

Entomopathogenic Nematodes (Steinernematidae and Heterorhabditidae) for Biological Control of Soil Pests

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Abstract: Several species of entomopathogenic nematodes in the families Steinernematidae (*Steinernema*) and Heterorhabditidae (*Heterorhabditis*) are being produced commercially and used as biological control agents against many soil insect pests and insects in cryptic habitats in many parts of the world. These nematodes, which are mutualistically associated with bacteria (*Steinernema* with *Xenorhabdus* bacteria and *Heterorhabditis* with *Photorhabdus* bacteria), offer a number of advantages because they have a broad host range, kill their hosts within 48 h, can be easily produced in vivo and in vitro, can be applied with standard spray equipment, are safe to humans and other non-target organisms, have no known negative effects on the environment, and do not require registration in many countries. We present a general overview on the current state of knowledge of entomopathogenic nematodes and their mutualistically associated bacteria. In addition, we examine the potential of these nematodes, which are commonly found in Turkish soils, as biological control agents against insect pests in Turkey.

Key Words: *Steinernema*, *Heterorhabditis*, Biological control, Entomopathogens, Insect pests, *Xenorhabdus*, *Photorhabdus*

Toprakta Yaşayan Zararlıların Biyolojik Kontrolü için Entomopatojenik Nematodlar (Steinernematidae ve Heterorhabditidae)

Özet: Steinernematidae (*Steinernema*) ve Heterorhabditidae (*Heterorhabditis*) familyalarına ait entomopatojenik nematodların bazı türleri ticari olarak üretilmekte ve dünyanın pekçok bölgesindeki cryptic habitatlar ve toprakta yaşayan pekçok zararlıya karşı biyolojik kontrol ajanı olarak kullanılmaktadır. Bakterilerle mutualistik ilişki içerisinde olan bu nematodlar (*Steinernema* *Xenorhabdus* bakterileriyle, *Heterorhabditis* *Photorhabdus* bakterileri ile) pekçok avantaja sahiptir. Çünkü bu nematodlar geniş bir konak dağılımına sahiptir ve konaklarını 48 saat içerisinde öldürürler. In vivo ve in vitro olarak kolayca üretilebilirler, standart spreyleme ekipmanlarıyla uygulanabilirler, insanlar ve diğer hedef olmayan organizmalar için güvenlidirler, çavre üzerinde negatif bir etkiye sahip değildirler ve birçok ülkede kullanım iznine gerek yoktur. Biz burada Entomopatojenik nematodlar ve mutualistik olarak ilişkide oldukları bakteriler hakkında bilinenlere genel bir bakış sunuyoruz. Bunlara ilave olarak, Türkiye topraklarında yaygın olarak bulunan bu nematodların Türkiyedeki zararlı böceklerle karşı biyolojik kontrol ajanı olarak kullanım potansiyellerini gözden geçiriyoruz.

Anahtar Sözcükler: *Steinernema*, *Heterorhabditis*, Biyolojik kontrol, Entomopatojenler, Zararlı böcekler, *Xenorhabdus*, *Photorhabdus*

Introduction

Biological control is defined as the action of natural enemies (arthropod predators, insect parasitoids and microbial pathogens) that maintains a host population at levels lower than would occur in the absence of those enemies (1). It can be divided into 2 broad categories, natural biological control and applied biological control. Natural biological control occurs where native or co-evolved natural enemies reduce native arthropod

populations, whereas applied biological control involves human intervention to enhance natural enemy activities. Applied biological control can be further separated into (a) classical biological control, where exotic natural enemies are introduced against an exotic or native pest, or (b) augmentative biological control, where human intervention occurs to enhance the effectiveness of the natural enemies already present in an area through manipulation of the environment (i.e. conservation) or

the natural enemies themselves. In augmentative biological control, where the natural enemies are manipulated, 2 broad approaches, which are not mutually exclusive, can be employed. These are (1) inoculative release, in which relatively few natural enemies of the same species are released and the progeny of the natural enemies is expected to effect biological control, and (2) inundative release, in which large numbers of natural enemies are released with the expectation that these enemies will effect immediate control (i.e. acting as a biopesticide).

Biological control had its scientific beginning in 1889 with the introduction of the vedalia beetle, *Rodolia cardinalis*, and a parasitic fly, *Cryptochaetum iceryae*, from Australia to control the cottony-cushion scale, *Icerya purchasi*, in California citrus groves (1,2). Since that time, arthropod predators and parasites have been the primary natural enemies used against insect pests. However, microbial control agents (i.e. viruses, bacteria, fungi, protozoa, and nematodes) have also become major players in biological control of pests (3).

Our focus in this paper is on entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae. Steinernematidae is represented by the genera *Steinernema* and *Neosteinernema* and Heterorhabditidae is represented by the genus *Heterorhabditis* (4-6). They are associated with mutualistic bacteria in the genus *Xenorhabdus* for Steinernematidae and *Photorhabdus* for Heterorhabditidae (7). Thus, it is a nematode/bacterium complex that works together as a biological control unit to kill an insect host. These 2 nematode families belong to the order Rhabditida and are not closely related (8), but because they possess many biological similarities, they will be considered together.

Soil has been one of the most difficult environments in which to achieve biological control of insect pests. These nematodes are adapted to soil and have been especially effective as inundative biological control agents against a number of soil insect pests (4,9,10). They are also effective against a number of insect pests that occur in cryptic habitats (e.g. tree boring insects). Other positive attributes of these nematodes as biological control agents are that they have a broad host range, are safe to vertebrates, plants and other non-target organisms, have no known negative effect on the environment, are easy to mass produce in vivo and in

vitro, are easily applied using standard spray equipment, can search for their host, kill rapidly (i.e. within 48 h), have the potential to recycle in the environment, are compatible with many chemical and other biological pesticides, are amenable to genetic selection for desirable traits, and are exempt from registration in many countries (4,11). Negative attributes include their broad host range (although no negative effects on non-target hosts have been observed, this broad host range may include some beneficial insects), narrow tolerance to environmental conditions (e.g. moisture requirement), poor long-term storage, poor field persistence, and relatively high cost in comparison to chemical pesticides (11).

The intense interest in entomopathogenic nematodes as biological control agents of insect pests has resulted in a plethora of research efforts and subsequent publications. Accordingly, the following sources can be consulted for more detailed information on these 2 nematode families and their mutualistic bacteria. These include books edited by Gaugler and Kaya (12), Bedding et al. (13) and Gaugler (14) and review articles by Kaya and Gaugler (4), Forst and Neelson (15), Forst et al. (16), Lewis et al. (17), Barbercheck and Millar (18), Burnell and Stock (6), Liu et al. (19) and French-Constant (20). Kaya and Stock (5) and Koppenhöfer (21) cover techniques for use with entomopathogenic nematodes, and the book edited by Lacey and Kaya (22) covers field techniques with various cropping systems. There is an extensive bibliography compiled by Smith et al. (23) and an updated web-based version is also available at

http://128.146.54.216/nematodes/insect_parasitic_nematode_public.htm

Biology of the Nematode/Bacterium Complex

Steinernematids and heterorhabditids, obligate pathogens in nature, have the non-feeding, free-living, third stage infective juvenile or dauer juvenile that infects the insect host in the soil environment (Figure 1). The infective juvenile, the only stage that occurs outside of an insect, is ensheathed in the second-stage cuticle that is easily lost in the steinernematids (Figure 2a) but is retained in the heterorhabditids (Figure 2b) until just prior to or shortly after host infection. In addition, the nematode/bacterium association is highly specific. In the infective juvenile, the bacterial cells are housed in a vesicle

