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Evaluation of Entomopathogenic Nematodes as Biological Control against the Banded Ash Clearwing Borer¹

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Abstract

Three species of entomopathogenic nematodes were evaluated for suppression of the banded ash clearwing borer (BACB; *Podosesia aureocincta*) attacking green ash (*Fraxinus pennsylvanica*). Field trials were located in Howard County, Maryland (shopping mall parking lot), utilizing *Steinernema carpocapsae* and *S. feltiae*, and Ocean County, New Jersey (suburban street trees), utilizing *Steinernema carpocapsae*, *S. feltiae*, and *S. glaseri*. Nematodes were applied as bark sprays in June (NJ), July (MD), and October (NJ) using a backpack sprayer. Examination of pupal exuviae and/or adult emergence revealed reduced BACB populations in 7 of 9 nematode treatments, with control ranging from 12–54 percent. Nematode sprays applied to dry bark did not provide acceptable control.

Index words: biological control, clearwing moth borer, cryptic habitat, entomopathogenic nematodes, frass, *Lepidoptera*, *Podosesia*, pupal exuviae, *Sessidae*, pupal exuviae, treatment window.

Insecticides used in this study: Dursban (chlorpyrifos); 0,0-diethyl 0-(3,5,6-trichlor-2-pyridinyl) phosphorothioate.

Species used in this study: green ash (*Fraxinus pennsylvanica*); *Steinernema carpocapsae* (Exhibit, Vector T/O); *Steinernema feltiae*; *Steinernema glaseri*.

Significance to the Nursery Industry

Ash trees grown for use as shade trees and street trees are a valuable landscape and nursery crop. A primary pest attacking green ash is the banded ash clearwing borer, which causes structural weakening, wounds, dieback and even death of infested trees. Traditional synthetic, long residual insecticide controls have relatively narrow treatment windows. This field study demonstrates that entomopathogenic nematodes offer an effective and economically viable biological control option for controlling the banded ash clearwing borer. Nematode use may also widen the treatment window.

Introduction

Ash trees, *Fraxinus* spp., are grown extensively in nurseries and used frequently in urban landscapes. They are easy to propagate, transplant readily, grow rapidly, and are tolerant of adverse growing conditions (3). In the urban landscape, green ash is extensively used as a street, shade, and specimen tree. However, a shortcoming of green ash is the susceptibility to attack by clearwing moth borers (*Lepidoptera*: *Sesiidae*; genus *Podosesia*).

Borers have been recognized as a leading impediment to ash culture in the nursery and landscape (7). As primary or

secondary invaders, clearwing borers have a tendency to attack open-grown, exposed, urban trees (19), such as shade and landscape trees (7, 16). Trees planted in adverse site conditions, such as compacted, restricted, or poorly drained soil, also seem to be preferentially infested by the clearwing borers (7), as are trees injured by improperly installed wire staking, lawn mowers or string trimmers (1). Once attacked, ash is prone to reinfestation (19), and may be disfigured, scarred, seriously weakened, or killed.

A 1971 survey showed that approximately 50 percent of the green ash in the cities of the Canadian Prairies were attacked by clearwing borers (4, 13), and 33 percent of the boulevard trees in Grand Forks, North Dakota were attacked (4, 15). Economic losses approached \$5,000 per acre per cropping cycle in damage in Ohio nurseries (15).

The banded ash clearwing borer (BACB), is a key pest of green ash (*F. pennsylvanica*). Although the lilac borer (*Podosesia syringae*) also commonly attacks ash, the morphologically similar BACB is now known to be a distinct sibling species (15, 17). Its distribution ranges from New York to Florida, and west to Indiana and Oklahoma (8).

The period of BACB adult emergence is from late August through September in Ohio, Virginia, and Maryland (7, 13, 15). Females deposit eggs on tree branches and trunks, throughout the entire tree canopy, principally at crotches and wounds from previous pruning cuts (1). Larvae tunnel through the bark into the cambium (phloem), forming splotch mines, before feeding and excavating inward and upward into the sapwood, where they overwinter (8, 19). Feeding galleries of individual borer larvae can extend from 7–32 cm (2.8–12.6 in) long, and may intersect the heartwood. Galleries are extended almost to the bark surface prior to pupation the following summer (19). Mining causes branch dieback, disfiguration, structural weakening, and death of trees. There is one generation a year.

