# Biocontrol: The Potential of Entomophilic Nematodes in Insect Management[1]

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*Abstract:* A review of the development of entomophilic nematology and a commentary on the potential of entomophilic nematodes in controlling insect pests. The paper considers some of the major contributions to our knowledge of entomophilic nematology; factors involved in insect pest management and how they are applicable to the use of nematodes; nematodes which are most promising as biological control agents; and problems to be solved to facilitate the use of entomophilic nematodes in insect management. *Key Words: Mermis nigrescens, Neoaplectana* spp., *Romanomermis culicivorax, Deladenus siricidicola, Tetradonema plicans.*

> "A modern endeavour is to bring insect pests under natural control . . . . . The 'natural control' of insects will be most effective if all possible agencies and factors are utilized; among these agencies nemas are by no means negligible."

This is a quotation from Nathan Cobb (12) in the 1927 USDA Yearbook of Agriculture. In recent years, pest management philosophy has developed in ways that avoid harm to useful organisms. As nematodes that kill pest insects are usually harmless to other organisms, they have enormous potential for use in place of chemical pesticides in pest management systems. Nevertheless, more than 50 years after Cobb's statement, this potential has yet to be fully realized. Why? In the following discussion I will give some of the reasons.

## MILEPOSTS

*Mermis nigrescens,* though atypical of the family, is often considered the type organism for entomophilic nematology and was described by Dujardin in France 137 years ago (16). About 20 years later, Sir John Lubbock, in Edinburgh (34), provided biological and taxonomic information on *Sphaerularia bombi* that widened contemporary perspectives on the nature of nematode-insect associations. Numerous publications in entomophilic nematology followed, especially in Germany, and this was crowned in the early part of the 20th century by a detailed taxonomic account of the mermithids by Hagmeier (27) of the University of Heidelberg. Cobb (10) entered the arena in 1920 with descriptions of several new species of entomophilic nematodes and followed by his famous observations on the biology and taxonomy of mermithids (11,13). During the first half of the 20th cen-

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tury Cobb in the United States, Filipjev in Russia, Steiner in Germany, and Bovien in Denmark made significant contributions to our understanding of the taxonomy of entomophilic nematodes, and Tom Goodey's presentation (22) to the Royal Society in 1930 on the biology and taxonomy of the tylenchid *Tylenchinema oscinellae,* a parasite of the frit fly, was, and still is, a fine example of detailed biological observation. Superimposed on these were Oldham's perspicacious comments in his paper "Helminths in the biological control of insect pests" published by the former Imperial Bureau of Agricultural Parasitology (39). Thereafter, interest in the subject waned for 20 years, except for occasional curiosity recordings such as that of Muspratt (35), who described mermithids in tree-hole breeding Culicidae.

Despite the mental perambulations of Cobb in 1927, and the enthusiasm and successes of the 1930s, little progress was made toward the application of entomophilic nematodes in the control of pest insects. An exception to this generalization was the work of Glaser and his colleagues, of the New Jersey Department of Agriculture (United States),who steadily pursued a line of research with rhabditid nematodes of the genus *Neoaplectana.* This was to provide a valuable base for subsequent applied studies. They spent several years examining the association of *N. glaseri* with the Japanese beetle; subsequently they developed *in vitro* culture techniques for this nematode and applied a similar species experimentally in the field (21). In 1955 Dutky (17), at the USDA Laboratory at Beltsville, pioneered the use of the DD-136 strain of *N. carpocapsae* for control of pest insects of orchards (17). This was followed in great anticipation by applications of this species to pests of vegetable and field crops, forest crops, and to turf crops.

During the same period Welch completed his studies, at Rothamsted Experimental Station, England on entomophilic nematodes of fruit flies and subsequently, at the Canada Department of Agriculture, Belleville Laboratory, published a sequence of papers (eg. 54, 56) that demonstrated the potential of nematodes for the control of pest insects. Into this gathering momentum of research stepped Rachael Carson, whose book (8) stimulated major shifts in the agricultural and social philosophies of the industrialized world. Since that time we have witnessed a plethora of publications in entomophilic nematology and this time, nematologists have focused more on the use of nematodes in insect control rather than on their taxonomy.