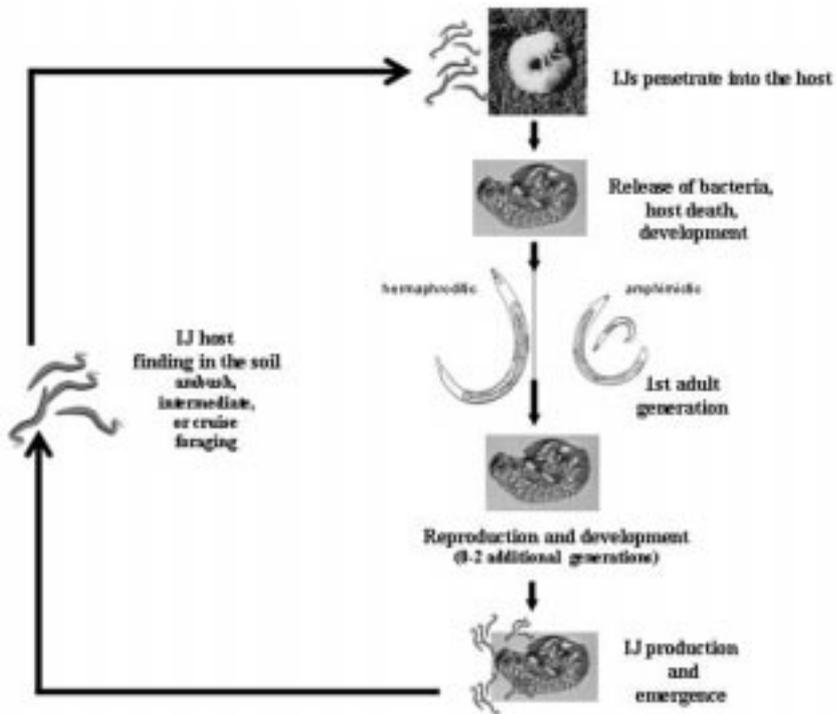


Figure 1. Generalized life cycle of steinernematids and heterorhabditids. IJ = infective juvenile.

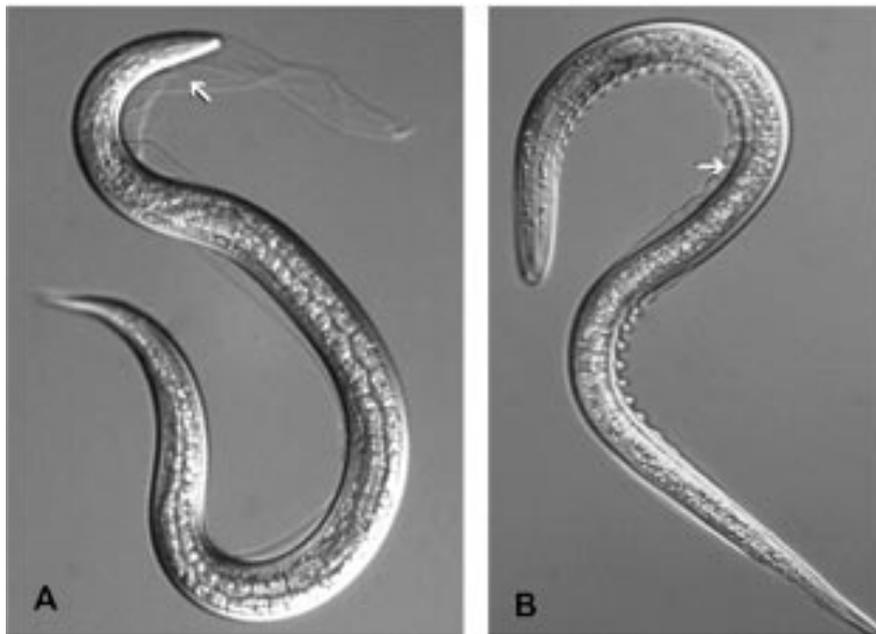


Figure 2. Third-stage infective juvenile of (A) *Steinernema carpocapsae* showing the loose second-stage cuticle (arrow) and (B) *Heterorhabditis bacteriophora* showing the close-fitting second-stage cuticle (arrow). Photos by Ursula Kölzer.

in the anterior part of the intestine for steinernematids and in the intestinal tract for heterorhabditids.

The infective juvenile infects the host through natural openings (mouth, spiracles, anus) or thin areas of the host's cuticle (common only in heterorhabditids) (4) and penetrates into the host's hemocoel. The infective juvenile then releases the bacterium through the anus for steinernematids (24) or through the mouth for heterorhabditids (25). The mutualistic bacterium propagates and produces substances that rapidly kill the host and protect the cadaver from colonization by other microorganisms. The nematode initiates its development, feeding on the bacterial cells and host tissues that have been metabolized by the bacterium and has 1-3 generations, depending on host size. As the food resources in the host cadaver are depleted, a new generation of infective juveniles is produced and emerges from the host cadaver into the soil to search for new hosts (Figure 1).

A major difference between steinernematids and heterorhabditids is that all but one species in the former group are amphimictic, whereas species in the latter group are hermaphrodites in the first generation but amphimictic in the following generation. Thus, steinernematids require a male and a female infective juvenile to invade an insect host to produce progeny, whereas heterorhabditids need only one infective juvenile to penetrate into a host as the resulting hermaphroditic adult is self-fertile. However, Griffin et al. (26) found an undescribed *Steinernema* species from Indonesia that consists largely of self-fertile hermaphrodites with a low frequency of males (1-6% of the population).

A given nematode species is specifically associated with one bacterial symbiotic species, but the bacterial species may be associated with more than one nematode species (Table 1). Akhurst and Boemare (27) state that the best nematode reproduction occurs with their natural symbiont, but in some cases, the nematode can develop on other bacterial species. The relationship between the nematode and bacterium is truly mutualistic for the following reasons: the nematode is dependent upon the bacterium for (1) quickly killing its insect host, (2) creating a suitable environment for its development by producing antibiotics that suppress competing microorganisms, (3) transforming the host tissues into a food source, and (4) serving as a food resource. The bacterium needs the nematode for (1) protection from

the external environment, (2) penetration into the host's hemocoel, and (3) inhibition of the host's antibacterial proteins.

Taxonomy of Entomopathogenic Nematodes

Over the past several years, numerous events have impacted entomopathogenic nematode systematics. These include standardization of criteria for species description (28), proposal of name emendations (28), interpretation of phylogenetic relationships in the phylum Nematoda based on molecular evidence (8), proposal of a phylogenetic species concept (29), and a new proposed classification for Nematoda (30). These changes have brought the status of the entomopathogenic nematode systematics into a "phase of stability and growth."

The number of newly discovered nematode species/isolates has significantly increased over the past decade. Accurate and prompt identification/diagnosis of these taxa requires the implementation of appropriate taxonomic tools. To meet these expectations, new technologies (molecular methods) have been incorporated into their traditional morphological approaches. Assimilation of molecular approaches into entomopathogenic nematode systematics has escalated dramatically in the last few years. Various molecular methods and markers have been used not only for diagnostic purposes, the sorting out of cryptic species, populations and strains, but also to assess evolutionary relationships among these nematodes. Stock (31) and Stock and Reid (32) have reviewed these methods.

At present, there are more than 40 recognized named species of entomopathogenic nematodes in the 2 families with 35 species in *Steinernema* (plus 1 nomen nudum species), 1 species in *Neosteinernema* and 10 species in *Heterorhabditis* (2 of these species are considered species inquirenda) (33) (Table 1).

Mutualistic Bacteria

Xenorhabdus and *Photorhabdus* are motile, Gram-negative, facultative, non-sporeforming, anaerobic rods in the family Enterobacteriaceae. In the genus *Xenorhabdus*, 5 species are associated with *Steinernema*, whereas in the genus *Photorhabdus*, 3 species are associated with *Heterorhabditis* (Table 1) (6,7,34,35) with 1 species, *P. luminescens*, divided into 5 subspecies

Table 1. Described species of entomopathogenic nematode species and their respective symbiotic bacterial species.

