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DERLEME/**REVIEW**

SOME ENTOMOPATHOGENIC NEMATODES

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ABSTRACT

Most of all nematodes studied for biological control of insect, those in the families Steinernematidae and Heterorhabditidae have aroused the most interest, and information about them is growing exponentially. These two families, mutualistically associated with bacteria in the genus *Xenorhabdus* are similar in their actions and are considered here together.

Key Words: Entomopathogen nematodes, Heterorhabditidae, Steinernematidae, *Xenorhabdus*.

BAZI ENTOMOPATOJEN NEMATODLAR

ÖZ

Böceklerin biyolojik kontrolü için çalışılmış nematodların çoğu Steinernematidae ve Heterorhabditidae familyalarına aittir. Bu canlılar arasındaki ilişki konuya olan ilgiyi artırdığı gibi bilgi arayışıda sürekli olarak artmaya devam etmektedir. Bu iki familya *Xenorhabdus* cinsi bir bakteri ile de karşılıklı olarak bir ilişki içinde olup, aktiviteleri birbirine benzerlik göstermektedir. Burada bu ilişkiler birlikte ele alınmıştır.

Anahtar Kelimeler: Entomopatojenik nematodlar, Heterorhabditidae, Steinernematidae, *Xenorhabdus*.

1. INTRODUCTION

The most urgent needs in the field of insect parasitic nematodes are to conduct more successful field trials and to activate private industry into mass producing these parasites for retail. We now know enough about identification rearing, life cycles and parasite associations to begin large scale field trials for controlling insects with these parasites. The free-living, non-feeding infective juveniles of these nematodes possess attributes of both insect parasitoids or predators, and microbial pathogens. Like parasitoids-predators, they have chemoreceptors and are motile; like pathogens, they are highly virulent, killing their hosts quickly, and can be cultured easily in vitro, have a high reproductive potential, and have a numerical but no functional response. They have a broad host range (Gaugler, 1981;1988), are safe to vertebrates, plants and other non-target organisms (Akhurst, 1990; Poinar, 1989), have been exempt from registration in the United States (Gorsuch, 1982), are easily applied using standard spray equipment (Georgis, 1990 a), are compatible with many chemical pesticides (Forschler et.al., 1990; Hara and Kaya, 1983; Rovesti et.al., 1988; Rovesti and Deso, 1990; Zimmerman and Cranshaw, 1990) and are amenable to genetic selection(Gaugler,1987). These nematodes, because they serve as vectors of *Xenorhabdus* bacteria, are termed entomopathogenic, reinforcing the link between insect nematology and insect pathology (Gaugler and Kaya, 1990). Numerous reviews have been written on entomopathogenic nematodes since 1985 including aspects of biology and biological control (Gaugler , 1988; Georgis and Hauge, 1991; Georgis and Poinar, 1989; Kaya ,1985; 1990 a; Popiel and Hominick,1992; Wouts, 1991), genetics and biotechnology (Gaugler,1987; Poinar, 1991), epizootiology (Kaya, 1987), techniques (Woodring and Kaya,1988) and safety (Akhurst, 1990; Poinar ,1989). Compilation of research conducted in Ja-

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pan (Ishibashi, 1987;1990) and an extensive bibliography (Smith et.al.,1992) of Steinernematid and Heterorhabditid literature are available. Moreover a comprehensive book on Steinernematids and Heterorhabditis and their mutialistic bacteria was recently published. Therefore, this review provides a brief background of the nematodes and bacteria and focuses on recent advances made with these nematodes and on current critical issues and research directions (Kaya and Gaugler, 1993).

1.1. Nematodes

Entomopathogenic rhabditid nematodes belong to two monogenetic families, the Steinernematidae and the Heterorhabditidae. The Steinernematids have 10 species (Douced and Douced 1990; Poinar, 1990) and the Heterorhabditids contain 3 species (Poinar, 1990) (Table 1).

We need to conserve these strains or isolates to prevent their loss or contamination with other strains and species, to preserve genetic diversity, and so that they can serve as a source to group and identify nematode species and strains through classical taxonomy and biotechnology. (Curran, 1990; Smits et.al., 1991). The liquid-nitrogen storage technique will be most helpful in reaching the goals (Popiel and Vasquez, 1991; Smith et.al., 1990).

1.2. Bacteria

Symbiotic bacterium *Xenorhabdus* spp., plays an essential role in subsequent stages of the life cycle of entomopathogenic nematodes. Bacterial cells are found in a modified ventricular part of the intestine in Steinernema and in this area, as well as throughout the intestine lumen, in Heterorhabditis. *Xenorhabdus* spp., which are gram negative, facultatively anaerobic rods, belong to the family Enterobacteriaceae. Five species have been described (Table: 1), but some undescribed organisms may represent new or different species of *Xenorhabdus* (Akhurst and Boemare, 1990). The vast majority of *X. luminescens*, associated with Heterorhabditis, luminesce (Akhurst et.al., 1992; Akhurst and Boemare, 1986) while *Xenorhabdus* spp. associated with Steinernematids do not luminesce. Dimorphism occurs in *Xenorhabdus* spp. and is referred to as phase one (primary form) and phase two (secondary form). Phase one and two have distinctly different colony morphologies but show no differences in pathogenicity to a *Galleria mellonella* host. Other differences are that phase one produces antibiotics (Akhurst and Boemare, 1990; Mc Inerney et.al., 1991; 1991; Nealson et. al., 1990) adsorbs certain dyes, and develops large intracellular inclusions, whereas phase two does not adsorb the dyes, does not produce antibiotics, and forms intracellular inclusions inefficiently. For detailed information on the associated bacteria, see Akhurst and Boemare (1990), Frackman and Nealson (1990), and Nealson et. al.. (1990).

Table 1. Steinernema and *Heterorhabditis* Species and Their Respective Symbiotic *Xenorhabdus* (bacterial) Species (Kaya and Gaugler, 1993).

