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Tabulation and Evaluation of Recent Field Experiments Using  
the DD-136 Strain of *Neoplectana carpocapsae*  
Weiser: A Review

115 MAY 1976

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(Submitted for publication, 1 August 1972)

BENHAM, G. S., JR., AND POINAR, G. O., JR. 1973. Tabulation and Evaluation of Recent Field Experiments Using the DD-136 Strain of *Neoplectana carpocapsae* Weiser: A Review. *Experimental Parasitology* 33, 248-252. A tabulation and discussion of trials utilizing the nematode, *Neoplectana carpocapsae* Weiser, against insect pests in the field are presented. Only those references not included in an earlier review article (Poinar, 1971) are considered here.

Moisture limits the immediate usefulness of these nematodes and this factor should be taken into consideration for field trials. The neoplectanid species, *N. carpocapsae*, seems best adapted for living on or just beneath the soil surface but can also parasitize deeper soil insects and even enter plant tissue to locate successfully potential hosts.

In conclusion, it can be stated that neoplectanid nematodes can play an important role in the control of insect pest populations and may be most successful in a program of integrated control.

INDEX DESCRIPTIONS: *Acrolepia assectella*; Control, biological; *Cirphis compta*; *Coptoermes formosanus*; *Dendroctonus frontalis*; Field experiments; *Graphognathus peregrinus*; Insects; Control, integrated; Control, microbial; Nematodes; *Neoplectana carpocapsae*; *Operophtera brumata*; *Pseudaletia separata*; *Pseudexentera mali*; Reviews; *Rhyacionia frustrana*; Soil.

In a recent review, Poinar (1971) listed several insect hosts in which *Neoplectana glaseri* Steiner or the DD-136 strain of *N. carpocapsae* Weiser were used to regulate or reduce field populations of those insects. Among the listed hosts were the codling moth, Colorado potato beetle, European corn borer, imported cabbage worm, banded cucumber beetle, winter moth, and corn earworm. Since this publication (Poinar 1971), there have been additional attempts at controlling field populations of

insects with *N. carpocapsae* strain DD-136.

The purpose of this paper is to tabulate and discuss these additional trials which are summarized in Table I.

#### LITERATURE REVIEW

The study by Jaques *et al.* (1968) was an attempt to control the pale apple leaf roller, *Pseudexentera mali* (Freeman), and the winter moth, *Operophtera brumata* (L.), by applying nematodes to the soil surface beneath apple trees. The nematodes were

TABLE I  
Trials Using the DD-136 Strain of *Neoplectana carpocapsae* Weiser  
for control of insect field populations

Host	Application dosage and site	Results	Reference
Pale apple leaf roller <i>Pseudexentera mali</i> (Freeman)	$3.4 \times 10^4$ – $1.6 \times 10^5$ Nemas to soil surface in ft <sup>2</sup> cages beneath apple trees	Reduced survival of larvae to one-third of control	Jacques <i>et al.</i> 1968
Winter moth <i>Operophtera</i> <i>brumata</i> (L.)	Same as above	Reduced survival of cocoon to 12%	Jacques <i>et al.</i> 1968
Cutworm <i>Pseudaletia separata</i> Walker	$1 \times 10^6$ Nemas to a $3.0 \times$ $6.5$ M <sup>2</sup> rice field plot	Significant reduction of larvae	Israel <i>et al.</i> 1969
Paddy cutworm <i>Cirphis compia</i> Mo.	Same as above	Significant reduction of larvae	Israel <i>et al.</i> 1969
Nantucket pine tip moth <i>Rhyacionia frustrana</i> (Comstock)	$4 \times 10^3$ Nemas/ml in 0.1% formalin and 10% glycerin and wetting agents to pine tips	5–15% Reduction in moth population	Nash and Fox 1969
White-fringed beetle <i>Graphognathus</i> <i>peregrinus</i> (Buchanan)	$4.3 \times 10^5$ Nemas/M <sup>2</sup> to 12 $30.5 \times 30.5$ m grassland	38% Reduction in larvae	Harlan <i>et al.</i> 1971
Formosan termite <i>Coptotermes</i> <i>formosanus</i> Shiraki	Infected termites released in termite colonies	Infection obtained	Reese 1971
Southern pine beetle <i>Dendroctonus</i> <i>frontalis</i> Zimmermann	740 Nemas/ft <sup>2</sup> in 0.1% formalin and wetting agent to pine bolt surface	40.1–49.6% mortality to brood and adults	Moore 1970
Onion borer <i>Acrolepia assectella</i>	Not stated	High mortality at pupation	Laumond 1972