Nematode genus	Nematode species	Symbiont species
<i>Steinernema</i>	<i>abbasi</i>	undescribed
"	<i>affine</i>	<i>Xenorhabdus bovienii</i>
"	<i>anatoliense</i>	undescribed
"	<i>arenarium</i> (= <i>anomali</i>) ¹	<i>Xenorhabdus</i> sp.
"	<i>asiaticum</i>	undescribed
"	<i>bicornutum</i>	undescribed
"	<i>carpocapsae</i> ²	<i>X. nematophila</i>
"	<i>caudatum</i>	undescribed
"	<i>ceratophorum</i>	undescribed
"	<i>cubanum</i>	<i>X. poinarii</i>
"	<i>diaprepesi</i>	undescribed
"	<i>feltiae</i> (= <i>bibionis</i>) ³	<i>X. bovienii</i> ³
"	<i>glaseri</i>	<i>X. poinarii</i>
"	<i>intermedium</i>	<i>X. bovienii</i>
"	<i>karii</i>	undescribed
"	<i>kraussei</i>	<i>X. bovienii</i>
"	<i>kushidai</i>	<i>X. japonica</i>
"	<i>loci</i>	undescribed
"	<i>longicaudum</i>	undescribed
"	<i>monticolum</i>	undescribed
"	<i>neocurtillae</i>	undescribed
"	<i>oregonense</i>	undescribed
"	<i>puertoricense</i>	undescribed
"	<i>rarum</i>	<i>Xenorhabdus</i> sp.
"	<i>riobrave</i>	<i>Xenorhabdus</i> sp.
"	<i>ritteri</i>	<i>Xenorhabdus</i> sp.
"	<i>sangi</i>	undescribed
"	<i>scapterisci</i>	<i>Xenorhabdus</i> sp.
"	<i>scarabaei</i>	undescribed
"	<i>siamkayai</i>	<i>Xenorhabdus</i> sp.
"	<i>tami</i>	<i>Xenorhabdus</i> sp.
"	<i>thanhi</i>	undescribed
"	<i>thermophilum</i>	undescribed
"	<i>websteri</i>	undescribed
"	<i>wesieri</i>	undescribed
"	undescribed	<i>X. beddingii</i>
<i>Nomen nudum</i>		
"	<i>serratum</i>	undescribed
<i>Neosteinerinema</i>	<i>longicurvicauda</i>	undescribed
<i>Heterorhabditis</i>	<i>bacteriophora</i> (= <i>heliotidis</i> , = <i>argentinensis</i>)	<i>P. luminescens</i> ⁴ and <i>P. temperata</i> ⁵
"	<i>baujardi</i>	undescribed
"	<i>brevicaudis</i> ³	<i>P. luminescens</i> ⁴
"	<i>downesi</i>	<i>Photorhabdus</i> sp.
"	<i>indica</i> (= <i>hawaiiensis</i>)	<i>P. luminescens</i>
"	<i>marelata</i> (= <i>hepialius</i>)	<i>P. luminescens</i> ⁴
"	<i>megidis</i>	<i>P. temperata</i>
"	<i>poinari</i> ³	<i>Photorhabdus</i> sp.
"	<i>taysearae</i>	undescribed
"	<i>zealandica</i>	<i>P. temperata</i>

¹In brackets previously used names and/or synonyms. ²The species "*carpocapsae*" has been referred to as "*feltiae*" in the literature primarily between 1983 and 1989. The name "*feltiae*" is valid and takes precedence over "*bibionis*" species *inquirenda*." ³Species *inquirenda*. ⁴Fischer-Le Saux et al. (1999) showed that *Photorhabdus* is a heterogenous group. *P. luminescens* is associated with *H. indica* with some but not all isolates of *H. bacteriophora*. The status of bacterial symbionts with *H. argentinensis*, *H. brevicaudis* and *H. marelata* is not clear and we have maintained the bacterial species as *P. luminescens*. This may change at a future date. In addition, one species, *P. asymbiotica*, is not associated with nematodes and has been isolated from human clinical specimens. ⁵Some subgroups of *H. bacteriophora* are associated with *P. temperata* (34).

(35). The subspecies of *P. luminescens* are subsp. *luminescens*, *laumondii*, *akhurstii*, *kayaii*, and *thraciaensis*. One species, *P. asymbiotica*, has also been isolated from human clinical cases and is not associated with nematodes (7).

Major differences occur between the 2 bacterial genera (7). For example, most *Photorhabdus* spp. are luminescent and catalase positive, whereas *Xenorhabdus* spp. have no luminescence and are catalase negative. Both bacterial genera produce phenotypic variant cell types called primary form (phase I) and secondary form (phase II) (36). The primary form is the cell type naturally associated with the nematodes, whereas the secondary form can arise spontaneously when the bacterial cultures are in the stationary non-growth stage. The *Xenorhabdus* secondary form can revert to the primary form, but this phenomenon has not been documented for *Photorhabdus* spp.

Differences between the primary and secondary forms occur. For instance, the primary form produces antibiotics, adsorbs certain dyes, and develops large intracellular inclusions composed of crystal proteins, whereas the secondary form does not or only weakly produces antibiotics, does not adsorb dyes, and produces intracellular inclusions inefficiently. The primary form is superior to the secondary form in its ability to support nematode propagation in vitro, although some evidence suggests that this is not always the case (37,38). The reason for the occurrence of the 2 forms is not known (7).

The association between the bacterium and nematode is essentially monoxenic, but other bacterial species have been isolated from the infective juvenile from various steinernematid (39-41) and heterorhabditid (42,43) species. Recent studies by Vivas and Goodrich-Blair (44) with *X. nematophila* and *S. carpocapsae* found that a bacterial gene serves to retain the specificity between the bacterium and nematode. Moreover, Martens et al. (45) have shown that few *X. nematophila* cells initiate the colonization of an infective juvenile and that these grow inside the lumen of the intestine in a reproducible polyphasic pattern during colonization.

A major breakthrough has been sequencing the entire genome of *P. luminescens*, strain TT01 (46). The complete genome sequence has 5,688,987 base pairs and contains 4,839 predicted protein-coding genes. As

expected, it encodes a large number of adhesions, toxins, hemolysins, proteases, and lipases and contains a wide assortment of antibiotic synthesizing genes. In addition, 2 paralogs that encode proteins similar to juvenile hormone esterases were found. Juvenile hormone maintains the insect in a larval state, and its occurrence in *P. luminescens* poses some interesting questions regarding the origin of these paralogs. The authors state that this bacterium will be a promising model for the study of symbiosis and host-pathogen interactions.

Host Range

In the laboratory, most entomopathogenic nematode species infect a variety of insects where host contact is certain, environmental conditions are optimal, and no ecological or behavioral barriers to infection exist (4,47). In the field, entomopathogenic nematodes attack a significantly narrower host range than in the laboratory (48-51), adding to their safety as biological control agents. Because these nematodes are adapted to the soil environment, the principal hosts are soil insects. The isolation of new nematode strains/species is usually done using larvae of the greater wax moth, *Galleria mellonella*, and therefore, the host range of known nematode species tends to be biased towards generalists or species adapted to lepidopterous insects. However, some nematode species that have been isolated from host cadavers in the field have a restricted host range with *S. kushidai* (52) and *S. scarabaei* (53) being adapted to scarab larvae. *S. scapterisci* appears to be adapted to mole crickets and poorly infects other insects (54,55), but Bonifassi et al. (56) demonstrated that a combination of *Xenorhabdus* strain UY61 and *S. scapterisci* readily infects the waxworm, *G. mellonella*.

Nematode/Bacterium Interactions with Hosts

Even with the broad host range of most entomopathogenic nematode species, their efficacy varies with many biological factors, including nematode species and strain and insect species and their developmental stage (57,58). One of the factors affecting efficacy is that many soil-dwelling insects have evolved behaviors resulting in reduced host finding, attachment, or penetration by the infective juveniles. Some of the documented insect behaviors include (1) a high defecation rate that reduces infection via the anus (scarab grubs) (2)

low CO₂ output or CO₂ released in bursts that minimize chemical cues (lepidopterous pupae and scarab grubs), (3) the formation of impenetrable cocoons or soil cells before pupation that serve as physical barriers (many lepidopterans and scarabs), (4) walling-off nematode-killed individuals that avoid or reduce contamination to other insects in a nest (termites), and (5) aggressive grooming or evasion behavior that reduces infective juvenile contact (scarab grubs) (59,60).

