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ENTOMOPATHOGENIC NEMATODES

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The nematodes establish various and characteristic relationships with the insects; in particular the EPNs belonging to the orders Mermithida, Rhabditida, Aphelenchida and Tylenchida, show interesting association with exapods and some species are used in controlling insect pests. In particular the Steinernematidae and the Heterorhabditidae that are widely widespread in numerous habitats in almost all continents, harbor in their gut symbiotic microorganisms pathogenic for many arthropod species.

KEY WORDS: facultative parasitism, obligate parasitism, symbiosis, phoresy, non-insect parasite.

INTRODUCTION

Nematodes and insects are highly capable of colonizing ecosystems. One predominant group of nematodes has been closely related to hexapods, ranging from phoresy to symbiosis, to facultative or to obligate parasitism. The present study deals specifically with nematodes EPNs (entomopathogenic or entomoparasitic nematodes) in particular, those belonging to the orders Mermithida, Rhabditida, Aphelenchida and Tylenchida, that have prompted greater interest due to their association with hexapods and their practical application in controlling harmful insects.

MERMITHIDA

Members of Mermithidae Braun 1883 are diffuse worldwide and evolve at the expense of invertebrates. The species vary in length, between a few millimeters to 1 m (e.g., in sea urchins) and can easily be seen in the integument of host larvae and especially when leaving the host, when they assume a whitish color. Given their parasitic behavior whereby causing mortality in the host, these species have long been considered as potential candidates for the biological control of pests.

The aquatic species of the Mermithidae family are found in mild and tropical areas, moist soils, swamps, rivers and lakes. Their parasitic activity varies considerably in relation to the pH of the habitat. PAINE and MULLENS (1994), for example, report that *Heleidomermis magnapapula* Poinar and Mullens, a parasite of the Diptera: Ceratopogonidae *Culicoides variipennis* (Coquillett) widespread in the habitats of California, very rich in organic nutrients and does not thrive in saline and alkaline habitats. Another Mermitide species is *Romanomermix culicivora* Ross and Smith. Originating in the southern regions of the US, where it attacks the larvae of many Diptera Culicidae, *R. culicivora* has been utilized as part of control strategies in various zones prior to being supplanted by *Bacillus thuringiensis* spp. *israelensis*. *Culex* larva is parasitized by dauer juveniles (DJs) penetrating with the stylet or by ingesting nematode eggs; in either case, the nematode grows in the host it feeds upon. The host continues to thrive and is abandoned before it reaches the pupal stage. The DJs become adults, copulate and lay their eggs. Usually, mermithids reach the adult stage immediately after entering host larvae (NICKLE 1972, 1984) and their growth interval varies greatly according to water temperature. In the biotope mermithids encounter numerous antagonists such as gammarids, pre-immaginal stages of the dragonfly, water beetles, with mainly copepods limiting their population (PLATZER & MACKENZIE-GRAHAM, 1980). The life cycle of mermithid parasites of terrestrial hexapods is similar to the life cycle of the aquatic species, but must overcome the risk of egg dessication. In order to parasitize grasshoppers, the females of *Mermis nigrescens* Dujardin spp. leave the soil to deposit their eggs on plants only when the humidity level is high (i.e., with rain or at night). Then the eggs are ingested by the grasshoppers where they hatch. A case in point regards Formicidae spp., which being "mermithized" undergo morphological alterations whereby the workers develop features typical of the intermediate stage between soldiers and queens. The queens are shorter than in the initial stage and have a smaller thorax; they are brachyptera, physogastric and microcephalic. In contrast, males develop shorter wings, a larger head, larger eyes and other morphologic features that are typical of workers (NICKLE, 1974). The degree of deformity depends on the time period in which parasitization occurs and is more evident in specimens which have previously undergone nematode infestation.

RHABDITIDA

Among the EPNs associated in various ways with insects, the order Rhabditida Oerly, 1880, and in particular the families Steinernematidae (TRAVASSOS, 1927) and Heterorhabditidae (POINAR, 1975), have been found to be widespread in numerous habitats in every continent, except for the Antarctic whose colder and temperate zones are preferred by Steinernematidae and the warmer and tropical regions are populated by Heterorhabditidae (HOMINICK *et al.*, 1996). The members of this family, commonly known as EPNs, are easily reproduced in the