Chemical control options exist during a relatively narrow window of time, just prior to egg deposition. Residual insecticides, such as Lindane or Dursban (chlorpyrifos), are commonly applied as a protectant bark spray 10–14 days

¹Received for publication July 24, 1995; in revised form March 18, 1996. Support for this project was provided by research grants from The Horticultural Research Institute 1290 I. Street, N.W., Washington, D.C. 20005, and the Agricultural Agents Association of New Jersey. The authors thank J. Fiola and P. Gross for statistical assistance, Ocean County Master Gardeners for data collection assistance, and M. Raupp, J. Fiola and J. Davidson for thoughtful review of this manuscript.

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after first adult male capture in pheromone traps. A single, properly timed insecticide application provides effective control (1); however, once larvae are under the bark, pesticide treatments are ineffective.

The use of entomopathogenic nematodes in the family *Steinernematidae* has been shown to be effective in controlling clearwing borers, including the dogwood borer, peachtree borer, western poplar clearwing borer, and clearwing borers in alder and sycamore (2, 6, 9, 11) in landscape settings. The moist, humid larval galleries are ideal for nematode searching and survival (11). Nematodes are applied directly on the woody portions of trees with conventional spray equipment. The nematodes enter the borer feeding gallery, search, and attack borer larvae. Larvae are typically killed within 48 hours of attack by the *Xenorhabdus* bacteria symbiotically sustained by the nematode (12).

This study was initiated to determine the effectiveness of three entomopathogenic nematodes, *Steinernema carpocapsae*, *S. feltiae*, and *S. glaseri*, as possible biological alternatives to chemical control of BACB larvae on green ash. Data was evaluated in two states, Maryland and New Jersey, to determine the effectiveness of different application timings, species of nematodes, and techniques.

Materials and Methods

Maryland trial. The Maryland trial was located at a shopping mall in Columbia, Maryland where *F. pennsylvanica* trees were growing in grass planting islands. The Brickman Landscape Company maintained the site and donated the trees for the field study. The trees had exhibited borer damage for at least the previous three years, and had exhibited numerous entry and exit wound sites on the trunk and major branches. Dieback of the upper canopy occurred in the majority of the trees. In May 1993, the trees were examined for signs of active infestation, including fresh frass and oozing of sap.

Eighteen trees which had at least two major scaffold branches with fresh frass/ooze were selected for the trial. Two branches ranging from 5–10 cm (2–4 in) in diameter at the trunk juncture and with fresh frass extruding were selected. To determine the area of the branches to be treated, branch diameters were measured at the juncture with the trunk, 1 m (3 ft) from the trunk, and 2 m (6 ft) from the trunk. The area of the bark surface was calculated based on the average of these three measurements multiplied by the length of the branch.

The nematodes were supplied by Biosys, Inc. (10150 Old Columbia Rd, Columbia, MD) and Ciba, Inc. (PO Box 18300, 410 Swing Rd, Greensboro, NC), under the brand name 'Exhibit', and contained 250,000,000 nematodes immobilized in an alginate gel. Three treatments were evaluated; two nematode species, *Steinernema carpocapsae* and *S. feltiae*, and a water treated control. The trial design was a randomized, complete block with six single tree replications. Nematodes were observed for mobility prior to application.

Both nematode treatments were applied at the rate of 500 nematodes per 6.54 cm² (1 in²) of bark area. Treatments were applied on July 20, 1993, between 8:00 am and 10:00 am. The temperature and relative humidity were measured at the start of the treatments using an Omega RH-20 portable meter. Temperature was 24C (75F) with relative humidity of 79% at the start of applications. By 10:00 am the temperature was 27C (81F) with relative humidity of 76%.

The weather was overcast with continued high humidity throughout the afternoon.

Nematodes were mixed with well water of pH 6.0. One thousand ml of water was sufficient to treat the two individual branches on each tree. Applications were made to the point of runoff using a Birchmeyer backpack sprayer with a trombone arm extension.

A baited pheromone trap was placed in one of the trees in early August. When the first adult BACB was captured, the branches were removed from the trees, labeled, cut into bolts of roughly 75 cm. (30 in.) in length and placed in individual rearing cages. The number of emerging adults and pupal exuviae protruding from the wood were recorded from early August until October 15, 1993.

The efficacy of the nematode treatments was analyzed by ANOVA in which islands were considered blocks. The abundance of borers was estimated by the number of pupal exuviae found in the branch samples. Nematode treatment means were compared with planned orthogonal contrasts (18).