Infective juveniles can penetrate into insects using several routes, depending on which are accessible (57). In some insects, the usual routes of entry may be inaccessible because the mouth may be obstructed by oral filters (wireworms) or be too narrow (insects with sucking/piercing mouthparts or small insects with chewing mouthparts). The anus may be constricted by muscles or other structures (i.e. in wireworms), or the spiracles may be covered with septa (wireworms) or sieve plates (scarab grubs) or simply be too narrow for nematode entry (some dipterans and lepidopterans).

The infective juveniles have to penetrate through the cuticle (including the trachea) or gut to enter the hemocoel. To enter through the cuticle, the nematodes employ physical force such as body thrusting to rupture through the thin trachea or, as with *Heterorhabditis*, use an anterior tooth to penetrate directly into the hemocoel. To enter through the gut, they use physical force and/or proteolytic secretions to digest the midgut tissues to gain access into the hemocoel (61,62).

Within the insect's hemocoel, the nematodes and bacteria overcome the host's immune response (4,63) that involves interacting humoral and cellular factors.

To counteract the bacterial cells, the insect may use antibacterial proteins and/or phagocytosis followed by nodule formation, and to inactivate the nematodes, the insect's hemocytes may encapsulate them followed by melanization. In some cases, the nematodes can overcome the insect defenses. Thus, *S. glaseri* is initially encapsulated by larvae of the Japanese beetle, *Popillia japonica*, but it escapes from the capsule and successfully infects its host (64) because the nematode has surface coat proteins that suppress the host's immune response and destroy the hemocytes (65). A *Heterorhabditis* species avoids encapsulation in tipulid larvae by exsheathing from the second-stage cuticle during host penetration (66). Moreover, the invading nematodes can

produce immuno-inhibiting factors that destroy the antibacterial factors produced by the insect and allow the mutualistic bacteria to produce insecticidal toxins that rapidly kill the host (67). Nematodes may also produce paralyzing exotoxins and cytotoxic and proteolytic extracellular enzymes. The above reactions are dependent on the insect host and nematode/bacterium complex (68) and contribute to the variable efficacy of entomopathogenic nematodes against different insect species.

Biogeography

Entomopathogenic nematodes have been recovered from soils from many parts of the world (69). Some nematode species appear to have a global distribution and are essentially ubiquitous (69). *S. carpocapsae* and *S. feltiae* are widely distributed in temperate regions, *H. bacteriophora* is common in regions with continental and Mediterranean climates, and *H. indica* is found throughout much of the tropics and subtropics. Other species such as *S. rarum*, *S. kushidai*, *S. ritteri* and *H. argentinensis* appear to have a much more restricted distribution, but as more surveys are conducted these species may be found more widely.

In Turkey, 3 surveys have been conducted with the isolation of several known species and at least 1 new species (70-72). Özer et al. (70) recovered *S. feltiae* from the coast of the Black Sea, and Susurluk et al. (71) isolated *H. bacteriophora*, a *Heterorhabditis* sp., and *S. feltiae* from Ankara. Hazir et al. (72) did an extensive survey throughout Turkey and isolated *H. bacteriophora*, *S. feltiae*, *S. affine*, and an undescribed *Steinernema* species. (Hazir et al. (73) subsequently described the new species as *S. anatoliense*). In this survey, the most common species was *S. feltiae*, which was isolated from 10 sites in 6 regions, *H. bacteriophora* from 7 sites in 5 regions, *S. affine* from 4 sites in 2 regions, and the newly described *S. anatoliense* from 1 site (Figure 3). (For more information, see the section on the Research on Turkish Entomopathogenic Nematode/Bacterium Complex.)

Behavioral Ecology

A major factor restricting the entomopathogenic nematode host range is the foraging behavior of the infective juveniles. These nematodes employ different



Figure 3. Map of Turkey showing the positive sites where entomopathogenic nematodes were recovered. References: ■ *H. bacteriophora*, ● *S. affine*, ◆ *S. feltiae*, ★ *Steinernema anatoliense*, n=number of soil samples taken within regions.

foraging strategies to locate and infect hosts, which range from one extreme of sit-and-wait (ambush) to the other of widely foraging strategy (cruise) (74,75). Most nematode species are situated somewhere along a continuum between these 2 extremes, placing them as intermediate foraging strategists (e.g. *S. riobrave* and *S. feltiae*) (76-78). These intermediate strategists are adapted to infecting insects that occur just below the soil surface, such as prepupae of lepidopterous insects, fungus gnats, or weevil larvae. The sit-and-wait strategists or ambushers (e.g. *S. carpocapsae* and *S. scapterisci*) are characterized by low motility and a tendency to stay near the soil surface. They tend not to respond to volatile and contact host cues unless presented in an appropriate sequence and efficiently infect mobile host species such as the codling moth, cutworms and mole crickets near the soil surface. At the other extreme, the widely foraging strategists or cruisers (e.g. *S. glaseri* and *H. bacteriophora*) are characterized by high motility and are distributed throughout the soil profile. They orient to volatile host cues and switch to a localized search after host contact and are well adapted to infecting sedentary hosts such as scarab and lepidopterous prepupae and pupae.

Another behavior of infective juveniles is their typical body-waving where 30-95% of their body is raised off the substrate for a few seconds. Most nematode species

that have an ambush or intermediate foraging strategy can body-wave by raising >95% of their body off the substrate, standing on a bend in their tail and assuming a straight posture or alternating periods of no motion and active waving (77,78). Cruisers can body-wave but cannot stand on their tails. Infective juveniles that can stand on their tails and body-wave (i.e. ambushers and some intermediate foragers) can also jump. This jumping behavior can be used for host attachment or be non-directed where it may play a role in dispersal (78).

Ecology

Dispersal - In addition to jumping for some nematode species, the infective juveniles can disperse in soil up to 90 cm in both the horizontal and vertical directions within 30 days (79). This dispersal, especially for cruiser nematodes, allows the entomopathogenic nematodes to actively seek out hosts. Factors influencing the motility of infective juveniles are moisture, temperature, and soil texture, of which moisture is the most critical because the nematodes need a water film in the interstitial spaces of soil for effective propulsion. When this water film becomes too thin (i.e. in dry soil) or the interstitial spaces become completely filled with water (i.e. in saturated soil), nematode movement is restricted (80). Different nematode species/strains have different temperature optima and ranges (81,82) that affect their survival and

hence motility. Nematodes lose motility at low temperatures (<10-15 °C) and become inactivated at high temperatures (>30-40 °C). Soil porosity affects nematode dispersal with less dispersal occurring as soil pores become smaller (79). Nematodes can also be dispersed great distances passively by water, wind, phoresis, infected hosts, human activity, etc., which may, in part, account for their widespread global distribution.

Survival - After field application of infective juveniles, their persistence is generally short-lived (83). Abiotic factors such as extreme temperatures, soil moisture, osmotic stress, soil texture, RH, and UV radiation (83-85) and biotic factors such as antibiosis, competition and natural enemies (86,87) are the primary extrinsic causes that affect infective juvenile survival.

Infective juveniles can survive low moisture conditions by lowering their rate of metabolism. Gradual water removal from the infective juveniles gives them time to adapt to the desiccating conditions (88,89). Thus, natural soils allow the infective juveniles to persist longer in dry soil. Infective juveniles may survive desiccating conditions by remaining inside the host cadaver until the soil moisture situation improves (90,91).

Nematode survival at different temperatures varies with species and strains (82,92). Extended exposure to temperature extremes (below 0 °C or above 40 °C) is lethal to most species of entomopathogenic nematodes (93). In the soil environment, infective juveniles are normally buffered from temperature extremes. For storage, the best longevity of infective juveniles is between 5 and 15 °C. At higher temperatures, the infective juveniles have increased metabolic activity and deplete their energy reserves, shortening their life span.

UV can kill nematodes within minutes (94). UV is most important when nematodes are applied as biological insecticides. Direct exposure to UV light (i.e. sunlight) can be minimized by applying infective juveniles early in the morning or evening, or using sufficient amounts of water to wash the infective juveniles into the soil.

Soil texture affects infective juvenile survival, with the poorest occurring in clay soils (at the same water potentials). The poor survival rate in clay soils is probably due to the lower oxygen levels in the smaller soil pores. Oxygen is also a limiting factor in water-saturated soils and soils with high organic matter content, but pH does not have a strong effect on infective juvenile survival. Soil

salinity also has a negligible effect on infective juvenile survival even at salinity above the tolerance levels of most crop plants (95). Seawater has no negative effects on the survival of several *Heterorhabditis* species/strains (96) as they have been frequently isolated from soils near the seashore.

