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PREFACE

Insecticidal nematodes are increasingly being used in IPM programs for managing a wide array of pest species in diverse cropping systems. Field efficacy of these nematodes is often inconsistent and is affected by parameters such as nematode choice, temperature, application method, storage and handling, formulation quality, irrigation frequency, and soil type. Insecticidal nematodes like other biological control agents require a knowledgeable user to achieve optimal results.

A group of researchers and extension specialists at Rutgers University were funded by USDA Northeast region Sustainable Agricultural Research and Education (SARE) program to develop educational materials for using insecticidal nematodes. One of the goals of this grant was to develop comprehensive multi-media educational tools such as a video program, fact sheets, slide set, and a website. Once these educational tools become available the plan called for organizing a national workshop to provide comprehensive information to Extension Specialists, County Agricultural Agents, pesticide suppliers, field development representatives, private IPM consultants, and other end-users. This workshop is the culmination of all these efforts in disseminating science-based information to the end-users on the appropriate use of insecticidal nematodes in pest management.

This volume provides a summary of most of the presentations at the National Workshop on "*Optimal Use of Insecticidal Nematodes in Pest Management*", to be held in New Brunswick, New Jersey, August 29-30. Throughout this volume, various authors have used "insecticidal nematodes", "entomopathogenic nematodes" and "insect parasitic nematodes" interchangeably. No effort was made to standardize the terminology except that "insecticidal nematodes" was used more often while referring to nematodes in the context of pest management.

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Biology and Ecology of Insecticidal Nematodes

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ABSTRACT

The insecticidal nematodes in the genera *Steinernema* and *Heterorhabditis* are symbiotically associated with bacteria in the genera *Xenorhabdus* and *Photorhabdus*, respectively. The bacterial cells are housed in the intestine of the infective juvenile of the nematode, which is the only free-living stage. In nature, the infective juvenile forages for an insect host in soil, enters through natural openings of the host, penetrates into the host's body cavity, releases the bacterial cells that kill the host within 2 days, and completes its life cycle within the cadaver. At least 32 species of insecticidal nematodes have been described of which 9 are commercially available or being considered for commercialization. Insecticidal nematodes are used as biological insecticides against a number of insect pests in soil and cryptic habitats. Because the infective juveniles are susceptible to a number of abiotic factors including temperature extremes, rapid desiccation and ultraviolet light and biotic factors such as antagonists and competitors, the user needs to understand the biology and ecology of these nematodes as the first step to an effective pest control program.

INTRODUCTION

Nematodes (Phylum: Nematoda), often referred to as round-, eel-, or threadworms because of their more or less cylindrical and elongated bodies, are non-segmented, invertebrate animals that range in size from 0.1 mm to several meters in length. They have digestive, reproductive, muscular, excretory and nervous systems but lack visual and auditory, circulatory and respiratory systems. The sexes are separate in most species (i.e., amphimictic), although parthenogenesis (females only) or hermaphrodism (both sex organs in the same individual) occur in some species. Nematodes are ubiquitous and are found in aquatic (marine and fresh water) and terrestrial environments. Most nematodes are free-living, but some species are parasitic on plants or animals including insects.

Nematode association with insects ranges from fortuitous to parasitic (Kaya & Stock, 1997). Some nematode species have an obligate, commensal relationship with their insect hosts where no deleterious effects are obvious. In other cases, the association may be facultative or obligate parasitism where deleterious effects do occur. In the facultative association, the nematode species has a free-living life cycle that is independent from its insect host and enters a parasitic life cycle phase when the insect host occurs in its environment (e.g., *Deladenus siricidicola* and *Sirex* wood wasps) (Bedding, 1993). In the obligate association, the nematode species cannot survive in the absence of its insect host. In either facultative or obligate associations, the deleterious effects of nematode parasitism on their insect hosts include sterility, reduced fecundity, reduced longevity, reduced flight, delayed development or other behavioral, morphological or physiological aberrations. These parasitic nematode species can be important natural control agents of insect pests, and one species, *D. siricidicola*, has been successfully introduced as a classical biological control agent (Bedding, 1993).

Since the early 1980s, a group of obligate, parasitic nematodes in the families Steinernematidae and Heterorhabditidae has received considerable attention from researchers in academia, government, and industry because they possess many favorable attributes as biological control agents (Kaya & Gaugler, 1993). Most species have an extremely broad host range and exhibit foraging strategies that can be matched against a given pest species. They are adapted to the soil environment and have been employed primarily as biological insecticides against a number of soil insect pests and insect pests in cryptic habitats. Furthermore, they have the ability to kill their hosts within 2 days. Because of this "quick-kill", they are called entomopathogenic nematodes or can be referred to as "insecticidal nematodes." The rapid mortality of the insect hosts is due to the mutualistic bacterial species that are associated with these nematodes. A thorough understanding of the biology and ecology of these nematodes and their associated symbiotic bacteria is essential in using them effectively as biological insecticides. Our chapter presents the basic information on the biology and ecology of these nematodes as the first step in using them effectively as biological insecticides.

BIOLOGY OF THE NEMATODE/BACTERIUM COMPLEX

The steinernematid and heterorhabditid nematodes have similar life histories but do not appear to be closely related phylogenetically based on molecular evidence (Blaxter *et al.*, 1998). However, more morphometric and molecular research is needed before a definitive statement can be made on the phylogenetic positions of these two families. Currently, in the Steinernematidae, there are 25 described species represented by one species in the genus *Neosteinernema* and 24 species in the genus *Steinernema*. In the monogeneric Heterorhabditidae, there are 7 described species in the genus *Heterorhabditis*. Many more new isolates are being recovered yearly, and some of them probably represent new species. Of the known species, a few are commercially available or being contemplated for commercialization (Table 1).

The only free-living stage is a non-feeding, developmentally arrested, third-stage infective (dauer) juvenile that is ensheathed in the second stage cuticle. It occurs in soil and its sole function is to search for new hosts and initiate an infection. The infective juvenile of both steinernematids and heterorhabditids enters its insect host through natural openings (e.g., mouth, anus, spiracles), eventually reaching the insect's body cavity (hemocoel) (Fig. 1). The heterorhabditid infective juvenile, because it possesses an anterior tooth for scraping, can also penetrate through thin cuticle where it directly enters the host's body cavity. Once in the body cavity, the infective juvenile that has the mutualistic bacterium in its intestine releases the associated *Xenorhabdus* bacterium for steinernematids or *Photorhabdus* for heterorhabditids.

The symbiotic bacteria multiply rapidly causing host mortality within 48 hours, but in smaller insects, host mortality can occur in minutes, presumably due to mechanical damage (LeBeck *et al.*, 1993). The nematodes feed upon the bacterial cells and degrading host tissues, mature, mate, and may produce up to three generations within a single host. [However, one species, *Steinernema kushidai*, produces only one generation per host regardless of its size (Mamiya, 1988).] The infective juveniles of steinernematids develop into either males or females, whereas those of heterorhabditids become hermaphroditic adults. In subsequent generations, both steinernematids and heterorhabditids are amphimictic (i.e., males and females). As the nutritional quality within the cadaver deteriorates, the nematodes develop into infective juveniles sequestering the mutualistic bacteria in their intestines. The infective juveniles emerge from the cadaver and seek new hosts. Under ideal conditions, infective juveniles of steinernematids emerge from the cadaver from 6-11 days and of heterorhabditids 12-14 days after infection.

Table 1. Nematode species and their mutualistic bacterium that are commercially available[1] or being considered for commercialization.

Nematode species	Bacterial symbiotic species	Commercial status
Steinernema carpocapsae	*Xenorhabdus nematophilus*[2]	Available
S. feltiae	*X. bovienii*	Available
S. glaseri	*X. poinarii*	Available
S. kushidai	*X. japonicus*	Not yet available
S. riobrave	*Xenorhabdus* sp.	Available
S. scapterisci	*Xenorhabdus* sp.	Currently unavailable[3]
Heterorhabditis bacteriophora	*Photorhabdus luminescens*[4]	Available
H. marelatus	*P. luminescens*[4]	Not yet available
H. megidis[5]	*P. luminescens*[4]	Available

[1]Information for commercially available nematode species obtained from website below.
http://www2.oardc.ohio-state.edu/nematodes/nematode_suppliers.htm
[2]Currently, 5 species of *Xenorhabdus* are recognized. Not listed in the table is *X. beddingi* associated with an undescribed *Steinernema* sp.
[3]This species was available at one time but not under current commercial production.
[4]The bacterial species associated with heterorhabditid species is under review and may be separated into more than one species.
[5]Available in European Union.

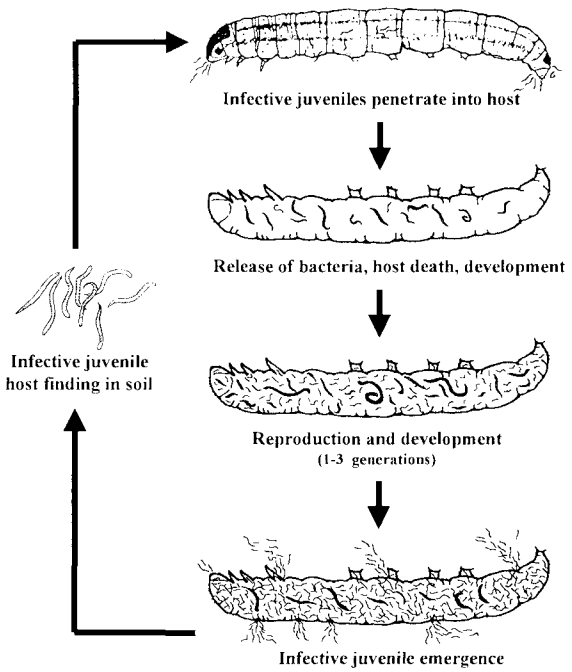

Figure 1. A simplified life cycle of insecticidal nematodes.

The relationship between the nematode and associated bacterium is one of classical mutualism as both derive benefits from the association. The bacterium receives the following benefits from the nematode.

(1) Because it is unable to survive in the soil, the bacterial symbiont requires the infective juvenile nematode for protection by being housed in its intestine.
(2) Lacking invasive ability, it is dependent upon the infective juvenile to transport it into the host's hemocoel.
(3) It receives protection from the nematode that inhibits the host's antibacterial defenses.

The nematode receives the following benefits from the bacterium.

(1) The bacterium kills the host quickly and creates a suitable environment for the nematode to develop by producing antibiotics that suppress competing microorganisms.
(2) The bacterium transforms the host tissues into a food source for the nematode.
(3) The bacterium serves as a food source for the nematode.

The bacterial symbionts, *Xenorhabdus* spp. and *Photorhabdus* spp., are motile, Gram-negative, facultative anaerobic rods in the family Enterobacteriaceae. At present, 5 species of *Xenorhabdus* are recognized (Table 1). Each bacterial species is associated with a given nematode species, but a given bacterial species may be associated with more than one nematode species. However, many of the bacterial species associated with steinernematid species, especially of the newly-described ones, remain to be identified. In contrast, all symbionts isolated from heterorhabditid species are currently assigned to *P. luminescens*, but recent research suggests that they will be separated into different species (Liu *et al.*, 1997; Ehlers & Niemann, 1998).

Differences between the two bacterial genera (Forst & Nealson, 1996) include the following:

(1) most *Photorhabdus* isolates bioluminesce whereas *Xenorhabdus* isolates do not bioluminesce,
(2) *Photorhabdus* isolates turn the host cadaver red, purple, orange, yellow, brown or sometimes green whereas *Xenorhabdus* isolates turn the cadaver tan, ochre, gray, or dark gray,
(3) *Photorhabdus* isolates are catalase positive whereas *Xenorhabdus* isolates are catalase negative, and
(4) *Photorhabdus* isolates produce antibiotics such as hydroxystilbenes and anthroquinones whereas *Xenorhabdus* isolates produce antibiotics such as indoles, xenorhadins, and xenocoumacins.

In addition, both bacterial genera produce phenotypic variant forms referred to as phase I and phase II. Phase I is always isolated from the insecticidal nematodes in nature and is the form normally associated with the nematodes. Phase II can arise spontaneously in the laboratory when the bacterial cultures are in the stationary stage. Differences occur between phase I and II with phase I producing antibiotics, adsorbing certain dyes, and developing large inclusion bodies composed of crystal proteins and phase II not producing antibiotic, not absorbing the dyes, and forming intracellular crystals inefficiently. Moreover, differences in colony morphologies occur between the two phases, and in general, phase I is more effective in producing nematodes in vitro than phase II.

HOST RANGE

Most insecticidal nematode species (e.g., *S. carpocapsae, S. feltiae, S. riobrave, H. bacteriophora,* and *H. megidis*) attack a wide spectrum of insects in the laboratory where host contact is ensured, environmental conditions are optimal, and ecological and behavioral barriers are removed. In the field, the range of insects infected by these nematodes after inundative application is considerably narrower with impact greatest on the target insect and little impact on nontarget insects (Georgis *et al.*, 1991, Bathon, 1996). Because isolation of new nematode strains/species is usually done using larvae of the greater wax moth, *Galleria mellonella*, the host range of these nematodes tends to be broad or biased towards lepidopterous insects. However, some nematode species that have been isolated from cadavers in the field have a restricted host range. Thus, *S. scapertisci* is adapted to mole crickets (Parkman & Smart, 1996) and

infects other insects poorly (Grewal *et al.*, 1993). Similarly, *S. kushidai* appears to primarily infect scarabaeid (white grub) larvae (Mamiya, 1989).

ECOLOGY

Natural occurrence

Insecticidal nematodes have a worldwide distribution as they have been isolated from every inhabited continent and many islands (Hominick *et al.*, 1996). They have been isolated from different soil types, from sea level to high altitudes, and from natural habitats to disturbed agroecosystems. Thus, in California, they have been found in many natural habitats (Stock *et al.,* 1999); in New Jersey, they have been found in nearly 22% of soil samples taken throughout the state (Gaugler *et al.*, 1992b); and in Hawaii and Ireland, they have been isolated primarily in sandy soils along the seashore (Hara *et al.*, 1991; Griffin *et al.*, 1994). High prevalence of nematode disease (i.e., epizootics) can occur in the soil environment, but these are difficult to document (Kaya, 1990). However, Akhurst *et al.* (1992) reported a heterorhabditid epizootic in a white grub larval population in sugar cane fields in Australia. Similarly, a heterorhabditid epizootic has been observed in high populations of white grubs in a northern California golf course (Kaya, unpublished data).

Abiotic factors

Abiotic factors in the soil environment, such as texture, moisture, temperature, aeration, and ultraviolet light, can affect insecticidal nematode survival. For example, when infective juveniles of *S. carpocapsae* and *S. glaseri* were placed in sterilized sand, sandy loam, clay loam or clay soils for 16 weeks, the lowest survival for both species was recorded in the clay soil (Kung *et al.*, 1990). This lower survival rate is probably related to the lower oxygen levels because of the small pores in clay soils. Nematode survival is also poor in soils with high organic matter or water-saturated soils because oxygen becomes the major limiting factor. Yet, adequate soil moisture is central to infective juvenile survival. Infective juveniles can survive desiccation to relatively low moisture levels if water is removed gradually providing sufficient time for them to adapt to an inactive state (Womersley 1990). However, when nematodes are applied to foliage during the day, nematode survival is for a few minutes to hours unless the relative humidity is close to or at 100% (Baur *et al.*, 1997). The nematodes will die from desiccation (Baur *et al.*, 1997) or ultraviolet light (Gaugler *et al.*, 1992a).

The effects of temperature on survival vary with nematode species and strains (Griffin, 1993; Grewal *et al.*, 1994). Extended exposure to temperatures below 0°C and above 40°C is lethal to most nematodes, but the lethal effect is dependent upon exposure time (Brown & Gaugler, 1996) and species (Kaya, 1990). Nematode species isolated from temperate regions tend to be more tolerant of low temperatures than species isolated from tropical or subtropical regions (Kung *et al.*, 1990). In general, infective juveniles survive best between 5 and 15°C. At higher temperatures, the infective juveniles have an increased metabolic rate and deplete their energy reserves faster shortening their life span. In the soil, the infective juveniles are buffered from temperature extremes and can move from areas of unfavorable to more favorable temperatures (or moisture). From a practical viewpoint, application of insecticidal nematodes is recommended early in the morning or late in the afternoon or on cloudy days to minimize detrimental effects of desiccation, ultraviolet light and extreme temperatures.

Other abiotic factors that the infective juveniles may encounter such as extreme pH and salinity or pesticides probably have minimal effect on their survival or infectivity. Soil pH values between 4 and 8 have little or no effect on nematode survival (Kaya, 1990). Soil salinity also seems to have limited negative effects on survival or infectivity even at a salinity well above the tolerance levels of most crops (Thurston *et al.*, 1994). Seawater has no negative effect on survival of heterorhabditids; many species have been isolated near the seashore (Griffin *et al.*, 1994; Liu & Berry, 1996). Although direct placement of infective juveniles into chemical pesticides can be detrimental (Kaya, 1990), in the soil environment,

the pesticides are probably sufficiently "diluted" that the effects will be minimal. In some cases, the exposure of insects to low concentrations of certain pesticides can stress them, making them more susceptible to insecticidal nematodes (Akhurst *et al.*, 1992; Koppenhöfer & Kaya, 1998).

Biotic factors

A number of biotic factors can influence the survival and infectivity of insecticidal nematodes in soil (Kaya & Koppenhöfer, 1996). Allelochemicals from plant roots are known to have an adverse effect on host finding. Competition, both intraspecific and interspecific, can affect nematode fitness and recycling. In intraspecific competition, nematode fitness, and hence progeny survival and recycling, may be affected by the presence of too many infective juveniles of one species in a single host. In interspecific competition, presence of different bacterial symbionts may reduce fitness of the nematode progeny and affect recycling. Although two or more nematode species may occur in the same soil habitat, they co-exist by having different foraging strategies or infecting different hosts (Koppenhöfer & Kaya, 1996). With other competitors, such as fungi, bacteria, or viruses, the timing of infection, and environmental factors such as temperature or soil moisture will determine the outcome.

Insecticidal nematodes have their own natural enemies (Kaya & Koppenhöfer, 1996). The infective juveniles are preyed upon by collembollans, mites, tardigrades and predatory nematodes. Nematophagous fungi, such as *Hirsutella rhossiliensis, Arthrobotrys* spp., and *Monacrosporium* spp., infect or "trap" the infective juveniles. However, the impact of these predators and nematophagous fungi on infective juveniles has not been evaluated under field conditions. In addition to these mortality factors of infective juveniles, developing nematodes within the cadaver are susceptible to various invertebrate scavengers that can affect recycling of the nematodes (Baur *et al.*, 1998).

Recycling

Because they are obligate parasites, natural populations of these nematodes need to recycle in their hosts to maintain their presence in the environment. The distribution of nematode populations is patchy at any given site (Stuart & Gaugler, 1994; Strong *et al.*, 1996; Campbell *et al.*, 1998) and may depend on various abiotic and biotic factors including seasonal variations, foraging strategy of the nematodes, host abundance, alternate hosts, natural enemy complex of the nematodes, etc. From a practical point, after inundative release of the nematode, recycling is a highly desirable attribute because it can provide additional and prolonged control of the pest and avoid or reduce further applications. Numerous studies have shown that nematode recycling in the soil environment does occur after inundative releases (Kaya, 1990; Klein, 1993), but factors favoring sufficient recycling to provide continuous control remain unknown. Abiotic and biotic factors that influence survival and infectivity also influence nematode recycling, but until we can understand them thoroughly, the approach will be to use these nematodes as biological insecticides. However, one species, *S. scapterisci*, has been successfully introduced as a classical biological control agent against the mole crickets in Florida (Parkman & Smart, 1996), suggesting that proper conditions prevail for this nematode to recycle.

CONCLUSIONS

Understanding the biology and ecology of insecticidal nematodes is the first step of many in using them for inundative releases. Clearly, insecticidal nematodes being living animals cannot be treated like inert, chemical compounds. Although they can be applied like a chemical insecticide, knowing their positive attributes and their limitations will enhance their successful use against target pests. They cannot tolerate temperature extremes, rapid desiccation, or ultraviolet light, and therefore, applications should be made early in the morning, late in the afternoon, or on cloudy days. Yet, many other issues such as foraging strategies and matching nematode species with the target insect, biology and ecology of the pest species, the cropping system, formulations, quality control, and application techniques require your attention to optimize pest control with the insecticidal nematodes.

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Matching Nematode and Insect to Achieve Optimal Field Performance

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ABSTRACT

More failures in the field using entomopathogenic nematodes are attributable to poor matches (e.g., *S. carpocapsae* vs. citrus root weevil) than any other factor. The broad host range of entomopathogenic nematodes like *S. carpocapsae* is largely a myth limited to the laboratory. Only when considered as a *group*, that is when the full suite of species is considered, may entomopathogenic nematodes be considered to possess a broad host range. Exploiting this biodiversity requires the ability to make proper matches between nematode and host species. A better understanding of the host selection process (host habitat finding, host finding, host acceptance, host suitability) permits better matches and enhanced field performance.

INTRODUCTION

In 1979, only a single strain of a single species of entomopathogenic nematode, the All strain of *Steinernema carpocapsae*, was commercially available. The hopes and dreams of this fragile infant industry, not to mention those of academic researchers, rested entirely on what proved to be a very narrow foundation. Today, 20 years after *S. carpocapsae* was first introduced, seven nematode species have been commercialized, six of which are still available, five in the U.S. What drove this expansion? Just as with chemical insecticides, consumers need different nematodes to match different pest biologies.

But in the beginning, the conventional thinking among many, especially in industry was that *S. carpocapsae* was some sort of biological "silver bullet." And, indeed, this nematode was well known to be lethal to hundreds of diverse pest species, from termites to caterpillars, fleas to black widow spiders. This nematode seemed to have a host range not unlike the organophosphates and carbamates that then dominated the insecticide scene, and this coupled with their exemption from government registration sparked keen interest from industry.

Yet when field tested against these same insects, the nematode worked sometimes but more often failed, usually miserably. The conventional wisdom began to grow that nematodes "don't work" when the only truth actually revealed was that nematodes don't work for that particular insect. Just as imidacloprid is a wonderful chemical agent against many white grub species but is nearly useless against carpenter ants, *S. carpocapsae* is effective against webworms but ineffective for mushroom flies -- yet *S. feltiae* is an excellent match against these flies.

The extraordinarily wide spectrum of activity attributed to *S. carpocapsae* is largely based on experimental infections (Gaugler, 1988). That is, lab exposures conducted in petri plates where host-parasite contact is assured, host escape is impossible, and environmental conditions of temperature, moisture, and light are optimal for infection. In the field, behavioral and environmental barriers come into play and restrict host range. In short, an experimental (i.e., lab-derived) host range is not to be confused with field activity. Experimental host ranges can be huge. But in the real world there are barriers that can disrupt the infection process, frustrating control efforts and resulting in a far narrower spectrum of insecticidal activity. It is these barriers that require careful matching of nematode and insect.

HOST SELECTION

Infection barriers are all part of the host selection process for entomopathogenic nematodes, which consist of four sequential steps (Fig. 1): 1) host-habitat finding, 2) host-finding, 3) host acceptance, and 4) host suitability (Doutt, 1964). Each step acts as a sort of biological sieve, narrowing an experimental to a field host range. If our goal as practitioners is to match target insects with the nematode species best able to parasitize it, we must understand and appreciate each step.

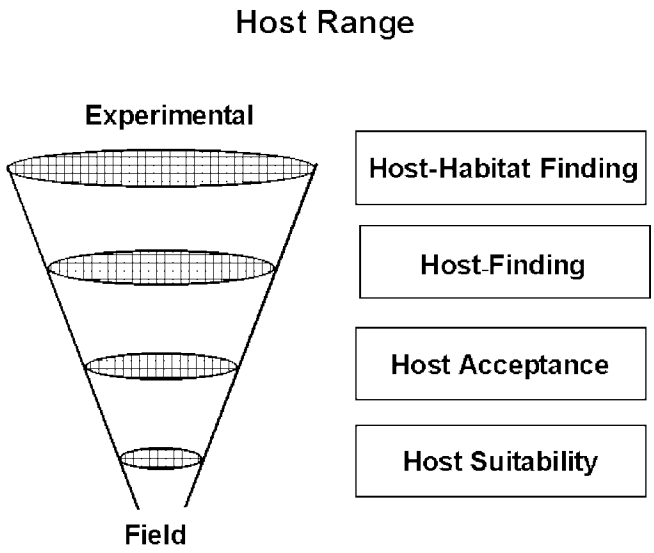

Figure 1. Host selection process in entomopathogenic nematodes.

Host-Habitat Finding

This simply means that parasite and host must coincide in time and space. For example, mosquito and blackfly larvae are good experimental but poor field hosts because nematodes are not adapted to the aquatic environment and quickly settle out of the host-feeding zone (Finney & Harding, 1981; Gaugler *et al.*, 1983). Cabbage loopers are easy for nematodes to kill in the lab but they can rarely tolerate the physical extremes characteristic of exposed foliage: rapid desiccation, high surface temperature, and exposure to solar radiation. Nematodes are soil adapted (Gaugler, 1988). The soil environment buffers them against extremes of the above ground world. Overwhelmingly, nematodes are most effective when soil insects are the target pests.

Every rule has exceptions. As soil organisms, nematodes certainly did not evolve for life within stems; however, this is an example of a microhabitat that coincidentally offers the same shelter from environmental extremes as the soil. Thus nematodes injected into wood galleries and other cryptic habitats tend to perform their insect-killing role well (Kaya & Gaugler, 1993). Such applications bypass the host habitat barrier. In addition, habitats can be modified to make them more favorable. Thus, Begley (1990) has demonstrated field efficacy against foliage-feeding caterpillars when commercial chrysanthemums were sheltered under shade-cloth, eliminating use of three conventional chemical insecticides. Although nematodes applied outside their natural reservoir, the soil, have no prospects for establishment and recycling (e.g., long-term control), they do have utility where a short-term knockout blow must be delivered.

Host-finding

Once in the proper habitat, infective-stage nematodes must locate insect hosts. Host-finding strategies can be divided into two broad categories: ambushing and cruising (Gaugler *et al.*, 1989; Campbell & Gaugler, 1993; Lewis *et al.*, 1992; 1993). Ambusher and cruiser strategies can be distinguished by their contrasting host search behaviors. Cruiser nematode species such as *S. glaseri* and *Heterorhabditis bacteriophora* tend to be highly mobile in searching comparatively large areas for hosts, whereas ambusher species tend to remain stationary. The key reason for this dichotomy in behavior is nictation. Ambushers nictate, that is they search by standing on their tail, elevating most of their bodies free in the air. This sit-and-wait approach to find hosts serves as a mechanism for host attachment. Ambushers are unable to detect hosts resting only a few millimeters away. By contrast, cruisers are unable to nictate but are highly responsive to host-released volatiles like carbon dioxide, which they use to orient toward insects. Ambushing is clearly a surface-adapted behavior, as it is not possible to nictate effectively within the soil. And, indeed, soil sampling reveals that ambush species tend to be found in the upper soil stratum especially near the soil surface litter and duff (Campbell *et al.*, 1996). Cruiser species are found distributed throughout the soil profile as would be predicted from their search behavior.