Nematode genus	Nematode species	*Xenorhabdus* species
Steinernema	*affinis*	*bovineii*
	anomali	undescribed
	carpocapsae	*nematophilus*
	feltiae (=bibionis)	*bovineii*
	glaseri	*poinarii*
	intermedia	*bovineii*
	kushidai	undescribed
	rara	undescribed
	riteri	undescribed
	scapteris	undescribed
	Undescribed	*beddingii*
Heterorhabditis	*Bacteriophora(=heliathidis)*	*luminescens*
	Megidis	*luminescens*
	Zealandic	*luminescens*

1.3. Biology of the Nematode Bacterium Complex

Knowledge of the bacteria-nematode interactions is based mainly on the *S. carpocapsae* and *X. nematophilus* model, which does not necessarily apply to other species and is unlikely to apply to Heterorhabditis and *X. luminescens*. When the nematodes and bacteria are within the haemocoel, they must cope with the host's ability to neutralize infectious agents. The infective third-stage (J3) juvenile, ensheathed by the second-stage cuticle (Poinar, 1979) carries the bacterial cells of *Xenorhabdus* in its intestinal tract. Once a suitable host is found, the infective juvenile enters the host through natural openings (mouth, anus or spiracles) or possible wounds and penetrates into the hemocoel. The infective juvenile of Heterorhabditids also possesses a dorsal tooth that may assist in the direct penetration through the host's integument, particularly around the thin intersegmental areas (Bedding and Molyneux, 1982) although cuticular penetration appears to be rare (Mracek et. al., 1988). The bacterial cells, voided from the nematode's intestine into the hemolymph, propagate and kill the host by Septicemia within 48 hours. The nematodes feed on the bacterial cells and host tissues, produce two or three generations, and emerge from the host as infective juveniles to search for new host (Kaya and Gaugler, 1993).

Mutualism occurs between the nematode and bacterium (Akhurst and Boemare, 1990). Each nematode species has a specific association with only one *Xenorhabdus* species, although a *Xenorhabdus* species may be associated with more than one nematode species (Table 1). In this association, the nematode relies upon

the bacterium (symbiont) for killing its insect host, creating a suitable environment for its development by producing antibiotics that suppress competing secondary microorganisms, break down the host tissues into usable nutrients, and serve as a food source. The bacterium requires the nematode for protection from the external environment penetration into the host's haemocoel and possibly inhibition of the host's antibacterial proteins such as cecropins.

In nature, the infective juveniles invariably contain only phase-one bacteria. Even where the majority of *Xenorhabdus* are in phase two during the time of *S. carpocapsae* infective juvenile formation, the infective juveniles only carry phase one cells (Akhurst, 1980).

1.4. Host Range

Nematodes have parasitized insects for a long time. Nematodes have evolved to parasitize just about every kind of plant and animal in the world, so it is not too suprising to find them killing, srerilizing or otherwise debilitating millions of insects such as mosquitoes, black flies, chironomids, grasshoppers, moths, ants, bees and many other insects and invertebrates. Entomopathogenic nematodes and their bacterial partners kill insect so quickly that they do not form the intimate, highly adapted host-parasite relationships characteristic of other insect-nematode infections like mermithids and allantonematids (Kaya and Gaugler, 1993). This rapid mortality permits the nematodes to exploit a range of hosts that spans nearly all insect orders (Poinar 1979), a spectrum of activity well beyond that of any other microbial control agent. This extraordinary host range is one of the main reasons for the current intense interest in nematode development for biological control. Entomopathogenic nematodes are routinely isolated from soil without determination of their host associations (Akhurst and Bedding, 1986; Akhurst and Brooks, 1984; Hara et.al., 1991; Hominick ,1990).

Like other biological control agent, Steinernamatid and Heterorhabditid nematodes attack a far wider spectrum of insects in the laboratory were host contact is assured, environmental condition are optimal and no ecological or behavioral barriers to infection exist (Gaugler, 1981, 1988). Foliage feeding lepidopteran larvae may provide the best illustration. These insects are highly susceptible to infection in petri plates but are seldom impacted in the field, where nematodes tend to be quickly inactivated by the environmental extremes like desiccation, radiation and temperature, characteristic of exposed foliage (Gaugler et.al., 1992). Infective juveniles are similarly highly lethal to mosquito and black fly larvae in the laboratory, but in pools or streams they cannot swim or quickly settle out of the host feeding zone (Finney and Harding, 1981; Gaugler et.al., 1983; Gaugler and and Molloy, 1981).

Field studies demonstrate that entomopathogenic nematodes do possess an unusual ability to exploit different insect species as hosts. Infective juveniles of *S.carpocapsae* can provide acceptable control of field populations of fungus gnats artichoke plume maths, cutworms, sod webworms, strawberry root weevils and citrus root weevils (Georgis, 1990 b) a list encompassing representatives from three orders of insects. But these successes, complied after decades of field testing, are trivial relative to the long list of insects that nematodes have not controlled in the field (Kaya et.al., 1981). Although field infections often result from these trials, impact on target populations has most frequently been judged modest or negligible, even when concentration equivalent to billions of nematodes per hectare are applied. Even in as analysis of field tests resulting in significant suppression of soil and aquatic target insects, non targets were not adversely affected (Georgis and Kaya, 1991).

1.5. Behavior

The behavior of the nematode depends on the species involved, but they are able to locate insect hosts with varying degrees of efficiency. They usually enter the host through natural openings such as mouth, anus, spiracles but the J3 stages of Heterorhabditid nematode species possess a tooth and can penetrate through the body wall. Predators and parasitoids respond to physical and chemical stimuli that lead them to potential prey. These cues are also essential in recognition and in decisions on whether or not to feed or oviposit. This ability to find and attack suitable prey is a key determinant of biological control performance and is likely a major source of inconsistent field result (Lewis et. al., 1990; Nguyen and Smart,1994; Parkman et.al., 1994). Consequently an extensive body of literature provides a theoretical framework on the efficient location and identity of prey by predators and parasitoids (Bell, 1990; Nordlund et. al., 1988; O'brien et. al., 1990). This framework is largely based on multiple feedings (predators) or ovipositions (parasitoid). Field efficacy could be improved simply by matching nematode species and strains against those insects for which they are best adapted, an approach that requires understanding how entomopathogenic nematodes locate, identify, and asses potential hosts. That is the infection process must be understood as it relates to host selection, behaviors (Kaya and Gaugler, 1993).

