grub species including *Cyclocephala* spp., *A. orientalis*, *R. majalis*, or Asiatic garden beetle, *Maladera castanea* (Arrow) appear to be less susceptible to the commonly used entomopathogenic nematodes (Cappaert and Koppenhöfer, 2003; Grewal et al., 2002; Koppenhöfer et al., 2000a,b, 2002, 2004; Koppenhöfer and Fuzy, 2003a,b; Shapiro-Ilan et al., 2002; Simard et al., 2001).

Grewal et al. (2006) give an extensive summary of studies on the efficacy of entomopathogenic nematodes against white grubs. In the following we consider good control as >70% control at a rate of $\leqslant 2.5 \times 10^9$ IJs/ha in the field. Nematodes that have provided good control of *P. japonica* include *S. scarabaei* Stock and Koppenhöfer (100%) (Koppenhöfer and Fuzy, 2003a), *H. bacteriophora* (GPS11) (34–97%) (Grewal et al., 2004), *H. bacteriophora* (TF) (65–92%) (Koppenhöfer and Fuzy, 2003a,c; Koppenhöfer et al., 2000a,b, 2002), and *H. zealandica* (X1) (73–98%) (Grewal et al., 2004). *S. scarabaei* is the only nematode species that has provided high field control of *A. orientalis* (87–100%) (Koppenhöfer and Fuzy, 2003a,b), *M. castanea* (71–86%) (Koppenhöfer and Fuzy, 2003b), and *R. majalis* (89%) (Cappaert and Koppenhöfer, 2003). Against northern masked chafer, *Cyclocephala borealis* Arrow, *H. zealandica* (X1) (72–96%), *S. scarabaei* (84%), and *H. bacteriophora* (GPS11) (47–83%) appear to be the most promising nematodes (Grewal et al., 2004; Koppenhöfer and Fuzy, 2003a).

White grub larval stage also can affect nematode efficacy, and the effect can vary with white grub species and nematode species. Koppenhöfer and Fuzy (2004) observed that *A. orientalis*-susceptibility to *H. bacteriophora* but not to *S. scarabaei* or *S. glaseri* (Steiner) decreased from second to third instars and from young third instars to older third instars. A decrease in susceptibility from *A. orientalis* second to third instars has also been observed for *Heterorhabditis* sp. Gyeongsan, *S. carpocapsae* Weiser, *S. glaseri*, and *Steinernema longicaudum* Shen and Wang (Lee et al., 2002). For *P. japonica* the decrease in *H. bacteriophora*-susceptibility was not significant in one study under laboratory conditions (Koppenhöfer and Fuzy, 2004) but was significant in another study under laboratory and field conditions (Grewal, unpublished data). Grewal et al. (2004) observed higher mortality of second instar than third instar *P. japonica* with *H. bacteriophora* (54–97 vs. 34%) but no clear difference for *H. zealandica* (73–98 vs. 75%). In other white grub species nematode-susceptibility has been observed to increase from second to third instars, e.g., *S. scarabaei* vs. *M. castanea* (Koppenhöfer and Fuzy, 2003b), *H. bacteriophora* vs. *Maladera matrida* Argaman (Glazer and Gol'berg, 1989, 1993), and *S. glaseri*, *Heterorhabditis* sp. NW-European group, and *H. bacteriophora* vs. *Phyllopertha horticola* L. (Smits et al., 1994).

Various biotic and abiotic factors can affect nematode efficacy against white grubs. Thatch, an accumulation of organic matter between the soil and turfgrass foliage, restricts nematode downward movement and its thickness is negatively related to nematode efficacy (Georgis and Gaugler, 1991). Nematodes, especially *H. bacteriophora*, become increasingly ineffective for white grub control as soil temperature drops below 20 °C (Georgis and Gaugler, 1991). *H. bacteriophora* has been observed to be more effective against *P. japonica* in fine-textured soils, probably because finer soils retain moisture better and restrict nematode movement to the upper soil layers where most of the white grubs can be found (Georgis and Gaugler, 1991). Irrigation volume and frequency and soil moisture are positively related to efficacy (Georgis and Gaugler, 1991; Grewal et al., 2002) with a minimum of 0.74 cm of post-application irrigation required for establishment of the nematodes in turfgrass (Shetlar et al., 1988).