Many claim that tylenchids are possible biological control agents (37,45). However, the only major success has been that of Bedding (1,2,4,5), with the Commonwealth Scientific and Industrial Research Organization in Australia, using *Deladenus,* a tylenchid parasite of the *Sirex* woodwasp.

The mermithid parasites of insects have emerged as the most favoured nematological tool for insect control, and significant advances have been made in all aspects of our knowledge of this group—on their taxonomy by Rubtsov (48,57) and Welch (55,57), on their biology and ecology by Petersen (40, 43), and on the physiological and biochemical relationships with their hosts by Gordon and Webster (23,24), Rutherford (49), and Wülker (59). However, only one species is on the verge of commercial application, namely *Romanomermis culicivorax* on mosquitoes. Our knowledge of this nematode comes largely from 10 years of observations by Petersen (40-44) and colleagues at the United States Department of Agriculture Laboratory at Lake Charles. Laird, of the Research Unit on Vector Pathology in Newfoundland, Canada has been a strong advocate of the use of mermithids to control biting flies in temperate and tropical climates (32).

## INSECT PEST MANAGEMENT

Despite the long history of agriculture and its success in progressively increasing crop productivity, the goal of optimum economic production of a crop of high quality remains elusive. To achieve optimum quality and economic production, the development of a sound pest management scheme is essential for each crop. To do this, a systems approach constructed on a large data base should be used to enable simulation and forecasting of the various biological, economic, and environmental events that provide information upon which the pest manager can make his decision.

Entomologists have been prolific in their studies of pest biology, but there is a danger that research on modeling insect pest problems might terminate in the publication of a set of monographs rather than in the utilization of the results in an interactive, applied programme. In nematology we have just begun the modeling approach (30) on plant-parasitic nematodes. Nothing has been done using this technique with entomophilic nematodes to aid in an insect pest management system.

Pest management is usually adopted only when the traditional chemical control methods have failed. Belated attempts in difficult situations are not those likely to be successful. The use of pest management techniques, as distinct from those of pest control, is still not widespread, probably for the following reasons:

1) Growers have relied on the apparent instant success from repeated use of relatively cheap, convenient chemical pesticides that have been marketed by companies willing to provide help and advice on their application.
2) Good growers have used sound cultural practices that have helped to minimize pest damage, but do not, on their own, facilitate optimum crop production.
3) Biological control methods which have been recommended have not always been integrated with other control methods or with the interactive ecology of the crop and pest. Their consequent failure extended the poor reputation of such techniques.
4) Our knowledge of the interaction of insect pest populations and associated nematodes with their environment is insufficient to provide the data base necessary for pest management decisions.
5) The goal of integrated control is to obtain a high sustained yield with the least use of energy whereas, unfortunately, most agricultural policies aim at maximizing short-term yields with the associated great use of energy. As Corbet (14) pointed out at the 1972 International Congress of Entomology, the fundamental obstacle to integrated control is epitomized in this conflict of short- and long-term goals.

We are now conscious of the energy problem but insufficiently responsive to its solution. An energy shortage will hamper our ability to provide adequate nutrition for the world's growing population. The greater the energy shortage, the greater the destruction of arable land and the higher the costs of chemical pesticides. This provides an additional stimulus to increase our understanding of biological control and the use of pest management (51). Biological control agents may be added either by "inundation," whereby the introduced parasite is applied once in large numbers, or by "inoculation" (or augmentation), whereby the parasite is added to the pest's environment which is manipulated so as to enhance establishment of the parasite as a long-term factor influencing the insect population. Few cases of crop protection have occurred as a result of inundative application. This is probably because a successful parasite exhausts its supply of hosts, suffers high mortality, and then is unable to suppress the multivoltine pest individuals that were at the nonsusceptible stage at the time of release. Using nematodes, the inundation method may have an advantage where there is an inexpensive labour force to produce very large numbers of parasites at a relatively low cost. The inoculation method using nematodes has been hampered by our inadequate knowledge of the environmental factors governing the establishment of such a parasite population within the pest insect population.