If ambushing is a stationary behavior that occurs at or near the soil surface, then it follows that ambusher nematodes are best adapted to parasitize highly mobile, surface-adapted hosts (e.g., cutworms, armyworms). How effective, for example, could *S. carpocapsae* or S. *scapterisci* be expected to be against white grubs when both parasites and hosts are relatively sedentary and inhabit different parts of the soil profile? If cruising is a mobile behavior that occurs below ground, then cruiser nematodes must be best adapted to parasitize sedentary, below ground hosts such as white grubs. Thus, understanding host-finding strategies increases our ability to make efficacy predictions, thereby optimizing host:parasite matches.

Again, there are exceptions to this generalization. Host finding is a continuum. Ambusher species such as *S. carpocapsae* and *S. scapterisici* form one end of the continuum and cruisers such as *H. bacteriophora* and *S. glaseri* form the opposite end. Other species, notably *S. riobravis* and *S. feltiae*, are intermediate, doing a bit of both ambushing and cruising (Campbell & Gaugler, 1997). We do not yet know where most of the more than 30 species of entomopathogenic nematodes fall on the continuum.

Host Acceptance

An entomopathogenic nematode can parasitize only a single host, so each infective nematode must carefully assess an insect before committing irreversibly. That is, nematodes must be able to recognize their hosts so they don't make an irreconcilable mistake and attack a host that's unsuitable. In short, if they don't recognize a host, they shouldn't attack under most conditions.

Lewis *et al.* (1997) demonstrated that entomopathogenic nematodes are able to discriminate among potential hosts. This study showed *S. carpocapsae* to be highly responsive to caterpillars, a modest response to white grubs, and unable to differentiate between millipedes and plastic. This correlates positively with the suitability of these insects as hosts, thereby providing an excellent measure of adaptation and an excellent means for making more accurate nematode:insect matches.

Once a potential host has been contacted and recognized, the insect is not defenseless. Consider white grubs. The spiracles are a key portal of entry of *S. carpocapsae* attacking caterpillars, but white grub spiracles are covered with sieve plates that preclude invasion via this route (Forschler & Gardner, 1991). The alternate penetration route for this nematode tends to be the gut; but whereas the highly susceptible wax moth has a thin, loose peritrophic membrane lining the gut, white grubs possess a thick, multi-layered protective membrane. Therefore only highly adapted nematodes such as *S. glaseri* are a good match against these insect pests.

Host Suitability
Once a host has been located, recognized, and penetrated, the nematode's attack still may not succeed if the insect is able to respond with an effective immune response. The immune response also provides us with clues for making the most appropriate host:parasite matches, since a strong immune response suggests a low level of adaptation. Thus, *S. carpocapsae* is a poor match for Japanese beetle larvae where encapsulation begins immediately and melanization is complete in a few hours (Wang *et al.*, 1995). By contrast, *S. glaseri* invasion elicits a week immune response that is quickly defeated by the nematode-released anti-immune proteins. This would indicate that the latter nematode is the best match for control purposes, a prediction borne out by extensive field testing. So consideration of host suitability provides another measure useful in avoiding incompatible matches.

SPECIES CHARACTERISITICS

Five entomopathogenic nematode species are currently commercially available in the U.S. Each species is a very different organism. The following brief synopsis on each species is intended to guide users in making predictions regarding field performance.

Steinernema carpocapsae
The most studied, available, and versatile of all entomopathogenic nematodes. Important attributes include ease of mass production and ability to formulate in a partially desiccated state that can provide several months of room-temperature shelf life. Particularly effective against caterpillars, including webworms, cutworms, armyworms, girdlers, and wood-borers. This species is a classic sit-and-wait or "ambush" forager, standing on its tail in an upright position near the soil surface and attaching to passing hosts. Consequently, *S. carpocapsae* is most effective when applied against highly mobile surface-adapted insects. Highly responsive to carbon dioxide once a host has been contacted, the spiracles are a key portal of host entry. It is most effective at moderate temperatures ranging from 22 to 28°C.

Steinernema feltiae
Attacks primarily immature dipterous insects (i.e., fly larvae), including mushroom flies, fungus gnats, and crane flies. This nematode is unique in maintaining infectivity at low soil temperatures, even below 10°C. *S. feltiae* offers lower stability than other steinernematids.

Steinernema glaseri
The largest entomopathogenic nematode at twice the length but eight times the volume of *S. carpocapsae* infective juveniles, *S. glaseri* attacks beetle larvae, particularly those of scarabs. This species is a cruise forager, neither nictating nor attaching well to passing hosts, but highly mobile and responsive to long-range host volatiles. Thus, this nematode is best adapted to parasitize hosts possessing low mobility and residing within the soil profile. Field trials, particularly in Japan, have demonstrated that *S. glaseri* can provide good control of several scarab species. Large size however reduces yield, making this species more expensive to produce than other species. A tendency to occasionally "lose" its bacterial symbiont is troublesome. Moreover, the highly active and robust infective juveniles are difficult to contain within formulations that rely on partial nematode dehydration (e.g., clay granules). In short, additional technological advances are needed before this nematode is likely to realize its full potential.

Steinernema riobravis
This highly pathogenic species, isolated to date only from the Rio Grande Valley of Texas, possesses several novel features. Its effective host range runs across multiple insect orders, a versatility likely due in part to its ability to exploit aspects of both ambusher and cruiser means of finding hosts. Trials have demonstrated its effectiveness against corn earworms, citrus root weevils, pink bollworms, and mole crickets. This is a high temperature nematode, effective at killing insects at soil temperatures above 35°C. Persistence is excellent even under semi-arid conditions, a feature no doubt enhanced by the high lipid

levels found in infective juveniles. Its small size provides high yields whether using *in vivo* (up to 375,000 infective juveniles per wax moth larvae) or *in vitro* methods. Only formulation improvements that impart increased commercial stability are needed for this parasite to achieve its full potential.

Heterorhabditis bacteriophora
Among the most important entomopathogenic nematodes, *H. bacteriophora* possesses considerable versatility, attacking caterpillar and beetle larvae among other insects. This cruiser species appears most useful against root weevils, particularly black vine weevil where it has provided consistently excellent results in containerized soil. A warm temperature nematode, *H. bacteriophora* shows reduced efficacy when soil temperature drops below 20°C (Georgis & Gaugler, 1991). Poor stability has limited the usefulness of this interesting nematode: shelf-life is problematic and most infective juveniles persist only a few days following application.

Heterorhabditis megidis
Isolated in Ohio, researched and developed in Europe, and now sold in the U.S., this nematode is marketed primarily against black vine weevil. Field results have been highly favorable in containerized soil although its large size, characteristic heterorhabditid instability, and dearth of field efficacy data against other insect pests limit its utility at present.

THE NEED TO KNOW MORE

Additional nematodes species are the subject of vigorous research and development efforts and may be considered to be in the "pipeline." *Heterorhabditis indicus* is a small, efficiently produced nematode said to be effective against white grubs and certain root weevils. *Steinernema kushidai*, a Japanese nematode, shows good efficacy against white grubs and has excellent stability and persistence potential, but mass production so far is problematic. *Heterorhabditis marelatus* is efficacious against root weevils and active at cool soil temperatures. *Steinernema scapterisci*, once wielded against mole crickets may make a comeback. Other species are also being evaluated in one or more of the 80 entomopathogenic nematode laboratories located around the world. Moreover, new strains of currently commercialized species are the subject of studies aimed at improved pathogenicity, stability, yields, and more.

Nematode end-users will need to know more as new strains and species come "on-line" and further technological advances are made. Tools are available to assist users in staying current and making optimal nematode-insect matches. The best place to find relevant new information is the SARE Entomopathogenic Nematode Website (www2.oardc.ohio-state.edcu/nematode). Particularly useful here is a comprehensive bibliography of the research literature that permits quick accessing of all published papers, particularly field trials, for any target insect of interest. The site's Electronic Expert Panel is an alternative means of getting answers to questions by tapping into the site's stable of international nematode authorities. Be prepared, however, for instances of inconclusive data or no data for a particular nematode-insect combination. This is best seen as an opportunity for end-users to cut new ground by experimentation.

CONCLUDING COMMENTS

It all looks so complicated; chemicals seem so much easier to use. Certainly most organophosphate and carbamate insecticides are sufficiently broad spectrum that matching is not a major aggravation. Well, those chemicals, many developed from military chemical warfare programs, are just beginning to disappear as the federal Food Quality Protection Act sees implementation. They are on the "chopping block" precisely because of their broad range of activity. The latest generation of chemicals requires a

level of "matching" previously unimagined for insecticides. Agents like imidacloprid are far narrower in activity than their predecessors. Imidacloprid has low activity for caterpillars but sees wide use for white grubs. Interestingly, it is extremely effective against many grubs (Japanese beetle, masked chafer), yet it does not provide good results against many other species within the same family (e.g., oriental beetle, asiatic garden beetle). Similarly, timing was not a significant issue for older generations of broad-spectrum chemicals, but many of the new chemicals like imidacloprid and halofenozide are used as preventatives against young insects, narrowing the application window. In short, chemical agents are no longer fool-proof -- the "ease-of-use" gap between chemicals and biologicals like nematodes is beginning to narrow. Whether chemical or biological, increasingly the ability to make optimal matches will enhance field performance.

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Production, Formulation, and Quality

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ABSTRACT

Mass-production and formulation of insecticidal nematodes have seen phenomenal success in the past two decades. Liquid fermentation processes have been established that allow production at 300 - 80,000L scale. Five steinernematid (*Steinernema carpocapsae*, *S. riobrave*, *S. feltiae*, *S. scapterisci*, and *S. glaseri*) and three heterorhabditid (*Heterorhabditis bacteriophora*, *H. indicus*, and *H. megidis*) nematodes have been successfully produced in liquid culture with yields ranging from 0.6×10^5 to 4×10^5 infective juveniles per ml. Easy-to-use wettable powder and water dispersible granular formulations with ambient storage stability have been developed. Formulated *S. carpocapsae* can be stored for 4-5 months at room temperature and *S. feltiae* and *S. riobrave* for 2-3 months. A new wettable powder formulation allows ambient storage of the first *Heterorhabditis* product for 2-3 months. Quality of nematode products is measured as nematode viability, total viable nematodes, virulence, age, and quantity of stored energy reserves. Cottage industry that uses *in vivo* nematode production lacks rigorous quality control.

INTRODUCTION

Insecticidal nematodes (Steinernematidae and Heterorhabditidae) have emerged as excellent biological control agents for soil-dwelling stages of many insect pests (Gaugler & Kaya, 1990; Kaya & Gaugler, 1993). They possess many positive attributes including their broad host range, safety to non-target organisms and the environment, exemption from registration in many countries, ease of mass-production, ease of application, ability to search for pests, rapid host mortality, potential to recycle in the environment, and compatibility with most agricultural chemicals. These positive attributes and the need to find alternative methods of pest control to chemical insecticides have led to the rapid commercialization of the nematodes. Progress has been made that insecticidal nematodes are now available commercially for large-scale application in citrus groves, strawberry plantations, cranberry bogs, artichokes, mint, mushrooms, ornamentals (both greenhouse and outdoor), and turfgrass (Grewal & Georgis, 1998). Advances in our understanding of the *in vitro* mass-production techniques involving solid substrates and liquid media (Friedman, 1990) have been so rapid that it is now feasible to consider the use of nematodes to control pests damaging one million hectares of apple orchards in China. During the past few years a distinct cottage industry has emerged which utilizes the *in vivo* mass-production and caters to the home lawn and garden markets. In this paper, the developments in nematode mass-production, formulation, and quality assessment are briefly reviewed.

MASS-PRODUCTION

Insecticidal nematodes can be mass-produced by *in vivo* or *in vitro* methods. The *in vivo* process is very simple and requires only minimal initial investment. The equipment used is also simple: trays and shelves. The wax moth larvae are most commonly used to rear nematodes because of their commercial availability. The methods of nematode infection, inoculation, and harvesting have been previously

described (Dutky *et al.*, 1964; Howell, 1979; Lindegren *et al.*, 1993; Flanders *et al.*, 1996). Using the *in-vivo* process, yields between 0.5 x 10^5 - 4 x 10^5 infective juveniles per larva have been obtained (Table 1). However, the *in vivo* process lacks any economy of scale; the labor, equipment, and material (insect) costs increase as a linear function of production capacity. Perhaps even more important is the lack of improved quality while increasing scale. The *in vivo* nematode production is increasingly sensitive to biological variations and catastrophes as scale increases (Friedman, 1990).

As early as 1931, Rudolf Glaser recognized the value of developing artificial culture methods for insecticidal nematodes and devised the first such method for *Steinernema glaseri*. However, Glaser was unaware of the significance of symbiotic bacteria in the nutrition and pathogenicity of nematodes which was recognized much later (Poinar & Thomas, 1966). Therefore, the first successful commercial scale monoxenic culture was developed by Bedding (1981) and has come to be known as "solid" culture. In this method, nematodes are cultured on a crumbed polyether polyurethane sponge impregnated with emulsified beef-fat and pig's kidneys along with symbiotic bacteria. Using this method approximately 6 x 10^5 - 10 x 10^5 infective juveniles/gram of medium were produced (Bedding, 1984). Since then, this method has been commercially used in Australia, China, and USA. In a scale-up model, Friedman (1990) reported that the solid culture method is economically feasible up to a production level of approximately 10 x 10^{12} nematodes/month. Labor costs increase significantly for nematode production beyond this level, making a less expensive method of large-scale production a necessity.

Friedman (1990) reported the development of a liquid fermentation technique for large-scale production of nematodes. In this method, costs of production decrease rapidly up to a capacity of approximately 50 x 10^{12} infective juveniles/month. Using this method, five steinernematids, *S. carpocapsae, S. riobrave, S. scapterisci, S. feltiae,* and *S. glaseri* have been produced at 80,000L scale. Liquid culture procedures have also been established for three heterorhabditid species, *Heterorhabditis bacteriophora*, *H. indicus*, and *H. megidis* which have been produced successfully at 300 - 2,000L level. Liquid culture is now used to mass-produce nematodes in USA, UK, Italy, Germany, and the Netherlands. Recent improvements in the fermentation and media formulation processes have resulted in further improvements in nematode yields and quality (Grewal, unpublished data). The current yields of different nematode species in the liquid culture are given in Table 1.

Table 1. Infective juvenile (IJ) yields of *Steinernema* and *Heterorhabditis* species in *Galleria mellonella* larvae and in liquid culture

	Mean IJ yield (x 10^5)	
Nematode species	Larva^{-1} (*in vivo*)	ml^{-1} liquid culture (*in vitro*)
S. carpocapsae	0.5-1.2	2.0 - 2.8
S. feltiae	0.8-1.2	1.2 - 1.6
S. glaseri	0.3-0.6	0.8 - 1.2
S. riobrave	2.8-3.2	3.0 - 3.5
S. scapterisci	0.3-0.5	2.4 - 3.0
H. bacteriophora	2.0-3.0	3.5 - 4.0
H. indicus	2.0-2.8	3.0 - 4.0
H. megidis	0.8-1.5	0.6 - 1.2

FORMULATION

Following recovery from production substrates, the nematodes can be either stored in bulk for extended periods or formulated immediately. When the nematodes are to be stored as bulk, nematode concentration, temperature, aeration, and contamination control are important considerations for the maintenance of high viability and quality. Differences in storage stability among nematode species can be attributed to their thermal and behavioral adaptations. Each nematode species has a well defined thermal niche (Grewal *et al.*, 1994) and an optimum temperature for the longest storage stability. For instance, *S. feltiae* stores better at 5°C whereas *S. scapterisci, S. riobrave* and *H. bacteriophora* are more stable at 10°C (Grewal, unpublished data). The nematode species (e.g. *S. carpocapsae* and *S. scapterisci)* that adopt a quiescent posture during storage generally store better than the more active species such as *H. bacteriophora* and *S. riobrave*. The latter species also tend to be more prone to bacterial contamination during storage. Antimicrobial agents may be used to suppress contamination, and the choice of the antimicrobial should be based on its safety to nematodes and symbiotic bacteria.

Infective juveniles (IJs) can be stored in refrigerated bubbled tanks for few days to several weeks. However, difficulties to store concentrated nematodes (>6 x 10^5 IJs/ml) due to high oxygen demand and contamination increases costs of storage and reduce nematode shelf-life and quality. Therefore, infective juveniles are formulated immediately after harvest from the fermentation media in either inert carriers or active materials including gels, granules, and powders (Table 2).

Inert carriers

Placement of nematodes on inert carriers provides a convenient way to store small quantities of nematodes under refrigerated conditions. Among various inert carriers, the polyether-polyurethane sponge and vermiculite are most commonly used for commercial nematode storage and shipping. An aqueous nematode suspension is applied to the sheets of sponge usually at 500-1000 IJs/cm^2 of surface area. Normally 5-25 x10^6 IJs are placed on a single sheet of sponge that are then placed in plastic bags. Nematodes on sponges can be stored for 1-3 months at 5-10°C (Table 2). Sponges are placed on ice packs for shipping, and the nematodes are removed by soaking and hand squeezing the sponges in water prior to application. Nematodes on sponges can be stored for 1-2 months. This method of nematode storage and shipping is convenient for small-scale home garden and lawn applications, but not for large acreage application due to the large volume of product required.

Vermiculite formulation is a significant improvement over the sponges. The advantages include a more concentrated nematode product, longer storage stability, and more convenient application. Normally, an aqueous nematode suspension is mixed homogeneously with micronized vermiculite. This mixture is placed in thin polythene bags for storage. In the vermiculite formulation, *S. feltiae* could be stored for 4-5 months and *H. megidis* for up to 3 months at 3-5°C (Table 2). The vermiculite-nematode mixture is added to the spray tank directly, mixed in water, and applied either as spray or drench. The only drawback of this formulation is the lack of ambient storage stability.

Active materials

Gels. Encapsulation of insecticidal nematodes in calcium alginate gel beads was first reported by Kaya & Nelsen (1985). It was originally developed as a means for the slow release of nematodes, but had only a limited success. This discovery subsequently led to the development of a commercial nematode product that used thin sheets of calcium alginate spread over a plastic screen to trap nematodes (Georgis, 1990). For application, the nematodes had to be released from the alginate gel matrix by dissolving it in water with the aid of sodium citrate. The alginate based *S. carpocapsae* products were the first to possess room temperature shelf-life of about 3-4 months (Grewal, 1998a), and led to an increased

acceptability of nematodes in the high and medium value niche markets. However, the time consuming extraction steps and the problematic disposal of large numbers of plastic screens and containers, rendered this formulation unsuitable for large-scale application.

A flowable gel formulation was developed to improve the ease of use by the consumer. In this formulation the nematodes are suspended in a viscous flowable gel that can be squeezed out of the paper tubes directly into the spray tank. This development did improve the ease of application, but nematode shelf-life in the flowable gel was shorter than the alginate gel (Table 2). Bedding and Butler (1994) developed another gel formulation in which nematode slurry is mixed in anhydrous polyacrylamide so that the resulting mixture attains a water activity of 0.80 to 0.995. This formulation also had a limited ambient shelf life.

Activated charcoal. Yukawa and Pitt (1985) described a system for nematode storage and transport wherein nematodes are homogeneously mixed with absorbent materials such as powdered activated charcoal. This formulation had several disadvantages including, high cost, unpleasant to handle, and no ambient storage stability.

Table 2. Expected shelf-life of some commercially available formulations containing *Steinernema* and *Heterorhabditis* species

Formulation	Nematode species	Shelf-life (Months)	
		Room	Refrigerated
Inert carriers			
Sponge	*S. carpocapsae*	0	2.0-3.0
	H. bacteriophora	0	1.0-2.0
Vermiculite	*S. feltiae*	0	4.0-5.0
	H. megidis	0	2.0-3.0
Active materials			
Alginate gels	*S. carpocapsae*	3.0-4.0	6.0-9.0
	S. feltiae	0.5-1.0	4.0-5.0
Flowable gels	*S. carpocapsae*	1.0-1.5	3.0-5.0
	S. glaseri	0	1.0-1.5
Water Dispersible Granules	*S. carpocapsae*	4.0-5.0	9.0-12.0
	S. feltiae	1.5-2.0	5.0-7.0
	S. riobrave	2.0-3.0	4.0-5.0
Wettable Powder	*S. feltiae*	2.0-3.0	5.0-6.0
	S. carpocapsae	2.5-3.5	6.0-8.0
	H. megidis	2.0-3.0	4.0-5.0
Liquid Concentrate	*S. carpocapsae*	5-6 day	12-15 day
	S. riobrave	3-4 day	7-9 day

Clay sandwich. Bedding (1988) reported a formulation in which infective juveniles are mixed in clay to remove excess surface moisture and to produce partial desiccation. He described the formulation as a sandwich consisting of a layer of nematodes between two layers of clay. This formulation was commercialized by Biotechnology Australia Ltd., and had following drawbacks: (i) no consistent room temperature shelf-life (ii) difficult to dissolve, (iii) frequently clogged spray nozzles, and (iv) a very low nematode to clay ratio. Therefore, this product was later discontinued.

Granules [Pellets, Pasta, WDG]. Capinera & Hibbard (1987) described a pellet nematode formulation in which the pellets contained alfalfa meal and wheat flour. Later, Connick *et al.* (1993) described an extruded or formed granular products in which nematodes are uniformly distributed throughout a wheat gluten matrix. This formulation was called "Pesta" and also included a filler and a humectant to enhance nematode survival. The process involved drying of granules to low moisture to prevent nematode migration and reduce risk of contamination. However, granules become very hard due to drying, and are difficult to dissolve. Furthermore, reported nematode survival is low.

A significant advancement was made with the advent of a water dispersible granule (WDG) in which infective juveniles are encased in 10-20 mm diameter granules consisting of mixtures of various types of silica, clays, cellulose, lignin and starches (Georgis *et al.*, 1995; Silver *et al.*, 1995). These granules are prepared through a conventional pan granulation process in which droplets containing a thick nematode suspension are sprayed on to fine dry powders on a tilted rotating pan. As soon as nematode droplets come in contact with the powders, the granules start to form, and roll over the dry powders adsorbing more powder around them. The granules are then sieved out of the powders and packaged into shipping cartons. The granular matrix allows access of oxygen to nematodes during storage and shipping. Under appropriate temperature regimes, the nematodes in the granules undergo a physiological desiccation process and enter into a partial anhydrobiotic state. This is usually evident by the decline in oxygen consumption by the nematodes within 4-7 days after granulation (Grewal, unpublished data).

The development of the water dispersible granular formulations offered several advantages over the existing formulations. These included: (i) extended nematode storage stability at room temperature (Table 2), (ii) enhanced nematode tolerance to temperature extremes enabling easier and less-expensive transport, (iii) improved ease-of-use of nematodes by eliminating time consuming and labor intensive preparation steps, (iv) decreased container size/coverage ratio, and (v) decreased amount of disposal material (i.e., screens and containers). In the WDG formulation, *S. carpocapsae* could be stored for 4-5 months at 25°C, and *S. feltiae* and *S. riobrave* for 2-3 months (Table 2).

Initial water activity and moisture content, temperature, and rate of water loss are the most important factors affecting nematode survival in the granules. For example, a less than optimum initial moisture content can substantially reduce nematode longevity in the granules (Grewal, 1998a). Temperature directly influences nematode longevity by regulating the use rate of stored energy reserves. Temperature is also important in the initial desiccation phase. For example, exposure of a warm adapted nematode *S. riobrave* to 5°C immediately after granulation can have catastrophic affect on nematode survival. The nematodes exposed to 5°C during the initial 24-48 h after granulation become melanized and die within 7-10 days (Grewal, unpublished data).

Wettable powder. Heterorhabditid nematodes normally do not store well, but a newly developed wettable powder formulation allows 2-3 month storage of *H. megidis* at ambient temperature (Table 2). This formulation is very similar to the vermiculite-based formulations and is very easy to apply due to its high dispersibility in water. It is expected that this technology will provide the much needed boost to the commercialization of heterorhabditid nematodes.

Liquid Concentrate. A cost-effective delivery system for highly concentrated nematode suspensions (7-8 x 10^5 IJs/ml) has been developed that allows transport at ambient temperatures. A proprietary metabolic inhibitor and an antimicrobial agent are added to the nematode suspension. The metabolic inhibitor reduces nematode O_2 demand allowing nematode survival under almost anoxic conditions for extended periods. Approximately 7-7.5 x 10^9 *S. carpocapsae* IJs can be stored for up to 6 days in a 10 L container at ambient temperatures (Table 2). This method can also be used for shipping liquid *S. riobrave*.

QUALITY

Consumer acceptance of nematode products is determined by their price, ease-of-use, and efficacy. The development of mass-production technology has led to the availability of nematode products at prices comparable to the standard insecticides in several markets. The ease-of-use of nematode products is constantly being improved through formulation research. Efficacy perhaps is the most important factor determining quality of nematode products. Efficacy depends on many factors, but maintenance of high nematode viability and virulence during production and formulation forms the backbone of an effective quality control (QC) strategy. QC begins with the selection of inoculum and ends with the satisfied customer (Table 3). Major QC procedures used during nematode manufacturing are described below.

Master culture

Genetic drift or inadvertent selection due to passage effect may cause deterioration in nematode quality. No alterations were found in nematode dry weight, lipid content, IJ length or virulence after constant sub-culture of *S. carpocapsae* for 2 years (Friedman, unpublished data). However, a decline in virulence of *H. bacteriophora* following repeated sub-cultures was observed. Therefore, as a precaution, the master cultures of nematodes and bacterial symbionts are usually frozen in liquid nitrogen and are used to initiate every new production batch. Master cultures are usually replenished by sub-culturing in a suitable insect host, usually *G. mellonella* larvae, every six months.

Bacterial phase

Bacterial symbionts of insecticidal nematodes (*Xenorhabdus* for *Steinernema* spp. and *Photorhabdus* for *Heterorhabditis* spp.) may shift into a secondary phase that reduces nematode production and quality. Therefore, checks to identify the presence of secondary phase are routine (Table 3). The techniques to distinguish secondary phase bacteria from the primary phase are described by Kaya & Stock (1997).

Infective juvenile formation

Completion of a fermentation run is marked by the conversion of the majority of nematode population into infective juveniles. The infective juvenile formation is induced by the accumulation of a population factor and the depletion of the food resource. Nematode batches may vary in IJ conversion. A lower than 'normal' conversion rate for a nematode species indicates sub-optimal fermentation and results in poor quality nematodes. Therefore, % IJ is used as a fermentation quality indicator.

