

# Application technology and environmental considerations for use of entomopathogenic nematodes in biological control

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Received 9 June 2005; accepted 13 September 2005

Available online 24 October 2005

## Abstract

A wide range of technology is available for application of entomopathogenic nematodes including various irrigation systems and spray equipment. The choice of application equipment, and manner in which the nematodes are applied, can have substantial impact on pest control efficacy. For example, nozzle or pumping system types are some of the parameters that can affect nematode performance following spray applications. Operating pressures for some nematode species may reach up to 2000 kPa without notable damage, whereas other species may require lower pressure limits, e.g., 1380 kPa for *Heterorhabditis megidis*. In addition to application equipment, a variety of other abiotic and biotic factors must be considered. In general, a rate of 25 infective juvenile nematodes/cm<sup>2</sup> is required for successful pest suppression. Critical environmental factors include avoidance of ultraviolet radiation, adequate soil moisture, and appropriate temperature. Certain fertilizers and chemical pesticides can have positive effects on entomopathogenic nematode efficacy, whereas other agents may have neutral or negative effects. Similarly, certain biotic agents present during soil applications can be expected to be detrimental to nematode applications (e.g., nematophagous mites and fungi), whereas other organisms may be beneficial (e.g., some combinations with *Bacillus thuringiensis*). With some exceptions foliar applications have been less successful than soil applications due to nematode susceptibility to desiccation and UV; recent research, however, indicates that frequent low-rate applications of nematodes to foliage can result in substantial suppression of greenhouse pests such as thrips. Further innovation in application technology will undoubtedly contribute to the expansion of entomopathogenic nematodes as biocontrol agents.

Published by Elsevier Inc.

**Keywords:** Application technology; Biological control; Entomopathogenic nematode; *Heterorhabditis*; *Steinernema*

## 1. Introduction

Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) are parasites of insects that kill their hosts with the aid of bacteria carried in the nematode's alimentary canal; steinernematids carry *Xenorhabdus* spp. whereas heterorhabditids carry *Photorhabdus* spp. (Adams and Nguyen, 2002; Poinar, 1990). These nematodes can be

used as biological control agents to suppress a variety of economically important insect pests (Grewal et al., 2005; Grewal and Georgis, 1999; Kaya and Gaugler, 1993; Klein, 1990; Shapiro-Ilan et al., 2002; Shapiro-Ilan, 2004). No matter how well suited an entomopathogenic nematode is to a targeted pest, the application will fail if the agent is not delivered in a manner that enables access to and infection of the host. Nonetheless, the technical aspects of biopesticide application in the field are often neglected. Effective and efficient delivery of entomopathogenic nematodes can only be achieved with careful consideration of available application technology coupled with an understanding of the attributes and limitations of the biocontrol agent. Entomopathogenic nematodes

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have been applied using practically every device available in the agricultural and urban environment, from knapsack sprayers and tractor equipment to sprinkler systems and aircraft. Numerous studies indicate that some methods of application are superior to others in facilitating pest control efficacy. In this paper, we review research on application equipment and methodology for soil and aboveground application of entomopathogenic nematodes; additionally, we offer analysis of the current state of the art and prospects for the future.

## 2. Application equipment

Entomopathogenic nematodes can be applied with nearly all commercially available ground or aerial spray equipment, including pressurized sprayers, mist blowers, and electrostatic sprayers (Georgis, 1990). The application equipment used depends on the cropping system, and in each case there are a variety of handling considerations including volume, agitation, pressure and recycling time, system environmental conditions, and spray distribution pattern (Grewal, 2002).

