APPLICATION OF ECOLOGICAL INFORMATION FOR PRACTICAL USE OF INSECT PATHOGENIC NEMATODES

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ABSTRACT

The use of pathogenic nematodes from four families to control insect pests is discussed. Explanations for the success or failure of specific programmes are proposed and related to the need for detailed understanding of the ecology of the nematodes and their hosts. Our conclusion is that for the forseeable future, pathogenic nematodes will continue to provide novelty and will prove to be increasingly necessary.

INTRODUCTION

Nine families of nematodes have adverse effects on insects and four, the Mermithidae, Allantonematidae, Steinernematidae and Heterorhabditidae, have been investigated for insect control programmes. This short review concentrates on ecological issues that pertain to the success or failure of the nematodes in control programmes. We concentrate on Europe and steinernematids and heterorhabditids, the so-called entomopathogenic nematodes, because these have received a great deal of scientific and commercial attention. Mermithids and allantonematids are covered only to the extent that they provide lessons that should be learned in applying ecological information to practical control of insect pests.

MERMITHIDAE

Mermithid nematodes are obligate and lethal parasites of invertebrates, particularly terrestrial and aquatic arthropods. They gain entry to their host, usually an early instar, by being ingested as eggs or penetrating across the cuticle as second stage juveniles. Once in the haemocoele, they undergo phenomenal growth, absorbing nutrients from the haemocoelic fluid and storing them in a modified intestine called a trophosome. Eventually, they occupy most of the haemocoele while the insect continues to live and feed. When they emerge from the host as post parasitic juveniles, the insect dies within hours. The nematodes complete their development in the environment, living on their stored nutrients. Depending on the species, they emerge from late instar larvae, pupae or even adults.

Application of mermithids for biological control has been generally aimed at classical techniques, whereby the nematodes are released into an environment, become established, and exert acceptable levels of control thereafter. Inundative methods, using the parasites as bioinsecticides, have also been proposed, providing mass rearing techniques could be developed. Hominick and Tingley (1984) assessed the limitations of mermithids for vector control. Based on population biology models, the conclusion was that mermithid populations are controlled by such strong density dependent constraints (environmental sex determination, parasite-induced host mortality and reduced fecundity of crowded females), that they can cause only moderate long-term depressions in their host populations. Their long generation times compared to their hosts will tend to produce cycling in abundance of the insects, with periodic breakdown in control. While there are no good quantitative data to determine the effect of mermithids on populations of agricultural pests, the conclusions are probably the same. Thus, the lesson is that even though these parasites are lethal and there are numerous reports of insect populations with high levels of mermithid parasitism at particular times, ecological information relating to regulation of the parasite population indicates that it is unlikely that these parasites exert significant long term control over host populations.

Mermithids may have use in inundative programmes, as part of an IPM approach, but this requires careful monitoring of host populations so that releases are at appropriate times. Also, hosts are not killed immediately, and can do substantial damage while the parasite is developing inside. In any case, these nematodes cannot be mass produced *in vitro,* and *in vivo* production is labour intensive, while storage and formulation are also problems yet to be solved. This is the second lesson from mermithids - unless parasites can be produced reliably and cheaply, their potential cannot be realised. Petersen (1985) and Popiel & Hominick (1992) can be consulted for detailed reviews of mermithids and biological control.

ALLANTONEMATIDAE (syn. NEOTYLENCHIDAE)

The most successful example of a nematode being used as a classical biological control agent against an insect pest is that of *Deladenus siricidicola* for the woodwasp *Sirex noctilio*. This European insect was accidentally introduced into Australia and New Zealand and caused extensive damage to *Pinus radiata* forests. CSIRO scientists were sent to Europe to search for natural enemies of the wasps, and a remarkable nematode was discovered, with a life cycle showing incredible adaptations to the wasp life cycle. When female *Sirex* oviposit into a tree, they also supply a symbiotic fungus and a toxic mucus. *Sirex* larvae feed on the fungus, which eventually permeates the whole tree. The nematodes are facultative parasites, which means that they have two possible life cycles. One depends on the fungus, and the nematode can go through a number of generations, feeding on the fungus. The other is a parasitic life cycle whereby the nematodes penetrate into a woodwasp, reproduce, penetrate into the eggs of the wasp and sterilise them, and are deposited by the female when she oviposits. The nematodes then emerge from the sterile egg and feed and multiply on the fungus. Hence, the female wasp is not only sterilised, but also disperses the nematodes and provides them with food in a new tree.