Various biotic factors (i.e. natural enemies) affect nematode survival (86,87). Among the natural enemies of nematodes, nematophagous fungi have received the most attention. For example, *Hirsutella rhossiliensis* causes a higher mortality of *S. glaseri* infective juveniles than of *H. bacteriophora* infective juveniles. This differential mortality is associated with the retention of the second-stage cuticle by *H. bacteriophora* infective juveniles. Other natural enemies of infective juveniles include collembolans, mites, tardigrades and predatory nematodes, but their impact under field conditions is not well understood. Scavengers such as ants will feed less on nematode-killed insects (97). The difference in feeding activity by ants is associated with a "deterrent" factor(s) produced by *Photorhabdus* and *Xenorhabdus* (98).

Recycling of nematodes - Recycling is desirable after an application of entomopathogenic nematodes because it can provide additional and prolonged control of a pest. The abiotic and biotic factors that affect persistence, infectivity, and motility of infective juveniles influence nematode recycling. Because they are obligate pathogens, the availability of suitable hosts is a key to recycling of the nematodes. Recycling is rather common (79,99) after nematode application but is probably not sufficient for prolonged host suppression, and the nematodes have to be reapplied to maintain adequate control of soil insect pests.

In natural populations of entomopathogenic nematodes, recycling occurs in their insect hosts, but only a few studies have examined the dynamics of nematode populations and the factors affecting them. Within-site distribution of nematode populations is patchy (100-103), and biotic and abiotic factors such as seasonal fluctuations, foraging strategy of the infective juveniles, host population dynamics and alternate hosts play a key role in nematode recycling.

Genetics

Entomopathogenic nematodes are obligate pathogens in the field, but in the laboratory they can be maintained

in vivo or in vitro. During their laboratory maintenance, the genetic diversity may be lost, or genetic variation may have been limited during collection or lost during importation and rearing. On the other hand, preservation of genetic variation for nematodes is affected by founder effect, inbreeding, and inadvertent selection (104). A serious problem for entomopathogenic nematodes is founder effect because only a limited number of insect cadavers are collected at single geographical sites, resulting in reduced genetic variance. To maintain or enhance genetic diversity, the same nematode species should be collected from as many geographical sites as possible and the isolates should be hybridized. If laboratory-adaptation occurs or is suspected, the nematodes can be outcrossed with new field isolates or with other sources to maintain or infuse genetic diversity.

Entomopathogenic nematodes may benefit from genetic improvement through selective breeding or genetic engineering. Examples of successful selective breeding are selection for cold tolerance (105,106), improved control efficacy (107), and nematicide resistance (108). In addition, genetic engineering to improve beneficial traits of entomopathogenic nematodes and their associated bacteria has been done on a limited scale. Hashmi et al. (109,110) incorporated a plasmid containing heat-shock protein genes from the free-living nematode *Caenorhabditis elegans* into *H. bacteriophora*, and the resulting transgenic strain had a higher tolerance to short temperature spikes than did the wild type (111). Field trials showed no increased persistence of the transgenic strain compared to the wild-type nematode indicating that the transgenic form has no advantage over the wild type (112). Thus, the transgenic nematode had an advantage over the wild type in storage and application because of its higher tolerance to short temperature spikes. However, regulatory issues in various countries may affect the commercialization and eventual field release of transgenic nematodes.

For the mutualistic bacteria, some of the main targets for genetic improvement include pathogenicity, host specificity, symbiont specificity, resistance to environmental extremes, and control of phase variation (113). A number of genes from these bacteria such as outer membrane protein genes, low-temperature induced genes, lux genes, extracellular enzyme genes, and crystalline protein genes have been cloned (36). Proteins with insecticidal activities have been isolated and the

genes identified, and they show potential to be incorporated into plants for insect control (67,114).

Research on the Turkish Entomopathogenic Nematode/Bacterium Complex

In Turkey, 3 surveys have been conducted with the isolation of several known species (70-72) and at least 1 new species (73). Özer et al. (70) initially stated that they had recovered *S. carpocapsae* from the coast of the Black Sea, but this species was later identified as *S. feltiae* (115). Susurluk et al. (71) found 2 *H. bacteriophora* isolates and *S. feltiae* from Ankara. Hazir et al. (72) did an extensive survey throughout Turkey taking 1080 soil samples, which produced 22 positive sites. The isolated nematodes included *H. bacteriophora*, *S. feltiae*, *S. affine*, and a new *Steinernema* species. This new species has been described as *Steinernema anatoliense* (73). The most common species were *S. feltiae*, which was isolated from 10 sites in 6 regions, *H. bacteriophora* from 7 sites in 5 regions, *S. affine* from 4 sites in 2 regions, and the newly described *S. anatoliense* from 1 site (Figure 3). The soils of the positive nematode sites were classified as sandy, sandy loam, or loam (68.2%) and sandy-clay-loam or clay loam (31.8%). The habitats from which the nematodes were isolated were broadly classified as disturbed (59.1%), which included agricultural fields and poplar planted for lumber or wind breaks, and undisturbed (40.9%), which included pine forests, grassland, and marsh and reed locations.

As indicated in the previous paragraph, the survey by Hazir et al. (72) led to the discovery of a new steinernematid species, *S. anatoliense*, from grassland in Kars, East Anatolia. It is characterized by the infective juvenile having a body length of 507-580 µm and a lateral field pattern with 6-8 longitudinal ridges (73). The tail shape of the first generation male has a cuticular mucron, spicules that are arcuate and robust with a rectangular or oval manubrium, a short calomus, and a very thin velum. *S. anatoliense* is most similar to *S. abbasi*, *S. carpocapsae*, *S. rarum*, *S. scapterisci*, *S. siamkayai*, and *S. tami*, but can be distinguished from these species by various morphological features of the males and infective juveniles. Moreover, attempts to cross hybridize *S. anatoliense* with *S. abbasi*, *S. carpocapsae*, *S. rarum*, *S. scapterisci*, and *S. siamkayai* failed to produce progeny, whereas control males and females of the same

species produced progeny. Restriction fragment length polymorphism (RFLP) profiles of the ITS region of rDNA from *S. anatoliense* showed that they were distinct from 3 other morphologically similar species (*S. carpocapsae*, *S. rarum*, and *S. siamkayai*).

Susurluk et al. (71) did an identification and ecological characterization of 3 nematode-bacterium complexes (1 steinernematid and 2 heterorhabditid isolates) from Turkey that were isolated from the University of Ankara campus. *S. feltiae* was identified based on the morphometrics and shape of the spicules, whereas the 2 heterorhabditid isolates (TUR-H1 and TUR-H2) were identified by molecular methods and by cross breeding with a known *H. bacteriophora* isolate. The cross breeding study showed that both isolates mated with the known *H. bacteriophora* isolate, but one Turkish isolate (TUR-H2) produced viable progeny and the other (TUR-H1) produced non-fertile infective juveniles. However, the RFLP analysis showed that these 2 isolates were members of the species *H. bacteriophora*. The bacterial symbionts shared a >99% similarity in the 16S rDNA sequence with *Photorhabdus luminescens* subsp. *laumondii*. Moreover, both Turkish isolates reproduced in monoxenic cultures of the symbionts from known *H. bacteriophora* and *H. megidis*.

The infectivity at various soil moistures and heat tolerance of the 3 Turkish nematodes (TUR-H1 and TUR-H2 of *H. bacteriophora* and *S. feltiae*) were compared (71). Using a nematode penetration assay into hosts, a significantly higher infection rate was observed for all 3 nematode isolates at a 10% soil water content. From a water content of 7% upwards, more *S. feltiae* invaded the insect hosts than did the 2 heterorhabditid isolates. Above a 10% soil water content, there was a reduction in all 3 nematode species invading the insect host. The heat tolerance study was done with the infective juveniles of the 3 isolates suspended in water at 28, 32 and 36 °C. The results showed that *S. feltiae* was most tolerant nematode at 32 °C, but that no nematodes could survive at 36 °C after a 4- or 5-h exposure.