### 2.1. Comparative effects of application equipment

Previous studies have indicated that application technique has a significant effect on efficacy of entomopathogenic nematodes in field trials (Bullock et al., 1999; Curran, 1992; Hayes et al., 1999; Shields et al., 1999). For example, Curran (1992) reported that trickle irrigation was inferior to surface spray or multiple injection, and Hayes et al. (1999) reported that sprinkler irrigation was inferior to a boom sprayer. Settling of nematodes in slow moving irrigation systems (e.g., trickle) can be a significant cause of poor distribution (Conner et al., 1998). Duncan et al. (1999) suggested that unequal distribution in some irrigation systems (i.e., microjet) can be overcome through addition of extra emitters at the end of the lines. These studies mainly focused on entomopathogenic nematode placement and distribution within the soil when comparing application methods and efficacy. While dispersal information is important, differences in the application equipment components and their potential to be detrimental to entomopathogenic nematodes have been largely ignored. Application equipment effects could be a contributing factor to inconsistent results in field studies.

There have been a few studies that have considered the direct influence of application equipment on entomopathogenic nematodes (Klein and Georgis, 1994; Nilsson and Gripwall, 1999). Klein and Georgis (1994) found that no adverse effects were observed for *Steinernema* spp. and *Heterorhabditis bacteriophora* Poinar after flow through several different pumps (piston, centrifugal, roller, and diaphragm), nozzle types (Spraying Systems XR8001VS, TK-VS2, FL-5VS), and strainers (100 mesh, 50 mesh, and 50 slotted). However, Klein and Georgis (1994) did not report any data explicitly and the criteria for determining effective survival and infectivity were not given.

Nilsson and Gripwall (1999) investigated the influence of application technique on the viability of *Steinernema feltiae* (Filipjev) with a backpack sprayer (200 kPa, diaphragm pump, Hardi 4110-12 fan nozzle) and a high-pressure sprayer (1000 and 2000 kPa, piston pump, 1.2 mm Wanjet pressure swirl solid cone), and reported no significant influence on nematode viability. However, they noted a tendency of reduced viability of the nematodes in all the high-pressure sprayer treatments, and a significant decrease in viability as the length of the pumping period in the high-pressure sprayer increased. They reasoned that the decreased viability was probably due to mechanical stresses from the piston pump and nozzle and the rise in temperature in the liquid after multiple passes through the pump.

A general recommendation for entomopathogenic nematodes has been common nozzle type sprayers with openings larger than 50  $\mu\text{m}$  and operating pressures less than 2000 kPa (290 psi) (Georgis, 1990). However, no studies were cited to support these recommendations, which are most likely based on information from *Steinernema carpocapsae* (Weiser), the most widely studied and commonly available entomopathogenic nematode, and may not be representative for all entomopathogenic nematode species. Clearly, more in-depth studies on effects of spray equipment are needed for optimizing their application.

### 2.2. Stress factors influencing nematode application in spray equipment: an in-depth study

In a conventional hydraulic spray system, the liquid suspension is pumped from a tank, through a pressure regulator and flow valves, to a nozzle where the suspension is forced under pressure through an orifice to the atmosphere. Entomopathogenic nematodes can experience a variety of physical stresses during flow through the spray system. Understanding the effects of the different physical phenomena within a spray system is important to begin identifying the equipment characteristics and operating conditions that are least detrimental to entomopathogenic nematodes. Recent work by Fife (2003) and Fife et al. (2003, 2005) evaluated several important physical factors that act on entomopathogenic nematodes within a spray system. Specifically, this work considered the effects of pressure differentials, hydrodynamic stress, and temperature increase (due to pump recirculation) on several entomopathogenic nematode species. These studies are highlighted below.