The first behavior specifically host search, attachment, and recognition is host search, which begins with habitat selection, presumably the most important barri-

er restricting entomopathogenic nematode host range (Schmaedick and Shelton, 1999). Here some species appear to prefer to search for hosts at or near the soil surface (*S. carpocapsae*) (Moyle and Kaya, 1981) whereas others are adapted to search deeper in the soil profile (*H. bacteriophora*) (Choo et.al., 1989). Once the habitat has been selected, entomopathogenic nematodes are believed to adopt one of two search strategies: ambush or cruise (Gaugler et. al., 1989; Lewis et.al., 1992). Ambushers, like *S.carpocapsae*, take an energy conserving approach, remaining nearly sedentary white waiting for mobile surface adapted hosts. Ambushers, ought to be poorly adapted to attack immobile hosts deeper in the soil such as scarabaeid larvae. They should however, be adapted for mole crickets, because these insects are highly mobile and feed at the soil litter interface. Cruisers, like *S. glaseri*, are highly mobile and respond strongly to host chemoattractants (Lewis et.al., 1992). They would be best adapted to parasitize sedentary subterranean hosts (Kaya and Gaugler, 1993).

The second phase in host selection is nematode attachment to the host. Although attachment must be a prerequisite to infection, this important phase remains unstudied. Even Ishibashi and Kondo's (Ishibashi and Kondo, 1990) detailed review of infective stage behavior does not deal with attachment. These authors do describe nictation, the behavior of lifting all but the posterior portion of the nematode from the substrate and waving the extended body from side, but do not ascribe a function. This behavior is believed to be involved in nematode orientation to host insect (Gaugler et.al., 1980).

The third phase of host selection is recognition. Surface carbohydrates have attracted attention as being plausibly essential to the specificity of host recognition in other nematode species (Cook, 1986). In the case of phytonematodes, contact between heterosaccharides on the nematode body wall (Spigel et. al., 1982) and their binding sites (lectins) on the root surface (Peumans et.al.,1982) may lead to recognition. Amphid secretions also appear to play a role in recognation. Blockage of *Heterodera roctochiensis* amphid apertures by detergents interfered with host recognition (Forrest et.al., 1988) As for entomopathogenic nematodes, the role of the sensory organs and the mechanisms of host recognition during search and attachment remain unknown.

Following host selection, the remaining behavior in the infection process is penetration into the host. Entomopathogenic nematodes using oral or anal portals of entry must penetrate through the gut wall, a behavior that has not been well described. Invasion through the spiracles and escape from tracheal tubes into the hemocoel have been associated in *S. scapterisci* with movement to a point where tube diameter is only slightly greater than the nematode, followed by vigorous thrashing movements that rupture the tube (Nguyen and Smart, 1991). Post penetration behaviors that may be involved in nematode response to the host defense response have not been documented (Kaya and Gaugler, 1993).

1.6. Ecology

Use of *S. glaseri*, the first nematode applied as a soil colonization agent against the larval stage of the Japanese beetle, *Popillia japonica*, produced mixed results in the 1930s. In part, a lack of understanding of the complex biology of the nematode-bacterium symbiotic relationship impeded efforts to effectively use this nematode (Poinar, 1979). The discovery of *S. carpocapsae* and other species resulted in renewed interest in using nematodes for biological control programs. Excellent control was obtained against insect pest in moist, cryptic habitats in the late 1970 s and early 1980s (Bedding and Miller, 1981; Lindegren et. al., 1981; Miller and Bedding, 1982) but failures against foliage feeding insects from the 1950s to early 1980s clearly demonstrated that these nematodes were poorly adapted to the foliage environment (Begley, 1990; Kaya,1985). Because the number of insect pests in cryptic habitats are limited and these nematodes provided poor efficacy against foliage feeding insects, the priority of using these nematodes shifted back to the soil, which is their natural habitat (Schroder et al., 1996). Moreover %90 of insect pest species spend at least part of their life cycle in soil, providing further impetus in redirecting the research emphasis (Akhurst, 1986; Klein, 1990). The successful application of these nematodes as inundative agents against various soil insect pests continues to be somewhat elusive (Georgis and Gaugler, 1991; Klein, 1990). Several studies have examined one or two components of abiotic and biotic soil factors to understand nematode ecology (Kaya, 1990b).

1.7. Dispersal and Host Finding

Nematode dispersal is necessary for host finding. Although nematodes may disperse passively (Epsky et.al., 1988; Timper et. al., 1988) active dispersal, particularly by cruiser nematodes, appears to be the primary means for host finding. Even where water assists in the passive movement of nematodes through thatch (Zimmerman and Cranshaw, 1991) active dispersal probably plays a major role in host finding (Perez et al., 1995).

Factors affecting active nematode dispersal and host finding in soil include small pore spaces (Blackshaw and Senthamizhselvan, 1991; Choo and Kaya, 1991; Georgis and Poinar, 1989; Molyneux and Bedding, 1984) , moisture (Molyneux and Bedding, 1984)

temperature (Molyneux and Bedding, 1984) and plant roots (Choo et. al., 1989). Clay soils have small pores limiting nematode movement , and nematodes require a water film to disperse in soil (Walker, 1984). In field studies, nematodes have been most efficacious against the Japanese beetle at soil temperatures at 20°C (Georgis and Gaugler, 1991). Many insect pests, however are active at lower temperatures and cold adapted nematodes and *Xenorhabdus* bacteria are required to kill such pests. Heterorhabditid isolates from temperate regions are active and can infect a host at 7°C (Griffin and Downes 1991) and an undescribed steinernematid species from England infects hosts in sand from 5 to 25°C with optimal infection occurring at 15-20°C (Fan and Hominick, 1991). Plant roots may also affect nematode dispersal (Bird and Bird, 1986; Choo et al., 1989; Ishibashi and Choi, 1991) *S. glaseri* accumulates around roots in response to CO_2 (Bird and Bird, 1986) and this behavior may bring this nematode in closer contact with a sedentary host feeding in the root zone.