Nematode yield

IJ yield also serves as a quality indicator. A less than 'normal' IJ yield indicates poor fermentation and usually results in poor nematode quality. Therefore, a record of total IJ yield is kept for each nematode batch and is compared with the established standard for each nematode species.

Table 3. A typical quality control process during nematode product manufacturing

Process	Quality assessment event
Selection of inoculum (nematodes and bacteria)	Source check Virulence Contamination Bacterial phase
Establish flask culture (1 L)	Contamination
Seed fermentation (7,500 L)	Contamination
Fermentor production (80,000 L)	Contamination % IJ IJ yield ml^{-1}
IJ separation	Viability Virulence Contamination Stored energy reserves
IJ bulk storage	Contamination Viability and Total viable ml^{-1} Virulence
Formulation	Viability and Total viable g^{-1} Virulence Dispersibility Contamination
Product storage	Viability and Total viable g^{-1} Virulence Age Contamination
Product shipping	Shipper reliability
Use by Customer	Price, Ease-of-use, and Efficacy

Viability and total viable nematodes

Viability (% viable) and quantification of total viable nematodes are central functions of manufacturing and quality control. Each harvested batch, each minimum unit of packaging and subsequent product application, is dependent upon accurate viability assessment and quantification. These two functions contribute greatly to product consistency. Overpacking is a method of ensuring minimum total viable nematodes over a period of storage. It may be used to extend shelf-life of a product in certain situations.

Virulence

Nematode virulence is the most important component of nematode quality. Virulence/pathogenicity can be measured by different methods including, 1:1 bioassay (Miller, 1989), LC_{50}'s (Georgis, 1992), invasion or establishment efficiency (Hominick & Reid, 1990; Epsky & Capinera, 1994), invasion rate (Glazer, 1992), and number of bacteria per infective juvenile (Kondo & Ishibashi, 1986). One-on-one bioassay is perhaps the most versatile method of virulence assessment (Grewal *et al.*, 1998). Most of the

infectivity assays using multiple nematodes against single or multiple hosts are considered inappropriate for quality control purposes due to the host-parasite interaction effects such as recruitment and over dispersion of natural parasite populations. The one-on-one filter paper assay and its modifications compare virulence of any nematode species with a pre-determined 'standard' against a very susceptible host. This method measures the proportion of infective nematodes in a population and is sensitive to 'impaired' nematodes. This method is appropriate for nematode species that have a lethal level of one infective juvenile per larva. The 1:1 method could be used effectively to assess quality of both ambush and cruise foragers by using different bioassay arenas. The filter paper arenas are used for ambushing nematodes such as *S. carpocapsae* and sand columns are used for nematodes that utilize a cruising approach such as *S. glaseri* and *S. riobrave* (Grewal, 1998b). A 15:1 sand-well assay has been developed for *S. scapterisci* (Grewal *et al.*, 1998) and a 5:1 assay for *H. bacteriophora* (Grewal, 1998b).

Stored energy reserves

Another major aspect of nematode quality is the maintenance of consistent viability for a minimum desired period (i.e., shelf-life claim). This aspect of quality is determined by analyzing the stored energy reserves of non-feeding infective juveniles. Total dry weight and total lipid content of nematodes are used routinely to determine batch-to-batch variation (Grewal & Georgis, 1998). Total lipid content, which constitutes about 40% of the dry weight of infective juveniles, may be used as a predictor of shelf-life.

Nematode age

Nematode age plays an important role in product performance. As the product gets older, the depletion in nematode stored energy reserves and changes in other physiological processes may reduce nematode performance (Lewis *et al.*, 1995; Selvan *et al.*, 1993). Therefore, time from production to formulation, formulation to packaging, packaging to receipt by end user, and likely end user storage time, is usually controlled. The use of batch codes and expiration dating are useful methods of tracking and controlling the inventory life (refrigerated storage time prior to application).

Contamination

Microbial contamination is a persistent problem in nematode formulations due to relatively high moisture content. Although, the direct effect of microbial contamination on nematode viability is not always evident, it significantly reduces formulation dispersibility, sometimes leading to clogging of nozzles. The commonly occurring contaminants include several different bacteria (*Entoerobacter cloacae, E. glomerans*, and *E. gergovae*), yeast (*Candida guilliermondi*), and fungi (*Penicillum expensum*, *P. chrysogenum*, and *Mucor circinelloides*). Antimicrobial agents can be used to suppress microbial growth in nematode formulations.

Nematode Quality and the cottage industry

Cottage industry that supplies *in vivo* produced nematodes through mail orders lacks rigorous quality control. In a recent study, Gaugler *et al.* (1999) found that; (i) most shipments did not contain the expected nematode quantity, (ii) pathogenicity of several products was not equivalent to the 'standard' controls, (iii) *H. bacteriophora* was not always available when ordered, (iv) a few products contained mixed populations of *S. carpocapsae* and *H. bacteriophora*, and (v) application rate recommendations provided by several suppliers were unsound. This lack of sound quality control may be due to the lack of effective means of self-regulation as the consumers are rarely able to provide feedback.

Product validation

All of the above measurements are relatively meaningless unless field efficacy data are developed for labeled targets at labeled rates. The most efficient, and perhaps believable, means of developing such

data are with the third party researchers, preferably governmental (USDA), university and industry consultants. Reaching a consensus on the efficacy of a given rate on a specific target by third parties will give the end user a clear message about the product. Lacking such credibility, the "quality" of a given product will be ambiguous.

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Handling, Transport and Storage of Insecticidal Nematodes

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ABSTRACT

Insecticidal nematode-based products are used for management of many different insect pests in several different commodities. Application methods and insect targets vary significantly in the field, but general rules for handling, transport and storage of insecticidal nematodes apply to most commercial products. Insecticidal nematodes are live organisms which are not in a resting stage. This means that their quality declines with age. They are also very susceptible to temperature extremes, ultra-violet light, anoxic conditions, and other extreme environmental circumstances. Chemical insecticides, and even some other microbial insecticides, are not as stringent in their environmental requirements. Insecticidal nematodes also have strict limitations as to their shelf life that vary significantly among different products. In this chapter, I point out potential risks that occur to insecticidal nematode products after they leave the manufacturer and before they are applied. Techniques to either deal with these periods of risk, or to assess viability afterward are the focus of the following text.

INTRODUCTION

Unpredictable levels of efficacy have been cited as one of the primary obstacles to enabling the widespread adoption of biological control materials for pest management (Georgis and Gaugler 1991). For insecticidal nematodes, one source of this variation originates from quality control issues during manufacture of the product applied. In a previous chapter, Grewal (1999) addresses variation that may occur due to manufacturing processes. Once the product leaves the manufacturer, proper storage and handling by transporters, the retailer and the end user are essential to minimize the likelihood of applying nematodes that have been subjected to conditions that have damaged them. Lethal or damaging conditions include storage at warm temperatures, freezing, exposure to UV light, anoxic conditions or simply storage for too long a duration. Insecticidal nematode products are exposed to potentially lethal or damaging environmental conditions at these four times after they leave the manufacturer and before they are applied:

(1) During transit from the manufacturer to the vendor

(2) During storage by the vendor prior to sale

(3) During transit from the vendor to the end-user

(4) During storage after purchase.

This chapter explains storage requirements and handling techniques that will maximize insecticidal nematode survival during these periods of risk, and enable the end user to assess the quality of a purchased product. I will proceed using these four periods of risk as subject headings. While these general rules will apply to most cases, some products may have special requirements not addressed here. In all cases, specific directions on product labels should take precedence over the general rules-of-thumb

provided in this chapter. When in doubt about how to store or handle a product, contact the manufacturer or University Cooperative Extension personnel. Retail personnel may not be familiar enough with these products to provide correct and up-to-date information.

The first and most important thing to remember about transporting, handling and storing insecticidal nematode products is that the nematodes are living organisms and need to be treated as such. Insecticidal nematodes are in the infective juvenile stage when purchased and applied. The infective juvenile stage of insecticidal nematodes is not considered a "resting stage". Unlike other microbial insecticides (e.g., Bt products, viruses or fungi), insecticidal nematodes use up their limited energy reserves even while they are in formulation. A new breakthrough in insecticidal nematode formulations, a dispersible granule, has extended the shelf life of insecticidal nematode products to up to six months (Georgis et al., 1995). Most formulations of insecticidal nematode products, including the dispersible granule, slow metabolism but do not stop metabolism entirely. Therefore, insecticidal nematode products have shorter shelf-life compared to chemical insecticides and many other microbial insecticides. Some formulations of insecticidal nematodes, especially granular ones, may appear very similar to chemical pesticides and it is tempting to treat them as chemicals. However, chemical insecticides are not affected by many environmental conditions that would be lethal to insecticidal nematodes. In short, insecticidal nematode products require specific conditions during transport and storage to remain viable and this information is provided below.

Transit from the manufacturer to the vendor

Purchasing a Product

The end user really has neither control over nor any real knowledge of the conditions that insecticidal nematode products experience during this phase of transit. However, there are some proactive efforts that can be made to avoid using products that have been damaged before products are purchased.

Assessment of a product's viability after purchase is not difficult, has minimal equipment requirements and can protect the end user from applying dead insecticidal nematodes.

Equipment needed:

(1) An inexpensive dissecting microscope or hand lens with at least 15X power

(2) A good light source

(3) A black or other dark colored surface

(4) A clear, shallow dish for the examination (a Petri dish is ideal)

To check for nematode viability, nematodes must be first released from their formulation. The methods for this are variable, depending upon what kind of formulation is purchased. Remember that the nematodes are, for the most part, about 0.5 mm long, so you need only check a very small amount of the purchased material. For example, a single granule will contain hundreds of individual nematodes, so a few granules are certainly enough to get a rough idea of viability.

General methods

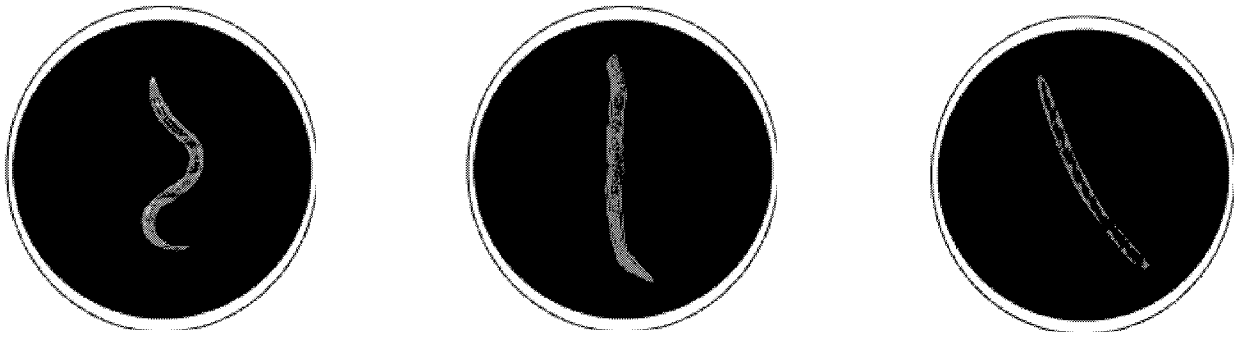
(1) Take a small amount of formulated product and weigh it.

(2) Prepare the sample for application following the instructions on the label and put the nematodes and water in the examination dish.

(3) Wait for the nematodes to revive from formulation. This duration will be written on the product label as the time to wait between preparing the product and application.

(4) Put the examination dish on the dark-colored surface and shine the light from the side, so you see white nematodes under a dark background. If the nematodes are difficult to see due to the formulation material making the water cloudy, dilute the sample. This will require some adaptation to formula (1) below.

Some formulations may require the addition of an "activator" to release nematodes from a gelatinous matrix. In this case, it may be necessary to check for viability after putting together the tank mix. After the allotted time for activation, all nematode species, except for *Steinernema carpocapsae*, should be moving in a sinusoidal ("S"-shaped) manner (Figure 1a). *Steinernema carpocapsae* infective juveniles will move when prodded using a pin or sewing needle. At rest, they assume a typical J-shape (Figure 1b). Dead nematodes appear to be straight, do not move and are often clear (Figure 1c). Another indicator of nematode viability is the density of lipid (fat) throughout the body. The nematodes should look "solid white" and not have the appearance of having bubbles within the body. These "bubbles" are actually water droplets that have replaced used-up energy reserves (Lewis *et al.*, 1995).



A **B** **C**

Figure 1. Representation of infective juvenile insecticidal nematodes. (a) All infective juveniles with the exception of *Steinernema carpocapsae* (*S. riobrave, S. feltiae, H. bacteriophora*, etc.). (b) *Steinernema carpocapsae*. (c) Any of the insecticidal nematode species after death. (Illustration by Lydia Ingrassia).

The actual number of viable nematodes in a product container can be calculated and then compared with the number specified by the manufacturer by using Formula 1. However, because the sample of nematodes taken from the package is a very small proportion of those in the entire container, this should be considered a "rule of thumb" approach. If the calculation comes close to the number stated by the manufacturer, the product is probably of adequate quality.

Formula (1)

$$\frac{NumberofLiveNematodes}{Package} = \left(\frac{NumberLiveNematodesCounted}{Wt.ofSampleCounted} \right) * NetWt.Package$$

Storage by the vendor prior to sale

Insecticidal nematode products are available for purchase at many retail locations that carry agricultural products or lawn and garden supplies. Another common source of insecticidal nematode products is to buy them through mail-order companies. Various products may have attributes and liabilities as to their shelf life and maximum storage capacity associated with their mode of manufacture, but that is beyond the scope of this chapter (see Grewal, 1999). Follow these guidelines to ensure that the product has not been damaged prior to purchase during storage by the retailer.

Every product should have some indication of the date by which they need to be used on the label. This information will vary among products. For example, indications may be a statement of "apply product within 30 days of receipt" or an actual expiration date of the product may appear. If the expiration date has passed, DO NOT PURCHASE THE PRODUCT. When products are packaged, the manufacturer typically will put more nematodes into the package than the amount stated on the label. The additional nematodes offset expected mortality that occurs during harvest and formulation, as a result of packaging or while the package is on the shelf. If the expiration date has passed, there is a good chance that the nematodes that you are buying are dead or dying and therefore, unable to infect insects when applied. Lewis et al. (1995) demonstrated the effects of storage of infective juvenile insecticidal nematodes. Though this study was conducted on nematodes stored in water, the effects of aging in formulations is likely to be similar. As they age, insecticidal nematodes become less able to infect insects successfully, they are less successful foragers, their symbiotic bacteria decrease in density, their cuticles become more permeable to water and their lipid reserves are depleted. These effects occur at different rates, depending on what species of nematode is considered, but with age it is obvious that nematode quality declines.

If possible, examine the storage conditions where the nematodes are purchased. Most product labels will state the optimum storage conditions for any insecticidal nematode formulation. Some products containing *Heterorhabditis* spp. (but not all), for example, may require refrigeration. Most insecticidal nematode products should be stored at temperatures between 40 and 75 F. Insecticidal nematodes should never be subjected to temperatures warmer than 90 F. Before purchase, it is worthwhile to read the storage requirements on the product label, and make sure that the establishment from which you are buying them is fulfilling these requirements. Take the time to look around the store to make sure that the product has been stored properly. If the product is stored improperly (the most common mistake is storage at too warm a temperature), then you will likely be buying sub-optimal nematodes.

If insecticidal nematodes are purchased through the mail, examining them for viability, as described in the previous section, is wise. It is also the only way to assure that the nematodes have been delivered in good condition. In this case, it is the responsibility of the supplier or the delivery company to make sure that you receive a quality product on time.

Transit from vendor to end-user

When transporting insecticidal nematodes, take care not to expose them to temperature extremes. As a rule of thumb, don't subject the nematodes to temperatures that would cause you discomfort. Thirty minutes in a 125° F truck cab is likely to render the product completely inviable. Freezing temperatures will also result in nematode mortality. To minimize nematode exposure to lethal conditions, do the following:

(1) Bring a cooler with ice or a freeze pack with you when you plan to purchase nematode products.

(2) Plan to deliver the product to the storage facility immediately after purchase.

(3) Limit direct contact between the product and the ice or freeze packs.

Storage after purchase

Two factors influence the success you will have storing insecticidal nematodes in the home. First, there are the conditions under which they are stored and second is the duration for which they are stored. The expiration date on a product label applies to the product in the home or storage facility the same way it applies to the retail vendor. The best practice is to purchase insecticidal nematode products as close to the time of use as possible. This will decrease the number of variables (e.g., those that were discussed above) that could potentially affect nematode efficacy. The less storage time the better. A storage facility that is perfect 90% of the time, but is too warm for the other 10% is not adequate.

If you need to buy nematodes in advance, storage directions are printed on the labels of most insecticidal nematode products. They usually instruct the user to store nematodes in a cool, dry place. Refrigeration may be required or suggested, but this is not always the case.

The only way to store insecticidal nematode products is while they are formulated and ideally still in their original containers. Once the nematodes have been released from their formulation, a number of changes take place. First, their metabolism will increase which will in turn cause them to use up their limited energy reserves more quickly. Second, with their increase metabolism, they will also use up available oxygen in the water in which they have been dispersed. Third, the products in which the nematodes were formulated may contribute to oxygen depletion in a tank mix. The best approach is not to keep a tank mix, even over night. If this is unavoidable, check nematode viability as described above.

CONCLUDING COMMENTS

Most biologically-based strategies for pest management require a more sophisticated user than do chemically-based management practices. The primary reason for this is that when biologicals of any kind are used, living organisms are applied to the target area. Living organisms usually require more specialized conditions than do chemicals, and to keep them viable requires greater expertise and some vigilance. This chapter has discussed ways to avoid potential pitfalls associated with handling, transport and storage of insecticidal nematode products.

I have divided these guidelines into two rough categories: Those over which the end user has no control, and those that are the responsibility of the end user. To address problems arising from the first category, I have provided a method by which product viability may be checked, and several suggestions of potential "trouble spots" to check in the retail outlet. These are ways to "check up on" retailers to make sure that they understand and comply with label recommendations. Other than an expiration date, there is no way to tell from looking at a package of insecticidal nematodes whether or not a product has been handled in the correct way. Therefore, it is necessary to ask about this. For the end user-based comments, I have tried to assemble some simple practices to follow that will allow anyone to avoid some of the most common pitfalls encountered during transport and storage. The best over-all advice is to READ THE PRODUCT LABELS and CALL THE MANUFACTURER AND EXTENSION PERSONNEL TO ASK QUESTIONS.

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Application Methods in Different Cropping Systems

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ABSTRACT

Insecticidal nematodes can be applied in almost any liquid application system that can deliver sufficient liquid to keep the nematodes in suspension. Because of the size of the nematodes, screens and filters need to be large enough to allow passage of the organisms. High pressure, extensive recycling through a pumping system, and mixing with certain chemical should be avoided. The principle of using sufficient water to move the nematodes to the pest target site should be paramount in considering any application method.

INTRODUCTION

Entomopathogenic nematodes can be applied using most conventional liquid application systems designed for delivering pesticides, fertilizers or irrigation. The major considerations involved with the selection and use of an application system should be: volume of application; agitation system (to keep the nematodes suspended); pump, pressure and recycling time; application distribution pattern; and, system environmental conditions (heat and cold). Though not technically part of the application system, other considerations that need to be addressed are: pre- and post-irrigation availability; compatibility of nematodes in mixes with other chemicals; and, compatibility of nematodes with previously applied pesticides.

APPLICATIONS IN VARIOUS CROP SYSTEMS

Field crops

Most field cropping systems rely on tractor drawn or self propelled boom sprayer systems (usually 10 to 30 feet wide). These consist of a tank, pump and boom system fitted with nozzles. In these systems, the main points that can cause problems are: excess tank agitation through sparging (recirculation of a portion of the spray mix) or mechanical stirring to keep the nematodes in suspension; pump pressure (usually well below the maximum of 300 psi) and temperature (should not subject the nematode suspension to temperatures above 30 °C); and clogging of nozzle filters or screens (should not be smaller than 50 mesh). (Note: If nozzle clogging is a problem, it is recommended that nozzles with larger orifices be used, but this will require recalibration of the system.

Large, spray-irrigation systems (pivot or linear) that have been fitted for fertigation or chemigation (i.e., an injection system that measures known quantities of fertilizer or pesticides into the water stream) are also suitable for applying nematodes to crops. In these systems, the most important factors are: injection holding tank agitation system to keep the nematodes suspended; liquid flow within the main distribution pipes must be sufficient to keep the injected nematodes in suspension; and the volume necessary to charge (get the necessary amount of nematodes to all the spray nozzles) and empty the system ("push" through the nematodes so that the system is cleared of nematodes in solution) must be known.

Subsurface applications (e.g., corn rootworm band or "at seeding") that can handle liquid fertilizers or pesticides can also distribute nematodes. The mechanical parts of these systems are similar to surface boom sprayers and the same details associated with a holding tank, pump and nozzles are encountered.

Specialty crops (large and small fruits, fresh vegetables, etc.)
Special booms with nozzles are often constructed for specialty crops to spray the sides and tops of the vines, canes, or trees. Without modification, these sprayers are usually not appropriate for nematode application since most of the target pests are soil dwellers. In some cases, caps or blanks can be used to block all the nozzles except for the bottom ones that direct sprays to the bases of the plants and the soil. Other than these modifications, the same attention to the details of using a spray tank, pump and nozzles is needed (agitation, pressure/heat, screens and size).

Many of these specialty crops are irrigated, fertigated or chemigated using trickle tube systems or permeable soakers. While on a smaller scale, these systems, equipped with an injector, are similar to the large, field crop systems. However, these smaller systems often have a problem of not having sufficient flow volume to keep the nematodes suspended. Such systems also need to be evaluated with a dye to determine the time needed to charge the system. Once nematodes are injected, samples should be taken from some of the end emitters at the beginning, middle and end of the injection cycle to ensure that nematodes are reaching the emitters. Permeable soakers also need to be tested to ensure that the pore sizes are sufficiently large to allow for passage of the nematodes. Since many of these application systems are constructed of black plastic or similar materials that are exposed to the sun during the day, temperatures can often exceed the tolerance of the nematodes. During hot, sunny weather, it is advisable to inject and distribute the nematodes at night or early in the morning.

In some smaller operations, back pack sprayers and even hand pump sprayers (= hand cans) may be used. These systems consist of tanks (usually one to five gallons), a lever arm attached to a low pressure, low volume pump (back pack sprayers) or an air compressing pump (hand can) and a single hose with an on/off valve and spray wand with nozzle. These systems should be shaken periodically to keep the nematodes in suspension and suitable filter screens should be used or removed.

Low-volume application technology using spinning disc spraying systems are increasing in popularity in specialty crops. With these systems, both the size of the droplets produced and spray coverage can be manipulated. Spinning disc systems can be calibrated over a wide range of nematode concentrations and sizes. Infective juveniles ranging in length from 500 to 1000 μm can be passed through these disc systems. Attention should be given to ensuring that the nematode suspension is agitated sufficiently to keep the nematodes from settling out.

Ornamentals and turf
Larger turf areas on golf courses, commercial sites and athletic fields are often sprayed using small boom sprayers (four to 10 feet long) that are dedicated units (self propelled or hooked to other turf maintenance equipment). Smaller turf areas, especially lawns, are usually sprayed with hand held booms or "shower droplet" style nozzle systems attached to a long length of hose that is connected to a tanker truck equipped with a pump. The boom systems have all the same components and problems as similar systems used in field crops.

The long hose systems appear to be much the same as boom sprayers (i.e., holding tank with agitator and pump), but the length of hose itself can cause unique problems. As the turf specialist moves from property to property, the hose must be reeled in. The reel containing the hose is often exposed to the sun and the time needed to move from one customer to another may exceed 20 minutes. This is ample time

for the nematodes to settle to the bottom of the hose loops. Spray residues remaining in the hose may heat to lethal levels, especially if the operator takes a lunch break and leaves the truck in the full sun. Operators of hose-based systems are encouraged to cycle the contents of the hose back into the tank upon arriving at a new site (often takes 90 to 120 seconds to accomplish this task). If the contents of the hose have been exposed too long to the sun or heat, the operator needs to know what volume of spray must pass (purge the hose) before fresh nematodes from the tank "charge" the system.

Subsurface applications of insecticides and nematicides are commonly made to southern turf. Two basic systems are used - slit injectors [a slit cutter (a sharp disk or rotating, vertical blade) followed by tubes that spray or dribble the solution into the slit], and high pressure injectors [that force the liquid mix through the turf canopy and into the soil]. The high pressure injectors are generally not suited to nematode applications because pressures up to 2000 psi are often used (nematodes should not be subjected to pressures exceeding 300 psi). The slit-inserting systems are highly suited for applying nematodes and have been the system of choice for applying nematodes for mole cricket control. These systems have all the mechanical parts of boom sprayers (holding tank, pump and tubes leading to the distribution nozzles or tubes). Therefore, spargers or mixers are necessary for the tank and any filters or screens need to be of sufficient size to allow passage of the nematodes.

On golf courses, liquid applications to greens and tee surfaces are usually not made using boom systems because the weight of the equipment can damage the delicate turf surfaces. In these cases, applications are made from the edges of the green or tee using high volume "hand guns" (nozzle systems that apply course sprays at relatively high pressures - 40 to 100 psi). These are attached via hoses to holding tanks with pumps. When using this type of equipment, pay attention to tank agitation and potential problems associated with long hoses as described above.

In and around ornamental plant beds, commercial applicators often use adjustable nozzle hand guns. The nozzles can be adjusted to produce a medium fine, cone-shaped spray (generally for spraying on the foliage of plants) or a coarse spray jet (generally for spraying tree trunks, taller trees or applications directed to the soil under the plants.

Many commercial tree/shrub care companies equip their technicians with backpack sprays or hand cans. These are especially useful for applying nematodes to targeted areas in the landscape. As with specialty crops, the sprayers should be agitated before spraying.

Tree and shrub soil injection systems can also be used to apply nematodes. Many of these systems use pressures in the 80 to 200 psi range while some may exceed the 300 psi limit. Nematode applications through such soil injectors are better suited to light or sandy soils that do not require high pressure systems.

Nematodes have been successfully injected into tree and shrub borer holes. Some moth and beetle borers have larvae that make access holes to the exterior. These are usually noticed as sap flows or small piles of sap-soaked sawdust like material. Injection of nematodes using a large gage hypodermic syringe has been able to introduce nematodes into these areas. Once inside the borer tunnel, the nematodes can seek out the insect larva. This process is not widely used by the tree/shrub care industry, but is available for difficult-to-control borer infestations.

One of the major problems encountered by commercial landscape and turf care firms is daily carryover. Many of these companies are used to mixing up large tanks of insecticide, fungicide, herbicide or fertilizer mixes and, because of the stability of these chemicals, they can use the mix for several days

before emptying the tank. This is not recommended for nematode use and fresh nematodes need to be used each day.