#### 2.2.1. Effects of pressure differentials

The extent of damage to three species of entomopathogenic nematodes (*H. bacteriophora*, *S. carpocapsae*, and *Heterorhabditis megidis* Poinar, Jackson, & Klein) in suspension due to the effects of a pressure differential, generated by a French pressure cell and press (Spectronic Unicam, Rochester, NY), was studied (Fife et al., 2003). Results from this study indicate that the magnitude of the pressure differential has an effect on the relative viability of entomopathogenic nematodes, and the effect is species dependent. For *S. carpocapsae*

*capsae*, the results were consistent with the common recommendation that operating pressures should not exceed 2000 kPa (290 psi). However, greater reductions in relative viability (i.e., damage) were observed for *Heterorhabditis* spp., in particular *H. megidis*, indicating that entomopathogenic nematode species is an important factor to consider when defining spray operating conditions. To maintain viability above 85%, the recommendation is to operate at pressures less than 1380 kPa (200 psi) for *H. megidis*, and less than 2000 kPa (290 psi) for *H. bacteriophora* and *S. carpocapsae*.

The differences in response of entomopathogenic nematode species to pressure differentials may be explained by several underlying factors. First, the ultrastructure properties of the nematode cuticle may be a factor. *S. carpocapsae* has a proportionately greater striated layer in the cuticle of infective juveniles (IJs) compared to several other *Steinernema* spp. (Kondo and Ishibashi, 1989; Patel and Wright, 1998). The enhanced structural integrity of *S. carpocapsae* may provide this species with the ability to withstand greater changes in pressure compared to other species. Even at 10,690 kPa (1550 psi), *S. carpocapsae* experienced a reduction in relative viability of only 50% compared to over 80% for the heterorhabditid nematodes. Another factor may be the size of the entomopathogenic nematodes. *H. megidis* is considerably longer than the other species, which may have contributed to greater damage of this species.

### 2.2.2. Effects of hydrodynamic stress

The effects of two common types of hydraulic nozzles (flat fan and cone) on damage to four entomopathogenic nematode species (*H. bacteriophora*, *H. megidis*, *S. carpocapsae*, and *Steinernema glaseri* [Steiner]) were investigated (Fife et al., 2005). Computational fluid dynamics (CFD) was used to numerically simulate the internal flows within a flat fan nozzle (Spraying Systems XR8001VS, Spraying Systems, Wheaton, IL) and a hollow cone nozzle (Spraying Systems TXA8001VK), and important flow field parameters from the CFD simulations were compared to the observed entomopathogenic nematode damage.

Overall, greater reductions in relative viability were observed for the flat fan nozzle compared to the hollow cone. The differences were due to the distinct characteristics of each nozzle's flow field. The internal shape of a flat fan nozzle causes liquid from a single direction to curve inwards so that the two streams of liquid meet at the elliptic exit orifice. The reduced flow area of the narrow, elliptic exit orifice generates an extensional flow regime where tensile forces are developed that are large enough to cause nematode damage. Within a cone nozzle, the liquid is forced through tangential slits into a swirl chamber giving the liquid a high-rotational velocity. The high-rotational flow component within the cone nozzle did not produce hydrodynamic conditions conducive to causing nematode damage. An exception was observed in *S. glaseri*, in which damage was observed from the cone nozzle; the length of *S. glaseri* is approximately the same size as the cone orifice and may have been a contributing factor.

Based on the flow field characteristics, the recommendation is that an appropriately sized (i.e., larger than the organism) cone nozzle is more suitable for spray application than a fan nozzle to avoid hydrodynamic damage to the entomopathogenic nematodes. However, the experimental flow rates in this study were considerably higher than those suggested by the manufacturer. When sprayed at 60 psi from an air-pressurized canister, no damage to *H. bacteriophora* nematodes was observed using either of these nozzles. Consequently, both nozzles are acceptable for spray application when following the manufacturer's recommendations. Larger capacity flat fan nozzles (Spraying Systems XR8002VS and XR8004VS) were also tested for the same range of experimental conditions, and no entomopathogenic nematode damage was observed. Larger capacity nozzles are more suitable for applying entomopathogenic nematodes, particularly for soil-applied treatments where a high volume of water is necessary to get the nematodes beyond the soil surface.

