FIELD APPLICATION OF ENTOMOGENOUS NEMATODES FOR BIOLOGICAL CONTROL OF CLEAR-WING MOTH BORERS IN ALDER AND SYCAMORE TREES

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Abstract. The entomogenous nematode, *Steinernema feltiae*, was field tested for its ability to control the sesiid borer, *Synanthedon culiciformis*, in alder in the fall. When 6.5 or 11.5 million nematodes were applied to the entire trunk, 77-84% borer control was obtained. When each gallery opening was treated with 18,000 or 36,000 nematodes, 86-93% control was obtained. The entomogenous nematodes, *S. feltiae* and *S. bibionis*, were tested by trunk spray applications to control another sesiid borer, *Synanthedon resplendens*, in sycamore in the fall and spring. The fall application was not successful for either nematode species and the spring application provided 61% control of the borer with *S. feltiae* only. *Synanthedon culiciformis* occurs in the moist heartwood habitat of alder trees which is advantageous to nematode survival and searching abilities. *Synanthedon resplendens* occurs in the drier bark galleries and has small gallery openings which prevent nematode penetration into the galleries. Other factors that affect the success of the nematodes against these borers are discussed.

Most sesiids (Order: Lepidoptera and Family: Sesiidae) are obligate borers of plants during their larval stage (Duckworth and Eichlin, 1978). The larvae bore into plant tissues and feed in galleries made in limbs, trunks, bark, or roots of trees, shrubs, herbs, and vines. Many species cause extensive damage by mining and tunneling in desirable plants. Some of the most important sesiids attack trees, resulting in wood defects or structural weakening that may lead to tree mortality. A few tunnel in bark and do not kill the tree but can give it an unsightly appearance.

Sesiid borers can be controlled with chemical pesticides, by mechanical means, or with biological control agents. Solomon (1985) summarized the recent information on the use of chemical pesticides for borer control. Chlorpyrifos, lindane, endosulfan, and diazinon are effective against these borers. Insecticides can be applied as trunk sprays or gallery injections. Fumigants are effective against the carpenterworm, *Prionoxystus robiniae*, and will probably be

equally effective against sesiids (Solomon, 1985). Appleby (1973) summarized the information on mechanical control which includes the insertion of a flexible wire into the gallery to puncture the larva, digging out the larva with a knife, or removal and burning of weakened trees and infested branches.

Recently, a biological control method using the entomogenous nematodes, *Steinernema feltiae* (=*Neoaplectana carpocapsae*) and *S. bibionis*, has been effective for borer control (Figure 1). *S. feltiae* controlled 85% or more of the carpenterworms in commercial fig trees in California (Lindegren *et al.*, 1981; Lindegren and Barnett, 1983). *S. bibionis* was successfully used against the sesiid borer, *Synanthedon tipuliformis*, a pest of commercial currants in Australia (Miller and Bedding, 1982), while *S. feltiae* was effective in controlling the western poplar clearwing, *Paranthrene robiniae*, in ornamental birch (Kaya and Lindegren, 1983). The successful use of steinernematid nematodes against these wood-boring larvae was in part due to the moist habitat which allowed the nematodes to seek their host.

The mode of action of *S. feltiae* and *S. bibionis* is as follows. The infective nematodes (Figure 2) enter through the mouth or anus into the midgut or spiracles of their host and then penetrate directly into the body cavity (Poinar, 1979; Kaya and Stimmann, 1983). Once inside the body cavity, the nematodes release the symbiotic bacterium, *Xenorhabdus nematophilus*, which is in the gut of the nematode. The bacterial infection proceeds very quickly within the insect host, killing it within 24 to 48 hours. The nematodes feed upon the bacterial cells and host tissues. The nematodes mate, progeny are produced, and a second or third generation of nematodes may occur. As the

resources within the dead insect are depleted, infective nematodes are produced which leave the host and seek other insects.

The steinernematids infect only insects or close relatives of insects. Extensive safety tests have shown that the nematode-bacterial complex is not harmful to plants or vertebrates. Consequently, this complex has been exempt from registration by the United States Environmental Protection Agency.

In 1983 and 1984, a study was initiated to evaluate control of the sesiids, *Synanthedon culiciformis* in alder and *S. resplendens* in sycamore, with steinernematid nematodes. *S. culiciformis* occurs in moist galleries in the heartwood and *S. resplendens* occurs in drier galleries in the bark. The purpose of the study was to determine the effectiveness of the nematodes against borers occurring in different habitats.

Materials and Methods

Nematode production. The nematodes were mass-produced in the laboratory on sterilized turkey offal according to the technique described by Bedding (1981). After harvesting, they were stored at 40,000 infectives/ml of water at 50°F with continual aeration and held for no more than 2 months before use.