For nematodes to be used successfully in pest management schemes in either the inundative or inoculative fashion, adequate understanding of a number of conditions are necessary (50):

1) Seasonal coincidence of the parasite and host.
2) Ecological coincidence of the parasite and host.
3) Physiological acceptance by the host of the parasite at the time of entry and during the parasite's endogenous development.
4) Environmental tolerance by the para-

site sufficiently long to locate and kill its host (inundative application) or to establish itself as a parasite (inoculative application).
5) Biological competitiveness with other parasites and predators in the host's ecosystem.
6) Dispersal of the parasite in the inoculative application adequate to ensure establishment.
7) Mass culture of the parasite inexpensively and rapidly, and the ability to store these cultures.

Four species of entomophilic nematode came close to satisfying these conditions, and at present are being exploited on an experimental or commercial basis.

1. *Deladenus siricidicola* is a parasite of the woodwasp, *Sirex noctilio,* which is a pest of *Pinus radiata.* Since its introduction into Australia from Europe, *Sirex* has become a serious pest in the states of Tasmania and Victoria, where it has killed thousands of hectares of trees. After 10 years of laboratory and field research, first in Europe and later in Australia, *Deladenus* was released at a *Sirex*-infested location in 1973 (2,4). The levels of parasitism of *Sirex* by this nematode increased rapidly, and this was followed by a decrease in the number of *Sirex*-killed trees. By 1977 no *Sirex*-infested trees were located in the original 400 hectare site (5). In forests within 2-13 kilometres of this site *Sirex* maintained its level of attack, but recently *Deladenus* has started to spread naturally into these infested areas. Bedding maintains (personal communication) that wherever *Deladenus* parasitism has reached 70-95% in the seeded areas no serious outbreaks of *Sirex* remain.

Favourable attributes of this nematode parasite are the following:

a) Large numbers are readily cultured in the laboratory on the fungus (*Amylostereum chailletii*) upon which it normally feeds during its alternate free-living cycle in the field.
b) It is easily transported to seeding sites either in *Sirex*-infested timber or as nematode cultures on fungal plates.
c) It is not exposed to desiccation as the nematodes are inoculated into the timber.
d) It maintains itself in the field for long periods in the absence of the insect host by feeding on its normal associate, the fungus. Hence, the problem of synchrony of parasite and insect host life cycles at application is minimized.
e) The natural dispersal of *Deladenus* is readily achieved by the parasitized host.

A relatively minor unfavourable attribute of this nematode is that culture must be established in the field by the rather time-consuming inoculation of individual trees. However, as a parasite introduced into a pest population by inoculation techniques, it shows considerable promise, and evidence to date suggests that *Deladenus* is associated with a decrease in *Sirex*-infested timber to an acceptable level.

2. *Neoaplectana carpocapsae* has, through its strains and closely related species, a very wide host range. Fourteen species of *Neoaplectana* have been recorded from natural infections in insects, and several of these have been cultured on a large scale by a variety of techniques (45). The ensheathed dauerlarvae of *Neoaplectana* species enter the insect's haemocoel and feed on the introduced bacterium (*Achromobacter nematophilus*) causing a lethal specticaemia in the insect.

Favourable attributes of this group of entomophilic nematodes are the following:

a) They can be cultured readily with their associated bacterium, on agar slants, or mass produced in *Galleria mellonella* larvae (3,4,5,53).
b) They may be readily stored for several months in an aerated, diluted formalin solution at about 4°C.
c) They can be conveniently applied as an "inundative" agent as either an aqueous spray (46,52,56) or by mixing with a bait and placing on infested foliage.
d) They are unharmed by many insecticides and in this respect are well suited for integrated control measures. For instance, when the effectiveness of *N. carpocapsae* was tested against *Hylemya* on tobacco, it was found to be as effective as diazinon

but somewhat less convenient to apply (9).