Home gardens and landscapes
Home gardeners generally can purchase and use hand cans or back pack sprayers, but hose-end sprayers are much more common. These consist of a spray concentrate holding jar attached via a siphon tube to a spray nozzle that is connected to a garden water hose. The stream of water passing over the siphon tube pulls up the concentrate and dilutes it in the resultant spray. These hose end sprayers produce large droplet sizes ideal for nematode application. However, the major problem encountered is calibration of these sprayers. Most hose end sprayers are marketed for a specific company's products and the calibration marks are usually for those specific products. Accurate calibration of these hose-end sprayers is difficult, even for professionals. However, if a known quantity of nematodes are placed in the sprayer and the size of the area to be treated is known, simply keep spraying the area until all the material in the holding jar is used up.

OTHER APPLICATION CONSIDERATIONS

Volume of application
For applying infective nematode juveniles, the larger the spray volume, the better. Most nematode labels suggest volumes of two to six gallons of spray per 1000 square feet (= 87 to 260 gallons per acre, or 133 to 400 liters per hectare). This is satisfactory for many boom sprayers and lawn "shower nozzle" sprayers equipped with sufficiently large nozzles. However, to save on the weight of tank volumes, most boom spray systems are being designed to use less spray volumes, usually in the range of 0.5 to 1.0 gallons of spray per 1000 square feet (= 20 to 45 gallons per acre, or 30 to 70 liters per hectare). Even lawn applicators are going to shower nozzles that deliver 1.0 to 1.5 gallons of spray per 1000 square feet.

When lower spray volumes are used, pre- and post-application irrigation can be adjusted to counteract the problems of low volume sprays and to assist in moving the nematodes to their targets and off exposed surfaces. Pre-application irrigations will assist in moistening the soil or turf thatch. Post-application irrigation is essential for washing any nematodes that may be on plant surfaces to the soil surface and the irrigation should be sufficient to provide enough water to allow the nematodes to move into the upper soil layers, out of the sun or drying air exposure. Most studies have indicated that 0.1 to 0.25 inches of post-application irrigation is sufficient to move the nematodes into the soil.

Post-application irrigation needs to be applied before the spray droplets dry. For many crop systems, this will mean treating smaller areas and then applying irrigation immediately before moving on to another section. For golf courses and home lawns that have irrigation systems, the zones of the irrigation system can be run on the "syringe" cycle (usually lasts for 10 minutes) to wash the nematodes off the grass blade surfaces, but a full irrigation cycle should follow after all areas are treated and syringed.

Where post-application irrigation is not available but the applicator has some ability to adjust when the nematodes are to be applied, make the application just before or even during a rainfall event.

Nematode suspension
Since the nematodes have mass and weight, they will settle to the bottom of any body of water containing them. Therefore, an agitation system is essential, especially if large spray tanks are used. Many tanks have recirculating systems (spargers) or internal paddles that stir the mix. Unfortunately, many of these systems do not operate unless the spray pump is active. Therefore, if the spray mix has been allowed to

set without agitation, allow sufficient time for resuspending the nematodes after starting the pump motor but before actually making applications.

Some irrigation systems, especially low volume trickle systems, may also not move water fast enough to keep the nematodes suspended. When in doubt, check such systems periodically by taking a sample at the emitters to determine if live nematodes are being moved through the system.

Unique problems with injections into irrigation systems

Most irrigation, fertigation, and chemigation systems do not empty when they are not in use. In other words they are usually filled with liquid and what is introduced at the beginning of the system takes time to reach the end of the system. Nozzles close to the pump source will begin releasing nematodes long before the end nozzle. Likewise, if the system is simply turned off after the last of the nematodes have been injected into the stream, they will remain in the piping and the end of the system will get much less nematodes than the beginning of the system. Therefore, these systems must be calibrated to determine how much water must enter the system to push something completely through the system. This can be calculated with complicated hydraulic equations, but it is much simpler to simply inject a harmless dye into the system and see how long it takes the dye to reach the end nozzle. This would be the MINIMUM time that the system must operate in order to "push" all the nematode solution through.

Pump systems

Most agricultural pumping systems use membrane or roller pumps. These usually develop low internal pressures and internal shear is low enough to not physically damage the nematodes as they pass through the system, often many times before being applied. Care should be taken when using higher pressure hydraulic pumps. Some of these will develop high internal pressures (within the piston cylinders) and they may have enough shear forces to shred the nematodes. If in doubt about the system, mix up some nematodes and run them through the system for as much time as you expect them to be in the tank before application. Then take a sample and determine the viability of the nematodes.

Application distribution pattern

While completely uniform distribution of the nematodes is not entirely essential to their success (the nematodes can move short distances on their own!), uniform distribution should always be a goal. Although sprayer and other equipment calibration and use techniques to achieve uniform distribution is beyond the scope of this article, some important factors will be mentioned.

It is always amazing how few sprayers are actually calibrated. Many users simply rely on manuals that indicate what "should" be coming out of the system if a certain pressure, nozzle size and speed is used. It is recommended that boom sprayers should be calibrated with actual calibration jars, two to three times during the season. Manuals on how to do this are usually available through most Cooperative Extension (county agent's) offices. Shower head type nozzles and spray guns should have a "bucket" check before each use. This only takes a few minutes (spray the material into a bucket for 10 to 30 seconds and measure the amount accumulated) and can assist the applicator recalibrate their walking or application speed.

To assist in developing more uniform patterns when using sprayers, run water through the application system and run the sprayer over a large pavement surface. Wait a few minutes and watch the pattern of drying. If streaks, zones or patches take much longer to dry, then the pattern is not uniform and the equipment or applicator needs to be adjusted or retrained.

Heat, Cold and Sun

It is always amazing how fast a tank of spray material can heat up when setting or running in the sun. Constantly be aware of this factor and check the tank mix periodically to determine its temperature. Likewise, even if the air temperature seems to be proper, be sure to check the soil temperature and irrigation water temperature. Exposure to the direct rays of the sun is rapidly lethal to the nematodes. Therefore, it is often advised to apply them in the morning, evening or on cloudy days.

Chemical Incompatibility

While the nematodes are quite tolerant of a wide range of water pH values, salt levels and minerals, there are several pesticides that can be lethal to them. These pesticides (including insecticides, fungicides and herbicides) can not be mixed with the nematodes and their residues in uncleaned spray systems. In fact, there are some of these pesticides that leave persistent residues in the soil that can adversely affect the nematodes for days to weeks after their application. Be sure to read the nematode labels provided by the supplier to determine those chemicals that are incompatible with nematode use.

In any case, it is always prudent to clean and triple rinse any spray system that has been used for pesticide application before mixing and applying the nematodes.

Table 1. The following chemicals have been found to reduce nematode efficacy when exposed directly. These chemicals should not be tank mixed with nematodes. To obtain best results, nematode applications should be made 1-2 weeks before or after application of these chemicals.

Chemical	Trade Name	Chemical	Trade Name
Anilazine	Dyrene	Fipronil	Chipco Choice
Azadirachtin	Azatin, Neem	Insecticidal Soap	Various
Bendiocarb	Turcam, Ficam	Methomyl	Lannate
Carbaryl	Sevin	Oxamyl	Vydate
Carbofuran	Furadan	Trichlorfon	Dylox, Proxol
Chlorpyrifos	Dursban	Triclorpyr	Turflon, Confront
Ethoprop	Mocap	2-4-D	Various
Fenamiphos	Nemacur		

Factors Affecting Efficacy

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ABSTRACT

Entomopathogenic nematodes have been extensively studied and used as commercial biological control agents for insects. However, their acceptance as conventional control strategies by the pest control community is somewhat disappointing considering the body of knowledge currently available. Much of this can be attributed to issues concerning efficacy, the perception of acceptable or poor control. Insecticidal nematodes are living organism and thus are subjected to stresses and conditions imposed on them by man or the environment. This presentation provides a summary that discusses some of these factors dealing with abiotic and biotic environmental issues.

INTRODUCTION

Insecticidal nematodes in the families Steinernematidae and Heterorhabditidae and their associated symbiotic bacteria (*Xenorhabdus* and *Photorhabdus* spp.) have been commercially utilized as biological insect control agents for about ten years. The attention they have received from the pest control community is due in part to the acceptable control they provide in certain commodities and that they can and do play a significant role in providing an alternative control strategy in IPM systems. They are extremely safe to use, easy to apply, have minimal impact on the environment and generally are efficacious on a wide range of different insect pest species. Developments in production and formulation stability have opened the doors to several markets especially those that could not support a high value product.

With all of these attributes in favor of insecticidal nematodes, why then are they not more widely accepted and used not just in the United States but in international markets as well? A lot has to do with the notion of "efficacy" in relation to insect pest control. The nematodes and their associated bacteria are living organisms associated with the soil environment and therefore are impacted by a variety of conditions both man-made and natural. These factors are both biotic and abiotic in origin and do have an influence on the nematode's ability to move, locate, enter and kill the target host. Having an understanding of these factors and the role they play in nematode efficacy, will greatly improve your ability to make decisions regarding their use as control agents in IPM programs.

BIOTIC FACTORS

Biotic factors are those concerned with the living components of the nematode/bacteria interaction with the host insect. There are a several different species and strains from both nematode families that are commercially available. How one chooses a species does depend on the target insect and the environment or cropping system in which the nematodes are to be used.

Each nematode species has its own unique biology resulting from evolutionary pressures. These have impacted its ability to move, search, enter, infect, kill and reproduce inside a potential host.

The behavior of the nematode in soil is closely tied to its host searching abilities. Lewis *et al.* (1992) divided these basic host searching behaviors into cruisers and ambushers. Cruisers tend to move actively both horizontally and vertically in search of a host. Nematodes such as *Steinernema riobrave, S. glaseri, Heterorhabditis bacteriophora* and *H. megidis* use such strategies. Ambushers such as *Steinernema carpocapsae* and *S. scapterisci* tend to minimize their active searching and instead wait for hosts to contact them.

Host susceptibility also plays a major role in the successful use of insecticidal nematodes for control. Under laboratory conditions researchers have demonstrated the susceptibility of different insect species and their life stages in artificial experimental arenas. However, field trials do not always demonstrate the same rate of susceptibility.

Insects have a variety of defense systems that are behavioral, morphological and physiological in nature. Drees *et al.* (1992) tested various *Steinernema* and *Heterorhabditis* spp. under laboratory and field conditions against the red imported fire ant *Solenopis invicta.* In petri dish bioassays, larvae, pupae and alates were very susceptible to *S. carpocapsae,* but the worker ants vigorously preened nematodes from the brood, alates and themselves. In field trials in which mounds were drenched with nematode suspensions, the entire colony vacated the treated mound in as little at 48 hours and created satellite mounds. Jouvenaz & Martin (1992) tried to utilize this relocation behavior in treating fire ant nests in nursery pots with *S. carpocapsae* drenches. However, the ants were not eliminated.

In some cases insect morphology can play a role in reduced susceptibility. Field trials against click beetles (Elateridae) are always negative (Sosa, 1990). This is unfortunate because this group of insects are serious pests on some crops. Eidt & Thurston (1995) demonstrated that several morphological factors including heavily scleritized spiracles and a well developed proventriculus inhibit penetration of infective juveniles.

Insect immune defenses or response can dictate susceptibility. Ehlers *et al.* (1997) and Peters & Ehlers (1997) examined the pathogenicity of *S. feltiae* and its symbiont *Xenorhabdus bovienii* to the crane fly species *Tipula oleracea.* Injection of axenic (bacteria free) nematodes into the hemocoel resulted in only 40% mortality within 8 days compared to 90% mortality of monoxenic dauer juveniles. Encapsulation rates were 80% for axenic versus 33% for monoxenic. Coinjection of the bacteria increased encapsulation of axenic nematodes, showing that *X. bovienii* is triggering the encapsulation response.

Insect susceptibility is also determined by the ability of the symbiotic bacteria to overcome host defenses and reproduction. Yamanaka *et al.* (1992) & Yamanaka (1993) examined pathogenicity of several species and strains of *Xenorhabdus* spp. against *Spodoptera litura.* Pathogenicity varied depending on "phasing" of the bacteria as well as production of biochemical exudates. Bowen *et al.* (1998) isolated insecticidal toxins from *Photorhabdus luminescens* that are as potent as the δ-endotoxins of *Bacillus thuringiensis.*

In addition there are several antagonists that will defeat the nematode from accomplishing its mission. Kaya & Koppenhofer (1996) have recently reviewed this topic. In summary, potential antagonists that can play a role are 1) antibiosis with plant allelochemicals such as α-terthienyl from several species of the Compositae family, 2) intra-specific and inter-specific competition between insecticidal nematode species, 3) competition with entomopathogenic viruses and bacteria, 4) nematophagous fungi such as the species *Hirsutella rhossiliensis*, 5) protozoan parasites, and 6) invertebrate predators such as protozoans, turbellarians, nematodes, tardigrades, oligochaetes, mites and insects.

ABIOTIC FACTORS

Abiotic factors generally play a more significant role in the ability of entomopathogenic nematodes to control insect pests. These can significantly limit nematodes' effectiveness to move, locate and enter a host. Many crop systems are not suitable for nematode use simply because production conditions are not favorable. As mentioned earlier, the literature has demonstrated that hundreds of insect families are susceptible to nematodes, however, many of the susceptible stages are found in environments incompatible for nematode use. This also includes pests in which the susceptible stage is not located in the soil environment but instead is found above ground.

Ultraviolet light

Insecticidal nematodes are very sensitive to sunlight, especially ultraviolet radiation. Gaugler *et al.* (1992) showed that exposure of *Heterorhabditis bacteriophora* to medium-wave UV radiation (302 nm) for only 4 minutes caused significant loss of pathogenicity. *Steinernema carpocapsae* had a similar response to exposures of 6 minutes. Fujiie & Yokoyama (1998) observed similar effects on *S. kushidai* and its symbiotic bacteria *X. japonicus.*

Desiccation

Nematodes are water loving organisms and as such are dependent on maintaining a state of hydration for movement and insect infection. One limiting factor with using nematodes for above ground applications is the rapid desiccation that can occur. Georgis & Hague (1988) evaluated *S. feltiae* against the web-spinning larch sawfly, *Cephalcia lariciphila.* Soil application resulted in 61% control while branch sprays gave results of only 3.4-29.4%. Several studies have been conducted in testing insecticidal nematodes against leafminer. Hara *et al.* (1993) and Williams & MacDonald (1995) found similar results in that infective juveniles (IJ's) can enter leaf mines and infect larvae. However, relative humidities >85% were required to achieve control better than 65%. This effect was also temperature dependent. Belair *et al.* (1998) conducted foliar sprays with *S. carpocapsae* against early-season apple pests. Their conclusion after four years of field study was "Although some efficacy of canopy sprays of nematodes was detected against early-season apple pests, the inconsistent results and high application costs preclude their use as a sole control tactic against these pests in commercial apple orchards."

Soil moisture

Soil moisture is probably the most important factor affecting nematode movement and survival. Moisture is not only necessary to prevent desiccation but nematodes require a film of moisture around soil particles to move (Kondo & Ishibashi, 1985). Generally if nematodes desiccate slowly, they can survive for much longer periods of time in the soil in a state similar to anhydrobiosis. Ames (1990) observed that IJ's of *S. scapterisci* can survive up to 13 weeks at wilting point (15 bars moisture tension) with survival better in sandy loam than pure sand. However, *S. carpocapsae* survival was less than 2% above wilting point. In turfgrass, Fujiie *et al.* (1996) found that the insecticidal activity of *S. kushidai* against the white grub *Anomala cuprea,* increased as soil moisture content increased from 10 to 40%. Similarly, Ehlers *et al.* (1998) concluded from a 4 year golf course study that *H. bacteriophora* provided excellent control against the garden chafer, *Phyllopertha horticola* if sufficient soil moisture was maintained. Koppenhofer *et al.* (1995 & 1997) again demonstrated that soil moistures are critical for nematode movement, establishment, persistence, and infectivity. On the other extreme, very saturated soils have been shown to inhibit nematode mobility and decrease their survival by creating an anaerobic condition (Molyneux & Bedding, 1984).

Soil texture/type

Both soil texture and sizes of the component soil particles greatly affect nematode survival, movement and host finding ability. Typically as soils become more clay in content the effectiveness of nematodes decreases. Hsiao & All (1996) studied the movement of *S. carpocapsae* in four different soils ranging from pure sand to sandy clay loam. Their findings concluded that the migration of IJ's decreases as the proportions of silt and clay increases. Kung *et al.* (1990a) studied survival and pathogenicity of *S. carpocapsae* and *S. glaseri* in four different soil types. Both parameters decreased as the proportion of clay increased. Choo & Kaya (1991) found that the host-finding ability of *H. bacteriophora* was significantly reduced in small pore size soils. Geographic surveys of native nematode species tend to show similar patterns. Zhang *et al.* (1992) surveyed soils near Beijing, China for insecticidal nematodes. 20.8, 15.1, 12.2 and 5.4% of soil samples contained nematodes for loose sandy, sandy loam, medium loam and light loam soils respectively. Nicolas *et al.* (1995) examined the susceptibility of a grasshopper species to insecticidal nematodes. They found that mortality and infectivity were positively correlated with soil moisture levels and soil type.

Soil temperature

Other than soil moisture, soil temperature plays a significant role in influencing the nematodes' ability to effectively control insect pests. This factor impacts the activity of the nematode itself but also plays a role on the symbiotic bacteria's ability to replicate within the host. Typically in field situations, efficacy diminishes rapidly at temperature below 16°C. These lower temperatures apparently reduce nematode metabolism thus diminishing the ability to move through soil. At the other extreme, temperatures higher than 30°C will greatly diminish nematode survival although some species such as *S. riobrave* can survive soil temperatures approaching 37°C.

Penetration rates of several *Heterorhabditis* and *Steinernema* spp. into larvae of *Galleria melonella*, *Spodoptera exigua* and *Otiorhynchus sulcatus* (Westerman, 1998) at 9 and 20°C showed that at 20°C, the numbers of IJ's entering a host increase significantly. Townsend et al. (1998) had similar results testing *S. carpocapsae* and *H. bacteriophora* against Green June Beetle. Significantly more larvae were killed at 25°C than 12°C. van Tol (1996) recently reviewed the prospects for biological control of black vine weevil in nursery stock. He concluded that the main problem of efficacy for many strains of nematodes is low soil temperatures. The discovery of so called "cold tolerant" species and applications restricted to when average soil temperatures are >16°C would improve grower acceptance of this control strategy.

Recent discoveries of canadian steinernematid isolates raises hopes for controlling insects at lower soil temperatures (Mracek *et al.*, 1997). In laboratory bioassays against *G. melonella*, several isolates provided 100% moralities in a few days at 10°C with one isolate from British Columbia providing 82% mortality at 4°C.

At higher temperatures some nematode species provide better control. Gouge & Hague (1995) tested susceptibility of different sciarid fly species to several *Steinernema* spp. *S. feltiae* was most effective at 22°C but *S. riobrave* was superior at 30°C. Lacey & Unruh (1998) had similar results against codling moth. *S. riobrave* was the most infective nematode at 35°C producing 68% mortality which was more than twice that observed for *S. carpocapsae* or *H. bacteriophora.*

Soil pH

Insecticidal nematodes can tolerate a wide range of pH conditions in the soil profile. Miduturi *et al.* (1996) surveyed native nematodes in Belgium soil. All of the positive samples for both steinernematid and heterorhabditid nematodes came from soils within pH range of 4.0 – 8.1. Cheng & Hou (1997) studied the survival of *S. carpocapsae*. When incubated in phosphage buffer at pH 4 – 12 for 10 days, the

IJ's maintained a 70% survival rate. However, all IJ's died when the pH was adjusted down to 2. Kung *at al.* (1990b) demonstrated that steinernematids survived poorly in soils with a pH of 10. Nematode pathogenicity and persistence did not decline when exposed in soil with a pH between 4 - 8 for 4 weeks.

Oetting & Latimer (1991) conducted an interesting study comparing *S. carpocapsae* with different potting media and horticultural practices. Potting media tested were aged pine bark, new pine bark, peat moss, aged cow manure, and a peat/vermiculite soilless medium. Horticultural practices tested were potting media pH level, fertilization, salt level and application of plant growth regulators. No significant differences were found indicating that under these diverse conditions *S. carpocapsae* would not be negatively affected.

Agrichemical compatibility

Although not a genuine abiotic factor, insecticidal nematode compatibility with agrichemicals nonetheless can play a significant role in using these nematodes for biological control of insects. I have been asked numerous times if nematodes can be tank-mixed with a chemical product. Multi-product mixes are common and understanding how these compounds can compromise the effectiveness of nematodes is crucial. However, if the nematode and other product(s) are not mix compatible, they can still be used in an IPM program if sufficient time has elapsed between application of the products. Sometime the simple degradation, uptake or movement of the compound is all that is necessary for nematode success. Table 1 (Georgis & Poinar 1994) includes several commonly used products by producers with recommendations on there use with insecticidal nematodes.

There are many published reports on agrichemical compatibility with insecticidal nematodes. A sample of these include compatibility with: 1) insecticides, fungicides and herbicides (Barbarossa *et al.*, 1996), 2) malathion (Baweja & Sehgal, 1997), 3) insecticides terbufos, fonofos, and tefluthrin (Nishimatsu & Jackson, 1998), 4) soil amendments (Bednarek & Gaugler, 1997), 5) *Bacillus thuringiensis* (Shamseldean & Ismail, 1997), 6) carbamates (Gordon *et al.*, 1996), 7) surfactants (Schroeder & Sieburth, 1997), 8) Neem (Stark, 1996), 9) fertilizers (Shapiro, *et al.* 1996), 10) diflubenzuron (Scheepmaker *et al.,* 1998), and 11) adjuvants (Baur *et al.*, 1997).

Another unique approach is the possible synergism of an insecticide (imidacloprid) and nematode (Koppenhofer & Kaya, 1998). This material did not effect the survival and infectivity of *H. bacteriophora.* Instead, application of the two together or with imidacloprid first followed by *H. bacteriophora* 14 days later had a strong synergistic effect on mortality on two different species of white grubs.

Sterilizing pink bollworm by irradiating parents is common control practice used by the USDA ARS in cotton growing areas of California and Arizona. Finding nematode species compatible with this system would be ideal. Gouge *et al.* (1998) investigated the susceptibility of larvae from native pink bollworm to those from irradiated parents. They found that *S. carpocapsae* appears to be an ideal species for this purpose because it was more likely to infect the mobile natives than the sedentary F_1 larvae from irradiated parents. Previous studies by the author show that *S. carpocapsae* is effective in controlling larvae in large scale field trials.

Table 1. Chemicals that can be used with *Steinernema carpocapsae* in turf and ornamentals.

Compounds	Chemical Class	Trade Name
	Tank Mix	
Biopesticides	Azatin	Margo-san
	Bacillus thuringiensis	M-One, Dipel
	Fatty acids	Safer soap
Insect growth regulators	Diflubenzurion	Dimilin
	Fenoxycarb	Logic
	Kinoprene	Enstar
	Methroprene	Apex
Insecticides	Acephate	Orthene
	Bifenthrin	Talstar
	Carbaryl	Sevin
	Cyfluthrin	Tempo
	Cythion	Malathion
	Diazinon	Knox-out
	Endosulfan	Thiodan
	Esfenvalerate	Asana
	Etridiazole	Terrazole
	Isofenphos	Oftanol
	Methidathion	Supracide
	Trichlorfon	Dylox
Fungicides	Benomyl	Benlate
	Bromine-chlorine	Agribrom
	Chlorothalonil	Daconil
	Copper hydroxide	Kocide
	Fosethyl-Al	Aliette
	Iprodione	Chipco 26019
	Metalaxyl	Subdue
	Oryzalin	Surflan
	Oxazoidinedione	Ornalin
	Pentachloronitrobenzene	Terraclor
	Thiophanate-methyl	Zyban
	Triademefon	Bayleton
Herbicides	Chlorthal dimethyl	Dacthal
	Glyphosate	Roundup
Miticides	Dienochlor	Pentac
Fertilizers	Most fertilizers are compatible with nematodes	
	Use 1 Week After Nematodes[a]	
Insecticides	Bendiocarb	Turcam
	Chlorpyrifos	Dursban
Fungicides	Anilazine	Dyrene
	Dimethyl benzyl ammonium chloride	Physan 20
	Fenarimol	Rubigan
	Mercurous chloride	Calo-Clor
Herbicides	2,4-D	2,4-D
	Triclopyr	Turflon
	Use 2 Weeks After Nematodes[a]	
Insecticides	Ethoprop	Mocap
	Isazophos	Triumph
Nematicides	Fenamiphos	Nemacur

[a] Laboratory bioassays. Days needed to assure that the survival and the pathogenicity of the nematodes are not affected by pesticides at recommended field dosages.

SUMMARY

This has been a brief overview concerning abiotic and biotic factors that can and do affect efficacy. In order to make intelligent decisions concerning insecticidal nematode use in IPM and sustainable agricultural systems, you must be aware of these factors. The bottom line is that nematodes are living organisms with a complex symbiotic association which have coexisted with insects for million of years. When used and treated appropriately, you will find that products containing these nematodes can provide a degree of efficacy comparable and in many cases superior to that of conventional control strategies. There is still much to learn both from a basic and applied approach. Working with researchers to better understand how best to utilize these unique biological control organisms will help to further beneficial nematode acceptance into the mainstream control arena.

There are numerous case studies that have accentuated the successful use of insecticidal nematodes and several more in which nematodes have failed out-right. In some cases, we suspect we understand the issues involved with nematode failure but unfortunately we do not have the answers in most of these. In my oral presentation I will discuss a few case studies showing where nematodes have become true champions in the eyes of researchers and growers alike, as well as some expensive attempts where nematodes just failed.

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The Worldwide Web and Nematodes

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ABSTRACT

A brief background on the growth of the internet is provided, along with a synopsis of early and current websites providing information on insect parasitic nematodes. A website developed as an integral part of a multimedia SARE* project for disseminating information on insect parasitic nematodes is introduced and a *guided tour* of the pages and their content is presented.

INTRODUCTION

The Internet, conceptually born at the Massachusetts Institute of Technology in 1962, did not become an essential information dissemination tool of academia and business until the 1990's. But as early as 1985, it was an established network, supporting a broad community of researchers, via a number of interconnected mainframe computers. The U. S Department of Defense (which had been using a form of e-mail for years), the National Science Foundation, and other Federal government agencies were instrumental in supporting the internet (as ARPANET, Advanced Research Projects Agency Network and NSFNET) in the late 1980's, and in the spirit of academia, promoted it's use for open publication of ideas and results (National Research Council, 1999). The true explosion of internet use began with the availability of graphically-based, hypermedia browsers, one of the first being *Mosaic* (Stross, 1996). With the release of the user-friendly, *Netscape*, the World-Wide-Web (WWW) became the most popular portion of the Internet, with an estimated excess of 58 million people worldwide cruising it's network of computers by 1998. All forms of information are now found on the Internet: text, graphic, video, audio. Future forecasts call for total integration of all digital technologies (television, cell phone etc.) – a truly super-communication highway with, ideally, access to information for all people, regardless of economic advantage or disability.