Despite considerable efforts in research and development, nematode use against white grubs is limited. The major reason for this has been competition from chemical insecticides that are easier to use and generally cheaper. In the USA, *H. bacteriophora* applied under the right conditions has provided good control levels of *P. japonica*, one of the major white grub pests. However, in vitro products containing *Heterorhabditis* spp. cost upwards from \$500 per ha, four times as much as similarly effective organophosphate and carbamate insecticides and twice as much as the preventatives, imidacloprid, clothianidin, and halofenozide. As a result, commercial use of nematodes has been extremely limited. Several small companies produce *Heterorhabditis* spp. for use against white grubs but the extremely high price of these in vivo produced nematodes (⩾\$1000/ha) restricts their use to small area applications such as in a home lawn setting. In Japan, *S. glaseri* has been successfully marketed for white grub control because of limitations on the use of chemical insecticides on golf courses, but since the registration of imidacloprid sales have declined. In Germany, where no insecticides are available for white grub control on golf courses, a product based on *H. bacteriophora* is commercially available.

The potential for improving nematode utility in the future (e.g., reduced production costs, more pathogenic nematode species and strains, and better understanding of white grub-nematode interactions) appears bright. However, the success of nematodes as biopesticides for white grubs is likely to remain limited by competition from chemical insecticides. *Steinernema scarabaei* has shown exceptionally high efficacy against a wide range of white grub species including many species that cannot be controlled effectively with presently available nematodes (Koppenhöfer and Fuzy, 2003a; Koppenhöfer et al., 2004). Unfortunately, attempts at in vitro production of this species have thus far been unsuccessful. A more promising future for nematodes in white grub management may lie in developing alternative approaches to their use as biopesticides. For example, conservation and, even better, manipulation of the widespread natural nematode populations in turfgrass could be used to buffer white grub outbreaks.

7.2. Mole crickets (Orthoptera: Gryllotalpidae)

Mole crickets, *Scapteriscus* spp., were accidentally introduced into Florida from South America around 1900 and have become the most important turfgrass insect pests throughout the coastal plain region of the southeastern USA. Damage is caused by adults and nymphs feeding on grass roots and shoots and through their extensive tunneling activity. There is one generation per year. After egg-laying in spring, the adults die off. The nymphs develop during early summer with the first adults appearing in late summer. Overwintering occurs primarily in the adult (tawny mole cricket, *Scapteriscus vicinus* Scudder) or nymphal stage (southern mole cricket, *S. borellii* Rehn and Hebard).

The best time to control mole crickets with conventional, short-residual insecticides is mid-summer, after most of the eggs have hatched but when the nymphs are still small. At this time the organophosphate acephate provides 50–60% control and the pyrethroid bifenthrin 60–70% control, with other pyrethroids being somewhat less effective. Imidacloprid provides 80–85% control when applied close to egg hatch. The present standard insecticide is the phenyl pyrazole fipronil that provides around 90% control and has 3–4 months of residual activity. The pyrethroids, imidacloprid, and fipronil are not likely to lose registration any time soon and are the major competitors for the nematodes.

First attempts at controlling mole crickets with nematodes were made with *S. carpocapsae* and provided an average of 58% control at 2.5×10^9 IJs/ha (Georgis and Poinar, 1994). Superior activity was later found with *Steinernema scapterisci* Nguyen and Smart and *S. riobrave* (average 75% control at 2.5×10^9 IJs/ha) (Georgis and Poinar, 1994). While *S. riobrave* only provides curative control of mole crickets because it does not reproduce in them, *S. scapterisci* proved to be an excellent agent for inoculative releases (Parkman and Smart, 1996; Parkman et al., 1996). In a classical biological control program, *S. scapterisci* was successfully established after inundative applications, application of *S. scapterisci*-infested cadavers, and using electronic mating callers to attract mole crickets to the site of application (Parkman et al., 1993b). In addition, *S. scapterisci* was dispersed by infected mole crickets to create new foci of infection (Parkman et al., 1993a).

Steinernema scapterisci efficacy is affected by mole cricket species and developmental stage (Parkman and Frank, 1992). *Scapteriscus abbreviatus* Scudder is less susceptible than *S. vicinus* and *S. borellii* in laboratory studies. In addition, *S. borellii* was found to be more susceptible than *S. vicinus* in field studies, probably because the greater activity arising out of its predatory behavior increased its chances of contact with the ambusher, *S. scapterisci*. Nymphal mole crickets are substantially less susceptible to infection than adults to *S. scapterisci*.

Steinernema scapterisci is an ideal control agent for pastures and turfgrass areas that can tolerate some mole cricket damage. A commercial product based on *S. scapterisci* was introduced in 2003. In pastures, the potentially biggest market, the nematodes are applied using slit injectors in strips covering 12.5% of the area. The nematodes then spread throughout the pasture over a period of several years. This approach reduces the cost to around \$62/ha, considerably lower than chemical insecticides that provide only short-term suppression. In the turf market, *S. scapterisci* is applied to low profile and environmentally sensitive areas on golf courses, sod farms, and recreational areas at a rate of 2.5×10^9 IJs/ha (cost \$500/ha). In more damage sensitive areas, *S. scapterisci* use is likely to remain limited due to the competition from the more effective but similarly expensive insecticide fipronil ($550/ha). The nematodes have to be applied in spring or fall when adults are present, whereas control measures are typically necessary in summer against nymphs.