A major disadvantage of this group is that if they do not penetrate their host immediately, they are vulnerable to desiccation unless evaporation retardants are added to the aqueous suspension. A wide range of such retardants—fire retardants (52), glycerine (36), and oil/wax mixtures (3)—have been tested to minimize nematode desiccation. There are a large number of records of field applications of *Neoaplectana* in a wide variety of habitats. Often this has resulted in statistically significant decreases in the number of pests but economically insignificant decreases in the pest population and a failure to prevent crop depredation (25). Despite our incomplete knowledge of the value of this genus in decreasing pest populations, *N. carpocapsae* is being produced commercially in France for application against insect pests.

3. *Romanomermis culicivorax,* or *Reesimermis nielseni* as it was formerly known, is lethal to most species of mosquitoes which breed in permanent and semi-permanent habitats. We now have a substantial pool of knowledge on *in vivo* culture methods and on the environmental conditions necessary for survival of the pre- and post-parasites and for optimum infection of the host (2, 41). Considerable progress has been made by Nickle to satisfy government legislative bodies in the United States so as to facilitate field application (38).

Favourable attributes of *R. culicivorax* as a biological control agent are these:

a) It can be readily cultured on a mass scale in mosquito larvae.
b) The gravid females and eggs are readily stored and transported to sites of field application. It is ideally used as an "inoculative" agent but can be effective as an "inundative" agent in isolated habitats.
c) It has a narrow host range, and its lethal potential is restricted almost entirely to the culicids. However, there are records of laboratory infections of blackflies.

An example of the level of production necessary was a recent field trial against anophelines in El Salvador where 700 kilograms of infective *R. culicivorax* material was required (44). Unfortunately, even this quantity did not kill all the mosquitoes in the area. Although some people may be satisfied with 60% control of some agricultural pests, medical entomologists require closer to 98% mortality of insect vectors of disease in order to obtain a period that is sufficient to break the transmission cycle. The ease with which mosquito larvae are cultured is the basis for the success of mass culture of *R. culicivorax*. However, *in vitro* culture of mermithids would be preferable because a nutrient balance at a level conducive to the optimum sex-ratio, rapid development, and high nematode fecundity can be more easily maintained in culture media. All these factors are likely influenced by the dynamic availability of nutrients. We know, for instance, that female locust hosts generally contain more nutrients than males and nymphs, and that developing *Mermis nigrescens* remain longer, grow larger, and are more likely to become female in female locusts. This implies that emergence from the host may be influenced by the availability of a limiting nutrient or by the build-up of a toxic product in the haemocoel because of the diminished ability of the parasitized host to excrete (23). Our knowledge of the nutrient requirements of mermithids (24) and of their effect on the physiology and biochemistry of the insect host (15,23,26,49) should ensure the successful culture of mermithids on a synthetic medium. *R. culicivorax* has been maintained in culture for several weeks on an *in vitro* medium (19), but growth and development was abnormal.

Like other mermithids, *R. culicivorax* frequently has a patchy distribution and greatly fluctuating population levels. Rubstov (47) maintains that this is associated with an inability to reproduce and disperse rapidly. Poor dispersion results because the mermithid usually kills its host before the host matures. Mermithids ensure survival of their species by balancing such factors as poor dispersal against innate ability to increase rapidly, and by responding to the influence of environmental factors, such as inadequate food supply, with sex determination during ontogeny. Our understanding of the mechanisms of population density regulation in mermithids remains incomplete. *Perutilimermis culicis* was considered a possible alternative to *R. culicivorax* because it is a parasite of adult mosquitoes which would overcome the dispersal problem. However, its potential is limited because its host range is restricted to one mosquito species.

There are many records of field application of this nematode, but our knowledge of its effect on the long-term decrease in mosquito populations is limited because observations were terminated too soon. However, Nickle (38) recently claimed that the *R. culicivorax* released in mosquito breeding areas in Maryland (United States) have perpetuated and caused 50-100% mortality in the mosquito population after surviving two winters and minimum temperatures of −19 C. Petersen (personal communication) reports field infections persisting up to 6 years after application.