### 2.2.3. Effects of pump recirculation

The effects of three common pumps (centrifugal, diaphragm, and roller) on damage to four entomopathogenic nematode species (*H. bacteriophora*, *H. megidis*, *S. carpocapsae*, and *S. glaseri*) were evaluated (Fife, 2003). No mechanical damage to the entomopathogenic nematodes occurred after a single passage through each pump at operating pressures up to 828 kPa (120 psi). This finding was consistent with previous work (Klein and Georgis, 1994), suggesting that reductions in nematode viability during pump recirculation are more likely the result of temperature influences and not mechanical stress.

A separate test was conducted in which a constant volume of water (54.5 liter) was recycled in the tank at 18.2 liter/min to evaluate change in water temperature with time. Within 1 h the temperature of water in the tank had increased from approximately 22 to 43 °C for the centrifugal pump, and to approximately 27 °C for both the diaphragm and roller pumps. The general recommendation is to avoid temperatures exceeding 30 °C within spray equipment (Grewal, 2002). However, it should be noted that the volume of liquid in the spray tank influences the temperature increase (i.e., the smaller the volume of liquid in the tank, the more passes through the pump during a pumping period). A large volume of liquid provides a heat sink in the tank which moderates temperature rise. Nonetheless, the heat added to the spray system by the centrifugal pump can, with time, produce conditions that are incompatible with entomopathogenic nematodes. Thus, the diaphragm or roller pumps are better suited for use with nematodes than the centrifugal type.

## 3. Soil application

The soil environment is the natural habitat for entomopathogenic nematodes, and thus offers great potential for successful biocontrol applications using these organisms. Nonetheless, numerous attempts to control soil insect pests

with entomopathogenic nematodes have failed (Klein, 1990; Shapiro-Ilan et al., 2002). To achieve successful applications in the soil environment, a variety of abiotic and biotic factors must be considered.

### 3.1. Application methods for soil

A wide range of technology is available for application of entomopathogenic nematodes to soil, from simple watering cans or hose end sprayers for small plot or home-garden use, to aerial applications over large fields or orchards (Georgis, 1990; Grewal, 2002). Other methods used in soil application include various irrigation systems such as overhead (Georgis, 1990), microjet (Georgis, 1990; McCoy et al., 2000a), irrigation channels (Gouge et al., 1996), center pivot (Wright et al., 1993), and trickle (Curran, 1992; Reed et al., 1986) as well as diverse spray or injection equipment (Georgis, 1990; Grewal, 2002; McCoy et al., 2000b). Advantages and disadvantages of different application equipment and parameters are reviewed in the previous section.

Various formulations for entomopathogenic nematodes may be used in soil application including activated charcoal, alginate and polyacrylamide gels, baits, clay, peat, polyurethane sponge, vermiculite, and water dispersible granules (WDG) (Georgis, 1990; Georgis et al., 1995). Formulations can extend shelf life through reduction of nematode metabolism and immobilization, which may be accomplished through refrigeration, partial desiccation, or both (Georgis, 1990; Georgis et al., 1995). Some formulations such as baits have the potential to enhance cost efficiency and may also extend nematode activity in soil (or other media) after application by protecting the nematodes from harmful environmental conditions (Kaya and Nelsen, 1985; Capinera et al., 1988; Georgis et al., 1989; Georgis, 1990; Renn, 1998; Navon et al., 2002). Formulations that are based on non-desiccated nematodes such as paste or sponge retain high viability (percentage live nematodes) but cannot be packaged at high densities and are therefore limited in their appropriateness for large-scale application. A successful, more concentrated, non-desiccated formulation has been developed for in vitro produced nematodes based on vermiculite, which, for example, allows a shelf life of at least 1 month for *H. megidis* and 2–3 months for steinernematids (Shapiro-Ilan et al., 2002). A liquid formulation that contains concentrated non-desiccated nematodes and maintains high viability has also been commercialized but must be applied within 48–72 h of receipt. A breakthrough in formulation technology was cited in the introduction of WDG, in which the steinernematids enter a partially anhydrobiotic state allowing them to survive up to 6 months at 4–25 °C (substantially longer than previous formulations) (Georgis et al., 1995). Viability of some nematodes in WDG, e.g., *Steinernema riobrave* Cabanillas, Poinar & Raulston, can be poor (below 50%) (McCoy et al., 2000a), but this is not necessarily an issue in regard to efficacy because the producer tends to over-pack to ensure excess viable nematodes remain in the product