7.3. Billbugs (Coleoptera: Curuclionidae)

Billbugs, *Sphenophorus* spp., are important turfgrass pests throughout much of the USA and Japan. Damage is caused by the young larvae feeding inside the stem and crown and the older larvae feeding externally on the belowground parts of the plant. The bluegrass billbug, *S. parvulus* (Gyllenhal), is an important pest of cool-season grasses, particularly Kentucky bluegrass and perennial ryegrass, in the northern half of the USA. It overwinters in the adult stage, becomes active around late April, and most egg laying is done between early May and early July. The older larvae are most abundant in the soil from around early July to early August, and damage usually becomes apparent from late June into August. Studies on other billbug species that may damage cool-season grasses are very limited. The hunting billbug, *S. venatus vestitus* Chittenden, is a pest of warm season grasses, in the southern USA, but in Japan, it is the most important insect pest on golf courses. In the northern parts of its range, *S. venatus vestitus* has one generation per year with a life cycle similar to that of *S. parvulus*. In the southern parts of its range, it primarily overwinters in the adult stage with some larvae overwintering, and it can have several overlapping generations per year.

For billbug control in the USA, the organophosphate, chlorpyrifos, and several pyrethroids are available for preventative applications against the overwintered adults, imidacloprid, clothianidin, and halofenozide are available for preventative applications against the young larvae inside the plants, and carbaryl, is available for curative control against the older larvae in the soil. In Japan, no chemical insecticides were available until the recent registration of imidacloprid for preventative larval control.

No detailed studies on billbug-nematode interaction have been published, but it appears that the larvae are more susceptible to nematode infections than the adults. In field tests in Ohio, USA, targeted against the larvae in the soil, control of *S. parvulus* by *S. carpocapsae* (average 78%) and *H. bacteriophora* (average 74%) was similar to that by standard insecticides (Georgis and Poinar, 1994). In Japan, *S. carpocapsae* has been more effective for control of *S. venatus vestitus* than standard insecticides (average 84 vs. 69% control) (Yamanaka, pers. comm.). Use of nematode products containing *S. carpocapsae* and *H. bacteriophora* against billbugs is limited in the USA, whereas *S. carpocapsae* has been the primary means of billbug control on golf courses in Japan. The main reason for this difference is the availability of effective insecticides for billbug control in the USA and lack thereof in Japan until recently. In addition, favorable environmental conditions (temperature and rainfall) and the adoption of 'nematode-friendly' application protocols, i.e., immediate watering after spraying and generally very careful following of label instructions have optimized nematode efficacy in Japan (Yamanaka, pers. comm.). However, *S. carpocapsae* sales for billbug control have significantly declined since the registration of imidacloprid for turfgrass uses. In the USA, use of nematode products for billbug control is likely to remain limited by the availability of several chemical insecticides that are easier to use and generally cheaper.

7.4. Black cutworm (Lepidoptera: Noctuidae)

The black cutworm is a perennial problem on the close-cut bentgrass of golf course greens and tees throughout the world. The larvae dig burrows in the thatch or soil and emerge at night to eat the grass blades and stems around the burrow. Of primary concern is that the feeding activity of the larvae interferes with ball roll on greens. The black cutworm has multiple generations per year. In the USA several organophosphates and pyrethroids, carbaryl, and the biorationals halofenozide and spinosad are available for black cutworm control. Availability of insecticides in other countries varies. Georgis and Poinar (1994) reported that *S. carpocapsae* is highly effective for black cutworm control (average 95%). Nevertheless, nematodes are not widely used for black cutworm control because damage thresholds on golf course tees and especially greens are so low that golf course superintendents will prefer to use chemical insecticides that provide even better and more consistent control than *S. carpocapsae*. This will continue until expectations and attitude of their clientele changes.

8. Effectiveness against cryptic habitats insects

A multitude of insect pests utilize cryptic habitats for all or a portion of their life cycle. These habitats include leaf litter, under bark, within galleries, nut mummies, buds and flowers, fruit bins, cracks and crevices of structures, and several others (Mráček, 2002). In the protection of such locations entomopathogenic nematodes are less vulnerable to desiccation and more likely to find a host than in exposed habitats. In many crops and environments, nematodes have provided acceptable control against various insect species.