New Jersey trial. The New Jersey trial was located at a 142 acre retirement community in Toms River, NJ, where *F. pennsylvanica* is the predominant street tree. Trees were located in full sun, in irrigated lawns maintained by turfgrass professionals on 0.1 ha (1/4–1/5 acre) lots. Trees were 6.1–9.1 m (20–30 ft) in height, 5–13 cm (2–5 in) DBH, and had been planted in 1988. Approximately 80% of the trees were infested with borers, exhibiting upper canopy dieback, entry wound bark swelling and cracking, 0.6 cm (0.25 in) exit holes, and/or active frass extruded from the bark.

One-hundred-fifty-nine actively infested green ash trees were sampled and visually rated for the degree of decline, on a scale of 1 to 4 (1 = 0–10% decline; 2 = 10–39% decline; 3 = 40–59% decline, and 4 = 60–100% decline). One-hundred-twenty-six trees were selected for the trial and blocked according to the degree of decline. The trial design was a randomized, complete block (RCB) with 14 replications per treatment (Table 1). The individual tree was the experimental unit. There were nine treatments, including three nematode species, a traditional Dursban treatment and a non-treated control (Table 1). Eleven trees were eliminated from the trial (random) due to tree death, homeowner mis-handling, or change of ownership.

Nematodes were stored in the refrigerator prior to application, mixed in the shade according to label directions, and the mix kept in coolers prior to application. *S. carpocapsae* (strain 25) was immobilized in an alginate gel, agitated in water, and diluted; *S. feltiae* (strain 27) was immobilized in an gel polymer and mixed as a 'tea bag' extraction. *S. glaseri* (#326) was diluted from a liquid concentrate. All nematodes were supplied by Biosys, Inc. (10150 Old Columbia Rd, Columbia, MD). Nematode activity in the spray solution was observed under magnification prior to application.

S. carpocapsae and *S. feltiae* were applied on June 15, 1993, targeting mid-instar borer larvae beneath the tree cambium. Nematodes were applied from 11:00 am to 1:00 pm. The weather was overcast with spot drizzle, temperatures ranged from 27C to 29C (81F to 84F), and relative humidity of approximately 56% at noon, falling to 49% by 1:00 pm. Humidity readings were taken from Lakehurst Naval Base weather station, 10 miles from site. *S. carpocapsae*, *S. feltiae*, and *S. glaseri*, applied on October 7, 1993, targeted newly hatched, early instar borer larvae within the tree cambium.

Table 1. Control of the banded ash clearwing borer utilizing three nematode species at two times of year.

Treatment	Rate ^a nematodes/cm ²	n ^b	Time of treatment	W/ or W/O wetting bark ^c	No. pupal exuviae	Percent control
Dursban 4E (chlorpyrifos)	1 oz/gal	14	September 14	—	3.0d ^w	74
<i>S. glaseri</i>	3866	13	October 7	w/	5.2 cd	54
<i>S. feltiae</i>	3866	11	October 7	w/	6.1bcd	46
<i>S. carpocapsae</i>	3310	11	June 15	w/o	8.4bcd	26
<i>S. carpocapsae</i>	3310	14	October 7	w/	9.5abc	17
<i>S. feltiae</i>	3866	12	October 7	w/o	10.0abc	12
<i>S. feltiae</i>	3866	14	June 15	w/o	11.4ab	0
CONTROL	—	13	—	—	11.4ab	—
<i>S. glaseri</i>	3866	13	October 7	w/o	12.3a	—

^aCalculated for the ave. 8.9 cm DBH tree.

^bSome trees were eliminated during the trial due to death, homeowner mishandling, or change of ownership.

^cBark sprayed with water prior to nematode application.

^wMeans followed by the same letter are not significantly different at $P < 0.02$.

Nematodes were applied from 10:30 am to 12:30 pm. The weather was sunny, clear, with temperatures of 21C (70F), to 22C (72F) by noon, and relative humidity approximately 52% at 10:00 am.

All treatments were applied to runoff using a Solo backpack sprayer. Sprays were applied to a 1.2 m (4 ft) section of the main trunk, below major scaffold branches. October treatments of *S. glaseri* and *S. feltiae* were applied as 1) sprays directly to dry bark, and 2) sprays following the thorough wetting of the bark with water using a hand pump sprayer. Dursban was applied on September 14, 1993, 14 days following pheromone trap capture of the first adult male moth.