Infection levels of *Sirex* approach 100% and the population collapses. Bedding (1984) provides details of this remarkable nematode and its use in controlling the wasp.

It is worth considering the success of this programme in more detail, because it has important general lessons to teach us for biological control, regardless of the agent (Bedding, 1984). First, it is essential to understand the biology of both the host and the parasite in great detail, so that the parasite can be manipulated and used effectively on a large scale. Here, the parasite was found to be highly host specific, so it will not affect beneficials in the system. Also, it is highly adapted to its host, so much so that the host efficiently disperses the parasite and even unwittingly furnishes it with a food supply. This meant that it was not necessary to apply the parasite throughout a forest. Instead, it could be inoculated in part of the forest and the wasps acted as the dispersal agents. Second, it is critically important to be able to culture and store the nematodes. Even before commercialisation, this is necessary for experimentation and development of the system to the point of large scale application. In this case, the insects have a 1-3 year life cycle, so *in vivo* rearing of the parasites would be impractical. The alternative, fungal feeding life cycle facilitated mass production in the absence of the hosts. It also allowed the nematode to multiply in the environment of the host, but in the absence of host individuals, effectively increasing the numbers of infective agents. Ironically, this ability is a disadvantage from the point of view of a biological control company. A third general lesson that Bedding (1984) did not mention relates to *Sirex* as an 'outbreak' pest. Such pests are relatively unstudied in their native ranges because they are not pests, so it is the existence of their many natural enemies that must be established first, well before the biology of the enemies becomes an issue. This nicely leads us into a consideration of steinernematids and heterorhabditids, the two families which offer the most potential for biocontrol of insects from a practical and commercial viewpoint.

ENTOMOPATHOGENIC NEMATODES

Nematodes belonging to the Steinernematidae and Heterorhabditidae are referred to as Entomopathogenic Nematodes (EPNs). In contrast to the nematode species described above, EPNs have been commercially developed as bioinsecticides. EPNs are attractive candidates for commercial exploitation because several different species can be produced economically, formulated and applied to a range of soil dwelling insect pests. The resulting product is easy to use and can provide a 'one off' solution to a specific insect pest problem. Recent reviews providing details not covered in this paper, are those of Georgis (1992); Kaya & Gaugler (1993); Popiel & Hominick (1992); Georgis & Manweiler (1994); and Ehlers & Hokkanen (1996).

Europe has been viewed as an attractive market for EPNs sales following legislative changes reducing the use of pesticides. Political pressures have ensured that most of the major agro-chemical companies have made a commitment to environmentally friendly products. EPNs are manufactured by three main companies : Thermo Trilogy Ltd (formerly biosys), Microbio Ltd and Koppert Ltd. These focus on biological products which are distributed through a number of marketing partners who use the products to supplement their 'green range'. A survey by the European COST Action 819 named products in eleven European countries. These were based mainly on three species, *Steinernema feltiae*, *S. carpocapsae* and *Heterorhabditis megidis*. Pest targets have been limited mainly to the black vine weevil *Otiorhynchus sulcatus* in ornamental crops and sciarid fly species in ornamentals and mushroom crops. By contrast, *S. carpocapsae* and *S. riobravis* have been used extensively against a wider range of insects in citrus, cranberry and mint markets in the USA and in turfgrass markets in the USA and Japan.

Life cycle.

Commercial EPN products consist of the third stage juvenile (infective juvenile or dauer larva) of the nematode life cycle. It is the only stage of the life cycle that can survive in the environment. It does not feed, but searches for an insect host to infect. In general, infective juveniles are released from the formulation by adding the product to water which is then sprayed topically around the root zone of the crop. The infective juveniles move through the water film around soil particles, actively searching for an insect host and presumably following chemical gradients including carbon dioxide and excretory products released over an extended period. The insect larval stage is the usual target because:

- It is the stage causing damage to the crop.
- It is relatively static allowing chemical gradients to build up in the soil.
- The soft larval bodies tend to be more susceptible to infection.