8.1. Codling moth (Lepidoptera: Tortricidae)

Codling moth, *Cydia pomonella* (L.), is one of the most serious worldwide pests of apple, pear and walnut. It has traditionally been controlled using organophosphate and other broad spectrum insecticides. The need for alternative interventions has included development of biological control agents. Codling moth utilizes cryptic habitats for most of its developmental stages. Mature fifth instars exit the fruit and seek sites in which to spin their cocoons such as under and within the bark of trees, cracks in wooden supports, leaf litter and other cryptic habitats. Overwintering by mature larvae that leave the fruit in late summer or early fall takes place in these habitats and pupation ensues the following spring.

Field trials with *S. carpocapsae* and other nematode species against diapausing codling moth larvae in natural and artificial substrates have demonstrated the utility of nematodes for codling moth control (Kaya et al., 1984; Lacey et al., 2006a; Unruh and Lacey, 2001). Lacey et al. (2000) have developed protocols for the evaluation of nematodes in orchards using codling moth sentinel larvae. In orchards, the principal limiting factors of nematodes are low temperature (<15 °C) and desiccation. Nematodes have also been efficacious in controlling cocooned codling moth larvae in fruit bins which are treated by submersion or drenching, and subsequently kept damp and at 15–25 °C for 24 h (Cossentine et al., 2002; Lacey and Chauvin, 1999; Lacey et al., 2006b). Formulation to retard desiccation and utilization of cold-tolerant nematode strains has improved efficacy (Lacey et al., 2006a,b).

8.2. Navel orangeworm (Lepidoptera: Pyralidae)

The navel orangeworm, *Ameylois transitella* (Walker), is a serious pest of almond, pistachio and fig in California. It utilizes nut mummies (unharvested nuts left in the orchard) for larval development and pupation sites in the fall, throughout the winter and in the spring. Removal of the nut mummies from trees and subsequent flail mowing or plowing destroys many of the larvae, but significant numbers survive to infest nuts in the following season. Siegel et al. (2004) demonstrated efficacy of nematodes, especially *S. carpocapsae*, for control of the navel orangeworm exceeding that of sanitation and plowing.

9. Effectiveness against forest insects

9.1. Caterpillar and sawfly species

The use of entomopathogenic nematodes in forestry has produced contrasting results that generally depend on targeting the correct life stage of an insect. Often foliar applications against insects such as the spruce bud moth *Zeitraphera canadensis* Mutuura and Freeman have provided poor control. However, more encouraging results have been obtained by targeting life stages that develop in habitats that harbor more favorable conditions for nematodes. For example, nematodes were injected in a gel suspension into the winter nests of the pine processionary caterpillar, *Thaumetopoea pityocampa* (Denis and Schiffermuller), a serious pest of pines in the Mediterranean area, resulting in very promising control (Triggiani and Tarasco, 2002). Encouraging results were also reported against prepupae of the web-spinning larch sawfly, *Cephalcia lariciphila* (Wachtl), in Wales (Georgis and Hague, 1988).

9.2. Large pine weevil (Coleoptera: Curculionidae)

The large pine weevil, *Hylobius abietis* L., is a widely distributed pest of plantation forestry occurring throughout Europe and Asia and is often regarded as being the most serious pest in conifer plantation establishment. In the UK the weevil is the only forest pest for which prophylactic treatment with insecticide is routine. A similar pest status is occupied by *Hylobius congener* Dalla Torre in North America, where work targeting the adult weevil via treating conifer transplants with entomopathogenic nematodes has been undertaken (Eidt et al., 1995). After oviposition in spring, the large pine weevil develops in the stumps and roots of dying and dead conifers, passing through four or five larval molts before pupation (Bejer-Petersen et al., 1962). In the UK and southern Scandinavia, adults emerge about 18 months after oviposition. The adults may live for up to 4 years, feeding on the bark and cambium of any woody plant, with a preference for conifers.

In mature forest stands, weevils are typically present in low numbers, as damaged or fallen conifer trees are a relatively sparse resource. However, coniferous forests in northern Europe are harvested and regenerated via the clear-cutting of a site before re-planting. This practice intensifies the potential for damage by producing a large supply of stumps and roots for the insects' development while reducing material suitable as food for the adults. The volatiles released from cut tree attract adult weevils to oviposit in the stumps. As it takes at least a year for the larvae to develop (Bejer-Petersen et al., 1962), many adults will be present when the site is re-planted. The adults feed on the vulnerable transplanted seedlings, weakening or even killing the trees. In the absence of protection up to 100% of transplants die and it is estimated that such losses would cost the British forestry industry £12 million per year.