Alder tests. Nematodes were applied to alder trees located in an apartment complex and an adjacent park in Davis, California at different concen-

trations with a 2 gallon Hudson sprayer or directly into the gallery openings with a 1 pint squirt bottle. Rates were 4000 or 8000 infective *S. feltiae*/ml (see Table 1). When the sprayer was used, the trees were sprayed to the point of runoff. When the squirt bottle was used, each gallery received about 4.5 ml of the nematode suspension. The average number of nematodes applied per tree or per gallery opening was estimated and is shown in Table 1.

Nematodes were applied only to the main stem up to about 8 feet above ground level. Control trees were sprayed with water. There were four trees per treatment with each tree serving as a replicate. Trees of comparative sizes were used and averaged 6 inches in diameter at breast height. Application was made between 3:00 and 5:00 p.m. on September 30, 1983. The skies were overcast with a slight drizzle and the temperature was 71°F. Nematode infectivity was checked by bioassay prior to and after spray application. One thousand nematodes were placed in 1 ml of water in a petri dish lined with filter paper and five larvae of *Galleria mellonella* were added to the dish. There were three replicates per treatment.

Tree treatments were evaluated as follows. Frass production was used as the index for control of *S. culiciformis*. Prior to nematode application, the frass from each gallery was removed and frass appearing again indicated that the gallery
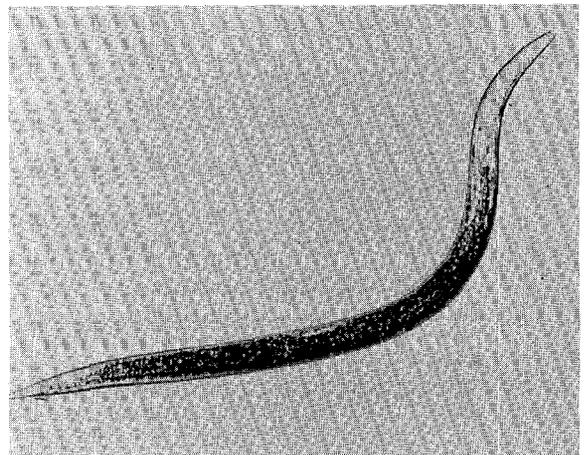

Figure 1. Commercial injection treatment of ash trees infested with the ash borer, *Podosesia syringae*, with *Steinernema feltiae* in an industrial park in Sacramento, California.

Figure 2. Infective nematode (.02 inches in length) of *Steinernema feltiae*.

was active. The gallery was marked with paint. One week after treatment, the frass again was removed from the gallery. If frass was found near the gallery opening after another week, the gallery was considered to be active. In addition, 20 gallery openings were selected randomly and measured.

Sycamore tests. Test trees infected with *S. resplendens* were located on the Riverside campus of the University of California. The sycamore trees averaged 7.5 inches in diameter at breast height. Nematodes were applied with a Hudson sprayer as described for the alders with the following exceptions. All trees were sprayed with water prior to nematode treatment. In addition to the 4000 and 8000 infective *S. feltiae*/ml, 4000 or 6000 infective *S. bibionis*/ml was included in the experiment.

There were two tests with one in the fall and the other in the spring. The first was initiated on October 20, 1983 when 32 sycamores (24 nematode treatments and 8 controls) were sprayed between 5:15 and 7:00 p.m. (see Table 2). The temperature was 60°F. The second test was initiated on April 12, 1984 when 15 sycamores (10 nematode treatments and 5 controls) were sprayed between 7:00 and 8:00 p.m. (see Table 3). The temperature was 64°F. The infectivity of the nematodes was checked prior to and after spray application by placing 1000 nematodes in 1 ml of water in a petri dish lined with filter paper. *Trichoplusia ni* larvae were used in this bioassay.

The success of the first test was assessed by gallery activity, as described for the alders. The second test was assessed by digging the *S. resplendens* larvae out of their galleries with a knife 1-2 weeks after treatment and examining each larva for nematode infection. The gallery openings were measured in October and April.

Results

Alder tests. Alders at Davis averaged 14.9 ± 5 active *S. culiciformis* galleries per tree and gallery

Table 1. Percent mortality of *Synanthedon culiciformis* larvae in alder trees based on frass activity after treatment with *Steinernema feltiae* at Davis, 1983.

Nematode treatment	No. trees	Active galleries pre-treatment	Active galleries post-treatment	% mortality
0	4	60	73	0
4000/ml (trunk)[a]	4	57	13	77
8000/ml (trunk)[a]	4	62	10	84
4000/ml (gallery)[b]	4	14	1	93
8000/ml (gallery)[b]	4	14	2	86

[a]Hudson sprayer—sprayed to runoff on tree trunks
    4000 nematodes/ml; trees received an average of 6.5 million nematodes
    8000 nematodes/ml; trees received an average of 11.5 million nematodes

[b]Squirt bottle—each gallery given ca 4.5 ml of nematode suspension
    4000 nematodes/ml per gallery received 18,000 nematodes
    8000 nematodes/ml per gallery received 36,000 nematodes

openings averaged 0.28 ± 0.07 inches in diameter. Microscopic examination of the nematodes prior to spray application showed that 98% were alive. Moreover, the nematode infectivity was not impaired before or after spray application because 100% of the *Galleria* larvae were killed in the bioassay by the nematode.