Hazir et al. (92) compared the development of 5 geographic isolates of *S. feltiae* at different temperature regimes. The isolates were from Mediterranean (Sinop from Turkey, SN from France, and Monterey from California), subtropical (Rafaela from Argentina), and tropical (MG-14 from Hawaii) regions. The 5 isolates were exposed to 5, 8, 10, 15, 20, 25, and 28 °C in wax

moth, *G. mellonella*, larvae, and mortality and progeny production data were obtained. All isolates caused 100% mortality of wax moth larvae and developed and produced progeny between 8 and 25 °C. At 28 °C, mortality was 100%, but no progeny was observed. The highest infective juvenile production was observed at 15 °C for all isolates. The time of emergence of the infective juveniles from the host cadaver showed some differences among isolates, with the Turkish Sinop isolate having the earliest emergence time from cadavers at 15 (10 days) and 20 °C (8 days). At 25 °C, the infective juveniles of the Sinop, SN and Rafaela isolates emerged from the cadavers in from 5 to 7 days. Host death occurred at 12 days at 8 °C, 9 to 11 days at 10 °C, 4 to 5 days at 15 °C, 3 days at 20 °C and 2 days at 25 and 28 °C. For penetration efficiency, Sinop, SN and Rafaela isolates penetrated their hosts at 5, 8, and 10 °C. Penetration of the infective juveniles was consistently high for all isolates at 15, 20, 25 and 28 °C, but it was significantly lower for the MG-14 isolate at 15, 25 and 28 °C. No progeny production occurred at 28 °C, but nematode penetration did occur with the MG-14 isolate having significantly lower penetration than the other isolates. When nematodes were produced at 8, 15, and 23 °C in wax moth larvae, all isolates had infective juveniles with longer body lengths at 8 °C followed by 15 and 23 °C. To verify the body length of the infective juveniles at the different temperatures, beet armyworm, *Spodoptera exigua*, larvae and dog-food agar medium were used, respectively, for in vivo and in vitro culture of the Sinop isolate. Infective juvenile body length showed the same trends with the longest being at 8 °C and decreasing in length from 15 to 23 °C. These data suggest that quality of food for the nematode and temperature (i.e. developmental time) influence the body length of the infective juvenile.

Oğuzoğlu Ünlu and Özer (116) conducted research with the TUR-H2 isolate of *H. bacteriophora* from Ankara and *S. feltiae* from the Black Sea region of Turkey. At 25 °C, their data showed that infective juveniles of *H. bacteriophora* and *S. feltiae* emerged from the *G. mellonella* hosts 6 and 9 days post infection. These data are contrary to other reports where steinernematids emerge earlier than heterorhabditids at the same temperature regime (117,118). For example, the infective juvenile of *S. feltiae* emerged from cadavers between 5 and 7 days post infection at 25 °C (92) and

heterorhabditid species emerge from cadavers 10 or more days post infection (118,119).

Oğuzoğlu Ünlü and Özer (116) also studied the reproductive potential of *H. bacteriophora* and *S. feltiae* with the former species averaging ca. 141,600 infective juveniles per cadaver (range 50,905-271,593) and the latter species averaging ca. 13,800 infective juveniles per cadaver (range 4365-27,510). The reproductive potential is based on exposing *G. mellonella* larvae weighing 200 mg to 100 infective juveniles. In competition studies between the 2 species, their results showed that the first species inoculated was responsible for the highest mortality rates.

Hazir et al. (35) have described 2 new subspecies of *Photorhabdus luminescens*, subspecies *kayaii* and *thraciaensis*, from Turkish isolates of *H. bacteriophora*. Seven bacterial isolates were obtained from *H. bacteriophora*, and using riboprint analyses and metabolic properties, 2 isolates showed differences to represent subspecies status.

Research with the Turkish nematode/bacterium complex has only recently been initiated. The many isolates and species found in Turkey provide vast opportunities for conducting fundamental studies with this complex and for using them in biological control programs against a number of soil insect pests and insect pests in cryptic habitats.

Mass Production, Formulation and Commercialization

Mass production - Entomopathogenic nematodes are easily cultured either in vivo or in vitro for laboratory tests or for commercial production (120). The waxworm, *G. mellonella*, is the insect of choice for in vivo production because it is produced commercially in large numbers in several countries for fish bait and bird and lizard food. The basic method for small-scale production is described in Kaya and Stock (5), whereas a large-scale method to produce nematodes is described by Gaugler et al. (121). In vivo production is labor intensive, lacks economies of scale, and is costly, but it is also simple and reliable and results in high quality nematodes (122). Industrial-scale in vivo production may be applicable in developing countries, and some cottage industries in developed countries also use this technology.

For large-scale production, in vitro methods using 3-dimensional solid media or liquid fermentation methods have been employed (123,124). The 3-dimensional solid media method, first described by Bedding (125), used crumbed polyether polyurethane foam coated with a nutritive medium and inoculated first with the symbiotic bacteria and then with nematodes, yielded up to 65 million infective juveniles per 500 ml flask (125) or 2 billion infective juveniles per aerated autoclavable plastic bag (126). Advantages of the solid media method are that capital costs are low, limited expertise is required (but more than in the in vivo method), and the logistics of production are flexible. Because of limited economies of scale, this production method is best suited for countries that have low labor costs or for serving high value markets (127).

The liquid fermentation method has economies of scale because the proportion of labor and capital costs decreases in scale as operating costs increase. This technology has the lowest mass-production cost and is the method of choice for larger companies with multiple products in industrialized countries. For successful liquid culture, key factors are suitable medium, monoxenicity (only the symbiotic bacteria present), and adequate oxygen (128,129). Typical components of a medium are yeast extract as a nitrogen source; a carbohydrate source such as soy flour, glucose or glycerol, lipids of plant or animal origin, and salts. Oxygen transfer within a bioreactor must not result in shear forces harmful to the nematodes. Conventional equipment (including flat blade impellers, bubble lift columns and internal loop bioreactors) has been successful (130,131). A number of nematode species have been successfully produced in 7,500-80,000 l bioreactors including *S. carpocapsae*, *S. riobrave*, *S. kushidai*, *S. feltiae*, *S. glaseri*, *S. scapterisci*, *H. bacteriophora* and *H. megidis*, with yield capacity as high as 250,000 infective juveniles/ml depending on the nematode species. With *Heterorhabditis*, liquid fermentation yields inconsistent numbers of infective juveniles. The production time can be prolonged due to the variable recovery of the infective juveniles inoculated into the cultures and the inability of the amphimictic adults to mate under liquid culture conditions (131,132).

Formulation and storage - Following mass production, nematodes can be either bulk stored in refrigerated aerated tanks for extended periods or formulated immediately. Infective juveniles can be stored

in an aqueous suspension at 4-15 °C (depending on nematode species) without much loss of activity for 6-12 months for *Steinernema* species and 3-6 months for *Heterorhabditis* species.

In the simplest type of formulation, nematodes are mixed with a moist substrate (e.g. sponge); such formulations require continuous refrigeration to maintain nematode quality for extended periods. To improve the shelf life and resistance to temperature extremes, formulations that reduce the infective juvenile's metabolism by immobilization or partial desiccation have been developed. These formulations contain alginate, vermiculite, clays, activated charcoals, polyacrylamide, and water dispersible granules (124,133). It is difficult to obtain an optimal formulation for all nematode species because they have different specific requirements for moisture and oxygen. One of the best formulations is the water dispersible granule that has been developed for steinernematids (e.g. *S. carpocapsae* and *S. feltiae*) as it combines relatively long nematode shelf life without refrigeration but with ease of handling. Partially desiccated infective juveniles in water dispersible granules have a shelf life at 25 °C of 5 to 6 months for *S. carpocapsae*, 2 months for *S. feltiae*, and 1 month for *S. riobrave* (134). This formulation is mixed with water prior to spray application, and the partially desiccated infective juveniles rehydrate after application to a moist soil environment. However, to achieve optimal infectivity the infective juveniles need to rehydrate for up to 3 days in soil (135).