1.8. Survival

Both *Steinernema* and *Heterorhabditis* spp. have a third stage juvenile (J3), termed a dauer larva, that is the infective stage. These juveniles are non-feeding and can survive in soil for extended periods the duration of which depends on the species and the physical conditions within the soil, especially temperature and moisture. Nematode survival is enhanced in soil where they are buffered from environmental extremes. They can survive slow desiccation at high relative humidites (Womersley, 1990) and can survive low temperatures (Molyneux, 1985; Schmidt and All, 1979) although a tropical heterorhabditid isolate does not survive at 10 C° (Molyneux, 1985). *S. carpocapsae* and *S. glaseri* survive best in sandy loam and sand soils, respectively (Kung et. al., 1990). Poor aeration probably is a significant factor in lover nematode survival in clay soils (Kung et. al., 1990).

Not all infective juveniles are infectious at the same time (Bednarek and Nowicki, 1991; Fan and Hominick, 1991; Figueroa et.al., 1990; Hominick and Reid, 1990; Ishibashi and Kondo 1986). Suggesting that nematodes have adobted a survival strategy by staggering their period of infectivity. The number of nematodes infecting hosts decreases after storage at low temperatures, but is followed by an unexpected increase with passage of time, indicating that the cold temperatures induce some nematodes to enter a noninfectious or "diapause" state (Fan and Hominick, 1991). Regardless of nematode strain, concentration, or exposure to one or several insect host under presumably optimal conditions, only %30–40 of the nematodes infect the host (Fan and Hominick, 1991). The reason for the lack of infectivity by the majority of nematodes remains unknown, but if nematodes are all infectious at the same time and no hosts are available, the nematodes may become locally extinct. Their dispersal range is limited and because a certain percantage of the nematode population enters a quiescent or diapause state, some of them will survive periods of host absence (Ishibashi and Kondo, 1986).

1.9. Interspecific Competition

Entomopathogenic nematodes are widely distributed throughout the world and nematode species may occur sympatrically (Akhurst et. al., 1992; Akhurst and Brooks, 1984; Beavers et.al., 1983; Ehlers et.al., 1991). In laboratory studies, heterorhabditid and steinernematid nematodes generally connet coexist in the same host (Alatorre and Kaya, 1990, 1991) whereas two steinernematid species (Kondo, 1989) may successfully parasitise the same host. An antagonistic relationship, however may also exist between steinernematid species (Kondo, 1989). The mutualistic bacteria are believed to be the basis for the incompatibility of the heterorhabditid and steinernematid species. The nematodes from one genus cannot feed on the mutualistic bacteria from another genus (Akhurst, 1983; Alatorre-Rosas and Kaya, 1991).

If two insect pest species occur simultaneously in different niches in the same habitat, however one should consider using two nematode species for a that are each adapted to one of the pests. The combination of a cruiser nematode species for a sedentary pest in the soil and an ambusher nematode species for a soil-surface pest may be more efficient than one nematode species alone (Alatorre-Rosas and Kaya, 1990).

Interspecific competition studies of the entomopathogenic fungus *Beauveria bassiana* and entomopathogenic nematodes show that they are not compatible within the same host (Barbercheck and Kaya, 1990). The nematodes out compete the fungus, the mechanism for fungal exclusion is based on antibiotic production by the symbiotic *Xenorhabdus*. For the fungus to be successful, the host must be infected one to several days before nematode exclusion occurs. In spite of this competition, the fungus and nematode may coexist in the same habitat because the nematodes are not attracted to fungal infected hosts (Barbercheck and Kaya, 1991) And the presence of both pathogens in the soil results in higher mortality than either pathogen alone (Barbercheck and Kaya, 1991).

1.10. Recycling and Epizootiology

Entomopathogenic nematodes are similar to arthropod predators and insect parasitoids in that they kill their hosts, but their bacterial associates make them notably different. Steinernematids and heterorhabditids, obligate pathogen in nature, require insect hosts to recycle. In many inundative application of these nematodes, persistence date are routinely taken but whether the nematodes merely persist or actually recycle is not known. In a year-long study, the addition of insect hosts into potted soil at prescribed intervals showed good persistence at high levels of the nematode population, whereas the lack of hosts resulted in a rapid decline of the nematodes within 50 days after application (Kaya, 1990 a). Inoculative releases have not been attempted yet, but this method of nematode introduction may provide long-term control.

Conservation and augmentation of naturally occuring entomopathogenic nematode populations through agricultural practices show promise for insect control (Brust, 1991). Using a wheat-corn rotation and cultural practices, greater yields, less root damage and weedy treatments compared with conventional tillage treatments. Apparenty, no tillage and weeds create a diverse environment providing alternate hosts and soil conditions conducive for nematode survival and recycling (Kaya and Gaugler, 1993).

Epizootics of entomopathogenic nematode diseases probably occure regularly in soil but they are difficult to detect and often go unrecorded (Kaya, 1987). Two epizootic, both from Australia involving underscribed *Heterorhabditis* species, have been documented (Akhurst et.al., 1992; Sexton and Williams, 1981). In the first case, a significant reduction of the whitefringed beetle, *Graphagnathus leucoloma* larvae and adults was observed in a lucerne field (Sexton and Williams, 1981). In the second, two undescribed species of *Heterorhabditis* were found infecting four scarabaeid species in three adjoining sugar cane fields (Akhurst et.al., 1992). Under laboratory conditions, the scarabaeid larvae are not susceptible to the nematodes but in the field, infected larvae are readily recovered. One mechanism is that larvae in the field may have been stressed, making them more susceptible to nematode infection. As more epizootics are reported, conditions for their development can be determined and these conditions can be manipulated to initiate artificial epizootics. Long-term studies of both the insect and nematode populations in the soil will provide the most useful information (Kaya and Gaugler, 1993).