Spot treatment of galleries with *S. feltiae* provided 86-93% control in the fall. The lower rate of 4000 nematodes/ml resulted in better control than the higher rate. The general spray treatment of the tree trunks provided 77-84% control of *S. culiciformis* (Table 1). The higher rate resulted in somewhat better control although the differences were not statistically different.

Sycamore tests. Sycamore trees were heavily infested with *S. resplendens*, averaging 51.9 galleries per tree. The gallery opening averaged 0.05 ± 0.03 inches in diameter in October and 0.17 ± 0.04 inches in April. The viability and infectivity in both nematode species were excellent.

The general spray application with *S. feltiae* or *S. bibionis* in the fall did not give significant control when frass activity was compared with the control trees (Table 2). Application with *S. feltiae* in the spring resulted in 61% larval mortality (Table 3). *S. bibionis* was less efficacious causing only 12.5% larval mortality. The high mortality recorded in the controls can be attributed in part to the method of removing the larva from the

gallery. Some of the healthy larvae apparently were squashed during this process and were recorded as dying from unknown causes.

Discussion

Plant-boring lepidopterous insects, in particular carpenterworms and sesiids occurring in moist galleries, have been controlled effectively with steinernematids. The moist galleries are ideal for the survival and searching of the nematode. If the gallery is not sufficiently moist or the gallery opening is too small, the nematode spray is not effective. Wetting of the sycamore trees prior to nematode application did not increase efficacy in the fall treatment although it may have enhanced the spring treatment for *S. feltiae.*

Careful consideration must be given to the nematode species used. In our study, *S. bibionis* was not effective against *S. resplendens* in sycamore when applied in the spring, while *S. feltiae* provided some degree of control. The reason for differences in the degree of control between the two species may be related to the size of the infective stage. The infective stage of *S. feltiae* measures 0.02-0.03 inches in length, while that of *S. bibionis* measures 0.03-0.04 inches. The larger infective stage of *S. bibionis* may have greater difficulty in entering the gallery opening.

There are several possible reasons for dif-

Table 2. Percent mortality of *Synanthedon resplendens* larvae in sycamore trees based on frass activity after treatment with *Steinernema feltiae* (sf) and *S. bibionis* (sb) at Riverside, October 1983. Eight trees per treatment.

Nematode treatment	Active galleries pre-treatment	Active galleries post-treatment	% mortality
0	488	423	7.0
4000/ml (sf)[a]	479	446	6.9
8000/ml (sf)[b]	307	277	9.8
4000/ml (sb)[c]	422	366	13.6

[a]2.3 million nematodes per tree
[b]5.6 million nematodes per tree
[c]2.7 million nematodes per tree

Table 3. Percent larval mortality of the borer, *Synanthedon resplendens*, in sycamore trees after trunk spray treatment with the nematodes *Steinernema feltiae* (sf) and *S. bibionis* (sb), April 1984.

			Percent	
			Dead	
Treatment	Total larvae recovered[a]	Alive	With nematodes	Without nematodes
0	37	67.6	0	32.4
8000/ml (sf)[b]	33	24.2	60.6	15.2
6000/ml (sb)[c]	40	77.5	12.5	10.0

[a]Larvae recovered from 5 trees
[b]11.3 million nematodes per tree
[c]8.6 million nematodes per tree

ferences in the control of the sesiid larvae by the steinernematid nematodes. The strain of the nematode may not be as efficacious against the borers or the sesiid larval size may have affected the nematodes' ability to seek out its host or infect it. It is known that the smaller insects are less susceptible to nematode infection (Gaugler and Molloy, 1981; Kaya, 1985).

The current cost of nematodes at $2.00/million undoubtedly will prevent their widespread use to control sesiid borers and will limit their application to high value trees by gallery treatment. At the rate of 20,000 nematodes per gallery used in our study, 50 galleries can be treated with one million nematodes. Assuming a high infestation of 15 active galleries per tree, three and one-third trees can be treated for $2.00, excluding labor costs. Spraying of the entire main trunk could cost $12.00 or more per tree and could be used for high value trees or in areas where chemical pesticides are restricted or undesirable.

Although the current cost of the nematodes is high, research indicates that the production costs can be reduced to less than one cent per million (Bedding, 1984). When this kind of production can be met by commercial companies, the cost of the nematodes also will be reduced and may be comparable with chemical pesticides.

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