throughout its shelf life (Shapiro-Ilan et al., 2002). Baur et al. (1997a) reported reduced efficacy of WDG formulated *S. carpocapsae* relative to non-desiccated nematodes. However, Shapiro and McCoy (2000a) and Grewal (2000) reported no effect of WDG on steinernematid virulence.

### 3.2. Soil abiotic factors

Several environmental factors are critical to successful application of entomopathogenic nematodes in soil including ultraviolet radiation, soil moisture, and temperature (Kaya, 1990). Ultraviolet radiation is detrimental to nematodes (Gaugler and Boush, 1978); thus, if nematodes are being applied to the soil surface, it is best to apply nematodes to soil in the evening or early morning hours. Alternatively, efficacy can be improved, and exposure to ultraviolet radiation avoided, through sub-surface application (Cabanillas and Raulston, 1995); the advantages to such approaches, however, have not been detected in all studies (Schroeder et al., 1996; Wilson and Gaugler, 2004). Entomopathogenic nematodes require adequate soil moisture for survival and movement, but too much moisture may cause oxygen deprivation and restrict movement (Kaya, 1990; Koppenhöfer et al., 1995; Wallace, 1958; Womersley, 1993). Optimum moisture levels will vary by nematode species and soil type. For example, in a sandy loam *S. carpocapsae* infected insects at moisture levels as low as –5 Mpa and had the highest host establishment rates between –0.1 and –0.01 MPa, whereas *S. glaseri* required a minimum water potential of –0.3 Mpa to infect (Koppenhöfer et al., 1995). Irrigation is recommended for maintaining adequate soil moisture and promoting establishment of nematodes in the soil sub-surface (Downing, 1994; Shetlar et al., 1988; Zimmerman and Cranshaw, 1991). Soil temperature can have a great effect on nematode activity (Kaya, 1990). Optimum temperatures for infection and reproduction vary among nematode species and strains (Grewal et al., 1994). Some nematodes such as *Heterorhabditis indica* Poinar, Karunakar & David, *S. glaseri*, and *S. riobrave* are relatively heat tolerant and can maintain efficacy at temperatures of 29 °C and above whereas others, such as *H. megidis*, *S. feltiae*, and *Heterorhabditis marelatus* Liu & Berry are more cold tolerant maintaining efficacy at 15 °C and below (Berry et al., 1997; Grewal et al., 1994; Kung et al., 1991; Shapiro and McCoy, 2000b).

Soil characteristics must also be considered. Soil pH in most agroecosystems, having a range of 4–8, is not likely to have any significant effect on entomopathogenic nematodes, but a pH of 10 or higher is likely to be detrimental (Kung et al., 1990a). Soil texture affects nematode movement and survival (Barbercheck, 1992; Kaya, 1990). Generally, compared with lighter soils, soils with higher clay content restrict nematode movement and have potential for reduced aeration, which can result in reduced nematode survival and efficacy (Georgis and Poinar, 1983; Kung et al., 1990b; Molyneux and Bedding, 1984). However, exceptions to this trend have been observed (Georgis and Gaugler, 1991; Shapiro et al., 2000a).