The internet has become one of the primary tools for agricultural information dissemination. The typical US Land Grant Institution has too few extension agents to serve the diverse, demanding and growing clientele-base. The maintenance of a comprehensive website allows for speedy information retrieval by the community, and as public libraries add computers for free web browsing, information on virtually any subject should be free for all users. However, this glut of free information does come at a "price". Because "anyone" with a computer can currently publish a website, any type of information, accurate or not, goes out into cyberspace.

A coordinated set of media, available free to the public, includes a video, slide set, Fact Sheets, and poster, all accessed through a website maintained at the Ohio State University. Aspects of this site, and how it is best used, is the topic of this presentation. Hopefully, institutional reputation will help insure that the internet user is receiving up to date and sound advice. The trend in website construction and maintenance during the late 1990's has been toward one-stop informational-intensive sites that offer multimedia downloads and interactivity. As a goal toward consolidating information for users of insect parasitic nematodes, a SARE-funded project developed an integrated package of information for specialists, extension agents, growers and any prospective user of these biological control agents.

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*Sustainable Agriculture, Research & Education (SARE) is a program of the USDA, CSREES.

EARLY WEBSITES AND INSECT PARASITIC NEMATODES

Many of the earliest informational sites on the World Wide Web (web) were found at institutions of higher education. The academic origins of the internet meant that university faculty were familiar with using email as a "free" method of communication and data transfer by the late 1980's. So, it was a natural progression to the web when graphical browsers appeared and the information pipeline, complete with photographs, soon began to flow. The Land Grant institutions, with their core mission of agricultural research and extension to the public, found a new and powerful tool to serve their clientele.

Among the many aspects of agricultural research published on the web, are a number of sites either partially or totally devoted to insect parasitic nematodes. Searching for these sites requires using a number of keywords, all of which describe nematodes that kill insects, such as; entomopathogenic nematodes, nematodes, and entomogenous nematodes. The earliest academic sites on the web for insect parasitic nematodes were descriptive of research groups, their members, and what type of work was in progress. Sections descriptive of nematode biology and ecology were often included, along with current publications and links to other nematode sites, which quickly included nematology societies and nematology and entomology departments as they were created. Sites devoted to taxonomy or identification of nematodes in general could also be found.

As the 1990's progressed, academic websites were joined by government, organizational, personal, and especially, business sites. Biocontrol production companies were no exception, and at this writing, a minimum of 14 producers or distributors of insecticidal nematodes have websites for requesting information or buying nematodes. The number of sites can vary daily and the accuracy of the information is just as variable. Marketing their product is the primary goal of the website, and an informed buyer should be able to distinguish promotional hype from true product specifications.

THE SARE WEBSITE

Insect Parasitic Nematodes: Tools for Pest Management

One of the goals of the SARE project on use of insect parasitic nematodes, was to provide a website that offered a one-stop, research-based, comprehensive resource for information on nematode use (Fig. 1). The goal of this site, currently located at http://www2.oardc.ohio-state.edu/nematodes/, is to offer "real world" practical information on nematode use, backed up by a free video, numerous photographs, fact sheets on specific crop uses, and video clips. A Frequently Asked Questions (FAQ) section, where internet users can email questions to Experts, check FAQs, and receive personal answers, is available if the users questions are not answered by scrolling through the pages. A 3,000 plus database of academic publications is maintained for the browser who would prefer to track his/her own information from past research. In short, this site either answers the users' questions immediately, or directs the user to the answer. The website maintenance team is a combination of researchers and extension specialists whose goals are to provide the most effective guidelines on how best to use insect parasitic nematodes. The following sections represent a "guided tour" of the website as it exists in 1999. Keep in mind that useful websites are "current" and reflect the on-going changes in internet development today. New technologies and software will change the site's interactivity and fresh new looks and designs will have the internet user periodically checking the site for new developments in nematode use.

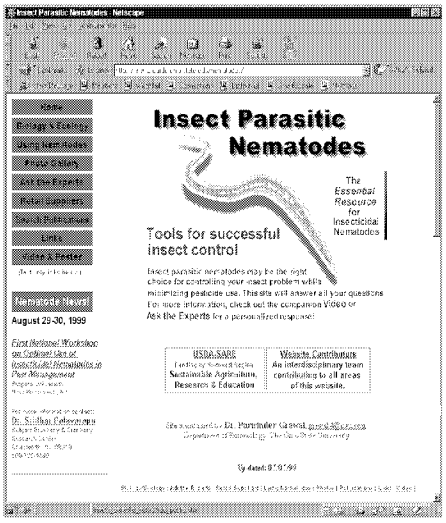

Figure 1. SARE project website at the Ohio State University.

The Home Page. http://www2.oardc.ohio-state.edu/nematodes/

The Insect Parasitic Nematode Home Page provides the internet user with an immediate idea of what the site is about and which page will start to answer a question or provide useful information on nematodes. Because users have different computers, modem speeds or ethernet connections, and browsing software, the site can be navigated by text links only. Turning off the graphics is an option to increase browsing speed, and most graphics will be represented by a descriptive sentence to give the user an idea of what the graphic illustrates. Buttons on the left hand side of the page are java applets, separate little computer programs that allow the button to light-up on mouse-over. These are fun but can be cumbersome, so turning off graphics may be a good option if you have an older computer or slow modem.

The Home Page has links for 90% of the pages on the site. The exception is the Using Nematodes page, which is the launch site for the fact sheets on specific commodities, and the Links page, which provides links to sites outside this particular web. Besides the standard information on where site maintenance takes place, a link to credits for the SARE sponsored project can be found here as well. The left hand side of the page is the current location for timely news, such as workshops, meetings, interesting new publications or fact sheets.

Biology and Ecology

The Biology and Ecology page is a great place to start for basic information on insect parasitic nematodes. Written for the lay person, yet detailed enough for a scientist from an outside discipline, this page has photos and video clips of nematode behavior to illustrate the interesting aspects of the most widely used genera.

Using Nematodes

Basic guidelines to properly using these living organisms in pest control, include storage, mixing and application techniques. The rules that apply to most situations are documented on this page, and as mentioned above, this is the launch page for fact sheets on specific pest use in different commodity situations. Current fact sheets linked off this page are for turfgrass, strawberry, and cranberry systems, however, other fact sheets will be made available for home and garden, and other situations as they are needed. The fact sheets are designed to be roughly 2 pages long, cover key pests, specific application techniques, and may be available someday as PDF files, so agents and specialists can print them as high quality handouts for distribution.

Publications Database

A wonderful resource for agents, specialists, and researchers, is the searchable publications database (Fig. 2). Developed and maintained by Dr. Kirk Smith, this database is up-dated routinely and contains in excess of 3,000 publications on insect parasitic nematodes. A list of keywords that will help in searching is provided, and an email address for Dr. Smith welcomes new citations that should be added. Searching the database is straightforward and results are output in tabular form.

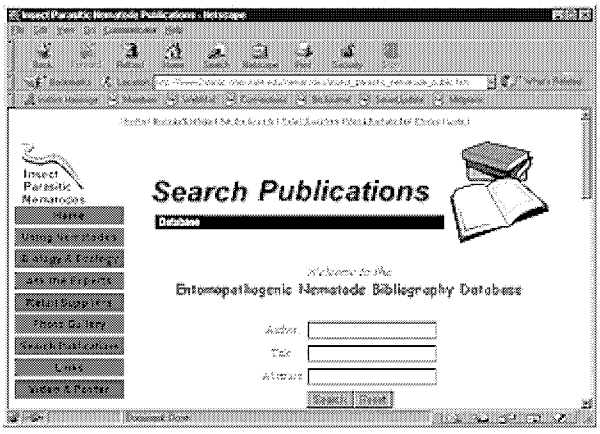

Figure 2. Publications database search page.

Links

A listing of some of the best outside links on insect parasitic nematodes can be found on this page. Links found here tend to be university-based or sites screened by the webmaster for accurate information. Because new sites are rapidly coming on-line, users finding great sites that are unlisted on this page, may send an email (via a link) to the webmaster for addition to this site.

Ask the Expert

Sometimes a specific question requires a specialized answer. The team that contributed to the SARE project (including the video, poster and other media), include a group of experts who are "on call" to answer specific questions that the website user cannot find somewhere at the site. From the Ask the Expert page (Fig. 3), questions can simply be submitted via email to the webmaster, and he will contact an expert and provide the answer to the user. Commonly asked questions and their answers will be posted in a Frequently Asked Questions (FAQs) section so the user can browse through and see what others are interested in. Repeatedly submitted questions will also signal the webmaster that certain concerns should be addressed on the site.

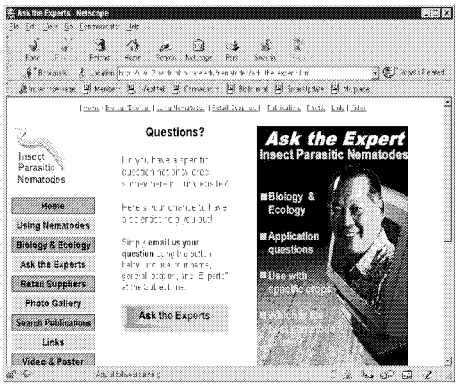

Figure 3. Ask the Expert page.

Retail Suppliers

Once internet users know more about insect parasitic nematodes, and how and when to use them properly, they need a place to purchase them. The Retail Suppliers page lists companies that produce insect parasitic nematodes. Information should include what species of nematodes they produce, along with a complete ordering address, phone, and FAX numbers. Most have email addresses for direct inquires, but a growing number have websites as well. This site will be up-dated as new information about companies or address changes become available.

Instructional Media

Extension agents, specialists, and instructors are always on the look-out for information to serve clientele and students. From the Video and Poster page, the user can find all the information needed to order the free video and educational poster. The video, *Nematodes: Tools for Pest Management*, is the heart of the SARE project. In 30 minutes it covers basic biology and ecology of insect parasitic nematodes, and shows application techniques for various crop situations. Ideal for grower meetings and extension agents, it is also a welcome addition for an academic course in biological control or insect pathology. The full-color, glossy poster representing key aspects of nematode biology and ecology, is also free.

Slide Collection

A slide collection, covering many aspects of insect parasitic nematode biology and use, completes the multimedia package that was a major goal of the SARE grant. While the 35 mm slides are available upon request, a visit to the Photo Gallery page will also allow the internet user to copy JPEG images of the collection for pasting into a slide making software or other media. Captions are added to each photo on this page, so it is also informative to just scroll through the images and read the descriptions.

Concluding Remarks

As the internet grows exponentially, and the public is bombarded with digital technology at a dizzying pace, it will be increasingly important to synthesize information into useful packages that require the least amount of time to access. For using insect parasitic nematodes wisely, it is hoped that this website will help coordinate what information exists and offer the public in a user-friendly format.

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Insecticidal Nematode Laboratory

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ABSTRACT

This laboratory is designed to provide hands on experience to workshop participants on the life history, behavior, quality assessment, and effects of biotic and abiotic factors on the survival of insecticidal nematodes. Demonstrations will include nematode life stages, signs and symptoms of infection, infective juvenile morphology, behavior, physiological requirements during storage and handling, and sensitivity to environmental extremes. Exercises will include estimation of the quality and quantity of nematodes in formulations, and evaluations of infectivity. Participants will also examine commercial formulations, products, and labels.

I. LIFE HISTORY, SIGNS AND SYMPTOMS OF INFECTION, AND INFECTIVE JUVENILE MORPHOLOGY AND BEHAVIOR

A. Life history and the signs and symptoms of infection

Insecticidal or entomopathogenic nematodes are in the families Steinernematidae and Heterorhabditidae. Their life stages include the egg, four juvenile stages (J-1 to J-4), and the adult. All of the life stages except the infective juvenile are found only in association with the cadavers of infected insects. Infective juveniles (or IJs) are specialized third stage juveniles that disperse from the host cadaver to locate and infect new hosts (Fig. 1). This free-living IJ is the active ingredient in nematode products. IJs locate insect hosts and enter through natural body openings (i.e., mouth, anus, spiracles) or thin cuticle, and release symbiotic bacteria from their intestine into the host. Infected insects usually die within 24-72 hours. In the host, IJs molt into fourth stage juveniles and molt again to form adults. In steinernematids, the invading IJs develop into males and females whereas in heterorhabditids they develop into self-fertilizing hermaphrodites. Reproduction occurs within the host cadaver, and two or three generations are often produced in large hosts before nutrients become depleted. Then infective juveniles are again formed and emerge into the soil to seek new hosts. After the first generation in the host, heterorhabditids also produce males and females.

Insects parasitized by insecticidal nematodes exhibit characteristic signs and symptoms of infection that are specific to the nematode species that caused the infection but are caused by the different bacteria species that are symbiotic with particular nematode species. The bacteria associated with heterorhabditids are in the genus *Photorhabdus*, and those associated with steinernematids are in the genus *Xenorhabdus*. Both bacterial genera are gram negative rods and are in the family Enterobacteriaceae. The bacteria produce a wide range of antibiotics that suppress the growth of other bacteria and create an environment within the host that facilitates nematode growth and reproduction. Insect cadavers infected by this nematode-bacterium complex are usually flaccid and do not putrefy (i.e., smell bad). The bacteria also produce pigments that give cadavers characteristic colors that vary among species. *Heterorhabditis* infected cadavers are usually red or reddish brown and are faintly bioluminescent. *Steinernema* infected cadavers range in color from light tan or cream to almost black

depending on the species, and are not bioluminescent. The color of infected insects varies somewhat depending on the characteristics of the insect cuticle.

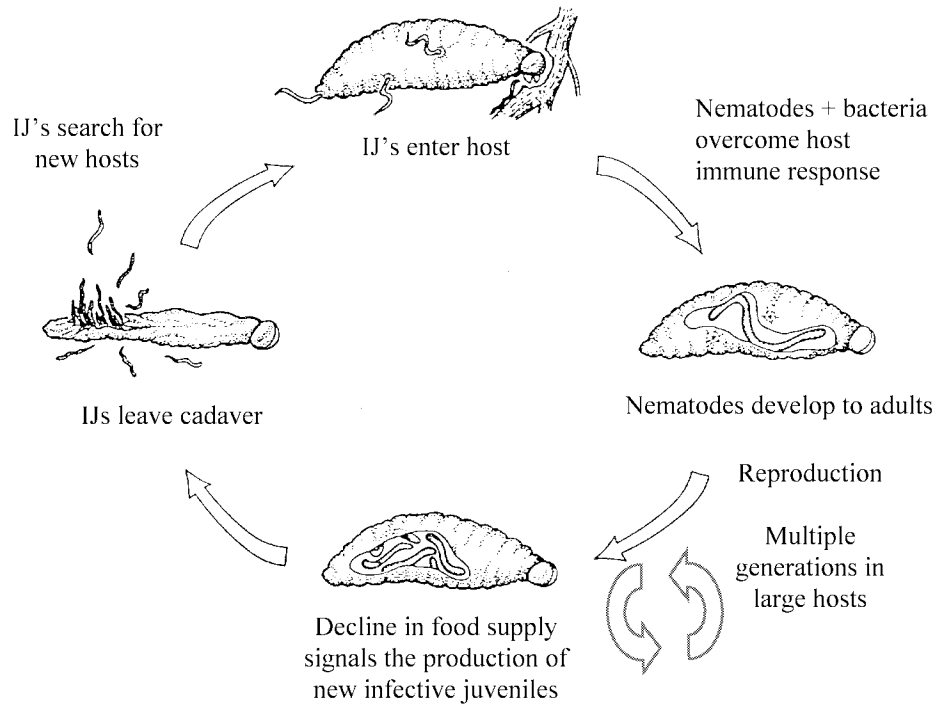

Figure 1. Diagram of the generalized life cycle of insecticidal nematodes.

Station 1. Signs of infection

Examine cadavers of the wax moth, *Galleria melonella* and other insects that have been infected with various steinernematid and heterorhabditid species. Note color differences, absence of bad smell, and flaccidity or limpness of the cadavers. *Heterorhabditis* killed wax worms tend to be less flaccid than those killed by *Steinernema*.

Petri Dish A. healthy wax moth larvae
Petri Dish B. wax moth larvae infected by *S. carpocapsae* (light tan or cream)
Petri Dish C. wax moth larvae infected by *S. feltiae* (chestnut brown)
Petri Dish D. wax moth larvae infected by *S. glaseri* (greyish-brown)
Petri Dish E. wax moth larvae infected by *H. bacteriophora* (brick red)
Petri Dish F. oriental beetle larvae infected by *H. bacteriophora*
Petri Dish G. other insects infected by insecticidal nematodes

Exercise 1. Life history of insecticidal nematodes
Observe various nematode life stages by dissecting *G. mellonella* larvae that have been infected with either *H. bacteriophora* or *S. glaseri* for various periods of time. Take an infected wax worm from each of the stock containers labeled A-C and place them into separate Petri dishes. Add enough water to just cover the bottom of each dish. Using dissecting needles, break open the cuticle of the larvae and spread out the body contents. Examine the dissections under the microscope and note various life stages as illustrated in Fig. 1.

Petri Dish A. Larva killed by *H. bacteriophora* (4-5 days post infection): The large nematodes in this dissection are adult hermaphrodites that developed from the infective juveniles that initially infected the host. Inside the hermaphrodites, note the large ovarioles and eggs.
Petri Dish B. Larva killed by *S. glaseri* (3 days post infection): The large nematodes similar to those observed in Petri Dish A are adult females. The smaller hook-shaped nematodes with the pair of spicules (or small spines) visible near the posterior end are males. The spicules are used for mating. All the nematodes in this dissection are first generation nematodes that developed from the infective juveniles that initially invaded the host.
Petri Dish C. Larva killed by *S. glaseri* (5 days post infection): This dissection contains a mixture of life stages including first generation adults as observed in Petri Dish B as well as second generation juveniles and adults. Second generation adults are generally smaller than first generation adults.

Station 2. Life history, infective juvenile emergence from the host
On demonstration is a standard laboratory emergence (or White) trap with wax moth larvae infected by *S. glaseri* (8 days post infection). These traps are used in the laboratory to collect IJs. Notice masses of IJs on and around the host cadavers. Some IJs might have already dispersed out of the inner Petri dish and become trapped in the water.

B. Specializations of infective juveniles
Infective juveniles have numerous morphological, physiological, and behavioral adaptations that enable them to survive in the soil environment and to locate new hosts. IJs have enhanced temperature and desiccation tolerance compared to other life stages, and substantial amounts of stored lipids (>40% dry weight) that serve as an energy reserve. They are a non-feeding stage with a collapsed digestive system and closed mouth and anus, and are therefore relatively slender compared to other juvenile stages. They also retain the cuticle from the previous molt, which forms an outer sheath that helps protect them against environmental extremes and microbial infections.

Infective juveniles of different species have a similar overall morphology but differ considerably in size and behavior (Table 1). The IJs of *S. carpocapsae* are among the smallest of the insecticidal nematodes whereas those of *S. glaseri* are among the largest. The IJs of different species also differ in activity. When stored in water, a common laboratory practice, *S. carpocapsae* IJs often become inactive and adopt a characteristic posture in which they lie straight with a curved tail, resembling the letter 'j'. The IJs of most other species (e.g., *S. glaseri* and *H. bacteriophora*) tend to remain active and do not assume this posture.

The foraging or host-seeking behavior of insecticidal nematodes can be categorized as either ambushing or cruising, although some species exhibit an intermediate or mixed foraging strategy (Table 1). Ambushing nematodes perform a behavior referred to as nictation in which they stand on their tails and extend >90% of their bodies into the air for long periods. Nictating nematodes will sometimes bend their bodies into a tight loop, and then suddenly fling themselves into the air or jump. Nictation and jumping enable these nematodes to contact and infect active, highly mobile insects on the soil surface. Cruising

behavior is exhibited by nematodes that actively search for hosts deeper down in the soil matrix. These widely ranging nematodes are more effective against sedentary, root-feeding insects. The differing foraging strategies of insecticidal nematodes illustrate how important it is to select the right nematode species to control a particular insect pest.

Table 1. Size, activity during storage, nictation and foraging strategy of infective juvenile *Steinernema* and *Heterorhabditis* spp.

Nematode species	Length x Width (µm)	Activity in water suspension	Nictation behavior	Foraging strategy
S. carpocapsae	438-650 x 20-30	Inactive	Present	Ambusher
S. feltiae	736-950 x 22-29	Active	Present	Intermediate
S. glaseri	864-1448 x 31-50	Active	Absent	Cruiser
S. riobrave	561-701 x 26-30	Active	Present	Intermediate
S. scapterisci	517-609 x 18-30	Inactive	Present	Ambusher
H. bacteriophora	512-671 x 18-31	Active	Absent	Cruiser
H. megidis	736-800 x 27-32	Active	Absent	Cruiser

Exercise 2. Infective juvenile morphology and environmental tolerance

We have provided a Petri dish containing various life stages of *H. bacteriophora* in Ringer's solution. Observe this dish under the microscope and note the behavior and activity of the various stages. Using an eye dropper, place a few drops of this suspension on a glass slide and observe it under high magnification. Look for the extra cuticle on the infective juveniles. Now, using the eye dropper, add 2 ml (i.e., about 2 droppers full) of 1.0% sodium hypochlorite (bleach) solution to the Petri dish. After 5 minutes, examine the various life stages as before. Notice that the infective juveniles are alive and active whereas other juvenile stages and the adults are dead. The thick cuticle and closed digestive system of the infective juveniles enabled them to survive the bleach treatment and similarly protects them against other adverse conditions in nature.

Exercise 3. Species differences in infective juvenile morphology and behavior

We have provided a Petri dish containing a mixture of *S. glaseri* and *S. carpocapsae* infective juveniles. Examine them under the microscope and note the size difference between the large *S. glaseri* IJs and the much smaller *S. carpocapsae* IJs (i.e., less than half as long). Also note that *S. glaseri* tends to be very active and occasionally rests in a tight "donut" shaped coil whereas *S. carpocapsae* is less active and often rests in a "J" posture. *S. carpocapsae* never forms tight coils like *S. glaseri*, and at most will form a loose circle with its head and tail just touching. Dead nematodes are often straight, needle-like, and uniformly cloudy, whereas live nematodes are opaque with small transparent regions at the anterior and posterior ends.

Exercise 4. Species differences in foraging behavior

To illustrate differences in nematode foraging behavior, we have prepared Petri dishes with agar and a light coating of sand, and inoculated them with mixtures of *S. carpocapsae* and *S. glaseri* IJs. *S. carpocapsae* IJs are relatively small and frequently exhibit nictation and jumping behavior. *S. glaseri* IJs are much larger and exhibit cruising behavior. Although *S. glaseri* IJs sometimes raise their bodies

above the substrate and "bridge" between sand grains, they do not nictate or jump. Handle the Petri dish carefully (since nictating nematodes are easily disturbed) and observe the differences in the behavior of these species under the microscope.

II. EFFECT OF ABIOTIC AND BIOTIC FACTORS ON NEMATODE SURVIVAL

Sensitivity to rapid drying
Insecticidal nematodes can often withstand drought under natural conditions but this ability varies considerably among species (Table 2). Nonetheless, they do not tolerate rapid drying on exposed surfaces. Consequently, nematodes should never be applied to dry soil, and they should always be washed off foliage and into the soil immediately after application.

Sensitivity to sunlight
Nematodes are killed by bright sunlight with the medium wavelengths of ultraviolet being the lethal component. Therefore, nematodes should not be applied during mid-day when UV radiation is high. To obtain the best results, nematodes should always be applied either during early morning or late evening.

Sensitivity to oxygen deprivation
Nematodes are sensitive to oxygen deprivation. This is important because nematodes could experience oxygen deprivation during storage, shipping, and application. In particular, when nematodes are mixed in water for application, they settle to the bottom of the tank where oxygen can become rapidly depleted. Thus, once nematodes are mixed in water, they must be constantly agitated. This maintains viability and also assures even distribution during application. Nematode species vary somewhat in their ability to tolerate oxygen deprivation with *S. carpocapsae* being the most tolerant (Table 2).

Temperature tolerance
Nematode species have different temperature activity ranges and lethal limits (Table 2), and this should be considered when selecting a nematode species for application. Although, most nematode species infect insects in a wide temperature range, insect mortality is generally slow at cooler temperatures. Nematodes that are native to cooler regions (e.g., *S. feltiae* and *H. megidis*) are better adapted to perform at cooler soil temperatures than are nematodes from warmer regions (e.g., *S. riobrave*). Both *Steinernema* and *Heterorhabditis* infective juveniles can be killed by excessive heat (Table 2). In general, nematodes should not be applied in extremely hot weather, and the temperature of the spray water should be checked. However, the warm-adapted *S. riobrave* can tolerate short exposures of 42°C whereas the cold-adapted *S. feltiae* and *H. megidis* do not tolerate temperatures above 35°C. Since nematodes may experience high temperatures in transit and storage, it is always a good idea to check the temperature of the 'ice pack' in the container upon arrival. Non-formulated nematodes (e.g., those shipped on sponges) are more sensitive to heat than formulated nematodes. The optimum temperature to store nematodes also varies among species (Table 2). Most species live longer at cooler temperatures, but some warm adapted nematodes do not store well below 10°C (Table 2). Insecticidal nematodes do not tolerate freezing and should never be stored in a freezer.

Nematode age
Newly emerged IJs are more active and opaque in appearance than older nematodes. The opaque appearance of young nematodes is due to their abundant lipid deposits. As IJs age, the lipids are consumed and the nematodes become increasingly transparent. In some species (e.g. *S. glaseri)* older individuals exhibit a distinctively striped or checkered appearance and are referred to as "zebras". Nematodes also become increasingly sluggish with age. In the laboratory, the proportion of *S. glaseri* found resting in a coiled posture in water storage increases with age. After prolonged storage in water,

some heterorhabditids tend to stick together at their tails to form "rosettes" and eventually die. Aging in water is exacerbated by the energy required by the nematodes to maintain a proper water balance. Low temperatures (4-10°C) slow aging by decreasing the metabolic rate. Granular and clay formulations attempt to slow aging by partial dehydration which induces a temporary quiescence (see section V). In general, larger (i.e., *S.glaseri* and *S. feltiae*) and less active (i.e., *S. carpocapsae*) species survive longer than smaller and more active species (i.e., *H. bacteriophora)* regardless of storage conditions.

Natural enemies

Various organisms including nematophagous fungi, collembolans, mites, tardigrades and predatory nematodes have been shown to reduce populations of insecticidal nematodes in soil under laboratory conditions. However, their impact under field conditions is poorly understood. In addition, these nematodes are also susceptible to scavengers 'preying' on nematode-killed insects. Interspecific competition for the same host resources between insecticidal nematodes and other insect pathogens (e.g., fungi, bacteria, viruses) may also affect nematode populations.