To determine efficacy, trees were sampled for exposed pupal exuviae, which noticeably protrude from the bark prior to adult emergence and remain exposed for a short time. Trees were sampled in June and September, 1993 and 1994, 20 days (average) following the first pheromone trap adult capture. Data collection in 1993, prior to treatment application, also determined specific *Podosesia* species activity. Visual decline ratings were taken at each sampling date. Final data collection occurred on September 15 and September 29, 1994; 15 and 29 days, respectively, following first pheromone trap adult capture. All data were subjected to analysis of variance; significant means were separated by LSD, ($P = 0.05$). (SAS)

Results and Discussion

Maryland trial. Nematode treatments caused approximately a 50 percent reduction in the mean number of BACB adults emerging per tree (Fig. 1). The difference in the mean number of exuviae per tree between treated and untreated trees was statistically significant ($F_{1,10} = 11.59$, $P = 0.0067$). However, the number of pupal exuviae emerging from trees treated with *S. carpocapsae* did not differ from the number emerging from trees treated with *S. feltiae* ($F_{1,10} = 0.30$, $P = 0.5953$).

New Jersey trial. Monitoring data was collected from 126 infested *F. pennsylvanica* trees to determine specific *Podosesia* species activity. Only 29 exuviae were found in June 1993–94, when adult lilac borers emerged, compared to 1,170 exuviae/126 trees in September 1993–94, when the BACB was active. This verified that the BACB was the primary pest on this site.

Both Dursban and *S. glaseri* (October treatment, with wetting of bark prior to application) had significantly fewer pupal exuviae than the control ($P < 0.02$) (Table 1). The Dursban treatment provided a 74 percent reduction in pupal cases emerging per tree, and *S. glaseri* a 54 percent reduction, relative to the control. Nematode treatments applied following the wetting of bark improved control. Nematode treatments applied to dry bark did not provide acceptable control.

All trees exhibited ample growth between 1993 and 1994, even with an unseasonably cold winter. Of the 148 trees visually pre-rated for decline in 1993, thirty two (22%) were visually rated to appear to have improved health from 1993 to 1994, following treatment. Many of these improved trees also displayed a new leader in 1994. Trees rated 1 (on a scale of 1 to 4; 0–10% decline) exhibited an average of 7.75 pupal exuviae/tree; trees rated 2 (10–39% decline) averaged 10.7 pupal exuviae/tree and trees rated 3 (40–59% decline) averaged 17.95 pupal exuviae/tree. Trees rated 4 (>60% decline) exhibited lower borer infestation (ave. 9.8 pupal exuviae/tree). Treatments were randomized within these trees, although 48 trees received no treatments.

A California study of clearwing moth borers attacking alder and sycamore (11) determined that *Steinernema* nema-

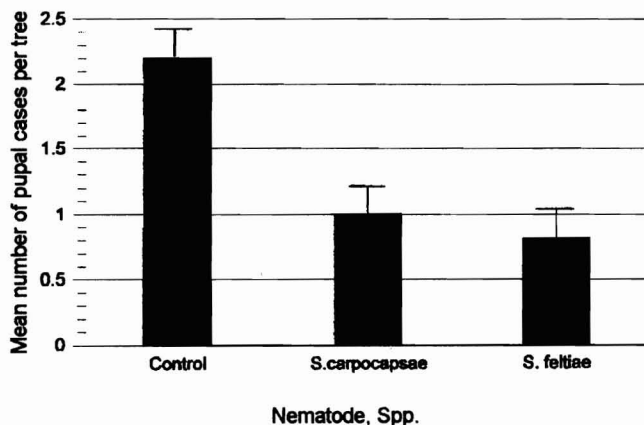


Fig. 1. Effect of nematodes on the number of banded ash clearwing borer pupal cases per tree.

tode bark sprays were not effective if the larval galleries were not sufficiently moist or the gallery opening was too small. The BACB appears to be subject to attack by entomopathogenic nematodes because of its large entry galleries, moist larval galleries, external bark openings, and frass expelling behavior, which aid nematode survival and host searching efficiency. Since the concealed habitat of the BACB larva protects it from conventional insecticide applications, entomopathogenic nematodes offer a safe, potentially economical, and relatively effective means of reducing BACB larval populations after trees have been attacked, decreasing the need for protective chemical sprays.

In Maryland, *S. carpocapsae* and *S. feltiae* treatments applied in July to shaded *F. pennsylvanica* branches significantly reduced the number of emerging adult BACBs, as compared to the control. In comparison, *S. carpocapsae* and *S. feltiae* exposure to direct sun on exposed, dry tree bark in New Jersey may have contributed to nematode mortality and reduced efficacy, although a much higher BACB infestation was present at this site. Note that nematode applications in both locations occurred during mid-day hours, to simulate commercial practices.