Fertilizers and chemical pesticides can have positive, neutral, or negative effects on entomopathogenic nematodes. Most fertilizers, when applied at recommended rates have little impact on entomopathogenic nematode efficacy (Bednarek and Gaugler, 1997; Shapiro et al., 1996). However, fresh manure or high rates of chemical fertilizers (e.g., urea) can be detrimental to entomopathogenic nematode survival and efficacy (Bednarek and Gaugler, 1997; Shapiro et al., 1996, 1999a). Some chemical pesticides (e.g., dodine, methomyl, and parathion) are quite toxic to entomopathogenic nematodes, others (e.g., chlorpyrifos and endosulfan) are quite compatible, and still others (e.g., tefluthrin, imidacloprid) act synergistically with entomopathogenic nematodes in pest suppression (Alumai and Grewal, 2004; Koppenhöfer, 2000; Koppenhöfer and Kaya, 1998; Mannion et al., 2000; Nishimatsu and Jackson, 1998; Rovesti and Deseö, 1991).

### 3.3. Soil biotic factors

Several factors related to the nematode's biology are critical for successful application; foremost is matching the appropriate nematode with the target pest. Proper match of the nematode to the host includes virulence, host finding, and environmental tolerance. If a nematode does not possess a high level of virulence toward the target pest, there is little hope of success. In some cases persistence may compensate for moderate virulence (Shields et al., 1999). Matching the appropriate nematode host seeking strategy with the pest is also essential (Lewis et al., 1992; Lewis, 2002). Nematodes that have an ambush strategy (e.g., *S. carpocapsae*) are most suitable for controlling mobile insects near the soil surface, whereas nematodes with more of a cruise strategy (e.g., *H. bacteriophora*) are most suitable for suppressing less mobile insects below the soil surface (Lewis et al., 1992; Lewis, 2002). Environmental tolerance to desiccation or temperature may also be important in choosing the best-adapted nematode for a particular pest.

To be effective, entomopathogenic nematodes must usually be applied to soil at rates of  $2.5 \times 10^9$  IJs/ha (= 25/cm<sup>2</sup>) or higher (Georgis and Hague, 1991; Georgis et al., 1995; Shapiro-Ilan et al., 2002). In cases where the pest is particularly susceptible or in controlled condition such as in the greenhouse, lower application rates might also be effective. For example, *S. carpocapsae* applied at the relatively low rate of 12.5 IJs/cm<sup>2</sup> reduced black cutworm, *Agrotis ipsilon* (Hufnagel) damage in field corn by more than 75%, which was as effective as or more so than the chemical insecticides tested (Levine and Oloumi-Sadeghi, 1992). On the other hand, some insects that are less susceptible or can be found deep below the soil surface may require higher rates to achieve sufficient efficacy, e.g., the Diaprepes root weevil (McCoy et al., 2000a; Shapiro-Ilan et al., 2002).

Generally, nematode populations can be expected to remain high enough to provide effective pest control for 2–8 weeks after application to soil under field conditions (Duncan and McCoy, 1996; Kaya, 1990; McCoy et al.,

2000a; Shapiro-Ilan et al., 2002). Thus, re-application of nematodes is often necessary in many cropping systems. In some cases, however, effective control has been reported over more than one season or even several years (Klein and Georgis, 1992; Parkman et al., 1994; Shields et al., 1999). The potential for nematode recycling and long-term pest suppression is dependent on various factors such as soil type, ground cover, host and host density, and the nematode species (Kaya, 1990; Klein and Georgis, 1992; Shapiro et al., 1999b; Shapiro-Ilan et al., 2002).