Exercise 5. Sensitivity to rapid drying

Your instructor will thoroughly spray a *Chrysanthymum* plant with a suspension of infective juveniles. Collect a few leaves from the sprayed plant and observe the infective juveniles at 10 minute intervals after application until the leaves are completely dry. Initially you will notice that the nematodes are trapped within the water droplets. As the water evaporates, the nematodes are able to move about on the moist leaf surface. However, once the water is completely evaporated, the nematodes quickly desiccate and die in a matter of minutes. This illustrates how important it is to wash the nematodes off the foliage and into the soil as soon as possible following application.

Exercise 6. Heat induced mortality

Transfer about 2 ml (i.e., 2 droppers) of *S. carpocapasae* suspension into a Petri dish. Examine the nematode suspension under the dissecting microscope and note the behavior and appearance of the infective juveniles. From the beaker on the hot plate, add about 5 ml (i.e., 5 droppers) of hot water (80°C) to the Petri dish containing the nematode suspension. Again observe the nematodes under the microscope. Note that nematodes are quickly killed at this temperature.

Station 3. Nematode aging and activity in water

Observe Petri dishes A-D under high magnification. These dishes contain *S. glaseri* IJs that emerged into White traps and have been stored in water at room temperature for various periods of time.

Petri Dish A – Fresh nematodes (less than 1 week after emergence). Fresh nematodes contain abundant lipid deposits. Therefore the whole body appears solid white, except for the anterior esophageal region and the tip of the tail. With careful observation you may be able to see the sheath (i.e., the second stage cuticle retained after the molt) at either the tip of the head or tail. The nematodes are very active and seldom stop moving.

Petri Dish B – One month after emergence. The nematodes still appear quite solid although in many the gut is beginning to show through the diminishing lipid deposits as a faint to clear line running down the center of the body. The nematodes remain quite active but increasing numbers rest in the tightly coiled "donut" posture in undisturbed plates.

Petri Dish C – Four months after emergence. All nematodes appear "thinner" and obviously more transparent or blotchy due to significant lipid reserve depletion. Older *S. glaseri* infective juveniles range in appearance from a uniform translucent (not solid) condition to the most starved individuals in which the remaining lipid deposits appear as two thin rough or broken lines running the length of the transparent body. In the majority of individuals the gut can be seen clearly as a wide band running the

length of the body. Most nematodes are still active, although obviously more sluggish than fresher individuals. In undisturbed plates, many rest in the donut posture.
Petri Dish D – A quizz. This plate contains nematodes of an undisclosed age. Compare these nematodes with the above plates and estimate their age. Check with your instructor for the correct age.

Table 2. IJ desiccation and oxygen deprivation tolerance and temperature (°C) activity ranges of *Steinernema* and *Heterorhabditis* spp.

Nematode species	Desiccation tolerance	O_2 deprivation tolerance	Inactivation temperature	Optimum storage temperature	Temperature infectivity range
S. carpocapsae	High	High	>40	2-5	10-32
S. feltiae	Moderate	Moderate	>35	2-5	8-30
S. glaseri	Moderate	Moderate	>40	10-15	10-37
S. riobrave	Moderate	Moderate	>42	12-15	10-39
S. scapterisci	High	High	>40	10-15	10-35
H. bacteriophora	Low	Low	>35	10-12	10-32
H. megidis	Moderate	Moderate	>35	5-10	8-32

III. ASSESSING NEMATODE QUALITY AND QUANTITY

Commercial shipments of formulated and nonformulated nematodes can vary considerably in nematode numbers and viability because of problems in production, storage, delivery, etc. Thus, for effective applications, it is important to check shipments for nematode quality and quantity. When receiving nematode shipments, always read and follow the manufacturer's instructions for storage, handling, and application since these can differ for different formulations and for different nematode species.

Nematode formulations (e.g., clays, granules, powders) are often non-homogeneous, and assessments of quality and quantity are best conducted by taking a series of small samples (e.g., 1-3 g) rather than a single large sample, with the actual number and size of samples depending on how rigorous an assessment is desired. Rehydrate samples according to the manufacturer's instructions and allow time for the nematodes to become active (usually 15-20 min.) before assessment.

Assessing nematode products delivered on sponges is more difficult. Quality can be assessed by taking a small sample and resealing the plastic bag, but quantity assessment can be done only after all of the nematodes on the sponge and inside the plastic bag have been released into water. The latter procedure is not recommended until application, which leaves little time for careful evaluation.

We have provided stock solutions made from commercial formulations for you to assess nematode quantity and quality. Nematodes rapidly settle out of suspensions, and samples should always be well mixed before evaluation. We bubble air through our stock solutions to maintain homogeneity. When mixing, avoid creating a vortex since this can produce concentration gradients of nematodes in the suspension and lead to erroneous counts.

Exercise 7. Estimating the total number of nematodes and percent viability

1. Using the pipetter provided, dispense six 20 µl aliquots into a counting dish as shown by your instructor.
2. Under the microscope, count the number of living and dead nematodes in each of your samples and enter the information on the data record sheet provided. Nematodes are sometimes inactive, especially in water, and an inactive nematode is not necessarily dead. Check to see if inactive nematodes are alive by gently prodding them with a probe.
3. Calculate totals for your counts of live and dead nematodes and for volume from your six replicates. Enter the data on your record sheet and on the board with data from the other groups. Record all group data on your data record sheet and calculate group totals.
4. Use the group totals and the formulas on your data record sheet to calculate the number of IJs in the stock solution and the percent viability.
5. Living nematodes are not always high quality nematodes. Assess the quality of the nematodes in your samples by evaluating their activity levels and the condition of their lipid deposits as directed by your instructor.

IV. TESTING NEMATODE INFECTIVITY

Nematode infectivity studies are conducted in various ways to test for nematode quality, to evaluate the effectiveness of nematodes against particular insects, to evaluate if an application has been effective, or to assess if entomopathogenic nematodes are active in soil samples. One way to test for the effectiveness of a nematode application is to look for nematode infected insects in the area of application 5-7 days after the nematodes were applied. This allows sufficient time for infection to occur and for the insects to show the classic signs and symptoms of infection as described above. To confirm that nematodes are present, suspect insects can be dissected and assessed directly, or incubated to await the emergence of infective juveniles.

To discover if entomopathogenic nematodes are active in particular areas, soil samples can be baited with a highly susceptible insect (e.g., larvae of the commercially available wax moth, *G. mellonella*), and evaluated for the classic signs and symptoms of infection, etc. To determine to what extent certain species of insects might be susceptible to particular nematodes, evaluations are often conducted in small Petri dishes or well-plates on filter paper where certain numbers nematodes are applied to the insect in question and infection assessed as indicated above. Commercial formulations of nematodes are often tested for quality by conducting these kinds of Petri-dish assays against a standard insect such as *G. mellonella*. Typical results for these kinds of assays using good quality nematodes are shown in Table 3.

Exercise 8 (optional). Infectivity bioassays with nematodes

To better understand infectivity assays, the signs and symptoms of nematode infection, and the nematode life cycle, we have provided everything you need to conduct your own infectivity study. Place a piece of filter paper or some moist soil into a Petri dish. Using a pipetter, apply nematodes to the filter paper or soil from one of the stock solutions. Add a *G. mellonella* larva and seal the Petri dish as directed by your instructor. Take these infected insects with you when you leave the laboratory and observe them periodically at your leisure.

Table 3. Recommended temperatures and IJ concentrations of *Steinernema* and *Heterorhabditis* spp. in the sand-well bioassay and expected *Galleria mellonella* larval mortality.

Nematode species	Bioassay temperature (°C)	*Galleria*:IJ ratio	Expected *Galleria* mortality after 72 h
S. carpocapsae	28	1:1	50-75
S. feltiae	25	1:1	35-50
S. glaseri	28	1:1	35-50
S. riobrave	28	1:1	50-75
S. scapterisci	28	1:15	30-70
H. bacteriophora	25	1:5	40-65
H. megidis	25	1:5	35-55

VI. EXAMINATION OF COMMERCIAL FORMULATIONS AND PRODUCTS

Brief descriptions of the major formulations of insecticidal nematodes are provided below.

Sponge-based products

Placement of nematodes on inert carriers provides a convenient way to store small quantities of nematodes under refrigerated conditions. The polyether-polyurethane sponge is most commonly used for commercial nematode storage and shipping. An aqueous nematode suspension is applied to the sheets of sponge usually at 500-1000 IJs/cm^2 of surface area. Normally 5-25 x10^6 IJs are placed on a single sheet of sponge and the sponge is sealed in a plastic bag. Nematodes on sponges can be stored for 1-2 months at 5-10°C. Sponges are placed on ice packs for shipping, and the nematodes are removed by soaking and hand squeezing the sponges in water prior to application. This method of nematode storage and shipping is convenient for small-scale home garden and lawn applications, but not for large acreage application due to the large volume of product required and time consuming preparation steps

Vermiculite

Vermiculite formulation is a significant improvement over the sponges. The advantages include a more concentrated nematode product, longer storage stability, and more convenient application. Normally, an aqueous nematode suspension is mixed homogeneously with micronized vermiculite. This mixture is placed in thin polythene bags for storage. In the vermiculite formulation, *S. feltiae* could be stored for 4-5 months and *H. megidis* for up to 3 months at 3-5°C. The vermiculite-nematode mixture is added to the spray tank directly, mixed in water, and applied either as spray or drench. The only drawback of this formulation is the lack of ambient storage stability.

Water dispersible granules (WDG)

In water dispersible granular formulations the infective juveniles are encased in 10-20 mm diameter granules consisting of mixtures of various types of silica, clays, cellulose, lignin and starches. These granules are prepared through a conventional pan granulation process in which droplets containing a

thick nematode suspension are sprayed onto fine dry powders on a tilted rotating pan. The granular matrix allows access of oxygen to nematodes during storage and shipping. Under appropriate temperature regimes, the nematodes in the granules undergo a physiological desiccation process and enter into a partial anhydrobiotic state.

The development of the water dispersible granular formulations offers several advantages over the existing formulations. These include: (i) extended nematode storage stability at room temperature, (ii) enhanced nematode tolerance to temperature extremes enabling easier and less-expensive transport, (iii) improved ease-of-use of nematodes by eliminating time consuming and labor intensive preparation steps, (iv) decreased container size/coverage ratio, and (v) decreased amount of disposal material (i.e., screens and containers). In the WDG formulation, *S. carpocapsae* could be stored for 4-5 months at 25°C, and *S. feltiae* and *S. riobrave* for 2-3 months.

Station 4

Observe the various formulations of insecticidal nematodes that are commercially available at this time.

SELECTED REFERENCES

Kaya H K; Stock S P (1997). Techniques in insect nematology. In: *Manual of techniques in insect pathology*, ed. L. Lacey, pp 281-324. Academic Press, San Diego.

Woodring J L; Kaya H K. (1989). *Steinernematid and heterorhabditid nematodes: a handbook of techniques*. Arkansas Agricultural Experimental Station Southern Coop Bulletin. Vol 331, 1-30.

Data Record Sheet for Exercise 7

Your Data:

Replicate	Live	Dead	Volume
1			
2			
3			
4			
5			
6			
Total			

Group Data:

Group	Live	Dead	Volume
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
Total			

$$\text{No. of IJs in Stock Solution} = \frac{\text{Total Living IJs + Total Dead IJs}}{\text{Total Sample Volume}} \times \text{Total Stock Volume}$$

$$= \frac{\qquad}{\qquad} \times \qquad =$$

$$\text{Percent Viability} = \frac{\text{No. of Living IJs}}{\text{No. of Living IJs + No. of Dead IJs}} \times 100$$

$$= \frac{\qquad}{\qquad} \times 100 =$$

The Insect Parasitic Nematode Slide Set

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ABSTRACT

The production of the insect parasitic nematode slide set is briefly discussed. The slide set contains 18 images to accompany the video "INSECT-PARASITIC NEMATODES: Tools for Pest Management" and the various other training materials and information available through the Insect Parasitic Nematode web site at *http://www2.oardc.ohio-state.edu/nematode/*.

INTRODUCTION

During the initial stages of the SARE project to produce a video on the biology and application of insect-parasitic nematodes, it was decided that other training materials, including a slide set should be produced. The slide set contains images to accompany and complement the video "INSECT-PARASITIC NEMATODES: Tools for Pest Management" and the various other training materials and information available through the Insect Parasitic Nematode web site at *http://www2.oardc.ohio-state.edu/nematode/*.

The slide set is intended to be a basic set of images that will be useful for giving introductory talks or training sessions on the biology, use and application of insect parasitic nematodes. The slide set will be especially useful for those trainers who do not have access to equipment for delivering computer-based presentations.

As with other aspects of the development of the video and training materials, a committee comprised of researchers, extension agents, and industry personnel was formed to develop the slide set. The images were collected from workers from the U.S. and Europe who have used or have conducted research on these nematodes. From the collected images the committee chose 18 that most clearly illustrate the biology, morphology, behavior, production and application methods. Aspects of the use of nematodes such as formulations or specific products that are likely to change over time were not included. The committee felt that this type of information would be more suitable for the web page, where information can be frequently updated. A descriptive text was developed to accompany the slide set. The source of each slide is acknowledged in parenthesis.

The hardcopy slide set is a subset of a larger set of images available for viewing in the Image Gallery on the Insect Parasitic Nematode web site. Any of the images from the Image Gallery can be downloaded so that trainers can produce a customized slide set.

Entomopathogenic Nematodes as a Component of Citrus Root Weevil Ipm

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ABSTRACT

The entomopathogenic nematode, *Steinernema riobrave*, is routinely used by many citrus growers in Florida as a component of IPM programs to control a root weevil, *Diaprepes abbreviatus*, because: 1) *D. abbreviatus* is currently the major biological threat to citriculture in the state; 2) until 1998, no effective chemical pesticides were registered for control of weevil larvae in soil; 3) the short-term efficacy of *S. riobrave* has been consistently documented; and 4) use of nematodes is relatively inexpensive. Although unexploited, natural control of root weevils by undescribed species of indigenous nematodes in Florida appears to be high. However, major questions remain regarding optimum use of *S. riobrave* and other entomopathogenic nematodes. Constraints on sampling methodologies have impeded the derivation of economic thresholds and of models of weevil population dynamics that could be used to estimate optimum timing and frequency of nematode treatments. The recommended nematode application rate appears to be adequate for treatment of young trees, but may be too low to provide consistent results in larger mature trees. Research is also needed to estimate: 1) the long-term efficacy of nematodes against weevils; 2) the relative efficacy of nematodes compared to insecticides in an IPM program, and 3) the profitability of *D. abbreviatus* IPM.

INTRODUCTION

Several insect species in the family Curculionidae are commonly referred to as citrus root weevils. In Florida, and throughout the Caribbean region, the West Indian sugarcane rootstalk borer weevil, *Diaprepes abbreviatus* L., is the root weevil of greatest economic significance to citrus. The insect was first detected in Florida in 1964, and currently infests an estimated 150,000 of the 845,000 acres of commercial citrus orchards. During the past decade, *D. abbreviatus* has become the most serious biological threat to the well-being of citriculture in Florida because of its high incidence, its devastating effect on trees, and because cost-effective IPM strategies have been elusive. Prior to 1998, attempts to intervene in the soil-borne phase of the weevil life cycle were hampered by the absence of registered, effective soil-applied pesticides, due to environmental concerns. For these reasons, the use of entomopathogenic nematodes to manage citrus root weevils has had a high priority for more than a decade among both researchers and citrus growers in Florida.

Adult *D. abbreviatus* feed and oviposit on the leaves of citrus and alternate host plants in orchards (Fig. 1). Newly-hatched (neonate) larvae drop to the soil where they develop for 4-9 months while feeding on the root systems of trees. Pupation occurs in the soil. Young larvae feed initially on the small fibrous roots (Fig. 2), but as they increase in size they feed on the cortex of increasingly larger roots. The insects create long lesions or channels in the bark of large roots, which are then infected by the root-rotting fungi *Phytophthora nicotianae* Dastur, and *P. palmivora* (Butler) Butler (McCoy, 1999; Graham & Menge, 1999). The interaction between root weevils and plant pathogenic fungi results in one of the most severe decline syndromes affecting citrus. Trees are sometimes killed by a resulting crown rot, but more typically trees decline severely and irreversibly due to cambium girdling and death of large structural roots.

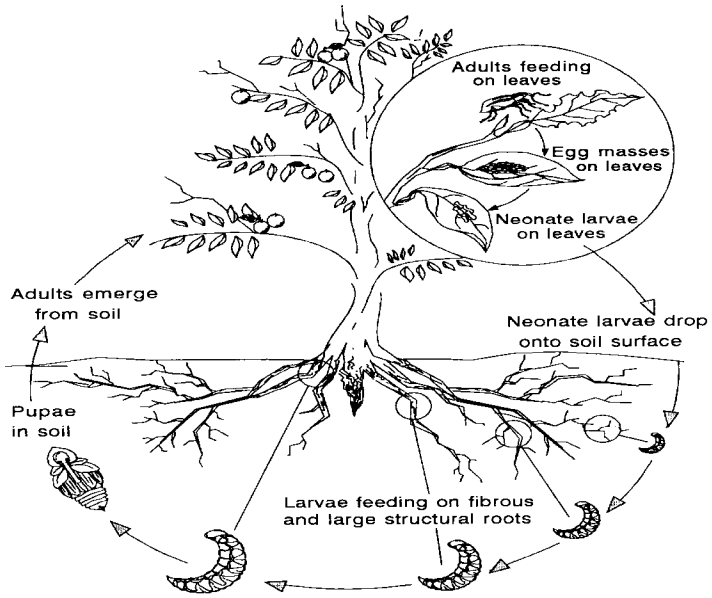

Figure 1. The generalized life cycle of *Diaprepes abbreviatus*. Reprinted by permission of APS Press and C. W. McCoy.

It is often necessary to remove and replant the majority of trees within root weevil-infested orchards. Large scale replanting has serious economic consequences because maximum fruit yield is not attained for 10-15 years. Moreover, weevil-resistant rootstocks are unavailable and the costs of managing weevils in replanted orchards may exceed $250/acre/year. Recommended IPM of *D. abbreviatus* currently consists of the use of insecticides (e.g., carbaryl) and ovicides (e.g., spray oil or diflubenzuron + spray oil to open adhering leaves that protect egg masses) for above-ground control of eggs and adult insects, fungicides (e.g., metalaxyl) for control of *Phytophthora* spp., and soil-applied insecticides (imidacloprid or bifenthrin) or entomopathogenic nematodes for control of soil-borne stages of the insect (Knapp, 1998). Increased application frequency of water and fertilizer is also recommended to improve the tolerance of trees to the loss of roots caused by root weevils.

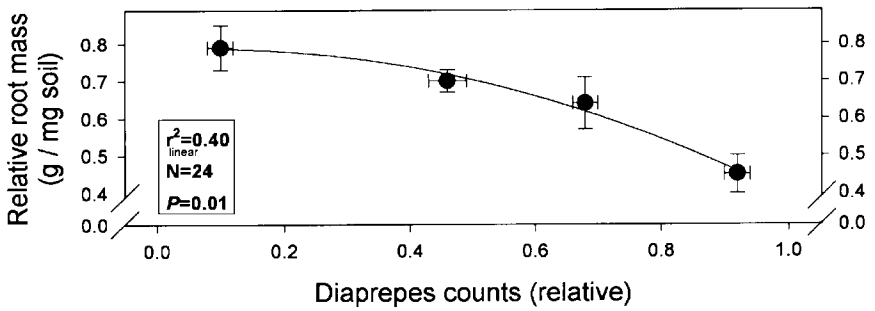

Figure 2. The relationship between population density of *Diaprepes abbreviatus* adults and citrus fibrous root density. Adult insects were monitored during 90 days using 12 Tedders traps in each of 12, 1.6 acre plots in an orchard. Fibrous roots in each plot were sampled with soil augers on 2 occasions and normalized by dividing root mass density by the highest mass density on each occasion.

When *Steinernema carpocapsae* Weiser was reported to have efficacy against *D. abbreviatus* (Schroeder, 1990), the nematode quickly became commercially available and widely used in Florida orchards. However, perceived failure of the commercial product in the field and subsequent research (Schroeder, 1994; Duncan *et al.*, 1996) resulted in changing the commercially available species from *S. carpocapsae* to *S. riobrave* Cabanillas, Poinar & Raulson and *H. bacteriophora* Poinar, Kanunakar, & David. Research during the past 5 years (Table 1) has confirmed the potential effectiveness of *S. riobrave* as a component in IPM of citrus root weevils (Bullock *et al.*, 1999; Duncan & McCoy, 1996; Downing *et al.*, 1991). Moreover, treatment costs ≈ $25 per acre are relatively modest compared to those for most soil-applied chemical pesticides.

Despite a widespread acceptance of entomopathogenic nematodes for use in weevil IPM, a number of concerns exist. Neither researchers nor growers consider current IPM programs to be adequate for economic management of citrus root weevils. There are no published studies of the profitability of these programs and anecdotal evidence suggests that long-term weevil control is variable and in some cases marginal. The research constraints to answering questions of efficacy and profitability are enormous. There are no methods to directly assess population densities of weevils in soil, chemical attractants for adults are unknown, and adult monitoring methods are inefficient. Root loss and root damage cannot be assessed non-destructively. The effect on yield of mitigating root damage is complex, requiring long-term study of several crop cycles. Methodology problems such as these have constrained research to determine the insect life cycle, the incidence and causes of natural control, optimum application timing and rate of entomopathogenic nematodes, root loss-yield relationships, and insect economic thresholds.

However, ongoing research is attempting to address some of these problems in order to provide information needed to optimize the use of tactics for IPM of citrus root weevils. The objectives of this paper are to use data from published and ongoing studies to describe how entomopathogenic nematodes are currently used by citrus growers, and to identify some of the questions that are being studied to improve the utilization of these organisms.

CHOICE OF NEMATODE SPECIES AND FORMULATION

Several indigenous entomopathogenic nematode species (some undescribed) that parasitize citrus root weevils occur in Florida citrus orchards (Authors & K. Nguyen, unpublished). For example, *Heterorhabditis indica* was recently described from South Florida and is now available commercially. Other nematodes available commercially for use in Florida citrus are *S. riobrave* and *H. bacteriophora*. Each nematode species has different search strategies that affect the horizontal and vertical distance it migrates (Kaya *et al.*, 1993), and each persists differently under different conditions (see below). Similarly, recent laboratory data indicate that *H. indica* may have higher virulence than other species against younger (ca. 4th instar) *D. abbreviatus* larvae (Shapiro *et al.*, 1999), whereas other experiments indicate *S. riobrave* is more virulent against older (7-11th instar) larvae (Shapiro & McCoy, unpublished). Published research suggests that *S. riobrave* performs as well as or better than other available species under conditions tested to date (Table 1). However, it should be noted that most studies have reported results of short-term evaluations, and did not measure long-term efficacy that could result from superior persistence in soil of a particular species. Similarly, further evaluation of indigenous nematode species may reveal characteristics that are advantageous compared to commercially available species.

The quality of formulated nematodes is also important when choosing a commercial product. Entomopathogenic nematodes differ in their ability to remain viable when commercially formulated and

Table 1. Reported efficacy of entomopathogenic nematodes against the citrus root weevils *Diaprepes abbreviatus* and *Pachnaeus litus*.

	Nematode	Approximate rate/cm^2	Method and no. of applications	Time of application	Site of trial	Target host	Percent reduction[b]	Reference
1.	*S. riobrave*	110	Watering can (2)	March/Sept.	Indian River County	*Diaprepes* *Pachnaeus*	98% 95%	Bullock et al. (1999)
2.	*S. riobrave*	110	Watering can (2)	March	Indian River County	*Diaprepes* *Pachnaeus*	82% 80%	Bullock et al. (1999)
3.	*S. riobrave*	110	Watering can (1)	March	Indian River County	*Diaprepes* *Pachnaeus*	85% 65%	Bullock et al. (1999)
4.	*S. riobrave*	110	Injection L.V.[c] irrigation (1)	March	Indian River County	*Diaprepes* *Pachnaeus*	No control	Bullock et al. (1999)
5.	*S. riobrave*	N.D. (2 million/tree)	Injection L.V. irrigation (1)	March	Indian River County	*Diaprepes* *Pachnaeus*	100% 90%	Bullock et al. (1999)
6.	*S. riobrave*	N.D. (2 million/tree)	Injection L.V. irrigation (1)	March	Indian River County	*Diaprepes* *Pachnaeus*	51% 100%	Bullock et al. (1999)
7.	*S. riobrave*	N.D. (1.6 million/tree)	Herbicide applicator (1)	March	Indian River County	*Diaprepes* *Pachnaeus*	98% 48%	Bullock et al. (1999)
8.	*S. riobrave*	120	Watering can (1)	May	Lake County	*Diaprepes*	90%	Duncan et al. (1996)
9.	*S. riobrave*	250	Watering can (1)	October	Polk County	*Diaprepes*	77-90%	Duncan & McCoy (1996)
10.	*S. riobrave*	N.D. (3-9/cm^3)	Watering can	N.D.	Greenhouse pot test	*Diaprepes*	77-86%	Schroeder (1994)
11.	*H. bacteriophora*	175-250	Watering can (1)	May	Lake County	*Diaprepes*	55%	Duncan et al. (1996)
12.	*H. bacteriophora*	250	Watering can (1)	October	Polk County	*Diaprepes*	No control	Duncan & McCoy (1996)
13.	*H. bacteriophora* (Otinem)	N.D. (7.8 million/tree)	(7 appl., in 3 yr) Injection via L.V. irrigation (1)	November	Indian River County	*Diaprepes*	No control	Adair (1994)
14.	*H. bacteriophora* (Otinem)	127 255 637	Injection via L.V. irrigation (1)	Early spring	Lake County	*Diaprepes*	83% 78% 69%	Downing et al. (1991)
15.	*H. bacteriophora* (Otinem)	127 255 637	Injection via L.V. irrigation (1)	Early spring	Lake County	*Diaprepes*	72% 47% 56%	Downing et al. (1991)
16.	*H. bacteriophora* (Otinem)	127 255 637	Injection via L.V. irrigation (1)	Early spring	Osceola County	*Pachnaeus*	17% 53% 76%	Downing et al. (1991)
17.	*H. bacteriophora* (Otinem)	160	N.D.	March	Lake County	*Diaprepes*	58%	Schroeder (1990)
	H. bacteriophora (Fl. strain)	160	N.D.	March	Lake County		26%	

[a]N.D. = no data.
[b]Efficacy defined as the percent reduction in larvae in soil or adults emerging from soil.
[c]L.V.= Low volume.

quality control can vary among products and production batches. Nematodes in liquid formulation cannot be stored by the grower for more than 2-3 days and their viability is generally evaluated just prior to shipment.

Alternatively, when using a granular formulation, it is advisable for the user to have a means to evaluate the motility of nematodes just prior to use. The proportion of motile nematodes is useful to estimate viability which sometimes deteriorates markedly during permitted storage times as long as 1 month.