*R. culicivorax* is unlikely to be an effective biological control agent in the vast mosquito-infested areas in the northern latitudes, especially for spring *Aedes* species. The temperatures in the snow-melt pools are below the threshold for preparasite activity. Hence, application rates of preparasites (50,000/$m^2$) to achieve even a 30% infection under favourable conditions are impractical with present mass culture techniques (20).

Mermithids should be fully integrated into pest management programmes if their full potential as biological control agents is to be realized. They are resistant to many insecticides and are more host-specific than chemical insecticides (33).

There are now two reports that laboratory-reared mosquitoes have developed resistance to a nematode biological control agent. *Anopheles quadrimaculatus* larvae showed a twofold reduction in susceptibility to *Diximermis peterseni* after about 104 generations (58), and *Culex pipiens quinquefasciatus* developed resistance to *R. culicivorax* after 300 generations (42) but not after 100 (38).

The World Health Organization of the United Nations has now developed a set of guidelines for screening the efficacy and safety of potential biological control agents. *R. culicivorax* is the only mosquito biological control agent to have cleared the whole screening process (31). The United States Environmental Protection Agency has allowed commercialization of this mermithid on the basis that it is not a pesticide; rather it is a multicellular parasitic organism that does not vector bacteria or viruses but kills its host insects by making a hole in the cuticle (during its own exit) resulting in loss of haemocoelic fluid (38).

4. *Tetradonema plicans* is a parasite of sciarid fly pests of mushrooms. It possesses many attributes favourable for a biological control agent. It is a member of the Mermithoidea, and, like the mermithids themselves, one nematode sterilizes and kills the host (18). The female produces up to 12,000 eggs; the life cycle is short; it can be readily cultured *in vivo* on sciarid flies reared on horse dung (29); and the eggs can be stored for several months at 10°C. Hence, it could be a useful agent against pests with short life cycles on rapidly growing crops, especially in the humid and intensively managed environment of mushroom houses (28). Despite the advantages of this nematode over many others as a biological control agent, it has not been used extensively on a commercial basis.

## THE FUTURE

In the last 50 years we have moved from species descriptions and biological recordings of entomophilic nematodes to a fund of knowledge on the taxonomy and biology of a wide range of species and the ability to culture and apply some of them in the laboratory and field to kill insects.

We know that these nematodes cause insect mortality in field populations and exert some control over insect populations, but it is difficult to determine accurately the effect on an insect population. The estimate of efficacy of the nematode as a biological control agent depends on determining either the number of nematode-parasitized insects or the changes in the insect population size over a given period. Neither of these methods is adequate to be of major use in the large data base needed for insect pest management.

Studying one factor at a time is the scientist's usual approach to a complex problem. In this way the contribution of each of the components of the complex can be evaluated and, hence, over time the whole problem understood. I suggest that it is time to start putting the pieces together to see how the parts interact. Future studies must be on pest and parasite populations and on their interactions with one another and with other factors in their ecosystem. Only then shall we learn enough about the checks and balances affecting a particular host-parasite population to make informed decisions as to whether to inundate or inoculate the host's environment with a nematode parasite, and when to do it. We are deficient in our knowledge of the biological competitiveness of nematodes when they are introduced into a pest population. The commercial and economic competitiveness between the production of chemical pesticides and the mass culturing of pathogenic nematodes is a significant obstacle. So also is the registration hurdle for all new insecticides, whether chemical or biological. Unfortunately, the great pressure on agricultural land and the need to rear disease-free plant and animal populations encourages an opportunistic short-term pest control route which ignores long-term implications. Such attitudes militate against the use of pest management and slow the introduction of nematode biological control agents of insects. An organized evolution from nationally financed projects to larger scale multinational ones may help overcome some of the human as well as biological problems (6).

If we can alleviate these man-made pressures the potential for the successful use of entomophilic nematodes in pest management is high. We know several candidate nematodes for insect management which we can mass produce and apply. Let's take up Cobb's (10) enthusiasm and do it!

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