Protection in the UK generally involves the use of synthetic pyrethroids that offer direct plant protection. Permethrin was the most widely used pyrethroid until the end of 2003 when its use in forestry was no longer allowed within the European Union. The most likely replacement to be adopted, α-cypermethrin, is considerably more expensive. There is no evidence that current control measures have any significant effect on overall insect populations as the insecticides merely act as anti-feedants (Leather et al., 1999) and the immature stages in the cryptic habitat are protected from chemical insecticides. In addition, the Forest Stewardship Council (FSC) provides a certification system for forestry and forest products that promotes the adoption of environmentally friendly, non-chemical pest management methods. In response to this, several years ago Forest Research UK (an agency of the Forestry Commission) initiated a research program to use entomopathogenic nematodes for biological control of this weevil.

Until recently, the majority of research in biological control of the weevil targeted the adult weevils (Collins, 1993) as they spend relatively long periods within the soil.

But this strategy proved ineffective because establishment of infection takes too long (Brixey, 2000) to prevent feeding damage and oviposition. Furthermore, larvae and particularly pupae are more susceptible to entomopathogenic nematodes than adults (Brixey, 2000; Pye and Burman, 1978); yet, and at least 25 days should be allowed to achieve maximum level of infection (Brixey, 2000). Targeting the larval/pupal population removes the potential for damage before the insects reach the adult stage. Entomopathogenic nematodes have shown much promise in controlling this weevil due to their ability to infect larvae within the galleries under the bark of roots and stumps (Collins, 1993).

Control of the large pine weevil is achieved via a single application of nematodes to each re-forestation site at a rate of 3.5×10^6 IJs in 500 ml of water around the base of every stump, equivalent to 7.5×10^9 IJs/ha. The spot treatment reduces non-target effects and keeps environmental impacts to a minimum. Pupae are targeted via applications during late spring to summer 1 year after felling. The nematodes are able to penetrate through a packed layer of wood fibers and grass into the pupal chamber that is located in the sapwood (Pye and Burman, 1978). The pupal stage lasts only a few weeks but occurs fairly synchronous throughout the UK regardless of the rates of larval development. Field trials have demonstrated that the window for effective application of nematodes is between mid-May and early July. Reducing the number of nematodes or the volume of water applied to each stump would lead to considerable cost savings and is the subject of further research.

Three nematode species that are commercially available in the UK can infect, kill and reproduce in the larvae. *Steinernema carpocapsae* and *S. feltiae* at 7.5×10^9 IJs/ha gave similar levels of infection in field populations (53–56%) but the efficacy of *H. megidis* was substantially lower (Brixey, 2000). Because *S. carpocapsae* is the easiest and cheapest to produce it has been chosen as the principal control agent for further trials.

Forest restocking sites pose many problems in terms of access, with sites often on soft ground containing ditches, substantial debris after the felling, and very high stumps. A system has been developed using a forwarder mounted spray rig and delivering nematodes to the target through hand-held lances. Any site felled by a harvester (ca. 70% of coniferous plantations in the UK) should be suitable for management using the current application system. On sites with firm dry ground, the use of large tractor units further reduces application costs significantly. Using this method, reductions in adult emergence in the region of 60–75% have been achieved. During 2003, around 150 ha of UK restocking were treated using this system, at an average application rate of 6.5 ha/day. This annual area treated is likely to increase rapidly once success has been demonstrated.

A number of factors will determine the rate at which the system is adopted. Once the success of nematodes has been demonstrated, the total cost of their use compared with alternative systems will be important. *Steinernema carpocapsae* will cost approximately $625–875/ha with an additional $120–150/ha for their application. The use of α-cypermethrin to protect plants is likely to cost around $500/ha. To reduce the overall cost of the use of nematodes in forestry, the Forestry Commission has invested in a different production system that should reduce the overall cost.

With careful consideration given to the timing of nematode treatment and application technique, an average 70% reduction in weevil emergence using *S. carpocapsae* has been achieved. The use of nematodes for weevil control should be part of an integrated management system including improving silvicultural techniques currently employed (Heritage and Moore, 2001). An effective biological control strategy will require the monitoring of weevil development to predict accurately the time of pupation. Nematode applications should occur at least 4 weeks before weevil emergence, and therefore, they cannot be applied as part of the felling operation. Weevil adults may live several years and move considerable distances, and as a result, may re-invade treated sites from adjacent untreated areas. Consequently, nematodes may be slightly less effective when used at the perimeter of the treated area. Where possible, entire forest blocks should be managed using nematodes to minimize this edge effect.

Recently, *Steinernema kraussei* Steiner, a nematode that favors coniferous woodlands (Stock et al., 1999), became commercially available as a biocontrol agent in the UK. This nematode is more effective at lower temperatures than the other available nematodes (Mráček et al., 1999) and may be of paramount importance for the Northern European climate. This similarity in habitat preference between weevil and nematode is very promising and could result in *S. kraussei* being very effective control agent against the large pine weevil.