Quality control - Before and after formulation, the quality of the nematodes should be checked. At a minimum, their viability and infectivity should be monitored. Several bioassay protocols are available, but assays using many nematodes are considered inappropriate for quality control purposes due to host-parasite interactions such as recruitment. Grewal (136) advocates the use of a one-on-one (one nematode to one *Galleria* larva) sand-well assay as a standard quality control tool. The one-on-one assay works well for steinernematids and five-on-one assay works well for heterorhabditids (137). Additional quality control parameters include assessment of energy reserves (dry weight or total lipid content) as a predictor of longevity.

Commercialization - The first step in the development of a commercial product is strain selection. Key properties of a commercial strain are high virulence

against the target pest(s) and ease of culture. Also desirable are superior shelf life and effectiveness against multiple insect pests (129). All traits must be embodied in the same strain. For example, although *S. glaseri* is effective against coleopteran larvae, it is difficult to market because of problems with formulation and storage.

Regulations on the use of entomopathogenic nematodes for insect control must also be considered. These regulations vary among countries (138-140). Indigenous nematodes are exempted from registration in many European countries, Australia, and the USA, while in other countries, they are subject to similar registration procedures as for a chemical pesticide. The importation and use of non-indigenous and transgenic nematode species are subject to strict regulations in most countries. Some countries consider foreign strains of endemic species to be exotic, and this can be a major obstacle to the commercialization of entomopathogenic nematodes.

Efficacy

Key target pests - Entomopathogenic nematodes have been tested against a large number of insect pest species with results varying from poor to excellent control (141). Many factors can influence the successful use of nematodes as biological insecticides, but matching the biology and ecology of both the nematode and the target pest is a crucial step towards successful application. Consideration has to be given to the foraging behavior and temperature requirements of a nematode species and to the accessibility and suitability of the pest to the nematode. Entomopathogenic nematodes have been most efficacious in habitats that provide protection from environmental extremes, especially in soil, which is their natural habitat and in cryptic habitats. Excellent control has been achieved against plant-boring insects because their cryptic habitats are favorable for nematode survival and infectivity (e.g. no natural enemies of the nematodes and adequate moisture). Low or highly variable control has been achieved in manure because of high temperatures in animal rearing facilities and toxic effects of manure contents (ammonia) on the infective juveniles. Control of aquatic insects has been unsuccessful because the nematodes are not adapted to directed motility (host finding) in this environment. On foliage and other exposed habitats, the infective juveniles face harsh

conditions that can be only marginally remedied by adjuvants. A list of some insect pests and the commodities in which they have been successfully controlled with entomopathogenic nematodes is provided in Table 2.

Application strategies - Entomopathogenic nematodes have almost exclusively been applied using the inundative approach where high numbers of infective juveniles are released in a uniform distribution and control of pest populations is expected to be quick and thorough. This approach is feasible for high value niche crops (greenhouse ornamentals and vegetables, citrus, cranberry, turfgrass, etc.). However, nematodes are a poor fit for an inundative approach (i.e. a chemical

pesticide paradigm) in many cropping systems (i.e. low value crops and/or wide acreage crops such as cotton and soybeans) because of their limited shelf life, susceptibility to environmental extremes, high cost, etc. Inoculative releases and conservation and management of endemic nematode populations need to be explored, as they may be more promising and feasible in many pest situations (17).

Inoculative release of entomopathogenic nematodes with the expectation that they will establish new populations or augment low populations for long-term pest suppression has only been attempted a few times and little is known about the optimal approach to this

Table 2. Target pests for commercially available entomopathogenic nematodes¹.

Pest group Order: Family	Common name	Life stage ²	Application Site	Commodity	Nematode sp. ³
Coleoptera					
Chrysomelidae	Flea beetles	L	Soil	Mint, potato, sweet potato, sugar beets, vegetables	Sc
Chrysomelidae	Rootworms	L	Soil	Corn, peanuts, vegetables	Sc, Sr
Curculionidae	Billbugs	L	Turf	Turf	Sc, Hb
Curculionidae	Root weevils	L	Soil	Berries, citrus, forest seedlings, hops, mint, ornamentals, sweet potato, sugar beet, banana	Sc, Hb, Hm, Sr
Scarabaeidae	White grubs	L	Soil, turf	Berries, field crops, ornamentals, turf	Sg, Hb, Hm
Diptera					
Agromyzidae	Leaf miners	L	Foliage	Ornamentals, vegetables	Sc
Ephydriidae	Shore flies	L	Soil	Ornamentals, vegetables	Sf
Sciaridae	Sciarid flies	L	Soil	Mushrooms, ornamentals, vegetables	Sf
Muscidae	Filth flies	A	Baits	Animal rearing facilities	Sc, Sf, Hb
Tipulidae	Crane flies	L	Soil, turf	Turf, ornamentals	Sc, Hm
Lepidoptera					
Carposinidae	Peach borer moth	L	Soil	Apple	Sc
Tortricidae	Codling moth	PP	Cryptic	Apple, pear	Sc
Noctuidae	Cutworms, armyworms	L/P	Soil, turf	Corn, peanuts, turf, vegetables, ornamentals	Sc
Cossidae	Carpenter worms	L	Cryptic	Ornamentals, shrubs, pear, apple	Sc
	Leopard moths	L			
Sesiidae	Crown borers	L	Cryptic	Berries	Sc
	Stem borers	L		Cucurbits, ornamentals, fruit trees	
Pyralidae	Webworms	L	Soil, turf	Cranberries, ornamentals, turf	Sc
Orthoptera					
Gryllotalpidae	Mole crickets	A/N	Turf, soil	Turf, vegetables	Ss, Sr
Siphonaptera					
Pulicidae	Cat fleas	L/P	Soil, turf	Pet/vet	Sc

¹After Klein (9), Georgis and Manwei (10), Georgis et al. (144).

²L = larva; PP = prepupa; P = pupa; N = nymph; A = adult.

³Sc = *S. carpocapsae*; Sf = *S. feltiae*; Sr = *S. riobrave*; Ss = *S. scapterisci*; Hb = *H. bacteriophora*; Hm = *H. megidis*.

strategy. *S. glaseri*, isolated originally from scarab grubs in New Jersey, was released in a massive inoculative control program from 1939 to 1942 against Japanese beetle larvae in turfgrass (142). Gaugler et al. (142) noted that the elimination of bacterial symbionts by the use of antimicrobials in the in vitro rearing procedure, and possibly poor climatic adaptation of this neotropical nematode limited the success of this program. More recently, *S. scapterisci* originally isolated from Uruguay was successfully introduced into Florida for the classical biological control of mole cricket pests (55).

Successful inoculative releases of entomopathogenic nematodes are dependent on long-term, multigenerational survival and recycling of the nematode populations. To achieve this goal, several conditions must be met including (1) the presence of moderately susceptible insect hosts throughout most of the year, (2) a high economic threshold level of the target insect pests, and (3) soil conditions favorable for nematode survival (79). Augmentative releases into established nematode populations and/or management of the susceptibility of the host/pest populations (for example using stressors such as other control agents) are 2 additional approaches that may be used to boost or manage established nematode populations and that warrant more attention (21,143).

Application methods - The most common application method for entomopathogenic nematodes is to use the same type of equipment used for spraying chemical pesticides. Thus, nematodes can be applied to the target site with most commercially available spray equipment such as hand or ground sprayers, mist blowers, and aerial sprayers on helicopters (144). Infective juveniles can withstand pressures of up to 1068 kPa and pass through common nozzle type sprayers with openings as small as 100 µm in diameter, but the screens in the nozzles should be removed to minimize damage to the nematodes. Nematodes have also been applied via irrigation systems including drip, microjet, sprinkler and furrow irrigation (144,145). Pre- and postapplication irrigation and continued moderate soil moisture are essential for good nematode performance. If water is limited, subsurface injection of nematodes can be an efficient delivery method (99).