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laboratory by utilizing, for instance, the larvae of *Galleria mellonella* L. (Lepidoptera Galleriidae) and *Tenebrio molitor* L. (Coleoptera Tenebrionidae) or on the industrial scale by employing fermentors. The intestine of these species contains symbiotic microorganisms that are pathogenic for many arthropod species; the symbiotic proportion nematode-bacterium determines whether the nematode protects the bacterium from the environment and transports it into the host, while the bacterium supplies food to the nematode promoting its growth. Both of the above-cited families are found in soil and thrive as parasites and saprophytes. The infectious stage (DJ) is the third stage of development which, depending on the species, actively searches for its victim ("cruiser strategy") or waits until the victim approaches in order to attack, even by leaping ("ambusher strategy"). Penetration of the insect occurs using the mouth, anus, spiracle or even by perforating the thin intersegmental membranes. The aim is to enter the hexopod's body cavity and release in the hemolymph the symbiont bacteria (*Xenorhabdus* spp. for Steinernematidae and *Photorhabdus* spp. for Heterorhabditidae) present in the intestine. The insects killed by the bacteria assume characteristic hues: the victims of *Xenorhabdus* spp. become grayish, whereas insects whose tissue is infested by *Photorhabdus* spp. become light to dark red or greenish and are luminescent in the dark. Once in the hemolymph the bacteria multiply rapidly, producing a wide range of toxins and exoenzymes that cause host mortality and transform the tissue into food for EPNs. In addition, antibiotic substances are synthesized and excreted favoring the growth of symbiont bacteria (AKHRUST, 1982; WEBSTER *et al.,* 2002). The species belonging to these two families have similar life cycles; however, in Steinernematidae the first generation is anphigonic, whereas in Heterorhabditidae the first generation is composed of hermaphrodite females that produce second-generation anphigonic populations. The life cycle is completed in a few days and hundreds of thousands of new DJs emerge from the host, which is by now entirely decayed, in search of new hosts. One sample of *Heterorhabditis* spp. entering the host is sufficient to produce a new generation, whereas at least two samples of *Steinernema* spp. are required, since these are gonochoric. As food becomes scarce, nematode growth is stunted at the DJ stage and the same DJs leave the host. The in-field discovery of insects parasitized by rhabditids is quite a rare phenomenon given that dead insects rapidly decompose in soil and become prey for other insects and vertebrates (PETERS, 1996). The probability for this occurrence becomes even greater when samples are taken from areas in which insect epizootic is in course. A recent finding concerns *Steinernema affine* (BOVIEN, 1937) WOUTS, MRÁCEK, GERDIN & BEDDING, 1982, in the region of Tuscany in the soil of a pine forest infested by *Traumatocampa* (*Thaumetopoea*) *pityocampa* (Den. et Schiff) (Lepidoptera Thaumetopoeidae). Among the various techniques used to extract EPNs from the soil (MRÁCEK, 1980), the most effective employs the larvae of *G. mellonella* in tea filters of metallic mesh placed in the soil. The larvae attract the rhabditids present in the soil and in several days cause their death and grow in the tissues. DJs are collected from the parasitized larvae placed in water traps and can be refrigerated for a few months at 4°C (for Steinernematidae) and at 10°C (for Heterorhabditidae); nitrogen refrigeration, however, is more advantageous.

APHELENCHIDA

Many species of the order Aphelenchida engage in phoretic behavior with insects, whereas other species such as *Parasitaphelenchus, Bursaphelenchus* and *Ektaphelenchus* are hexapod parasites and are tightly linked to the insect's habitat. The genus *Parasitaphelenchus* carries out its life cycle by feeding upon fungi and insects. The DJs penetrate the beetle larvae and turn into the fourth stage. Because the growth of this species is dependent on fungi and can only partially utilize the nutrients within the insect's haemocoel, *Parasitaphelenchus* is forced to leave the host and return to the environment to feed on fungi and complete its biological life cycle. For example, *Bursaphelenchus xylophilus* (Steiner and Buhrer) NICKLE *et al*., originating in southern regions of North America is the cause of mortality of pine trees both on the American continent as well as in Asia. In Europe *B. xylophilus* was detected for the first time in Portugal in 1999 on *Pinus pinaster*, and was intercepted in Austria, Finland, France, Norway and Sweden when found on raw wood crates introduced from North America and China. Till now this species has not been detected in Italy, yet "Regional Observatories" have been alerted to report its presence. This endoparasitic nematode carries out its life cycle by feeding on either fungi such as *Ceratocystis* spp., which is responsible for the bluing of wood, of the host plant; here the life cycle is strictly associated to that of the vector insect. *Ektaphelenchus* spp., which is normally present on the bodies of wood-boring beetles, was also detected internally. A substantial number of obligate parasites of the families Neotylenchidae, Sphaerularidae and Allantonematidae are tightly linked to hexapods and mites and belong to the order Tylenchida. Among the Neotylenchidae, now part of the family Beddingiidae (POINAR *et. al*., 2002), is worth including *Beddingia* (*Deladenus*) *siricidicola* (Bedding), a parasite of the larvae and adults of *Sirex noctilio* F. (Hymenoptera: Siricidae) that is very damaging to pines. The insect lays its eggs in the wood and also transfers the spores of *Amylostereum areolatum* (Chaillet) BOIDIN, 1958*,* which form the nourishment for the young nematodes. Adverse conditions, such as the drying of wood, generate females with longer stylets that penetrate the Siricidae larvae and reach maturity. The eggs hatch in the insect's body cavity and the nematode pursues the host in its growth until the adult stage is reached where it enters the reproductive system. In Australia remarkable results have been obtained controlling Siricidae by employing *B. siricidicola* (BEDDING & AKHURST, 1974). A peculiar behavior is noted with the Sphaerularidae family to which *Spherularia bombi* belongs, a parasite of the *Bombus* and *Psityrus* spp. The parasitized queen bee flies continuously without, however, being able to build its nest in the soil as do healthy queen bees. Frequently, the queens return to the first nest where third-stage nematodes abandon the host and mutate in the soil, copulate, and infest the queen bees as soon as they come out of hibernation. As a result, the queen bees become sterile and incapable of forming new colonies (TRIGGIANI, 1991). Of note is the species *Heterotylenchus autumnalis* Nickle of the family Allantonematidae; the fertilized females parasitize the larvae of *Musca autunnalis* De Geer (Diptera Muscidae) in escrements and manure. Having penetrated the haemocoel of the victim they deposit their eggs from which hatch young nematodes that evolve into parthenogenetic females which, in turn, lay hundreds of eggs. The young larvae thus migrate to the oviducts of flies that will deposit them in the manure together with their eggs. At this stage the life cycle complete.