10. Effectiveness against animal pests

Most arthropod pests of veterinary importance are controlled largely through spray and dip applications of chemical pesticides, but the development of resistance and the possibility of contaminating milk and meat are issues associated with most broad spectrum pesticides. Non-chemical approaches such as, controlling animal movement, quarantine and slaughter offer some level of pest reduction. Microbial control of arthropod pests of animals with several entomopathogens including nematodes was reviewed by Pinnock and Mullens (2000) with presentation of protocols for evaluation under field conditions.

10.1. Ticks, Ixodidae

Ticks are economically very important pests mainly as vectors of different animal and human disease organisms. They are obligatory blood-sucking arthropods with three blood-sucking stages: larvae, nymphs and adults. In certain species, all three stages drop to the ground when fully engorged, whereas in others only two stages or only fully engorged adults exhibit this behavior. For much of the time following the blood meal ticks rest on the soil surface which is an ideal situation infection by entomopathogenic nematodes.

To date there are no field test data available to demonstrate the practicality or cost effectiveness of nematodes for tick control. However, various laboratory and simulated field conditions tests have been conducted and the results are encouraging for future implementation of nematodes in tick management programs. Entomopathogenic nematodes are pathogenic to more than 16 ixodid tick species from six genera and three argasid species from two genera. In general, heterorhabditids were more virulent than steinernematids against ticks (Samish et al., 1998, 2001). Nematode virulence against ticks varies considerably among tick species and their developmental stages. For example, fully engorged argasid and ixodid female ticks were more susceptible to nematodes than the unfed adults, whereas preimaginal stages were the least susceptible (Samish et al., 1999b). Moreover, the females were more susceptible during their pre-ovipositional period than during oviposition.

Entomopathogenic nematode efficacy declined when soil moisture was below 8% (Hassanain et al., 1999) or when 25% cattle manure or 40–50% silt was added to sandy soil (Samish et al., 1998). Studies have indicated that ticks must be exposed to nematodes for extended periods (up to 32 h) to achieve the highest level of control (Samish et al., 1999a). Nematodes do not reproduce in infected ticks (Kaaya et al., 1999), and can therefore, only be used as biocidaql agents.

10.2. House fly (Diptera: Muscidae)

The house fly, *Musca domestica* (L.), is common in animal-rearing farms. The adults are a nuisance to the public and can transmit several disease organisms of humans and animals. Larvae develop in manure and other organic matter and the life cycle can be completed in as little as 7–10 days depending on temperature.

Geden et al. (1986) demonstrated that house fly larvae and adults are susceptible to entomopathogenic nematodes. In laboratory assays, nematodes that were applied to moist filter paper or animal manure were effective in killing this insect with the most susceptible stages being second and third instars and adults (Geden et al., 1986). However, the results of field application of entomopathogenic nematodes against this insect have been variable. Belton et al. (1987) demonstrated promising reduction of fly populations in chicken barns 10 weeks after the application of *H. heliothidis* (= *H. bacteriophora*) to manure. In contrast, Georgis et al. (1987) concluded that poor survival and limited movement of the nematode infective stage in poultry manure made them unsuitable as biological control agents against filth flies. In a later study, Renn (1995) demonstrated that formulation of the nematodes in calcium alginate partially helped to overcome the problem of nematode use in manure.

Targeting adult flies appears to offer more potential for controlling *M. domestica* using entomopathogenic nematodes. Nematodes placed in house fly bait were compared to a commercial bait of methomyl against the adults in a pig farm in the UK. Baits treated with either *S. feltiae* or *H. megidis* provided significantly greater control of the adult population compared to methomyl (Renn, 1998). High porosity bait substrates were more efficient than low porosity baits (Renn and Wright, 2000). Accordingly, advanced delivery systems that allow the nematodes to persist in the environment may further improve the chances of using the nematodes against filth flies.

10.3. Cat flea (Siphonaptera: Pulicidae)

Fleas are important pests of human and animals. The adult stage takes blood meals from dogs, cats, and human, but may spend considerable time away from the mammalian host. Eggs are deposited in organic matter, including the thatch layer of lawns. Larvae feed on organic matter and pupate within cocoons in or near the larval habitat. Silverman et al. (1982) reported the susceptibility of the cat flea, *Ctenocephalides felis* (Bouché), to entomopathogenic nematodes. Infective juveniles added to potting soil, sand, or gravel substrates inoculated with different developmental stages of cat fleas caused 70–100% reduction in adult emergence. Larvae and pupae or pre-emerged adults in the cocoon were also shown to be susceptible to nematodes by Henderson et al. (1995). Despite limited data, nematode-based products are labeled for the control of cat flea larvae and pupae in yard and garden habitats. Nematodes could play an important role in IPM programs along with other products such as shampoos, insect growth regulators and tablet or liquid insecticides.