Once a host has been located, the infective juveniles penetrate through the mouth, anus or spiracles. Infective juveniles of *Heterorhabditis* species may also penetrate directly through the cuticle. Eventually the infective juveniles enter the haemocoel and release a symbiotic bacterium (*Xenorhabdus* spp. from *Steinernema* spp. and *Photorhabdus* spp from *Heterorhabditis* spp). which proliferate and kill the insect through septicaemia. Once the bacteria become established, nematode development begins as the infective juveniles mature into males and females (*Steinernema* spp.) or hermaphroditic females (*Heterorhabditis* spp.). One or more generations of progeny develop, reproducing continually until the resource of the host is exhausted. At this time, the third stage juveniles retain their second stage cuticle and are released from the cadaver as the insect disintegrates. This cycle will continue in the presence of sufficient insect hosts. The initial application of infective juveniles, according to label recommendations, should have interrupted the insect life cycle and provided adequate crop protection. However, pest population control becomes less predictable with time because the reproductive rate, dispersal and persistence of infective juveniles are unknown. (See discussions of persistence of fungal pathogens (Thomas) and viruses (Hails) in these proceedings).

ECOLOGY OF ENTOMOPATHOGENIC NEMATODES

The label on a commercial product will state the target crop, insect pest and stage of its life cycle, the EPN rate, timing and application method and optimal environmental conditions. Compliance with the instructions should enable a grower to apply the product and achieve consistent crop protection. However, during the early stages of development of an EPN product, considerable ecological information is required to generate the label recommendation. Indeed, there are a number of key factors that potentially influence the interactions of entomopathogenic nematodes with their insect hosts. It is important to understand these if the nematodes are to realise their full potential. It is beyond the scope of this review to elaborate on each, and a number of comprehensive reviews already exist, so a summary with one or two key references is provided below:

Persistence (see Smits, Kaya & Koppenhofer, and Hominick *et al* in Ehlers & Hokkanen, 1996):

There are few long term studies of EPNs, but it is clear that persistence occurs. However, the population biology of EPNs is not understood and the effects on natural insect populations are unknown. The nematodes may regulate an insect population, but epizootics are difficult to detect and hence rarely recorded. It is equally likely that insect populations regulate the nematodes, that is, the nematodes persist and reproduce opportunistically as a susceptible host becomes available. It is difficult to quantify the nematodes in a soil sample, as results depend on the method of extraction used and the nematodes are very patchy in distribution. Standard soil extraction techniques for nematodes recover all nematodes, so EPNs need to be removed and identified separately, a laborious procedure. Bioassays utilising a susceptible host recover a portion of the population, and negative assays may reflect absence of nematodes or lack of infectivity of the resident population. It is known that infectivity of a population can vary or even cycle. Natural populations occur at low levels, with fewer than 10 individuals per 100 cc of soil the norm. The influence of abiotic factors on EPN persistence is documented to varying degrees while the effect of biotic factors is largely unknown.

With so many unknowns and variables, it is to be expected that entomopathogenic nematodes will be applied inundatively, at doses ranging around a half million per square meter, so that efficacy does not rely on their multiplication in the soil habitat. EPNs are biological insecticides and the pattern of the population change post-application is a rapid decline in the first few days, followed by a moderate decline over the next 2-6 weeks, and then a long period of recycling at a low level. That is, population density decreases to background levels within days or weeks after application.

Dispersal and geographical distribution (see Downes & Griffin and Hominick *et al* in Ehlers & Hokkanen, 1996):

To understand geographical distribution (and host and habitat specificity), it is important to identify accurately the nematodes that are isolated. Unfortunately, this is not always done, and so our knowledge is limited. Entomopathogenic nematodes can move only centimeters under their own power. However, the fact that some species such as *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* are essentially ubiquitous implies that dispersal for at least some species is highly efficient and probably occurs by a variety of means, including active methods by hosts and passive ones such as wind and water. It may be coincidence that these ubiquitous species are the three dominant species commercially. Is this because they were easy to isolate or most frequently isolated? Or, is it because they are generalists and hence useful in a wide range of applications? Also, commercial activities may have influenced natural dispersal because introductions could occur in areas where the nematodes do not occur naturally, but they become established after application. Only a small part of the globe has been surveyed for EPNs, but it does appear that some species are more restricted in their distribution than others. If the reasons for such differences were known, they could perhaps be exploited for particular biocontrol programmes.

Host and habitat specificity (see Peters, Simoes & Rosa, and Hominick *et al* in Ehlers & Hokkanen, 1996) :

Information on the natural host range of entomopathogenic nematodes is rare. Natural infections are transient and hence are infrequently observed. The literature implies a broad host range, but this is based on artificial laboratory bioassays and the image of EPNs as extreme generalists is being eroded. Clear distinctions must be made between laboratory host range and field host range, the latter involving the range of hosts successfully controlled by inundative release of nematodes, as distinct from the range of insects on which a naturally occurring nematode population propagates. The natural host range is the least understood.