2. CONCLUSIONS

Entomopathogenic nematology has a relatively short history dating back to the pioneering research of R.W. Glaser and his coworkers in the 1930s and 1940s (Gaugler and Kaya, 1990). The primary emphasis of their research and of others following them focused on developing and using these nematodes as biological insecticides. The reasons for success or lack of success controlling insect pests. Particularly in the soil environment, often remain unknown, underscoring the need to obtain basic information on the biology, behavior, ecology, and genetics of these nematodes. Recent advances made in nematode behavior and ecology clearly demonstrate that they are not generalist pathogens; their behavior, for example, restricts much of their activity to a certain soil stratum, eliminating many insect from infection. Understanding these behavioral patterns and their genetics will enhance the use and production of the most adapted species for insect control in the field (Kaya and Gaugler, 1993).

Survival mechanisms of nematodes are being unraveled. The occurrence of quiescent nematodes suggests that they have evolved effective survival strategies and the J2 cuticle on some nematode species seems to play a significant role in desiccation tolerance and protection against fungal antagonists. Although very little is known about other survival mechanisms or about their population dynamics, the nematodes are highly successful for they are ubiquitous in nature. As we begin to understand them, they can be used effectively and selectively as inundative agents against numerous insect pest and have the potential to be used as inoculative agents for classical biological control. Conservation and augmentation of natural nematode populations through proper management practices and periodic nematode releases offer exciting possibilities for insect pest suppression. Finally, these fascinating animals may contribute more to science than their use solely as biological control agents. To begin with, they may be useful tools in understanding evolution of parasitism and symbiosis and the mechanism of insect resistance of infection (Kaya and Gaugler, 1993).

This review explored some important aspects of the biology of the nematodes that require attention if progress in their use as biocontrol agents is to be maintained. Future efforts should be directed toward more field experimentation and the activation of the private sector to mass produce these parasites.

REFERENCES

Akhurst, R.J. (1980). Morphological and functional dimorphism in *Xenorhabdus* spp., bacteria symbiotically associated with the insect pathogenic nematodes *Neoplectana* and Heterorhabditis. *J. Gen. Microbial.*, 121, 303-309.

Akhurst, R.J. (1983). Neoplectana species specificity of association with bacteria of the genus *Xenorhabdus. Exp. Parasitol.*, 55, 258-263.

Akhurst, R.J. and Brooks, W.M. (1984). The distribution of entomophilic nematodes (Heterorhabditidae and Steinernematidae) in North Carolina. *J. Invertebrate Pathol.*, 44, 140-145.

Akhurst, R.J. (1986). Controlling insect in soil with entomopathogenic nematodes. *In.Fundamental and Applied Aspect of Invertebrate Pathology*, p.711.

Akhurst, R.J. and Bedding, K.A. (1986). Natural occurrence of insect pathogenic nematodes in soil. *J. Aust. Entomol. Soc.*, 25, .241-244.

Akhurst, R.J. and Boemare, N.E. (1986). A. non- numinescent strain of *Xenorhabdus luminescens. J. Gen. Microbial*, 132, 1917-1922.

Akhurst, R.J. (1990). Safety to nontarget invertebrates of nematodes of economically important pests. *In safety of Microbical Insecticidies,* 234-238.

Akhurst, R.J. and Boemera, N.E. (1990). Biology and taxonomy of *Xenorhabdus. See. Ref.*, 75-90.

Akhurst, R.J., Bedding, R.A., Bull, R.M. and Smith. D.R.J. (1992). An epizootic of Heterorhabditis spp. in sugar cane scarabaeids. *Appl. Nomatol.*, 15, 71-73.

Alatorre-Rosas, R. and Kaya, H.K. (1990). Interspecific competition between two entomopathogenic nematodes species in the genera *Heterorhabditis* and *Steinernema* for an insect host in sand. *J. Invertebr. Pathol.*, 55, 178-1.

Alatorre-Rosas, R. and Kaya, H.K. (1991). Interaction between two entomopathogenic nematodes species in the same host. *J. Invertebr. Pathol.*, 57, 1-6.

Barbercheck, M.E. and Kaya, H.K. (1990). Interactions between *Beauveria* bassiana and the entomogenous nematodes *Steinernema feltiae* and *Heterorhabditis heliothis. J. Invertebr. Pathol.*, 55, 225-234.

Barbercheck, M.E. and Kaya, H.K. (1991). Effect of host condition and soil texture on host finding by the entomogenous nematodes *Hetororhabditis bacteriophora* and *Steinernema carpocapsae. Environ. Entomol.*, 20, 582-589.

Beavers, J.B., McCoy, C.W. and Kaplan, D.T. (1983). Natural enemies of subterranean *Diaprepes abbreviatus* larvae in U.S.A. *Environ. Entomol.*, 12, 840-843.

Bedding, R.A. and Miller, L.A. (1981). Use of a nematode *Heterorhabditis heliotidis* to control black vine weevil *Otiorhynchus sulcatus* in potted plants. *Ann. Appl. Biol.*, 99, 211-216.

Bedding, R. A and Molyneux, A.S. (1982). Penetration of insect cuticle by infective juveniles of *Hetororhabditis* spp. *Nematologica*, 28, 354-359.

Bednarek, A. and Nowicki, T. (1991). New estimation method for the density of entomogenous nematodes in the soil. *Rev. Nematol.*, 14, 638-639.

Begley, J.W. (1990). Efficacy against insects in habitats other than soil. *See.Ref.*, 68, 215-231.