11. Slugs

Slugs are widespread pests of a variety of crops (wheat, oilseed rape, lettuce, Brussels sprouts and home gardens) when moisture is adequate. Eggs produced by adult slugs hatch into juveniles that resemble adults in all but size and sometimes color. In the absence of sufficient water they move deep into the ground in search of moisture and do not cause crop damage under these conditions (Wilson and Gaugler, 2000). *P. hermaphrodita* (Schneider) is the only parasite developed as a biological control agent of slugs (Wilson et al., 1993a) and has been available commercially in Europe since 1994. The nematode is a parasite of slugs, and only the infective juvenile can infect them in the natural environment (Tan and Grewal, 2001a). It can be mass-produced in vitro on the Gram-negative bacterium, *Moraxella osloensis* Bøvre and Henriksen (Wilson et al., 1993b, 1995c). This nematode–bacterium complex is a lethal combination against slug pests, especially the common gray garden slug *Deroceras reticulatum* (Müller). The infective juvenile penetrates into the slug through the shell cavity in the posterior mantle region of *D. reticulatum* (Tan and Grewal, 2001a). The juvenile develops into a self-fertilizing hermaphrodite and reproduces. As the disease progresses, the mantle region of the slug swells, and 7–21 days after infection, the slug dies. Nematode progeny then feed on the entire slug cadaver. When the food resources are depleted, the nematodes produce infective juveniles that leave the cadaver in search of new hosts.

11.1. Relationship of slug nematode and symbiotic bacterium

Moraxella osloensis produces an endotoxin, which is tolerant to heat and protease treatments and responsible for slug mortality (Tan and Grewal, 2002, 2003). Aged (e.g., 3 day) *M. osloensis* cultures were pathogenic to *D. reticulatum* after injection into the shell cavity or hemocoel. Co-injection of penicillin and streptomycin reduced the virulence of the bacteria to the slug. Axenic juveniles of *P. hermaphrodita* were non-pathogenic to the slug, and the virulence of the infective juveniles depended on the number of viable *M. osloensis* they carried. Thus, *P. hermaphrodita* serves as a vector for *M. osloensis* into the shell cavity and the bacterium is the main killing agent in the nematode–bacterium complex (Tan and Grewal, 2001b). The mutualism between *P. hermaphrodita* and *M. osloensis* is parallel to the association between the entomopathogenic nematodes in the genera *Heterorhabditis* and *Steinernema* and their associated bacteria in the genera *Photorhabdus* and *Xenorhabdus*, respectively.

11.2. Efficacy

Detailed protocols for evaluation of *P. hermaphrodita* under field conditions are presented by Wilson and Gaugler (2000). The infective stage is typically applied at a rate of 3×10^9/ha. As is the case with entomopathogenic nematodes, *P. hermaphrodita* are most efficacious when applied to moist soil followed by up to 5 cm of irrigation. Soil temperatures above 25 °C can be detrimental to the nematode.

Phasmarhabditis hermaphrodita has been field tested in Europe in many crops including wheat (Wilson et al., 1994, 1996), lettuce (Wilson et al., 1995a), oilseed rape (Wilson et al., 1995b), strawberry and sugar beet (see Wilson and Gaugler, 2000). Infective stages have been successfully applied with back pack sprayers and conventional hydraulic spray equipment with reduced pressure (100 kPa) and larger filter mesh and through irrigation lines (Wilson and Gaugler, 2000). In most cases, the nematode provided control equivalent to or superior to the chemical standards. Although the nematode may take more than 7 days to kill slugs, feeding by slugs is strongly inhibited within a few days of infection, providing rapid crop protection. Commercial formulations of *P. hermaphrodita* (Nemaslug) are produced by Becker Underwood.

12. Conclusions

Progress in nematode commercialization during the 1990s was substantial. Development of large-scale production technology and easy-to-use formulations led to the expanded use of nematodes. The emphasis to use proper rates and adopt standard quality control procedures provided opportunities for researchers and growers to generate reliable results. These developments led to the use of nematodes against various insect species. This progress was made possible by the collective effort of industries with universities and federal agencies, coupled with a socio-political atmosphere favoring a reduction in the use of chemical pesticides. Despite this progress, the reality is that arthropod- and slug-parasitic nematodes have limited share in most markets. Limited market is attributed to product cost, poor or inconsistent efficacy, refrigeration requirements for most formulations, use of suboptimal nematode species, and lack of detailed information on how to use them effectively. Kaya and Gaugler (1993) indicated that there is a need for more in-depth basic information on their biology, including ecology, behavior, and genetics, to help understand the underlying reasons for their successes and failures as biological control agents.