A number of biotic agents in soil can have positive or detrimental effects on entomopathogenic nematode applications. The nematodes are subject to infection or predation by certain phages, bacteria, protozoans, nematophagous fungi, predacious mites and nematodes, etc. (Kaya, 2002). Phoretic relationships have been indicated between entomopathogenic nematodes and other soil organisms such as mites (Epsky et al., 1988) and earthworms (Shapiro et al., 1995). Entomopathogenic nematodes have been reported to act synergistically with other entomopathogens such as *Paenibacillus popilliae* Dutky (Thurston et al., 1994), *Bacillus thuringiensis* (Koppenhöfer and Kaya, 1997), and *Metarhizium anisopliae* (Metsch.) Sorokin (Ansari et al., 2004), whereas other studies indicate antagonism such as with *Beauveria bassiana* (Balsamo) Vuillemin (Brinkman and Gardner, 2000) or *Paecilomyces fumosoroseus* (Wize) Brown & Smith (Shapiro-Ilan et al., 2004). The nature of interactions between nematodes and other entomopathogens (antagonism, additivity, synergism) can vary depending on the nematode species and relative timing or rate of application (Barbercheck and Kaya, 1990; Koppenhöfer and Kaya, 1997; Thurston et al., 1994). Similarly, interaction between different entomopathogenic nematode species in the same soil environment has been reported as competitive (Duncan et al., 2003a) as well as coexistence without apparent competition (Millar and Barbercheck, 2001). Duncan et al. (2003b) reported that free-living bacterivorous nematodes can increase insect mortality in the presence of entomopathogenic nematodes (e.g., *S. riobrave*) but decrease their reproductive potential.

### 3.4. Prospects for future technology in soil application

Additional studies and advances in soil application technology and formulation are likely to improve pest control efficacy with entomopathogenic nematodes. One potentially novel method of commercial application would be to apply the nematodes to the target site in nematode-killed hosts. Pest suppression would then be achieved by the nematodes that emerge from the host cadavers. Effective pest suppression has been reported in field trials using this method (Jansson et al., 1993; Parkman et al., 1993). Laboratory experiments indicated greater nematode dispersal (Shapiro and Glazer, 1996) and infectivity (Shapiro and Lewis, 1999) when the nematodes were applied in cadavers compared with aqueous application. Furthermore, greenhouse trials indicated superior pest suppression through

application of nematode in cadavers relative to aqueous (Shapiro-Ilan et al., 2003). The superior pest suppression observed in cadaver applications may have been due to metabolites present in the cadavers that enhance dispersal or infection (Shapiro and Lewis, 1999; Shapiro et al., 2000b). To facilitate storage and application of nematode-infected cadavers, and avoid rupture or sticking together, the cadavers can be coated with a protective formulation (Shapiro-Ilan et al., 2001), or hard-bodied insects (e.g., *Tenebrio molitor* L.) could be used (Shapiro-Ilan et al., 2003).

#### 4. Aboveground application

Due to the well-known limitations related to survival and efficacy of nematodes on a foliar target, commercialization of entomopathogenic nematodes for foliar pests has been rare and largely unsuccessful (Arthurs et al., 2004; Begley, 1990; Grewal and Georgis, 1999). In an analysis of 136 published greenhouse and field trials with *S. carpocapsae*, Arthurs et al. (2004) showed that nematode treatment efficacy depended on the insect's target habitat (bore holes > cryptic foliage > exposed foliage) and trial location (greenhouse > field). However, several research advances have moved the use of nematodes against foliar pests further down the road toward success. Attempts to improve the situation initially relied upon leaf flooding, together with the addition of surfactants to increase leaf coverage (Head et al., 2004; Williams and Walters, 2000), with some positive results. Additionally, there has been significant research attempting to characterize (and thus overcome) harmful environmental factors such as desiccation and ultraviolet light (Georgis, 1990; Glazer, 1992; Glazer et al., 1992). Desiccation is thought to be the key factor influencing nematode efficacy on foliage (Glazer et al., 1992). Desiccation survival of IJs varies markedly between species and isolates of entomopathogenic nematodes (Glazer, 1992), and with foliar application of IJs, there is inevitable exposure to increasing evaporative and osmotically driven water loss (Piggott et al., 2000).

In relation to the research discussed above, attempts were made to improve foliar pest control (e.g., Baur et al., 1997a,b; Broadbent and Olthof, 1995; Lello et al., 1996; Mason et al., 1998a,b), but in a number of cases control levels were variable or unsatisfactory. However, it has been demonstrated that nematodes can be applied against foliar pests such as *Spodoptera exigua* (Hübner) and leafminers in the genus *Lyriomyza* with a low-nematode rate and better placement of nematodes using polymeric formulations and adapted application equipment (Piggott et al., 2003).