Bell, W.I. (1990). *Searcing Behavior The Behavioral Ecology of Finding Resources.* London Chapman and Hall, p.358.

Bird, A.F. and Bird, J. (1986). Observations use of insects parasitic nematodes as a means of biological control of root-knot nematodes. *Int. J. Parasitol.*, 16, 511-516.

Blackshaw, R.P. and Senthamizhselvan, M. (1991). The effect of sand particle size on *Steinerma feltiae* activity against *Galleria mellonella* larvae. *Ann. Appl. Biol.*, 118, 637-643.

Brust, G E. (1991). Augmentation of an endemic entomogenous nematode by agroecosystem manipulation for control of a soil pest. *Agric. Ecosystems Environ.*, 36, 175-184.

Choo, H.Y. and Kaya, H.K. (1991). Influence of soil texture and presence of roots on host finding by *Heterorhabditis bacteriophora. J. Invertebr. Pathol.*, 58, .279-280.

Choo, H.Y., Kaya, H.K., Berlando, T. and Gaugler, R. (1989). Influence of plant roots on nematode host finding ability *Environ. Entomol.*, 18, 1136-1140.

Cook, G.M.W. (1986). Cell surface carbohydrates molocules in search of a function. *J. Cell Sci. Suppl.*, 4, 45-70.

Curran, J. (1990). Moleculer techniques in taxonomy. *See. Ref.*, 63-74.

Douced, M.M.A. and Douced, M.E. (1990). *Steinernema ritteri* with a key to the species of the genus *Nematologica*, 36, 257-265.

Ehlers, R.U., Deseo, K.V. and Strackebrandt,E. (1991). And their symbiotic bacteria Xenorhabdus spp. from Europa soils. *Nematologica*, 37, 360-366.

Epsky, N.D., Walter, D.E. and Capinera, J.L. (1988). Potential role of nematophagous microarthropods as biotic mortality factors of entomogenous nematodes. *J. Econ. Entomol.*, 81, 821-825.

Fan, X. and Hominick, W. (1991). Effects of low storage temperature on survival and infectivity of two *Steinernema* species. *Rev. Nematol.*, 14, 407-417.

Figueroa, W., Jansson,R.K., Cruz, C. and Acosta, N. (1990). Survey and effiency of entomopathogenic nematodes. *In Recontres Carajbus Biol.* p.145.

Finney, J.R. and Harding, J.B. (1981) Some factors affecting the use of *Neoplectena spp.* For mosquito control. *Mosq. News*, 41, 798-799.

Forrest, I.M.S., Spigel, Y. and Robertson, W.M. (1988). A possible role of amphids of potato cystnematode *Globodera rostochiensis* in host finding. *Nematologica*, 34, 173-181.

Forschler, B. T., All, J. N. and Gardner, W. A. (1990). *Steinernema feltiae* activity and infectivity response to herbicide exposure in aqueous and soil environments. *J. Invertebr. Pathol.*, 55, 375-379.

Frackman, S. and Nealson, K.H. (1990).The moloculer genetics of *Xenorhabdus. See. Ref.*, 271-284.

Gaugler, R. (1981). Biological control potential of neoaplectanid nematodes *J. Nematol.*, 13, 241-249.

Gaugler, R. (1987). Entomogenous nematodes and their prospects for genetic improvement. *Biotec. in Inverteb. Pathol. and Cell Culture*, 457-484.

Gaugler, R. (1988). Ecological considerations in the biological control of soil-inhabiting insect pests with entomopathogenic nematodes *Agric. Ecocyst. Environ.*, 24, 351-360.

Gaugler, R. and Kaya, H.K. (1990). Entomopathogenic nematodes in biological control. *Boca Raton, FL CRC*, 471-491.

Gaugler, R. and Molloy, D. (1981). Field evaluation of the nematode Neoplectana carpocapsae as a biologic control agent of blackflies. *Mosq. News*, 41, 459-464.

Gaugler, R., Bednerek, A. and Campbell, J.F. (1992). Ultraviolet inactivation of heterorhabditid and steinernematid nematodes. *J. Invertebr. Pathol.*, 59, 155-160.

Gaugler, R., Campbell, J.F. and Mcguine, I.R. (1989). Selection for host finding in *Steinernema feltiae. J. Invertebr. Pathol.*, 54, 363-372.

Gaugler, R., Kaplan, B., Alvarodo, J., Montap, J. and Ortaga, M. (1983). Assesment of *Bacillus thuringiensis* for control of the *Simulium* vectors. *Entomophaga*, 28, 309-315.

Gaugler, R., LeBeck, L.M., Nakagaki, B. and Boush, G.M. (1980). Orientation of the entomogenous nematode *Neoplectana carpocapsae* to carbon dioxide. *Environ. Entomol.*, 9, .649-652.

Georgis, R. (1990 a). Commercialization of steinernematid and heterorhabditid entomopathogenic nematodes *Brighton Crop Prot.*, 1, 275-280.

Georgis, R. (1990 b). Formulation and application technology. *See. Ref.*, 68, 173-191.

Georgis, R. and Gaugler, R. (1991). Predictability in biological control using entomopathogenic nematodes. *J. Econ. Entomol.*, 84, 713-720.

Georgis, R. and Hauge, N.G.M. (1991). Nematodes as biological insecticides. *Pestic. Outlook*, 2, 29-32.

Georgis, R. and Kaya, H.K. (1991). Effect of steinernematid and heterorhabditid nematodes on nontarget arthropods. *Environ, Entomol.*, 20, 815-822.

Georgis, R.and Poinar, G.O. (1989). Field effectiveness of entomophilic nematodes *Neoaplectane* and *Heterorhabditis. See. Ref.* 108a, 213-224.

Gorsuch, A. M. (1982). Regulations for the enforcement of the Federal Insecticide, Fungicide and Rodenticide Act exemption from regulation of certain biological control agents. *Fed. Regist.*, 47, 228-230.