Appreciation of habitat specificity is in a state similar to that of host specificity. That is, entomopathogenic nematodes were assumed to have little or no habitat specificity. This assumption is based on results where sample sizes or sampling strategies failed to allow for the aggregated distributions of EPNs, compounded by unreliable identification of the nematodes isolated. As more surveys occur, providing large sample sizes, and accurate identification occurs based on agreed morphological characters and DNA techniques, some habitat specificity is becoming apparent. This should not be surprising, as all organisms have niche requirements that will be satisfied only in particular habitats. Similarly, the soil habitat of the nematodes has three dimensions, so specificity may extend to occurrence at particular depths in the soil. Obviously, knowledge of host and habitat specificity is fundamental for matching the best nematode to a particular insect pest for a control programme.

Physiology and behaviour (see Downs & Griffin and Glazer, in Ehlers & Hokkanen, 1996) :

There are a number of studies and reviews on the behaviour of EPNs. Two basic strategies for host finding are possible, namely actively searching or passively waiting and presumably conserving energy until a host essentially contacts the nematode. These strategies are not mutually exclusive, so within population variation is possible. Thus, migrators and non-migrators may occur in a population, and the tendency to migrate or respond to hosts may change over time for a particular individual. In at least some species, infectivity may also vary, so that the population of nematodes exists as two sub-populations, one infective, the other not. The proportion that is infective can vary significantly over time. There is no indication of the relative importance of endogenous and exogenous cues in controlling these behaviours.

Since EPNs are found in a variety of habitats, from tropical to sub-Arctic and arid to moist, they have survival mechanisms to cope with particular environmental stresses. These include tolerance to extreme temperatures, desiccation, osmotic changes and lack of oxygen. The nematodes are also compatible with use of most insecticides and fungicides. Understanding the survival mechanisms could be a key component in eventually selecting optimal species or strains for particular programmes. Introduction of appropriate non-indigenous strains into particular habitats could be facilitated and result in increased efficacy. On the other hand, if long-term establishment is not desired, then a specific strain with a high susceptibility to a particular environmental stress, could be utilised. Certainly, understanding the mechanisms of survival are important for commercialisation, as these are fundamental for maximising survival during storage by appropriate formulation of the nematodes.

ECOLOGY AND COMMERCIAL CONSIDERATIONS

Regulatory Policy Issues (see Ehlers & Hokkanen 1996).

Regulatory policies for commercial products where the active ingredient is based on micro or macro organisms have been derived from a combination of the standard information requested from conventional pesticides and ecological information available at the time. In general, Europe and North America have the most established regulatory systems for biological products. While the need to standardise European legislation has been recognised, the reality is that commercial companies have to cope with a range of regulations country by country.

Where no registration is required, an EPN product may be introduced within two years subject to sufficient provision of data to convince the distributors and growers. In the UK no registration costs are incurred, but because EPNs are classed as 'animals' regulation occurs through the Wildlife and Countryside Act 1982. In this instance only indigenous 'kinds' of animals can be introduced. In Germany it is illegal to introduce exotic species of EPNs if they are likely to become established. However, exotic species can be introduced if they will not survive more than one season. It is not illegal to sell products based on exotic species of EPNs but it could be illegal to use them. A more extreme example has developed in Malaysia. The introduction of exotic EPN species is forbidden. The rational behind this decision is based on the concern that in laboratory bioassays the oil palm pollinating weevil *Elaeidobius kamerunicus* was susceptible to EPN infection. Palm oil production is a major industry in Malaysia. The weevil is an exotic species introduced to facilitate oil palm pollination. However, the oil palm weevils inhabit the foliage and do not enter the soil, the only environment where they would be susceptible to infection by EPNs. The naive interpretation of artificial laboratory data, without regard for the insect and EPN species biology, has regrettably prevented access to products for use by Malaysian growers of other crops who are obliged to compete in the international market without the benefits that such non-chemical pesticides might bring.