Furthermore, laboratory and field studies (Head et al., 2000; S. Piggott, unpublished data) indicated that concerns over and attempts to ameliorate desiccation and ultraviolet light may be circumvented by prudent use of nematodes and application equipment. This work led to the development of a commercial product for foliar thrips control. Prior work with the soil-dwelling stages of thrips (prepupae and pupae) showed that these stages were susceptible to

entomopathogenic nematodes (Chyzik et al., 1996; Ebssa et al., 2001a,b; Premachandra et al., 2003). For foliar application, issues relating to formulation, application equipment, and control strategy have been combined to allow the use of nematodes in thrips control programs. A new nematode formulation based on polymeric material has played a major part in the acceptance of foliar applications and the use of weekly, low volume, low-concentration sprays has proved more robust than one inundative release. For example, control of western flower thrips, *Frankliniella occidentalis* (Pergande), was observed in greenhouse chrysanthemums with weekly applications of specially formulated *S. feltiae* UK76 strain (Nemasys F; Becker Underwood, Littlehampton, UK) using 2.5 billion IJs/ha with 1000 liter water and with a suitable wetting agent. Although the approach and product are used throughout Europe (S. Piggott, unpublished data), published experimental greenhouse and field studies are needed to verify the use of this formulation against thrips.

In addition to foliar targets, aboveground nematode applications have also been directed toward control of insect pests located on or in plant stems or trunks (Begley, 1990). Cryptic habitats such as inside the plant are attractive for nematode application because they may offer protection from harmful environmental conditions (ultraviolet radiation). Indeed various studies have reported success in suppressing insect pests in or on the plant stem or trunk through injection or direct spray applications (e.g., Bedding, 1990; Begley, 1990; Kaya and Brown, 1986; Miller and Bedding, 1982; Treverrow et al., 1991; Unruh and Lacey, 2001).

#### 5. Conclusion

In some instances, entomopathogenic nematodes have proven to be safe and effective alternatives to chemical pesticides, but in numerous other cases they have failed to compete successfully. Certainly, expanded use of entomopathogenic nematodes in biological control can be brought about through the development of superior nematode species or strains that are more capable of suppressing the target pest. Another avenue to expanded use of entomopathogenic nematodes lies in improved delivery to the target site.

Practically all of the application systems used for entomopathogenic nematode application were originally developed and tested for application of other materials, primarily chemical pesticides. The adaptation of chemical application methods and equipment for entomopathogenic nematode application has proven to be substantially effective because application of both materials shares certain goals of efficiency in delivery, and because entomopathogenic nematodes are generally capable of withstanding many of the conditions generated when using equipment developed for applications of chemicals. The relationship is likely to continue, i.e., additional adaptation of entomopathogenic nematode uses to innovative systems originally

developed for chemical delivery will likely be fruitful. For example, the use of modern electronics such as global positioning systems (GPS) combined with direct injection or sensor controlled delivery has advanced the application of certain chemical pesticide applications (Miller and Paice, 1995; Vetter, 1994) and may offer opportunities for entomopathogenic nematodes as well.

On the other hand, due to limitations in the nematode's biology, direct adoption of chemical pesticide application technology for improvement of entomopathogenic nematode delivery can only be taken so far. The study of nematode application technology must be expanded in its own right. Clearly, increased understanding of entomopathogenic nematode and the target pest's biology and ecology will facilitate more efficient and effective application methodology. But the greatest advances in entomopathogenic nematode application technology are likely to come from development of novel delivery systems and more in-depth characterization of the effects of various systems on entomopathogenic nematode biology. Ultimately, research-based guidelines that define all operating conditions within each operating system are needed for optimizing entomopathogenic nematode application efficiency and increased acceptance and use by growers.

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