cultured in the laboratory on larvae of the greater wax moth, *Galleria mellonella* (L.). Beneath apple trees in a nonsprayed orchard, 1 sq ft open-bottomed cages were placed in the soil to a depth of 3 cm. A 500-ml aqueous suspension containing from 340,000 to 1,600,000 live infective third-stage nematodes was applied uniformly on the soil surface in each cage. One hundred full-grown apple leaf roller larvae and 75 winter moth larvae with apple foliage were placed in each cage, respectively. Both of these insects are defoliators. The apple leaf roller drops to the ground and forms cocoons during early summer. Pupae are

formed in the autumn and adults emerge next spring. The winter moth drops to the ground in June and almost immediately pupates on the soil surface. Upon examination of a 2.5-cm deep sample of soil from each cage, it was found that the DD-136 strain reduced the number of apple leaf rollers to less than one-third that of the nontreated cages. Treatment of the soil for the control of the winter moth reduced the number of cocoons to 12% of the nontreated cages. Although this may appear to be an insignificant amount of reduction to recommend these nematodes as a control measure, it is important to note that Jaques

*et al.* (1968) were unable to determine the exact mortality rate due to the deterioration of host cadavers. Also, less than 50% of the test insects survived in nontreated cages. As Jaques *et al.* (1968) pointed out, there must be important natural mortality factors in the soil and possibly also in the apple foliage. We need to know more about these factors contributing to the mortality of the winter moth before final conclusions can be drawn.

In an earlier experiment, Jaques (1967) found that nematodes applied to the foliage of apple trees had no effect on the winter moth because they apparently were killed by desiccation. In future tests concerning the winter moth, it may be advisable in new outbreak areas to consider a pest management program in which two introduced parasites, an ichneumonid, *Agrypon flaveolatum* (Gravely) and a tachinid, *Cyzenis albicans* (Fallen), are used together with a soil application of nematodes during the period when host larvae fall to the ground for pupation.

During the past 7 yr, the cutworm, *Pseudaletia separata* Walker, and the paddy cutworm, *Cirphis compta* Mo., have risen to pest status in Indian rice fields (Israel *et al.* 1969). Controlling the paddy cutworm by chemical means is difficult because the caterpillar is concealed near the soil surface in the rice clumps. Complete insecticide coverage is almost impossible and it is hazardous to control chemically the cutworms at the time of harvest. Therefore the Central Rice Research Institute of Cuttack, Orissa carried out laboratory and greenhouse experiments on the effectiveness of the DD-136 strain for the control of these two insect pests. Because of the encouraging results, the effectiveness of this nematode was tested under field conditions. Either 1,000,000 nematodes or a 0.04% concentration of parathion was applied to a 6.5-sq m rice field plot. Field cages were removed 60 hr after spraying. Live and dead cutworm larvae were counted after

removing them from sampled plants. Israel *et al.* (1969) did not state whether the nematodes penetrated the plant tissue or entered natural openings to contact the cutworms. They stated that the numbers of surviving larvae of both insect species were relatively low in the DD-136 strain treated samples as compared to the checks. There was no mention of the possibility of other existing mortality factors which may have been responsible for a reduction in the hosts. Israel *et al.* (1969) felt that their preliminary field experiments showed much promise for the use of this nematode.

Nash and Fox (1969) found that larvae of the Nantucket pine tip moth, *Rhyacionia frustrana* (Comstock), were killed under natural conditions by this strain of *N. carpocapsae*. A great difficulty in using most nematodes as biological control agents has been their rapid desiccation at low moisture levels. Nash and Fox (1969) were aware of this and added anti-desiccating agents such as glycerin, Emgard 2050, Soleonic CDDS, and IGEPON AP-78 to their suspensions of infective nematodes. Six different treatments were applied to the top whorl, including the terminal shoot and the first subterminal branches, of each of 420 loblolly pine trees. The suspension containing the 10% glycerin appeared to give the best control. However Nash and Fox (1969) felt that the control was not sufficient enough under the conditions of the Piedmont Region of South Carolina to recommend this nematode as a control agent. They felt that the high moisture requirement of the DD-136 strain was limiting the success of their experiment, since if the suspensions dry in too short a period, then nematode contact with the host is limited to a very short period.