Plant-boring insects have been successfully controlled by injecting nematode suspensions directly into the borer holes or blocking the holes with sponges soaked with

nematode suspensions (146). For the banana weevil, a nematode suspension can be placed into insect-attracting cuts in residual rhizomes of bananas (147). Although not commercially feasible at this point, baits containing infective juveniles may offer a cost-effective way of controlling mobile insects such as adult house flies (148).

The detrimental effects of desiccation and UV radiation often can be alleviated by the addition of adjuvants to the nematode formulation/suspension. Although the use of nematodes for foliar insect pests has not met with great success, some studies have shown that the addition of adjuvants improved nematode performance against foliage-feeding pests. Thus, solar radiation can be filtered with stilbene brighteners (149), whereas antidesiccants such as certain commercial oils, plant-based products, and glycerin (149-151) have provided short-term protection for nematodes in exposed habitats.

Effects of agrochemicals and biological control agents - Entomopathogenic nematodes are often applied to systems/substrates that are regularly treated with many other agents, including chemical or biorational pesticides, soil amendments, and fertilizers. Depending on the agents, application timing, and physico-chemical characters of the system, the nematodes may or may not interact with these other agents. If interactions occur, they may range from antagonistic to synergistic. In addition to these agents, intraguild predation between parasitoids and nematodes may occur (152), but Sher et al. (153) and Lacey et al. (154) have shown that some parasitoids are compatible with *S. carpocapsae*.

Entomopathogenic nematodes appear to be compatible with many, but not all, herbicides, fungicides, acaricides, insecticides, nematicides (133,155,156), azadirachtin (157), *Bacillus thuringiensis* products (158), and pesticidal soap (158). Negative effects of various pesticides on the infective juveniles have been documented (155,159). On the other hand, synergistic interaction between entomopathogenic nematodes and other control agents has been observed for various insecticides (21,160) and pathogens (95,143). In view of the diversity of available chemical and biorational insecticides, a generalization on pesticide-nematode compatibility cannot be made. The compatibility of each chemical pesticide and nematode species should be evaluated on a case-by-case basis.

Prospects for Biological Control of Insect Pests in Turkey

Entomopathogenic nematodes are being produced commercially and sold in countries throughout North America, Western Europe and Asia, as well as Australia. Many other countries are exploring the use of these nematodes for biological control of various insect pests. Currently, because of their high retail cost in comparison with other control agents, their use is restricted to high value crops in niche markets or to homeowners in developed countries.

The opportunities for using entomopathogenic nematodes against insect pests in the soil and cryptic habitats in Turkey are excellent. Table 2 identifies by common names the wide array of insect pests that are being controlled or have the potential to be controlled throughout areas where the nematodes are sold. Table 3 identifies by scientific names some of the insect pests that could be controlled by entomopathogenic nematodes in Turkey. In most instances, the insect pests in Table 3 are the same ones that have been targeted in Table 2.

Table 3. Insect pests in Turkey that could be potentially controlled by entomopathogenic nematodes.

Pest group Order: Family	Genus/Species	Common Name	Commodity	Application Site ¹	Life stage ²
Orthoptera					
Gryllotalpidae	<i>Gryllotalpa gryllotalpa</i>	Mole cricket	Cotton, tobacco, sunflower, corn, vegetables	Soil	N/A
Hemiptera					
Pentatomidae	<i>Eurygaster integriceps</i>	Stink bug	Grains	Litter	A
Coleoptera					
Buprestidae	<i>Capnodis</i> spp.	Flatheaded woodborer	Poplar, willow, pistachio, fruit trees	Borer	L
Chrysomelidae	<i>Leptinotarsa decemlineata</i>	Colorado potato beetle	Potato, egg plant	Soil	L
	<i>Phyllotreta</i> spp.	Flea beetle	Hemp, sugar beet, crucifers, carrot, spinach	Soil	L
Curculionidae	<i>Sphenophorus</i> spp.	Bill bugs	Turf	Soil	L
	<i>Ceutorhynchus</i> spp.	Weevils	Crucifers	Soil	L
	<i>Curculio elephas</i>	Chestnut weevil	Chestnut	Soil	L
	<i>Curculio nucum</i>	Nut weevil	Apple, cherry, pear, nut	Soil	L
	<i>Otiorhynchus</i> spp.	Weevil complex	Fig, quince, apple, walnut	Soil	L
Scarabaeidae	White grubs ³	White grubs	Turf, berries, field crops, ornamentals	Soil	L
	<i>Epicometis hirta</i>	Blossom beetle	Bitter orange, fruit, crop, grape	Soil	L
	<i>Melolontha melolontha</i>	European cockchafer	Forest	Soil	L
Diptera					
Agromyzidae	<i>Liriomyza trifolii</i>	Serpentine leafminer	Ornamentals, vegetables	Cryptic	L
Anthomyiidae	<i>Delia radicum</i>	Cabbage root maggot	Cabbage	Soil	L
Sciaridae	<i>Bradysia</i> spp.	Fungus gnat flies; sciarid flies	Mushrooms, ornamentals, vegetables	Soil, litter, casing	L
Tephritidae	<i>Bactrocera oleae</i>	Olive fruit fly	Olive	Soil	PP
	<i>Ceratitis capitata</i>	Mediterranean fruit fly	Apricot, quince, peach, fig, date palm, orange	Soil	PP
	<i>Rhagoletis cerasi</i>	Fruit fly	Cherry	Soil	PP
Tipulidae	<i>Tipula</i> spp.	Crane flies	Turf, ornamentals	Soil	L
Lepidoptera					
Arctiidae	<i>Hyphantria cunea</i>	Fall webworm	Apple, mulberry pear, cherry,	Soil	PP
Cossidae	<i>Cossus cossus</i>	Carpenter worm	Apple, pear, willow	Cryptic	L
	<i>Zeuzera pyrina</i>	Leopard Moth	Apple, plum, pear, walnut, cherry, olive, poplar	Cryptic	L
Noctuidae	<i>Agrotis ipsilon</i> , <i>A. segetum</i>	Cutworms	Sunflower, tobacco, sugar beet, cotton, vegetables	Soil	L
	<i>Heliothis armigera</i>	Bollworm	Cotton, tobacco, sesame, egg plant, tomatoes, potatoes, okra	Soil	PP
	<i>Peridroma saucia</i>	Variiegated cutworm	Sugar beet, strawberry, tobacco, mint, clover, vegetables	Soil	L
	<i>Spodoptera exigua</i>	Beet armyworm	Cotton, melons, vegetables	Soil	PP
	<i>Spodoptera littoralis</i>	Cotton leafworm	Cotton, okra, egg plant, cucumber, melons, tomatoes, pepper, sugar beet, cabbage	Soil	PP
Pyalidae	<i>Etiella zinckenella</i>	Lima bean podborer	Beans	Cryptic	L
Tortricidae	<i>Cydia pomonella</i>	Codling moth	Apple, pear, quince, walnut	Soil; cryptic	PP

¹ Refers to the site where the nematodes could be applied for the most effective control. For example, *Eurygaster integriceps* is a pest of sunn, but overwinters in forest litter.

² Target stage most susceptible to entomopathogenic nematode; L = Larva, PP = Prepupa (non-feeding, last larval instar before pupation), N = Nymph, A = Adult.

³ Many genera are involved that are commonly called "white grubs." The extent of genera and species in Turkey is not known but probably includes *Ataenius*, *Maladera*, *Rhizotrogus*, and *Phyllophaga*.

The challenges for Turkish scientists are many. These challenges include the (1) isolation of more native nematode species, (2) characterization of the native entomopathogenic nematode species and their symbiotic bacteria and matching them against the target pest species, (3) determination of the best application methods for these target insects (i.e. inoculative vs. inundative releases), (4) determination of the most appropriate nematode-bacterium complex for commercial production, and (5) production of the nematode economically for use by the growers. In addition, although the nematodes may be produced and formulated, they still must be shipped to the growers, who must be properly trained so that the nematodes are used effectively against the target pests.

Turkish regulations governing the production and use of entomopathogenic nematodes are not clear. In most countries, native entomopathogenic nematodes do not have to be registered with the federal government before use. Accordingly, Turkish scientists must work closely

with government regulators so that the use of entomopathogenic nematodes will be in the best interests of the people of Turkey.

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