APPLICATION TIMING AND FREQUENCY

Recommendations about when and how often to apply entomopathogenic nematodes have been inferred from seasonality of emergence of adult insects from the soil, from estimates of nematode persistence following application, from research on physical causes of nematode mortality, and by considering the cost of applying nematodes. However, significant gaps exist in our understanding of the insect population dynamics and the spatial/temporal relationships between nematode density and efficacy.

Nematode persistence and natural control by nematodes

When entomopathogenic nematodes are applied to soil in Florida, their population density declines rapidly (Fig. 3). Irrigation during and following nematode application increases the survival and efficacy of nematodes (Downing, 1994); however, large numbers of nematodes remain near the soil surface and die (Duncan & McCoy, 1996). Although recycling of exotic nematodes has been detected in experimental plots in the field, the level of long-term insect management does not appear to be significant. In a greenhouse experiment in which *S. riobrave* were applied at various intervals to potted citrus seedlings that were infested repeatedly with neonate larvae of *D. abbreviatus*, fibrous root weights of trees increased directly with the number of nematode applications (Duncan & McCoy, unpublished). Compared to trees not infested by weevils, fibrous roots of infested trees were reduced significantly even when treated monthly with nematodes. These data suggest that very limited feeding by the insect is likely to reduce fruit yield by diverting carbohydrates to fibrous root growth, and that low persistence by the nematode requires frequent application to mitigate the problem.

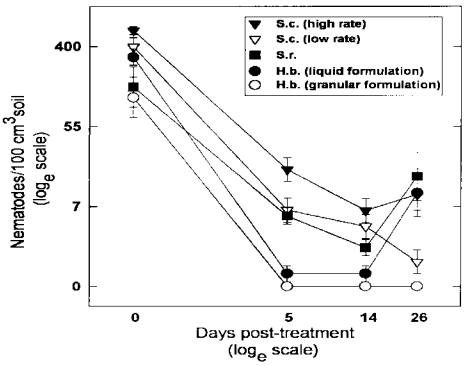

Figure 3. Average recovery of *Steinernema carpocapsae* (S.c.), *S. riobrave* (S.r.), and *Heterorhabditis bacteriophora* (H.b.) following application to soil beneath 4-year-old citrus trees. Reprinted by permission of Journal of Nematology.

Time of day is also important when scheduling nematode applications (Fig. 4). Nematodes applied beneath the canopy of a tree survive in direct proportion to their proximity to the tree trunk where evaporation of soil moisture and exposure to ultraviolet radiation are least (Duncan *et al.*, 1996; Molyneaux & Bedding, 1984; Gaugler & Boush, 1978). Application of nematodes in the evening provides the longest possible time for their establishment before being exposed to desiccation and sunlight.

Indigenous entomopathogenic nematodes are generally found to have highest activity during summer months in Florida (Beavers *et al.*, 1983) and elsewhere (Doucet & Giayetto, 1998). These natural control agents appear to be key mortality factors regulating the population dynamics of citrus root weevils. Recent experiments in Florida have found natural, nematode-induced mortality of *D. abbreviatus* during summer to be as high as 40-50% after just 3 days in the soil (Fig. 5). Increased activity of indigenous nematodes coincides with the onset of the characteristic seasonal depression in numbers of insects emerging from soil (Fig. 6). Important questions regarding these as yet undescribed species include understanding their incidence throughout the industry, whether the level of natural control is dependent on root weevil density, and whether they are good candidates for augmentation by virtue of their ability to persist under Florida conditions, or for other reasons.

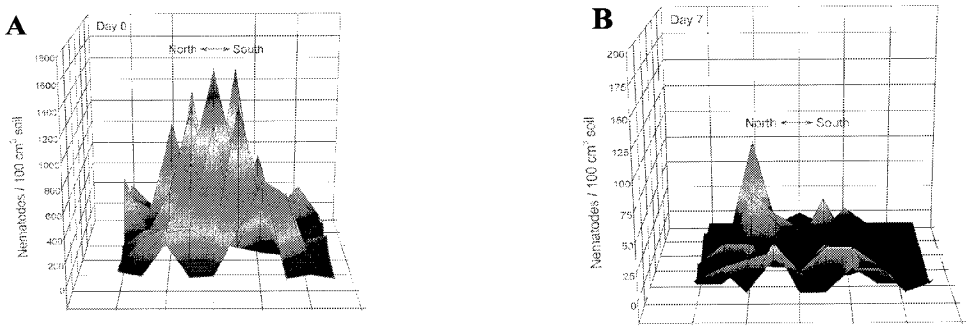

Figure 4. Population densities of *Heterorhabditis bacteriophora* recovered from 100 cores of soil from a grid centered on a mature citrus tree. Nematodes recovered 1 hour after application (A) and 7 days after application (B). Note the inverse relationship between persistence and distance from tree trunk (center of grid). Reprinted with permission of Journal of Nematology.

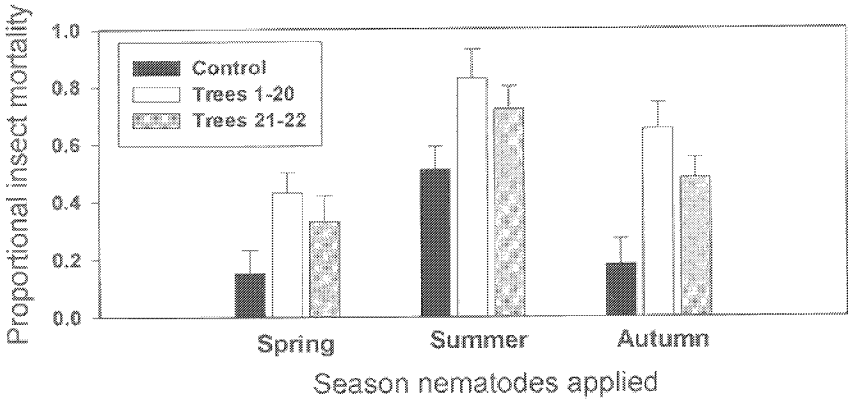

Figure 5. Efficacy of *Steinernema riobrave* against *Diaprepes abbreviatus* in three experiments in a Florida citrus orchard. Insects were caged and buried beneath trees for 3 days following nematode application. The first 20 trees in rows received higher numbers of larvae than did trees at ends of rows, as shown in Fig. 7. Mortality of weevils in untreated control plots was caused primarily by an undescribed species of *Steinernema.*

Insect population biology

Because nematodes attack insects in the soil and show little evidence of significant persistence, it is generally assumed that nematodes will have the greatest effect if applied when numbers of insects in soil are highest. Emergence from soil of *D. abbreviatus* and another species of root weevil, *Pachnaeous litus*, is seasonal in Florida, with maximum emergence in late spring (Fig. 6A). The data in Fig. 6A are from Tedders traps which are placed under the tree canopy and require insects to climb a dark colored base into an elevated trap. Tedders traps are more efficient than cone shaped traps placed on soil beneath tree canopies, and seasonal patterns of adult activity are the same for both types of trap (McCoy, unpublished). There are no comparable census data for densities of insect larvae in soil; however, the adult census data suggest that the rate of egg deposition in the tree canopy increases in early summer. Thus, by autumn the surviving larvae in the soil have likely reached a maximum density, because low winter temperatures greatly reduce ovipositional activity. As temperatures increase in the spring, larval and pupal development continues until adults emerge from the soil. Because larval development requires a minimum of 4 months, it is likely that most larvae which enter soil during mid-to-late summer emerge the following spring.

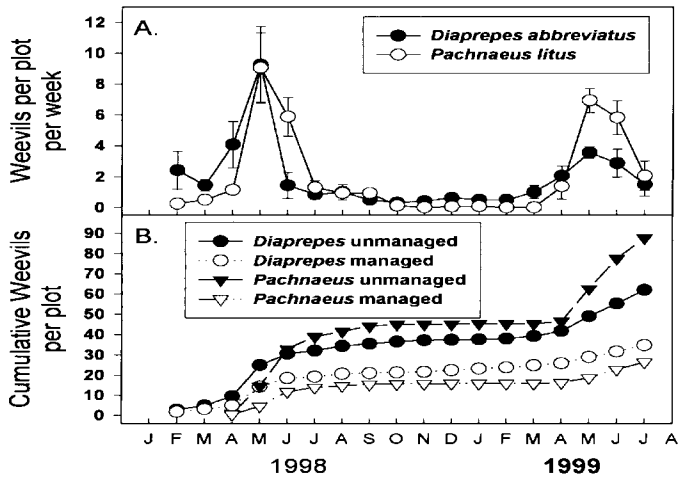

Figure 6. Mean monthly number of insects trapped per 1.6-acre plots that were managed or not managed to control citrus root weevils (A), and mean cumulative numbers of weevils trapped during 18 months in the managed and unmanaged plots (B). Weevil management consisted of use of foliar applied ovicides and adulticides and applications of *Steinernema riobrave* in June and October, 1998 and April, 1999.

Growers generally do not apply more than 2 applications of nematodes per year for economic rather than empirical reasons. Based on the pattern of adult emergence from soil, there is general consensus that an application of nematodes in the autumn presents the parasites with their greatest opportunity to locate insect prey. A second application of nematodes in spring when soil temperatures are high enough for nematode activity, but before adult emergence occurs, is practiced by many growers and researchers (e.g., Bullock *et al.*, 1999). To reduce the deposition of larvae into soil following a springtime nematode treatment, management of above ground stages of the insect is recommended at peak adult emergence. It has also been suggested that an application of nematodes in summer, when rainfall and soil temperatures are highest, provides the worms with ideal conditions for parasitism at a time when natural control is highest (Knapp, 1998).

Clearly, more realistic management models are needed to determine the optimum number and timing of nematode applications. Such models will require a great deal of additional basic information on insect population dynamics, and on questions such as effect of insect developmental stage on rate of nematode infection, and on positive or negative interactions between indigenous and exogenous nematode species.

APPLICATION METHODS

Entomopathogenic nematodes are applied to citrus either with herbicide application equipment or via under-tree, low-volume irrigation systems. The latter method has the advantages of eliminating the cost of driving equipment through the orchard and of depositing nematodes only in irrigated soil. However, the spatial pattern of nematode deposition throughout a grove is less consistent when applied by irrigation than with tractor-driven equipment. Nematodes tend to settle to the bottom of irrigation lines, particularly when the flow rate is low as in drip-irrigation systems (Conner *et al.*, 1998). Micro-sprinkler irrigation systems have higher flow rates and deliver nematodes more uniformly. Numbers of nematodes delivered to tree rows is reasonably uniform with distance from the injection point (Fig. 7). A similar pattern is seen within the tree rows, except that trees at the very ends of rows receive significantly fewer nematodes, due to changes in water flow as water reaches the ends of lines. In preliminary experiments, efficacy of *S. riobrave* against *D. abbreviatus* was only measurably affected at the ends of tree rows (Fig. 5). This deficiency can be corrected by adding additional emitters or emitters with increased water delivery at the ends of rows.

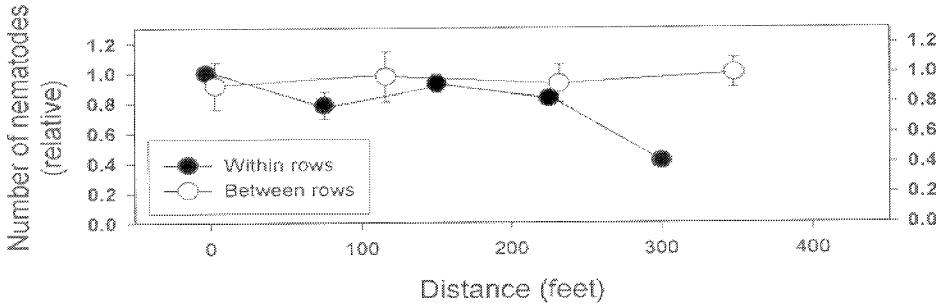

Figure 7. Relative numbers of nematodes delivered via micro-sprinkler irrigation to rows of citrus trees with increasing distance from the injection point (at the pump), and delivered within rows of trees from the beginning of the rows to the ends of the irrigation lines. Within rows, nematode delivery was measured at 4 equidistant trees, which included the first and last trees in the rows.

Various common-sense factors should be considered with regard to application equipment. Holding/mixing tanks should be thoroughly cleaned of nematode-detrimental chemical residues from previous operations. Nematodes should not be combined with other chemicals to be applied to trees. The pH of delivery water should not be excessively low or high. Artesian well water lacks sufficient oxygen for nematodes and should not be used. Pumps that generate excessive heat should not be used for injecting nematodes into irrigation systems or for maintaining nematodes suspended in holding tanks.

APPLICATION RATE

Label rates for *S. riobrave* and *H. bacteriophora* in citrus are 200 million and 100 million nematodes per acre, respectively. However, the actual rate of application varies with tree age, because the under-canopy surface area of young trees is an order of magnitude less than that of mature trees. Thus, reported application rates (using label recommendations) vary from more than 200 to fewer than 20 nematodes per cm^2 soil surface. At the higher rates, short-term efficacy against *D. abbreviatus* was found to be very high (85-95%; Duncan *et al.*, 1996; Duncan & McCoy, 1996; Bullock *et al.*, 1999). On mature trees at the lower application rates, efficacy has tended to be much lower (Figs. 5 & 6B). Results shown in Fig. 5 are typical of 2 ongoing experiments and indicate a dosage response to *S. riobrave* rate. Efficacy of the nematode against *D. abbreviatus* larvae buried beneath trees at the ends of rows was consistently lower than efficacy measured beneath other trees in those rows. Trees at the ends of rows were shown to receive far fewer nematodes than other trees (Fig. 7). These modest estimates of short-term efficacy are confirmed by the cumulative numbers of *D. abbreviatus* and *Pachnaeus litus* that were trapped in those plots during an 18 month period (Fig. 6B). Moreover, nematode applications in this experiment were used in combination with other tactics to manage the insect. It should be noted that these results are in marked contrast to those of a similar study in which cumulative numbers of adult *D. abbreviatus* trapped during one year were reduced by up to 95% by the application of the label rate of *S. riobrave* (Bullock *et al.*, 1999). Nevertheless, trees in the study by Bullock *et al.* (1999) were relatively young (7 years), and nematodes were applied by sprinkling can, suggesting that the area treated was small.

The recommended application rate for *S. riobrave* has consistently produced very high short-term efficacy against root weevils in young trees. Results in mature trees, where the effective application rate is lower, have been consistently measurable, but variable in magnitude. The data suggest that further research to determine an expected dosage-response based on surface area treated is warranted. In Florida, such trials should be conducted on the sandy soils in the central part of the state, and on the heavier soils along the coasts.

CONCLUSIONS

The nature of the life cycle of *Diaprepes abbreviatus* presents a serious and complex management challenge because insects are continually recruited from soil to the tree canopy and from the canopy to soil. Short-term suppression of either the aboveground or belowground stages of the insect, independently of one another, is unlikely to provide adequate control. However, in the absence of host resistance, IPM relies increasingly on non-persistent, narrow-spectrum tactics to manage insects with the least environmental disruption. Entomopathogenic nematodes have been found to have outstanding potential for use as a component of root-weevil IPM. Nevertheless, breaking the insect recruitment cycle requires a great deal of additional knowledge in order to intervene with the correct tactics at the appropriate time.

Future research should evaluate the relative contribution to overall insect control of current tactics to intervene in the above ground (mainly insecticides) *vs* the below ground (insecticides or nematodes) stages of the insect life cycle. Profitability of current IPM programs should be evaluated to provide a baseline for future management innovations. Population models and economic thresholds relating population density to root damage and damage to yield are urgently needed and require improved methods to monitor these insects. Finally the diversity of entomopathogenic nematodes should be exploited by further characterizing the biology and biocontrol potential of known and yet to be discovered indigenous and exotic species.

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Insecticidal Nematodes for Cranberry Pest Management

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ABSTRACT

Insecticidal nematodes are increasingly used in cranberries to manage cranberry girdler and the root weevil complex, primarily in Wisconsin, Massachusetts, British Columbia, Oregon, and Washington. A summary of available data on the efficacy of insecticidal nematodes against important cranberry pests is presented and appropriate choice of nematodes for selected pests suggested. Availability of high quality nematodes and cost are some of the major factors limiting nematode use in cranberries. Factors to be considered in the application of nematodes to achieve optimal efficacy are discussed. Future research should focus on the evaluation of newer species, strains and formulations against major target pests, investigation of timing and application strategies (single versus multiple applications), susceptibility of various larval stages for nematode infection, and nematode persistence in acidic cranberry environments.

INTRODUCTION

Several species of soil-dwelling immature insects mainly belonging to Lepidoptera and Coleoptera cause significant damage to roots and stems of cranberries throughout the cranberry production areas of North America. The resurgence of grub infestations in cranberries since the mid 1980's has been attributed to the waning residues of very long-lasting organochlorine insecticides which were in use until the mid 1970's (Averill & Sylvia, 1998). Currently, with the exception of a granular formulation of diazinon registered in some regions for the management of cranberry girdler, there are no effective chemical insecticides registered for managing the majority of the soil insect pests on cranberries. Maintaining a summer flood between mid-May to mid-July has been shown to be effective in managing several scarab grubs. However, this treatment will result in a total loss of crop for that year, and reduced yields the following year (see Averill & Sylvia, 1998). Although regular sanding at 3-4 year intervals is known to suppress cranberry girdler infestations, grower adoption of this practice has been limited to certain regions due to cost and logistics. Other cultural control options such as fall flooding in September, usually after harvest, for managing cranberry girdler requires precise timing before the formation of cocoons. The timing of this practice in the midst of harvest season is logistically difficult and therefore is not widely implemented.

Over the past decade, insecticidal nematodes have been found to provide acceptable control of several cranberry pests. Insecticidal nematodes are especially suitable for use in cranberries because of some unique environmental conditions in which cranberries are grown. The cranberry root zone has high soil moisture levels and relative humidity, is protected from direct sunlight (and from ultraviolet radiation), and temperatures rarely reach levels harmful to nematodes. Insecticidal nematode use in cranberries (Table 1) has been steadily increasing over the past 10 years (Weber & Henderson, 1998; and Don Weber personal communication). In recent years, nematode use has been mainly concentrated in Wisconsin, British Columbia, and Massachusetts for managing cranberry girdler and the root weevil complex. In this report, I have summarized laboratory and field evaluations of insecticidal nematodes against cranberry pests and suggested appropriate nematode species for each target pest based on pest biology and nematode behavior. Application methods to achieve optimal efficacy with nematodes and some of the constraints in deploying nematodes in cranberries are also discussed.

Table 1. Insecticidal nematode use in cranberries, 1989-1998

	Acres treated								
Region	1989	1990	1991	1992	1993	1994	1995	1996	1998
MA	3	164	159	452	246	71	171	418	365
WI	0	8	140	388	193	117	371	463	1260
OR	78	13	17	21	12	5	0	6	0
WA	120	219	234	204	218	203	120	10	0
BC	275	114	113	257	594	303	247	665	427
Total	476	518	663	1322	1263	699	909	1552	2052

TARGET PESTS, CHARACTER OF INJURY, AND APPROPRIATE CHOICE OF NEMATODE SPECIES

Target pests

The major pests of cranberries that are potential targets for management with insecticidal nematodes are listed in Table 2. Except for cranberry girdler, the majority of the potential target pests infesting cranberries are coleopterans belonging to the families Scarabaeidae, Curculionidae, and Chrysomelidae. In the Pacific northwest, cranberry girdler and the root weevil complex are the target pests for insecticidal nematodes whereas cranberry girdler is the target in Wisconsin. In Massachusetts, nematodes are applied for managing cranberry girdler, the rootweevil complex and scarab grubs. Cranberry rootworm is the most significant root infesting pest in New Jersey (Polavarapu & Stuart, 1997).

Character of injury

Several grub species that infest cranberries cause similar types of injury, mainly devouring the fine roots, often so extensively that the vines may be easily pulled up along with the surface soil and rolled back like a carpet. This feeding can cause severe stunting and spindling of vines and, in the most severe cases, vines may die, leaving patches of bog bare. Often, weeds take over these bare patches, making re-establishment of vines difficult and expensive. The damage caused by cranberry girdler is somewhat different in that the larvae mainly chew on the stems and runners and to a lesser extent on roots. Feeding often entails complete girdling of the stem resulting in the death of individual uprights rather than the more generalized decline seen over a larger area with root grubs. The root weevil grubs feed on both roots and the bark of stems. Often, the damage to the bark appears similar to cranberry girdler injury, but seldom is this damage as deep as cranberry girdler injury. Among the coleopteran pests, only root weevils, cranberry rootworm, and striped colaspis cause damage in the adult stage. In most cases, damage caused by adults is relatively unimportant.

Appropriate choice of nematode species

Insect parasitic nematodes exhibit diverse hunting strategies and have different tolerances to temperature. Certain species (e.g., *Steinernema carpocapsae)* are relatively inactive, remain near the soil surface and use an 'ambusher' strategy in which they stand on their tails and await passing insects (see Gaugler, 1999). Others (e.g., *Heterorhabditis bacteriophora*), penetrate more deeply into the soil matrix and use an active 'cruiser' strategy to locate and infect sedentary insects. Still others (e.g., *S. feltiae*), use an intermediate or mixed strategy. Species such as *H. marelatus* and *H. megidis* are cold adapted whereas *S. riobrave* is warm temperature adapted. Therefore, the selection of appropriate nematode species to match the biology and environment of the target pest species is very important to achieve effective control. Table 2 lists the recommended nematode species for each pest species based on target pest biology, nematode behavior, and field efficacy data. Convincing field efficacy data are not available for the use of insecticidal nematodes against other cranberry pests such as cranberry whitegrub, cranberry rootgrub, oriental beetle, and striped colaspis.

Table 2. Target pests, their distribution, and recommended nematode species based on field performance

Common name	Scientific name	Family	Distribution	Nematode species
Cranberry girdler	*Chrysoteuchia topiaria*	Pyralidae	WA, OR, WI, MA, NJ, BC	*Steinernema carpocapsae* *Heterorhabditis bacteriophora* *H. marelatus*
Black vine weevil	*Otiorhynchus sulcatus*	Curculionidae	WA, OR, MA, BC	*H. bacteriophora* *H. marelatus*
Strawberry root weevil	*Otiorhynchus ovatus*	Curculionidae	WA, OR, MA, BC	*H. bacteriophora* *H. marelatus*
Hoplia grub	*Hoplia modesta*	Scarabaeidae	MA	*H. bacteriophora*
Cranberry whitegrub	*Phyllophaga anxia*	Scarabaeidae	MA, NJ, WI	
Cranberry rootgrub	*Lichnanthe vulpina*	Scarabaeidae	MA	
Oriental beetle	*Exomala orientalis*	Scarabaeidae	MA, NJ	
Cranberry rootworm	*Rhabdopterus picipes*	Chrysomelidae	NJ	*H. bacteriophora*
Striped Colaspis	*Colaspis costipennis*	Chrysomelidae	MA	

FIELD AND LABORATORY EVALUATIONS OF INSECTICIDAL NEMATODES IN CRANBERRIES

Cranberry girdler

Initial work on cranberry girdler was mainly conducted with *S. carpocapsae.* Dapsis (1993) reported that in laboratory assays *S. carpocapsae* infective juveniles (IJs) infected approximately 60% of newly hatched girdler larvae in 15-cm diameter plastic arenas. In field trials, mortality of cranberry girdler larvae enclosed in 5 x 5 x 0.6 cm stainless-steel (40 mesh) cages placed in the field treated with formulated *S. carpocapsae* at 2 billion per acre ranged between 44-87.5%. In another field trial conducted in Oregon, Smith *et al.* (1993) reported that application of formulated *S. carpocapsae* at 2 billion per acre reduced the third and fourth instar larval population by 92%.

More recently, several novel species of heterorhabditids and steinernematids were evaluated against cranberry girdler (Berry & Liu, 1998; Henderson & Singhai, 1998). Several strains of *H. marelatus* and *H. bacteriophora* significantly reduced cranberry girdler populations in microplots (5,673 cm^3) treated at 0.5 – 1.0 billion IJs per acre (Berry & Liu, 1998). In small m^2 plots, *H. marelatus* significantly reduced cranberry girdler population at both 0.5 and 1.0 billion per acre rate (Fig. 1). *H. marelatus* was found to persist for at least 6 weeks in treated soil at both rates. Mortality of waxmoth larva (*Galleria melonella*) also increased significantly over the six-week period suggesting recycling in girdler larvae.

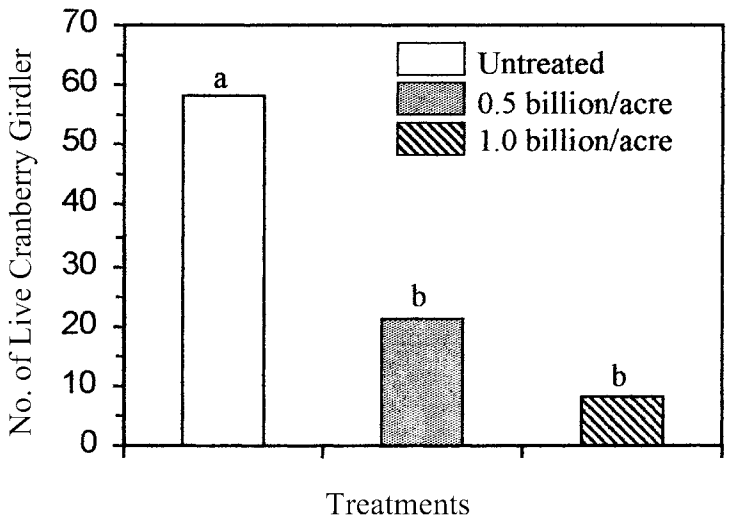

Figure 1. Efficacy of *H. marelatus* against cranberry girdler larvae in m^2 plots, Bandon, Oregon, 1997, (redrawn from Berry & Liu, 1998).

Henderson & Singhai (1999) compared the efficacy of *H. bacteriophora*, *S. carpocapsae* and *S. krausei* (all species provided by MicroBio Inc.) applied at 3 billion per acre (Fig. 2). Larval populations were significantly lower in plots treated with *H. bacteriophora* and *S. carpocapsae*, but, *S. krausei* had no effect on larval populations. Both these studies have convincingly shown that *Heterorhabditis* species have potential in managing cranberry girdler larvae. However, considering the difficulties involved in formulating *Heterorhabditis* species and shorter shelf life compared to *S. carpocapsae* formulations, there may not be any significant advantage in using *Heterorhabditis* species for managing cranberry girdler. Nonetheless, attributes such as cold tolerance and efficacy at lower rates exhibited by *H. marelatus* may provide the additional incentive for its further development for cranberry girdler management.