Griffin, C.T. and Downes, M.I. (1991). Low temperature activity in *Heterorhabditis* spp. *Nematologica*, 37, 83-91.

Hara, A. H and Kaya, H. K (1983). Toxicity of selected organophorphate and carbamate pesticides to infective juveniles of the entomogenous nematode *Neoaplectane carpocapsae. Environment Entomology*, 12, 496-501.

Hara, A.H.,Gaugler, R.,Kaya, H.K. and LeBeck, L.M. (1991). Natural populations of entomophilic nematodes. *Environ.Entomol.*, 20, 211-216. *heliotidis* to control black vine weevil *Otiorhynchus sulcatus* in potted plants. *Ann. Appl. Biol.*, 99, 211-216.

Hominick, W.M. (1990). Perpectives of entomopathogenic nematology. *See. Ref.*, 68, 327-345.

Hominick, W.M. and Reid, A.P. (1990). Perspectives on entomopathojenic nematology. *See. Ref.* 68, 327-345.

Ishibashi, N. (1987). Recent Advances in Biological Control of Insect Pests by Entomogenous Nematodes in Japon. *Culture and Science*, p.179.

Ishibashi, N. (1990). Development of Biological Integrated Control of Agricultural Pests by Beneficial Nematodes. *Culture and Science*, p.159.

Ishibashi, N. and Choi, D.R. (1991). Biological control of soil pests by mixed application of entomopathogenic and fungivorous nematodes. *J. Nematol.*, 23, 175-181.

Ishibashi, N, and Kondo, E. (1986). A possible quiescence of the applied entomogenous nematode *Steinernema feltiae* in soil. *J. Nematol.*, 16, 66-67.

Ishıbashi, N. and Kondo, E. (1990). Behavior of infective juveniles. *See. Ref.* 68, 139-153.

Kaya H.K. (1985). Entomogenous nematodes for insect control in IPM systems. *Biological Control in Agricultural systems*, 283-302.

Kaya, H.K. (1987). Diseases caused by nematodes. *Epizootiology in Insec. Diseases*, 453-470.

Kaya, H.K.(1990a).Entomopathogenic nematodes in biological control of insects. *See. Ref.*, 11a, 189-198.

Kaya, H.K. (1990b). Soil ecology. *See. Ref.*, 68, 93-115.

Kaya, H.K. and Gaugler, R. (1993). Entomopathogenic nematodes. *A.R.E.*, 38, 181-206.

Kaya, H.K. Hara, A.H. and Reardon, R.C. (1981). Laboratory and field evolution of *Neoplectana carpocapsae* against the elm leaf beetle. *Can. Entomol.*, 113, 787-793.

Klein, M.G. (1990). Efficacy against soil inhabiting insect pests. *See. Ref.*, 68, 195-214.

Kondo, E. (1989). Studies on the infectivity and propagation of entomogenous nematodes, *Steinernema* spp. in the common cutworm *Spodoptera litura Bull. Fac. Agric. Saga Univ.*, 67, 1-88.

Kung, S.P., Gaugler, R. and Kaya, H.K. (1990). Soil type and entomopathogenic nematode persistence. *J. Invertebr. Pathol.*, 55, 401-406.

Lewis, E., Gaugler, R. and Harrison, R. (1992). Entomopathogenic nematode host finding relevance of host contact cues to cruise and ambush foragers. *Parasitology.*, 10, 41-44.

Lewis, W.J., Vet, L.E.M., Tomlinson, J.H., Van Lenteren, J.C. and Papaj, D.R. (1990).Variations in parasitoid foraging behavior essential element of a sound biological control.theory. *Environ. Entomol.*, 19, 1183-1193.

Lindegren, J.E., Yamashika, T.T. and Barnet, W.W. (1981). Parasitic nematodes may control carpenterworms in fig trees. *Calif. Agric.*, 35, 25-26.

Mc Inerney, B.V., Gregson, R.P., Lacey, M.J., Akhurst, R.J. and Lyons, G.R.(1991). Biological active metabolites from *Xenorhabdus* spp., Part 1 Dithiolopyrrolone derivates with antibiotic activity. *J. Nat. Prod*, 54, 774-784.

Mc Inerney, B.V., Taylor, W.C., Lacey, M.J., Akhurst, R.J. and Gregson, R.P. (1991). Biological active metabolites from *Xenorhabdus* spp., Part 2. Benzopyran -1- one derivates with gastroprotective activity. *J. Nat. Prod.*, 54, 785-795.

Miller, L.A. Bedding, R.A. (1982). Field testing of the insects parasitic nematode, *Neoplectana bibionis* against current borer moth, *Synanthedon tipiformis* in black currants. *Entomophaga*, 27, 109-114.

Molyneux, A.S. (1985). Survival of the infective juveniles of *Heterorhabditis* spp., and *Steinernema* spp. at various temperatures and their subsequent infectivity for insects. *Rev. Nematol.*, 8, 165-170.

Molyneux, A.S. and Bedding, R.A. (1984). Influence of soil texture and moisture on the infectivity of *Heterorhabditis* spp. and *Steinernema glaseri* for larvae of the sheep blowfly *Lucilia cuprina. Nematologica*, 30, 358-365.

Moyle, P.L. and Kaya, H.K. (1981). Dispersal and infectivity of the entomogenous nematode *Neoplectena carpocapsae. J. Entomol.*, 13, 295-300.

Mracek, Z., Hanzal, R. and Kodrik, D. (1988). Sites of penetration of juvenile steinernematids and heterorhabditis into the larvae of *Galleria mellonella. J. Invertebr. Pathol.*, 52, 477-478.

Nealson, K.H., Schmidt, T.M. and Bleakley, B. (1990). Physiology and biochemistry of *Xenorhabdus See. Ref.*, 271-284.

Nguyen, K.B. and Smart, G.C. (1991). Mode of entry and sites of development of *Steinernema scapterisa* in male crickets. *J. Nematol.*, 23, 267-268.