An OECD workshop on scientific and regulatory policy issues for the use of non-endemic EPN species was held in 1995 and the proceedings are available (Ehlers & Hokkanen, 1996). The participants identified the following special pertinent features and ecological facts for EPNs:

1. Natural epizootics are rare.
2. The host range of EPNs in the field is limited, in contrast to the broader spectrum of activity obtained experimentally in the laboratory.
3. Effective control of susceptible insects by EPNs requires the application of large numbers of infective nematodes dauer juveniles ($<10^9$ ha $^{-1}$).
4. After inundative release (mass application), populations of EPNs decline rapidly to low levels that are comparable with natural densities.
5. There is little dispersal of EPNs after application.
6. EPNs have been widely used for many years in pest control without known detrimental effects.
7. EPNs pose much less threat to the environment than chemical pesticides. They substitute for some of the broad-spectrum pesticides currently used in soil.

Based on these and other considerations, the unanimous opinion of the workshop was that EPNs should not be subject to any kind of registration. However, in some cases their use should be regulated and the introduction of exotic species is a good example. Legislators who have access to such informed interpretation of ecological information are able to form useful policies protecting the interests of the environment without unfairly restricting commercial development.

Commercial Development

Commercial development has been a double edged blade. While it has stimulated and supported a great deal of research, it may have complicated interpretation of ecological information due to wide application of a few species.

The success of an EPN bioinsecticide depends not only on technical information based on ecological data, but also on business issues. Growers require a great deal of educating and support in the first instance to achieve the potential offered by a product. When they ask, 'Does it work?' they are making the last assessment of the product NOT the first. Prior to this, a manufacturing company will have achieved a number of product objectives from a specific EPN strain. From a commercial point of view the EPN strain should exhibit the following qualities:

1. It must be easy to produce on a large scale. Once a production specification has been established, the strain should be robust in production i.e. the yield of infective juveniles should be consistent and the quality (lipid levels and pathogenicity) reliable.
2. It should be suitable for the formulation available. That is, the infective juveniles should survive for the specified shelf life and remain pathogenic during the life of the product, delivered to the grower in optimum condition.
3. It should be pathogenic over a wide temperature range. This allows wide application against the same pest (for example, in Northern and Southern Europe).
4. It should not be host specific, allowing the development of the same bioinsecticide against a range of pests and for a number of crops and markets (retail, horticultural, agricultural). However, in direct contrast, the EPN strain should also have minimal impact on non-target organisms, reducing environmental impact.

From this, it is clear that ecological facts and business priorities may conflict. Thus while the ecological facts tell us that each species will perform optimally in specific habitats and against a few pests, commercially it may be preferred to produce one species and, if necessary, apply larger doses to achieve control of a wider range of pest species.

Once the label recommendation has been established and consistent crop protection achieved, additional ecological information on the EPN population persistence, multiplication and dispersal is of limited commercial interest. However, it is possible that applications may fail without explanation. The commercial pressure to limit costs and hence restrict expensive ecological studies could mean that no solution for the failure is available because relevant data do not exist. It is difficult for a company to know when to restrict work and what work must be done to protect the efficacy of a product and its place in the market.

CONCLUSIONS

At present, the cost of EPNs make them economic in high value crops. In Europe, with basically 3 producing companies and 54 products chasing a few markets, it may be that costs could be reduced if there were fewer steps between production and the grower. Distributors in Europe demand a large profit margin from the product. Often they set a high price and accept small volumes of turnover. This automatically limits the manufacturer's activity and ability to work towards economies of scale. Is it commercially sustainable to produce only a few products for a range of insects using huge fermenters in big business? Is it better to apply our ecological knowledge and produce a wider range of species, each for specific markets, essentially exploiting niche markets through small companies relying on low technology? In developing countries, where high cost biological products may not be appropriate, it may be that indigenous EPNs could be produced on a low technology basis for local use with local labour, providing new business opportunities and reducing input of inappropriate and expensive chemicals. This is the current focus of the work at IIP.

To relate this paper to the overall theme of the symposium, Microbial Insecticides - Novelty or Necessity?, we would end by saying that pathogenic nematodes are both novel and necessary. Only a small proportion of insects and environments has been examined for pathogenic nematodes. How many equivalents of *Deladenus siricidicola* remain to be discovered? How great is the biodiversity of EPNs and are there species yet to be discovered which will prove useful in particular environments? At IIP, we have over 25 species new to science, and hence with unknown biological attributes, and most of the world remains to be explored. Working from the opposite tack, colleagues from other laboratories are exploring genetic attributes that could lead to manipulation of currently used species to make them more effective. In any case, novelty cannot be questioned. Neither can necessity, as the need to reduce chemical pesticides in an IPM approach to insect control is a general goal in crop protection. Pathogenic nematodes do play a part and, in our opinion, promise to play an increasingly important part.

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