THYLENCHIDA

To the order Thylenchida also belongs the species *Praecocilenchus ferruginophorus* (RAO & REDDY, 1980). This species was found in India in the trachea, intestine and fat tissue of larvae and in the uterus and haemocoel of the adults of *Rhyncophorus ferrugineus*, a well-known weevil beetle harmful to palm trees. The nematodes are most likely released when the infected female deposits her eggs, but may also pass into the feces through the intestine. The female beetles' ovaries are stunted in growth and as a consequence the production of eggs as well. Research is under way to investigate the possibility of utilizing *P. ferruginophorus* to control *R. ferrugineus.*

EXAMPLES OF BIOLOGICAL CONTROL UTILIZING HETERORHABDITIDAE AND STEINERNEMATIDAE

Numerous predators and parasites have been and are currently being used to carry out biological control; some of these have obtained interesting results while others have failed. Various orders, families and genera of invertebrates have proved to be effective and efficient antagonists; however, only some are capable of predating or parasitizing soil-dwelling insects. Among such insects are the nematodes of the families Heterorhabditidae and Steinernematidae. Interest in these EPNs has been continuously increasing not only because they are highly effective and simple to employ but also because many pesticides have been withdrawn from the market due to the risk involved for humans and the environment. Frequently, new species of EPNs are discovered and described. Of all the European countries, Italy is endowed with the highest degree of biodiversity (TARASCO *et al.,* 2009). Some results obtained by us are worth mentioning and are briefly cited as follows: winter control of the larvae of *T. pityocampa* inside their own silk nests (TRIGGIANI & TARASCO, 2002), control of larvae of *Rhytidoderes plicatus* Oliv. (Coleoptera Curculionidae) in soil (TARASCO & TRIGGIANI, 2002), of *Corythucha ciliata* Say (Rhynchota Tingidae) colonies overwintering under tree bark (TARASCO & TRIGGIANI, 2006), of *Xanthogaleruca luteola* (Coleoptera Chrysomelidae) on the leaves of elm trees (TRIGGIANI & TARASCO, 2007), and control of *R. ferrugineus* (Coleoptera Curculionidae) (Triggiani, in press). Also worth mentioning is the species *Phasmarhabditis hermaphrodita* (Schnieder) (Nematoda, Rhabditidae), a "non-insect" parasite utilized successfully to control slugs; introduced in the British market in 1994, *P. hermaphrodita* has been employed in many European countries under its registered name Nemaslug© (WILSON & GRAWAL, 2005).

CONCLUSIONS

The present investigation reveals that EPNs play a very important role, of both scientific and practical interest, also due to the characteristic association they establish with insects. Though the action of *S. bombi* may be drastic for wild pollinators (in Apulia during the spring season parasitic activity greater than 90% was detected for the queens of *Bombi* spp. (TRIGGIANI & TARASCO, 1997) and although *B. xylophilus* is capable of decimating vast extentions of pine trees, species of Steinernematidae and Heterorhabditidae may be readily used in support of or even to substitute pesticides. The most suitable species can be found on the market and distributed utilizing the same tools as those used for pesticides. As living organisms it follows that the proper and successful use of EPNs be linked to rigorous values of T° and HR at the precise moment in which they are employed and also to the growth stage of the insect and its habitat. The application of EPNs in field must be assessed on an individual basis to obtain optimal results.

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