Nguyen, K.B. and Smart, G.C. (1994). *Neosteinernema longicurvicaudan* (Rhabditida: Steinernematidae) a parasite of the termite *Reticulitermes flavipes* (Koller). *J. Nematol.*, 26, 162-174.

Nordlund, D.A., Lewis, W.J. and Altieri, M.A. (1988). Influence of plant derived semiochemical on host-prey selection behavior of entomophagous insects. *In novel aspect of insect plant interactions*, 65-70.

O'Brien, W.I., Browman, H.I. and Evans, B.I. (1990). Search strategies of foraging animals. *A.m. Sci.*, 78, 152-160.

Parkman, J.P., Frank, J.H., Nguyen, K.B. and Smart, G.C. (1994). Inoculative release of *Steinernema*

scapterisci (Rhabditida: Steinernematidae) to suppress pest mole crickets (Orthoptera: Gryllotalpidae) of golf courses. *Environ. Entomol.*, 23, 1331-1337.

Perez. C.J., Shelton, A.M. and Derksen, R.C. (1995). Effect of application technology and Bt. Subspecies on management of diamondback moth. *J. Econ. Entomol.*, 88, 1113-1119.

Peumans, W.J., Stinissen, H.M. and Calier, A.R. (1982). Lectin synthesis in devoloping and germinating wheat and rye embryos. *Planta*, 156, 41-44.

Poinar, G.O. (1979). Nematodes for biological control of insects. *Boca Raton* FL CRC; p.277.

Poinar, G.O. (1989). Non-insect hosts for the entomogenous rhabditoid nematodes *Neoaplectane* and *Heterorhabditis. Rev. Nematology*, 12, 423-428.

Poinar, G.O. (1990). Taxonomy and biology of Steinernematidae and Heterorhabditidae. *See. Ref.* 68, 23-61.

Poinar, G.O. (1991). Genetic engineering of nematodes for pest control. *Biotec. for Biological control of pests and Vectors*, 77-93.

Popiel, I. and Hominick, W.M. (1992). Nematodes as biological control agents. *Part II. Adv. Parasitol.*, 31, 381-433.

Popiel, I. and Vasquez, E. (1991). Cryopreservation of *Steinerma carpocapsae* and *Hetororhabditis bacteriophora. J. Entomol.*, 23, 432-437.

Rovesti, K. and Deso, K.V. (1990). Compatibility of chemical pepticides with the entomopathogenic nematodes, *Steinernema carcapsae* and *Steinernema feitide. Nematalogica*, 36, 237-245.

Rovesti, L. Heinzpeter, E., Tagliente, W. and Deseo, K.V (1988). Compatibility of pesticides with the entomopathogenic nematode *Heterorhabditis bacteriophera. Nematologica*, 34, 462-476.

Schmaedick, M.A. and Shelton, A.M. (1999). Experimental evalution of Arthropod predation on *Pieris rapae* eggs and larvae in cabbage. *Environ. Entomol.*, 28, 439-444.

Schmidt, J. and All, J.N. (1979). Chemical attraction of *Neoplectana carpocapsae* to common excretory product of insect. *Environ Entomol.*, 8, 55-61. *See. Ref*, 285-300.

Schroeder, P.C., Ferguson, C.S., Shelton, A.M. Wilsey, W.T., Hoffmann, M.P. and Petzoltd, C.H. (1996). Greenhouse and field evalutions of entomopathogenic nematodes (Nematode: Heterorhabditidae and Steinernematidae) for control of cabbage maggot (Diptera: Anthomyiidae) on cabbage. *J. Econ. Entomol.*, 89, 1109-1115.

Sexton, S.B. and Williams, P. (1981). A natural occurrence of parasitism of *Graphagnathus leucolema* by the nematode *Heterorhabditis* sp. *J. Aust. Entomol. Soc.*, 20, 253-255.

Smith, B.S., Hodgson-Smith, A., Popiel, I., Minter, D.M. and James, E.R. (1990). Cryopreservation of theEntomogenous nematode parasite *Steinernema feltiae. Cryobiology*, 27, 319-327.

Smith, K.A., Miller, R.W. and Simser, D.H. (1992). Entomopathogenic nematode bibliography Heterorhabditid and Steinernematidid nematodes. *South. Coop. Ser. Bull.*, 370, 1-81.

Smits, P.H., Groenen, J.T.M. and Raay, G. (1991). Characterization of Heterorhabditis isolates using DNA restriction fragment length polymorphism. *Rev. Nematol.*, 14, 445-453.

Spigel, Y., Cohn, E. and Spigel, S. (1982). Characterization of the sialyl and galoctosyl resudies on the body wall of different plant parasitic nematodes. *J. Nematol.*, 14, 33-39.

Timper, P., Kaya, H.K. and Gaugler, R. (1988). Dispersal of the entomogenous nematode *Steinernema feltiae* by infected adult insects. *Environ. Entomol.*, 17, 546-550.

Walker, T.J. (1984). Mole crickets in U.S.A. *Fla. Agric. Exp. Stn. Bull.* 845, 54.

Womersley, C.Z. (1990). Dehydrogeneration survival and anhydrobiotic potential. *See. Ref.*, 68, 117-137.

Woodring, J.L. and Kaya, H.K. (1988). Steinernematid and Heterorhabditid nematodes. *Crop. Ser. Bull.*, 331, 1-30.

Wouts, W.M. (1991). *Steinernema* and *Heterorhabditis* species. *Manuel of Agriculturel Nematology*, 855-897.

Zimmerman, R.J. and Cranshaw, W.S. (1990). Compatibility of three entomogenous nematodes in a aqueous solutions of pesticides used in turfgrass maintenance. *J. Econ Entomol.*, 83, 97-100.

Zimmerman, R.J. and Cranshaw, W.S. (1991). Short term movement of *Neoplectana* spp. *Neoplectana* spp. *J. Econ. Entomol.*, 84, 875-878.

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