In a field trial by Harlan *et al.* (1971) to evaluate the effectiveness of the DD-136 strain against larvae of the white-fringed beetle, *Graphognathus peregrinus* (Buchanan), 430,000 nematodes were applied per square meter of soil. The nematodes

were applied to 12 plots in a Louisiana Crossland by a piston-type sprayer mounted on a jeep. Monthly samples were taken in each plot by removing a soil core 5 cm in diameter and 15 cm deep. The authors obtained 38% fewer larvae in the treated than in the check plots. They felt that this nematode could effectively control white-fringed beetle larvae if high enough concentrations of nematodes were used. The nematodes were recovered from plots 16 months after application, indicating their ability to continue the parasitic cycle.

An attempt to control the Formosan termite, *Coptotermes formosanus* Shiraki, in Hawaii using this nematode has been reported by Reese (1971). Apparently large numbers of termites are collected, infected with the DD-136 strain, and then returned to the colony. Hawaiian investigators found that it takes approximately 7 days to kill the termites and they assumed that this will allow an adequate time for the infected termites to disperse throughout the colony. There has been no record of percentages of reductions in termite numbers. The termites in some colonies were able to recognize infected individuals and wall them off behind earthen barriers. This behavior is similar to that which occurs when a portion of the colony is treated with chemical insecticides.

The work of Moore (1970) on infection of the southern pine beetle, *Dendroctonus frontalis* Zimmerman, by *Neoaplectana* shows the variety of habitats that have been used with these nematodes. These experiments were conducted in the laboratory but with an attempt to simulate field conditions. Seven hundred and forty nematodes in a 0.1% formalin solution containing Tween 20 as a spreader at 60% relative humidity were applied to each square foot of pine bark surface. Moore (1970) observed that soon after application, at least 70% of the nematodes moved into bark crevices and bark beetle galleries. The minimum distance through the bark to a host

was 3–4 cm. It was estimated that it would take a minimum of 30–45 min for the nematodes to enter bark beetle tunnels and encounter hosts. Since drying resulted within 1 hr after spraying, glycerin was added to the suspension and retarded drying up to 5 hr. From between 40 to 49% of the brood and adults were killed in the pine bolts, but the nematodes primarily attacked full-grown larvae. Moore (1970) found that even though the nematodes entered the bark beetle galleries more easily when glycerin was added to the suspension, there was little difference in the quantity of beetles infected.

Recently, Laumond (1972) reported on the successful use of both the DD-136 and agriotos or Leningrad strains of *N. carpocapsae* when applied to the field for the control of insect pests of artichoke. Control of the plutellid moth, *Acrolepia assectella*, on onion was also achieved with these nematodes.

#### DISCUSSION

In analyzing the above reports and discussing the future use of *Neoaplectana* nematodes for insect control, the following points should be considered: (1) the physical environment (temperature and moisture requirements) and its effect on the nematodes; (2) defense reactions or host immunity to nematodes, as Poinar (1969) has shown that reactions such as encapsulation and melanization occur against most groups of entomogenous nematodes, including *Neoaplectana*, by a wide-range of insect hosts; (3) long-term studies on the effect of natural populations of *Neoaplectana* species on insect hosts; (4) natural movement and migration of infective stage nematodes under various soil types; and (5) characterization and comparison of the symbiotic bacteria associated with various species and strains of *Neoaplectana* (see Poinar *et al.* 1971).

Poinar and Lindhardt (1971) have recently shown that *N. bibionis* Bovien in

Denmark is capable of maintaining itself 7 years in populations of its natural host species of bibionid flies. This indicates that species of *Neoaplectana* may be exerting more influence on insect populations than we now imagine. Further studies along this line should have considerable significance.

It is hoped that future studies will better illustrate how well *Neoaplectana* nematodes can be used in an integrated or biological control program against pest insects.

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