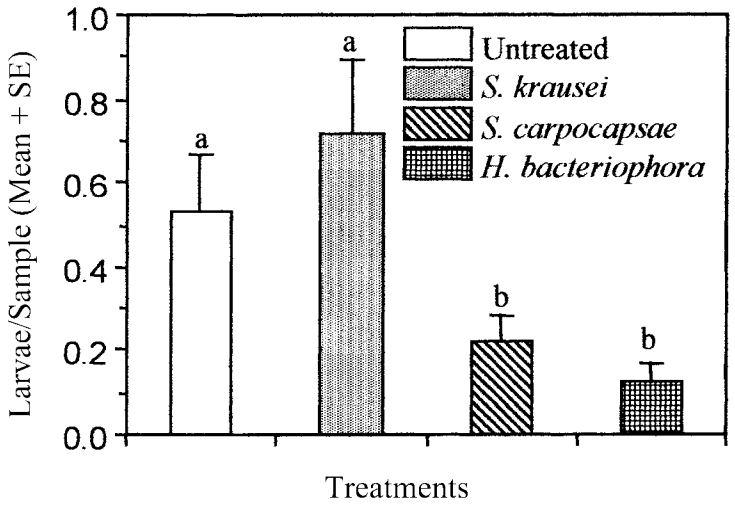

Figure 2. Comparison of steinernematids and *H. bacteriophora* against cranberry girdler, Pitt Meadows, British Columbia, 1998, (redrawn from Henderson & Singhai, 1999). Bars with different letters are significantly different (Tukey-Kramer HSD, P = 0.05).

Black vine weevil and strawberry root weevil

Larvae of black vine weevil and strawberry root weevil are pests of nursery crops, strawberries, and raspberries throughout the world. These species are major pests of cranberries in the Pacific northwest and Massachusetts. Numerous laboratory and field evaluations have been conducted to evaluate various species and strains of insecticidal nematodes against these two pests (Klein, 1990). Only work conducted on cranberries is reported here.

Shanks & Agudelo-Silva (1990) evaluated *H. bacteriophora* (NC and HP88 strains) and *S. carpocapsae* (All strain) against black vine weevil. Plots treated with *H. bacteriophora* at 6.4 billion per acre in the spring had 75% fewer grubs than the untreated control. *Galleria* baiting revealed nematode persistence for at least 10 months. In a second trial, both NC and HP88 strains of *H.bacteriophora* and *S. carpocapsae* (All strain) applied at 3 billion per acre approximately a month later than in the first trial (May 13) significantly suppressed black vine weevil populations. The later application date seemed to improve efficacy because of warmer temperatures. Laboratory bioassays with *H. bacteriophora* (NC-1 strain) and *S. carpocapsae* (All strain) against strawberry root weevil resulted in 51 and 62% mortality, respectively (Simser & Roberts, 1994). Under field conditions, the same treatments, however, showed only 32-38% mortality. In a second field trial, HP88 strain of *H. bacteriophora* and *S. carpocapsae* All strain reduced larval populations by more than 90%.

Recent studies under laboratory conditions have shown that *H. marelatus*, a cold temperature adapted nematode species, is more virulent than *H. bacteriophora* against both weevil species at 14°C (Berry *et al.*, 1997). Field experiments conducted in strawberries also indicated that *H. marelatus* is equally effective at rates 0-7 times lower than that of *H. bacteriophora.* In a field trial conducted in cranberries, Berry & Liu (1999) have also shown that *H. marelatus* and *H. megidis* (provided by Koppert Biological Systems) applied at 2 billion per acre provided effective control of black vine weevil grub populations (Fig. 3). The two isolates collected from Bandon, Oregon (BPN-8 and BPS-6), belonging in the *H. marelatus* species group, were not effective in suppressing black vine weevil larvae.

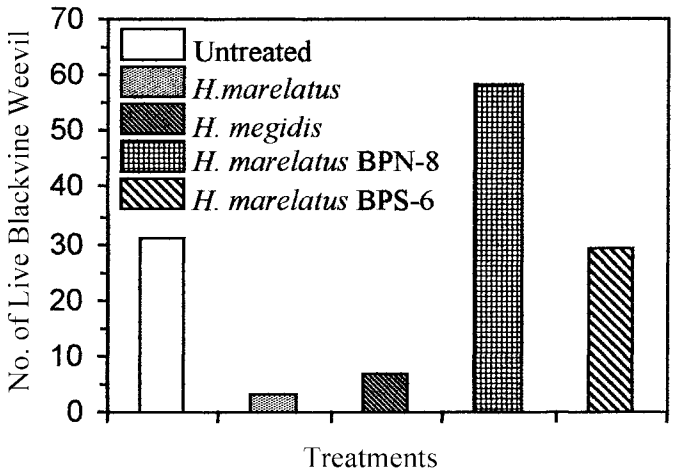

Figure 3. Evaluation of *H. marelatus* and *H. megidis* against black vine weevil, Grayland, Washington, 1998, (redrawn from Berry & Liu, 1999).

Cranberry rootworm

Several species of steinernematids and heterorhabditids were evaluated in the laboratory using Petri dishes (3.5 x 1.5 cm) filled with moist sand (Stuart & Polavarapu, 1997). Results indicated that various species and strains of insecticidal nematodes including certain strains of *H. bacteriophora* and *S. glaseri* are capable of infecting and killing cranberry rootworm larvae under controlled laboratory conditions (Fig. 4A & B). However, in some assays, the infection process for this insect appears to proceed relatively slowly with maximum mortality often not being achieved until about 15 days after the beginning of exposure (Fig. 4A). This delayed response appeared to vary depending upon species and strains of nematodes, with some strains achieving maximum mortality within 7 days after exposure to nematodes (Fig. 4B). Nonetheless, at a dose of 500 infective juveniles per larva, mortality rates of 85-100% were frequently achieved (Fig. 4A & B). Heterrhabditids were generally more effective than steinernematids under laboratory conditions.

In a separate field trial, *H. bacteriophora* supplied by Bio Integrated Technologies (BIT, Italy) and nematodes produced *in vivo* by Integrated Bio Systems (IBS) were compared with imidacloprid applied at 0.25-0.5 lb a.i per acre (Polavarapu *et al.*, 1999). Both *H. bacteriophora* treatments significantly reduced the grub populations, and the BIT product was as effective as imidacloprid treatments (Fig. 5).

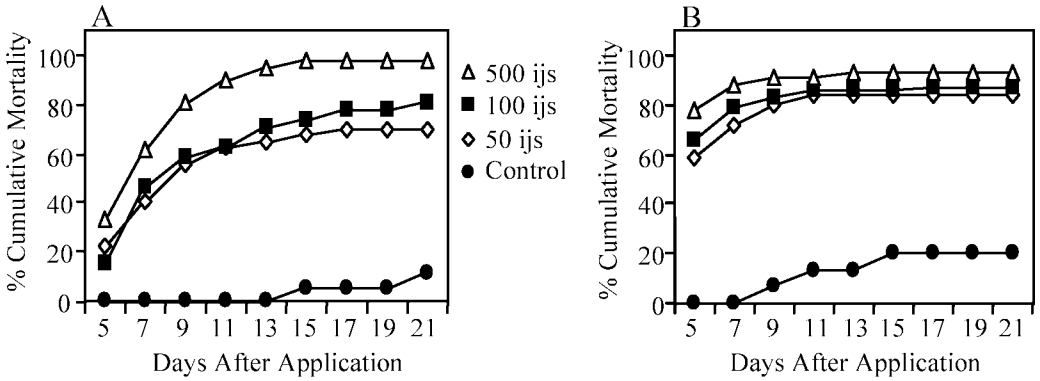

Figure 4. Cumulative percent mortality of cranberry rootworm larvae after exposure to various doses of infective juveniles (ijs) of entomopathogenic nematodes. A) Data are the pooled results for three heterorhabditid strains (n = 20/dose/strain), and B) for four heterorhabditid strains (n = 15/dose/strain).

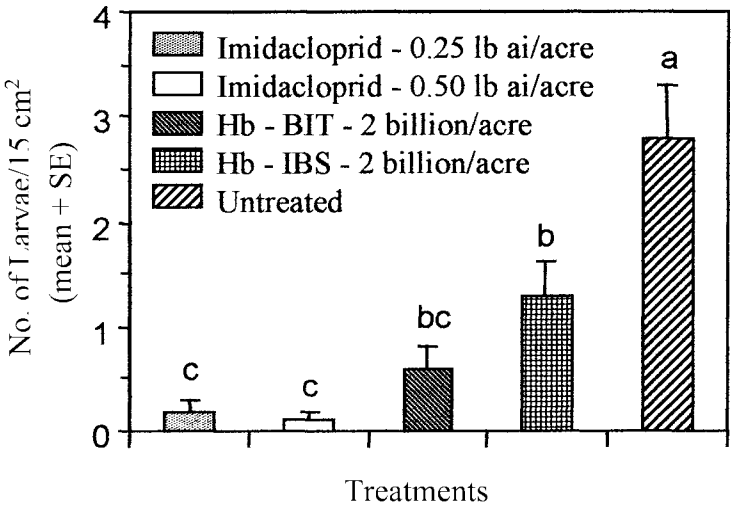

Figure 5. Evaluation of two *H. bacteriophora* products and imidacloprid against cranberry rootworm, Chatsworth, New Jersey, 1998. Figure redrawn from Polavarapu *et al.*, 1999. Bars with different letters are significantly different (Duncan's multiple range test, P = 0.05).

***Hoplia* grub**

Weber & Henderson, (1998) reported a small-plot field trial to evaluate a *H. bacteriophora* formulation (Cruiser, Ecogen, Pennsylvania) during 1996. The mortality of *Hoplia* grubs collected from treated plots was as high as 60% at the 2 billion rate (Fig. 6). At the 1 billion rate the mortality was only 30%. Management of *Hoplia* appears to be feasible. More work is needed to evaluate other commercially available heterorhabditids against this species.

Cranberry rootgrub

Dapsis (1991) reports evaluation of *S. feltiae* (strains 27 and 980) under field conditions against cranberry rootgrub at 1 and 2 billion per acre. At both rates *S. feltiae* was ineffective in suppressing the rootgrub populations. Weber &Henderson (1998) reported about 20% mortality of cranberry rootgrub with *H. bacteriophora* (Fig. 6) applied at 2 billion per acre, although as high as 60% of the recovered grubs had nematode infections. The high rate of infection and low rate of mortality suggests that cranberry rootgrub may have strong immune response against nematode infections.

Cranberry whitegrub

Cranberry whitegrub is the largest among the scarabid grubs infesting cranberries and probably the most difficult grub species to manage with insecticidal nematodes. Dapsis (1991) reported evaluation of *S.carpocapsae, S. feltiae,* and *H. bacteriophora* applied in July and August against cranberry whitegrub in Massachusetts. None of the applications in July provided significant suppression of cranberry whitegrub. In one of the two marshes treated in August, all three nematode species significantly reduced grub populations. Evaluations of *S. glaseri* strains 27 and 980 were inconclusive, with strain 980 providing significant suppression of grub populations in one of the two trials. *S.glaseri* (Biosys) and *H. bacteriophora* (Ecogen) were also evaluated at 1 billion per acre in Wisconsin during 1992 and 1995 populations (Dapsis, 1993; Dittl, 1996). In both years, these nematode species also failed to suppress cranberry whitegrub. More recently, Weber & Henderson (1998) also reported similar results with *H. bacteriophora* (Ecogen).

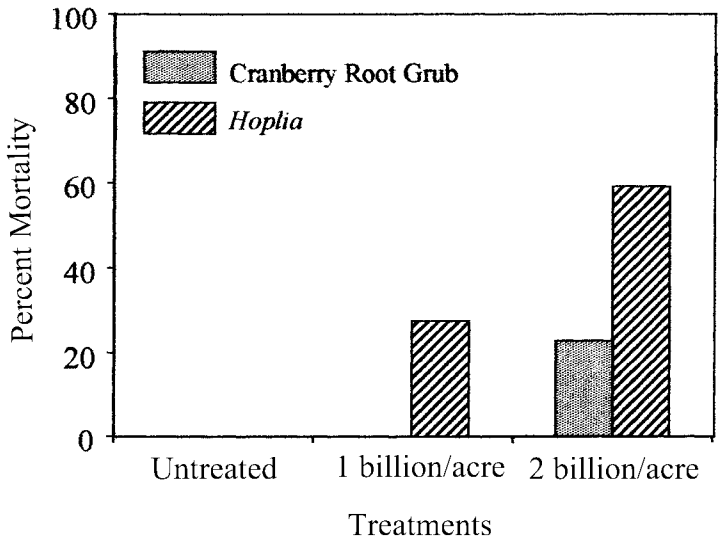

Figure. 6. Evaluation of *H. bacteriophora* (Cruiser, Ecogen) against *Hoplia* grub and cranberry root grub, Massachusetts, 1996 (redrawn from Weber & Henderson, 1998)

Oriental beetle

Although there is some evidence for the feasibility of using insecticidal nematodes for managing oriental beetle (see Yeh & Alm, 1995), insecticidal nematodes have not been evaluated against oriental beetle under field conditions in cranberries. Mortality in laboratory sand-dish (3.5 x 1.5 cm) assays with various strains of *H. bacteriophora*, *H. zealandica*, *S. feltiae* and *S. glaseri* ranged between 10 and 74% (Stuart & Polavarapu, unpublished).

APPLICATION METHODS

Insect parasitic nematodes can be applied in cranberries through irrigation systems (chemigation), by conventional boom sprayers or by air. Significant variability in nematode distribution has been reported in nematode applications in cranberries with both boom sprayers and irrigation systems (Hayes *et al.*, 1999). Irrespective of the application method used, uniformity in nematode distribution is one of the most important factors affecting efficacy.

Application rates in cranberries vary depending on the product and target pest, but generally are in the range of 1-2 billion per acre. Ensure that the application equipment is thoroughly cleaned before nematode application. Although insecticidal nematodes can be tank-mixed with many pesticides, this is not recommended because not all tank-mix combinations have been investigated for safety. In addition, there may be differences among nematode species in tolerance to various insecticides. Nematode applications should not be made for 10-14 days before or after the application of chlorpyrifos (Lorsban). Mix the formulated product in water and allow the mixture to stand for 20 minutes to hydrate the nematodes prior to application.

The following application guidelines should be followed for achieving optimal efficacy with nematodes in cranberries.

(1) Apply nematodes during evening hours to minimize exposure to lethal sunlight.
(2) Nematodes should be applied to moist soil, never to hot or dry soil. Apply 1/10 inch of irrigation prior to applying nematodes to increase soil moisture.
(3) Nematodes should not be applied if soil temperature at 1-2 inches below the soil surface is below 65 °F or above 85 °F.

(4) Provide continuous agitation in the spray tank or feeder tank to ensure proper mixing and uniform distribution of nematodes during application.
(5) Use high volume of water to prevent nematodes from drying out before post-application watering is initiated.
(6) Remove all screens and filters from the sprayer to prevent clogging prior to application. If you must use screens, use screens 50 mesh or coarser.
(7) Do not subject nematodes to pump pressures in excess of 200 PSI.
(8) Apply at least 1/4 inch of irrigation immediately following application to wash nematodes off the foliage and facilitate penetration through the thatch and into the soil.
(9) Maintain soil moisture by irrigating as frequently as possible for the first 10 days after nematode application. If possible, break the normal irrigation schedule of 1 inch per week into 4-5 equal portions.

The majority of nematode applications in cranberries are made through the chemigation system. Follow all best management practices (BMPs) recommended for best performance (Bicki, 1998). While chemigation parameters such as rinse out time or concentration are inconsequential for this kind of nematode application, all other practices that ensure uniform distribution of the injected product are very important. In addition to the guidelines listed above, the following points should be followed when nematodes are applied through the chemigation system.

(1) Mix the required amount of product in at least 4 gal of water per acre for uniform injection of mixture. Generally longer injection times than used for insecticides and fungicides are recommended for nematode applications.
(2) Pressure at the pump should be in the range of 50-55 PSI. Pressure losses in the range of 5-10 PSI can result over the length of the line. Pressures below 40 PSI are not recommended because lower pressures will significantly reduce the uniformity of nematode distribution.
(3) Sprinkler head pressure should be between 40-55 PSI at the farthest point.

Storage and handling precautions summarized elsewhere in this volume (Lewis, 1999; Shetlar, 1999) should be followed as they also apply for nematode handling in cranberries.

FACTORS AFFECTING NEMATODE USE IN CRANBERRIES

Efficacy

Robust data on the efficacy of insecticidal nematodes is currently available only for cranberry girdler, the root weevil complex, cranberry rootworm, and *Hoplia* grub. The cranberry whitegrub, cranberry root grub, oriental beetle, and striped colaspis have not been convincingly shown to be susceptible to insecticidal nematodes. Furthermore, more than one pest species may be the targets at the same time and at the same location (especially scarabaeid grubs) for the same nematode application. It is therefore essential to obtain field efficacy data with various commercially available species, strains, and formulations against important pests so that nematode species can be appropriately matched. Although *S. carpocapsae* has been shown to be consistently effective against cranberry girdler and black vine weevil, and has other favorable attributes such as long shelf-life, stability, and ease of formulation, it appears not to be suitable for other target pests especially the other coleopteran species. Heterorhabditids such as *H. bacteriophora* and *H. marelatus* have been shown to be effective against cranberry girdler and several coleopteran species. Moreover, these species may provide comparable efficacy to *S. carpocapsae* at lower rates. With improvements in formulations and better understanding of handling and other use parameters, these and other newer species should increase our options and improve field efficacy of nematode products.

Nematode persistence

Although *H.bacteriophora* and *S. carpocapsae* have been shown to persist upto 250-300 days in cranberries (Shanks &Agudelo-Silva, 1990; Hayes *et al.*, 1999), several other studies have documented only 4-6 weeks of persistence (Dapsis, 1991; Berry & Liu, 1998). Soil pH between 4 and 8 have little or no effect on nematode survival or infectivity (Kaya, 1990). However, cranberries are often grown in acidic soils with pH considerably lower than 4.0. It is therefore worth determining the effect of pH, especially below 4.0, on nematode persistence and infectivity.

Most of the research work has focused on species and rates of nematode application. Studies have not been conducted on the effect of split applications of nematodes. This is especially important for cruiser nematodes targeted against pests that are active closer to the soil surface. (e.g., cranberry girdler). In such cases, more contacts between nematodes and the pest will increase efficacy. Split application of nematodes may increase the probability of contact between cruiser nematodes and targets that are present near the soil surface. Of course this is possible, only if all larval stages are equally susceptible for nematode infection. Relative susceptibility of different larval stages to nematode infection has not been studied with the majority of cranberry pests.

Quality and product availability

Ready availability of consistently high quality nematodes is a major factor affecting nematode use. It is not uncommon for the end-user to receive purportedly fresh products with fewer than requisite numbers of viable nematodes. With companies coming and going, and products disappearing before researchers have completed the development process, end-users are not very enthusiastic to try products that have never been tested independently. While knowledgeable end-users are desired for achieving better results with nematodes, they also mean more scrutiny of nematode products and retailer storage and handling practices. It is therefore, very important to resolve the quality, and storage and handling issues so that nematode products retain credibility with end-users.

Cost

The nematode products cost significantly more than comparable traditional chemical controls if available. Growers will bear this price if there are no other options available to manage the pest in question. With newer chemicals such as imidacloprid and other chloronicotinyls becoming available in the very near future in cranberries, cost will become a major factor affecting nematode use. But higher quality products that perform well at lower rates (consequently lower cost) and cost savings from improvements in formulation should improve prospects for nematode products in cranberries.

CONCLUDING COMMENTS

In the past decade, insecticidal nematode use in cranberries has increased over four fold. Over this period, growers and IPM consultants have gained valuable experience in using the nematodes. Future work should concentrate on evaluation of newer species, strains and formulations against major target pests, investigation of timing and application strategies (single versus multiple applications), susceptibility of various larval stages for nematode infection, and nematode persistence in acidic cranberry environments. An aggressive research program coupled with outreach efforts in educating the end-user in the appropriate use of nematodes, should further improve the prospects for insecticidal nematodes in cranberries.

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Considerations for Using Insecticidal Nematodes to Control Root Weevils on Strawberry

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ABSTRACT

Insecticidal nematodes can effectively control root weevils infesting strawberry plantings. They can easily be applied to the crop using various kinds of spray equipment and through irrigation systems. Insecticidal nematodes can also be applied directly onto the straw mulch used in some strawberry production systems, thus, eliminating the need to remove then reapply straw to the crop during treatments against root weevil larvae.

INTRODUCTION

Insecticidal nematodes are one of the best control measures currently available for reducing populations of root weevil larvae infesting strawberries. These insecticidal nematodes have provided control of these pests that is comparable or even better than insecticides. Currently, there are no effective chemical insecticides registered for root weevil larvae in strawberry plantings making insecticidal nematodes an attractive tool for controlling these pests in the field. However, insecticidal nematodes are living organisms and cannot be treated as conventional insecticides. While successful control is not guaranteed, there are things that applicators can do to help ensure success. Presented below are some useful considerations when insecticidal nematodes are applied against root weevils on strawberries.

FACTORS TO CONSIDER FOR SUCCESSFUL USE

Target pests

It is important to understand the biology of the pests being targeted for control. There are several common root weevil pests that attack strawberry including the strawberry root weevil (*Otiorhynchus ovatus*), rough strawberry root weevil (*O. rugosostriatus*) and the black vine weevil (*O. sulcatus*). Any of these weevils can seriously damage strawberry plantings if left unchecked.

The main damage these insects cause is by feeding on the root system thus weakening the plant. Root feeding is done by the immature larvae (grubs) of these insects. Under heavy pest pressure, root feeding can severely stunt the plants causing yield reductions. Severely damaged plants can die during summer drought or during cold winters. Adult weevils will feed along leaf margins causing leaves to have scalloped edges. This damage is not considered serious unless there are large numbers of feeding adult weevils.

The black vine weevil overwinters as larvae near strawberry roots and crowns. Adults emerge during the early summer months and lay eggs primarily on or just below the soil surface under the plant canopy (Garth & Shanks, 1978). Eggs hatch during the late summer and larvae begin to feed on the plant roots and crowns. Applications of insecticidal nematodes should be applied against the larva of these pests during the late spring after the soil warms up or during the fall after egg hatch (Klingler, 1988).

Type of nematode
Choosing the right insecticidal nematode is an important decision because not all species or strains are effective against root weevil pests of strawberry. Several studies have demonstrated the effectiveness of *Heterorhabditis* species including *H. bacteriophora* and *H. marelatus* against black vine weevil larvae feeding on strawberry crowns and roots (Klingler, 1988; Backhaus, 1994; Berry *et al.*, 1997, Wilson *et al.*, 1999). Other *Heterorhabditis* species have also been used successfully as has *Steinernema glaseri* (Jackson, *et al.*, 1985).

Quality
There is an obvious time lag from when the nematodes are produced to when they are applied. During this time, the nematodes can be exposed to extreme temperature conditions which can have deleterious effects on the product (Gaugler *et al.*, 1997). Therefore, check to make sure that the nematodes are healthy and viable before they are used. Nematodes may have to be stimulated with a probe or gentle heat to make them move when estimating viability. Movement is an indication that the nematodes are alive but not necessarily an indicator that they are of good quality. Healthy nematodes have more dense and whitish appearance because of their higher lipid contents. Nematodes that are transparent (low lipid content) are of poor quality and are not as infective.

Storage and handling
Store the product according to the directions supplied by the manufacturer. In general, store nematodes in a cool dry place out of direct sunlight. Refrigeration is sometimes required. Do not allow them to freeze or be exposed to extreme temperatures. Do not store diluted products.

Application rate
Insecticidal nematodes are generally applied at the rate of 1-2 billion nematodes /Acre.

Application methods
Insecticidal nematodes can be applied against root weevil larvae on strawberry using various hand-held watering cans and hose-end sprayers; backpack and research plot sprayers; through drop-nozzles on a boom-sprayer; through drip irrigation tape and micro-sprinklers; and probably using other methods, as well.

With all methods, agitation is needed to keep the nematodes in suspension to allow even distribution and uniform flow through the application system. Some commercial spray equipment either have mechanical or hydraulic agitation to keep the nematodes in suspension. If your equipment is not self-agitated, which is typical with small hand-held sprayers, remember to shake the sprayer occasionally. Likewise, several types of injectors that pump solutions into irrigation systems do not have agitation, therefore, it is important to stir the water/nematode solution to prevent settling out of the nematodes.

Choosing the most suitable application method depends mostly on what type of equipment is available and the size of the planting to be treated. Obviously, backyard gardeners with small plantings can get by with any of the hand-held sprayers listed above. Big plantings require either larger equipment or some way to apply the nematodes through irrigation. Applying nematodes through irrigation systems has several advantages including getting the nematodes out of the sunlight and down into the soil where the target pests live. Additionally, nematodes applied through irrigation allows treatment of large plantings with minimum labor and effort.

Insecticidal nematodes can be applied through drip irrigation tape (Curren & Patel, 1988; Kakouli *et al.*, 1994). Applying them through drip irrigation tape is a very effective delivery method when the berries

are grown on raised beds covered with plastic mulch. Where strawberries are grown with straw mulch, any method that delivers the nematodes to the plant should work providing that irrigation is applied following application to facilitate nematode movement into the soil.

The following points should be followed when applying nematodes:

1. Make sure application equipment is clean,
2. Apply nematodes during early morning or late evening when lethal sunlight is minimal,
3. Apply nematodes to moist soil, never soil that is hot and dry (Scherer, 1987). Pre-irrigate with at least 1/4 inch of water before applying nematodes if soil is dry,
4. Apply nematodes when soil temperature is between 60 and 85°F,
5. Apply nematodes with a high volume of water to prevent nematodes from drying out before post-application watering is initiated,
6. Agitate the sprayer to ensure proper mixing and dispersion of the nematodes during application,
7. Use 50-mesh (or coarser) screens to prevent clogging or remove screens if necessary,
8. Apply at least 1/4 inch of irrigation immediately after applying nematodes to help move them into the soil.

Disposal
Spray remaining nematodes and sprayer rinse water directly on the crop or dispose of on-site or according to federal and local regulations.

Soil temperature
In general, efficacy of insecticidal nematodes are limited by temperature extremes and work best in warmer soil (Backhaus, 1994). Soil temperatures in the Mid-Atlantic States rarely get hot enough to negatively impact effectiveness but cool soil temperatures found in spring-time soils prevent insecticidal nematodes from working. Apply *H. bacteriophora* after soils have warmed up to about 15°C. *H. marelatus*, however, are effective in much cooler conditions (Berry *et al.*, 1997, Wilson *et al.* 1999).

Evaluating results
Control of grubs can be expected if the nematodes were in good condition, properly applied, and environmental conditions (good soil moisture and temperature) were favorable. Mortality is temperature dependent. It takes longer for the nematodes to kill grubs in cooler soil than warmer soil. However, nematode infested grubs should be visible within about a week after application. Dig up plants that you suspect are infested and look for the grubs near the roots and crowns. Nematode-infected grubs and pupae should have a rusty reddish-brown color compared with the creamy white color of healthy grubs and pupae.

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