When an entomopathogenic nematode is used against a pest insect, it is critical to match the right nematode species against the insect pest (Kaya and Gaugler, 1993). Some nematode species have a limited host range. For example, *S. scapterisci* is effective against mole crickets, but not against other insects (Nguyen and Smart, 1991). Proper match of the nematode to the host entails virulence, host finding, and ecological factors. If a nematode does not possess a high level of virulence toward the target pest, there is little hope of success. In rare cases, persistence may compensate for moderate virulence (Shields et al., 1999). Matching the appropriate nematode host-seeking strategy with the pest is also essential (Gaugler, 1999). Nematodes that have an ambush strategy are most suitable for controlling mobile insects near the soil surface (e.g., *S. carpocapsae*), whereas nematodes with a cruiser strategy (e.g., *H. bacteriophora*) are most effective for less mobile insects below the soil surface (Lewis et al., 1992). Ecological factors such as relative ability to withstand desiccation or temperature tolerance are also important in choosing the best-adapted nematode for a particular pest. Poor host suitability has been the most common cause of failure in entomopathogenic nematode application (Gaugler, 1999). Furthermore, high virulence under laboratory conditions has often been inappropriately extrapolated to field efficacy (Georgis and Gaugler, 1991).

The definitive test of commercial potential of nematodes is efficacy under field conditions. Consistent, efficacious control is critical for the successful commercialization of any technology. Replicated plot tests for three or more years under various environmental conditions should be performed before deciding on product introduction. Additionally, multiple on-farm commercial application tests using grower equipment and practices should also be conducted to understand the "efficacy gaps" that might exist between plot tests and on-farm tests. Field efficacy is one of the required components for commercialization. Other important factors are cost, storage, delivery, handling, mixing, coverage, competition, compatibility with grower practices, and profit margins to manufacturers and distributors. Careful assessment and consideration of each of these factors is essential for product development and market penetration. Historically one or more of these factors have prevented the commercial development of nematodes for some crops or markets (Georgis, 2002).

The development of nematodes for effective insect control in the context of sustainable agriculture will be a major challenge. A truly integrated approach is required, in which all agricultural practices, including other insect control options, should be considered to obtain maximum effect from a given intervention or practice without interfering with the effectiveness of other practices. Because of the low environmental impact and selectivity of nematodes, they have potential to be valuable components of integrated pest management and resistance management programs. The use of nematodes in rotation schedule programs with standard insecticides for the control of foliar immature stages of leafminers (Section 3.4) and thrips (Section 3.5) and for the control of codling moth (Section 8.1) and navel orangeworm (Section 8.2), as well as with pheromones for the control of oriental fruit moth *Grapholita molesta* (Busck) and peach fruit moth *Carposnia niponesis* (Yamanaka, unpublished data) are possible suitable implementations of nematodes in IPM programs. The use of nematodes in tank mixes or in rotation with standard pesticides, biological control agents and/or pheromones as well as cultural approaches and monitoring methods may expand the size of current markets (e.g., citrus weevils, black vine weevil, and fungus gnats.) or penetrate new markets (e.g., large pine weevil, sweet potato weevil, and cutworms in turf and borers in tree fruits). The implementation of nematodes in IPM programs will likely increase their usage in organic crops, a market that is rapidly growing in USA, Canada and Western Europe.

Discovery and development of new nematode species and strains and further improvement in formulation to enhance the biological control potential of entomopathogenic and slug nematodes will further expand the options for implementation of nematodes against a wider range of targeted pests. Improvements in production technology, distribution, and application will be key to reducing nematode costs and insuring quality. In this vein, Gaugler (1997) proposed local-level cooperatives to produce nematodes cheaply and effectively for on-site use. Application of nematodes in infected hosts instead of aqueous suspensions (Shapiro-Ilan and Lewis, 1999a,b; Shapiro-Ilan et al., 2003) is another approach with potential to reduce in vivo production costs by avoiding several labor-intensive steps.

Genetic improvements in entomopathogenic nematodes may be the solution to developing more stable formulations or expanding their potential as biological control agents (e.g., increasing search capacity, virulence, and resistance to environmental extremes). Progress may lead to reduction in cost by making it possible to achieve acceptable control at rates lower than current recommendations (Gaugler et al., 1997a,b). Increased emphasis needs to be placed on training of extension agents and end-users in the proper use of nematodes and their implementation in insect control strategies.

The benefits of utilizing entomopathogenic nematodes and other microbial controls agents compared to that of broad spectrum insecticides are less apparent when simply comparing efficacy and labor costs. However, the reduction of non-target impacts, potential benefit as tools for resistance management, safety for applicators, and no re-entry or preharvest interval grow in importance when a sustainable IPM system is considered.

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