High nematode application rates have shown decreased control for clearwing moth borer larvae (7, 11), although the California alder study determined that higher rates of *S. feltiae* did somewhat increase control, albeit not significantly (11). To facilitate application, *S. carpocapsae* is now formulated as a water dispersible granule (sold under the trade name VECTOR T/O), which can be measured out by volume as done with a wettable powder formulation pesticide, for better rate control.

Spring/summer treatments of *Steinernema* nematode tree sprays targeting larger clearwing borer larvae have shown moderate control levels, ranging from 61% for the sycamore clearwing borer (11), 66% for the peachtree borer in cherry laurel (6), and 47–66% for the BACB (7). Nematode treatments in the fall (October) in New Jersey tended to give better control than summer treatments. At this time larval galleries are closer to the bark surface (11), theoretically aiding host searching nematodes. It is also known that smaller insect hosts are more susceptible to nematode infection (10). Fall treatments may be preferable to summer treatments since trees have yet to sustain major damage, and cooler fall and early spring temperatures are not as hostile to nematodes. However, some present strains of nematodes, including *S. carpocapsae*, are sensitive to low temperatures, thus restricting control efforts to early fall (5). Additionally, nurserymen and landscape managers are not as busy at this time of year, and should welcome the opportunity to widen the spray window. These results are consistent with those from the California alder clearwing borer study (11), where *S. feltiae* trunk sprays in late September provided 77–84% control (frass index utilized). However, Kaya and Brown (11) found that an October *S. feltiae* trunk spray targeting the sycamore clearwing borer did not give significant control.

Wetting the tree bark prior to nematode application significantly enhanced nematode survival and control in the fall for *S. glaseri* in New Jersey. The moist bark and amount of water applied with the nematodes may influence nematode survival, or perhaps facilitate entry into the galleries of borers. In California, wetting the bark of sycamore trees prior to a fall nematode application did not increase nematode

efficiency against the sycamore clearwing borer, yet it may have enhanced spring nematode treatment results (11). It may be best to commercially apply nematodes on premoistened bark in the morning or evening, on overcast days, when humidity levels are higher, to reduce nematode dessication and mortality.

An apparent trend in New Jersey showed that declining trees with >60% decline, had fewer pupal exuviae (an average of 9.82/tree) than trees rated <60% decline (average 12.1/exuviae/tree). By 1994, following all treatments, 63% of these trees had improved in health and many had a new leader. Seventeen percent, however, were removed or died. Such severely weakened trees may not be readily reinfested by the BACB, a primary invader, and they may be able to improve in health under good conditions, such as the regular irrigation and fertilization at the site.

In conclusion, nematode biopesticides are gaining popularity for effective, economic and safe pest control, both for soil dwelling insects and insects in cryptic habitats, such as tree borers. This study demonstrates the validity of utilizing entomopathogenic nematodes for control of the BACB as trunk sprays under standard treatment conditions, with greater control achieved under conditions of moist bark. Nematode use can extend the treatment window compared to traditional pesticide treatment. Future research will pinpoint a more thorough understanding of nematode species selection, application technique, and control timing for specific insect hosts.

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A Comparison of Morphological Features Affecting Water Loss in Norway Maple and Washington Hawthorn Stems¹

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Abstract

Crataegus phaenopyrum (Washington hawthorn) stems are known to be more sensitive to water stress during cold storage than *Acer platanoides* (Norway maple). Histological examination revealed that maple stems had a highly suberized periderm, and uniform cuticle with few disruptions. Periderm suberization of hawthorn stems was variable and extensive peridermal cracking was evident. Cuticle wax decreased with increasing distance from the stem apex for both species. No differences in lenticellar characteristics were found between species. Results indicated that hawthorn stems had anatomical features that allowed for more water loss than maple stems, which may provide a possible explanation for differences in maple and hawthorn stem water loss rates.

Index words: cuticular wax, periderm, suberization, *Crataegus phaenopyrum*, *Acer platanoides*, lenticels.

Significance to the Nursery Industry

Tree species differ widely in their response to desiccating conditions. Washington hawthorn often have low post-transplant survival rates due to a high degree of desiccation sensitivity. Results of this study show that stem morphology may contribute to stem water loss differences observed in Norway maple (desiccation tolerant) and Washington hawthorn (desiccation sensitive). Hawthorn stems have anatomical features (stem fissures, reduced suberization and cuticular wax) which may allow for more water loss than Norway maple stems. For most species of bare-root plants, current storage and handling practices are acceptable and post-transplant survival rates are high. Desiccation sensitive species such as Washington hawthorn, however, may require modified cultural practices during extended cold storage and after transplanting to ensure acceptable survival rates. Storage conditions for sensitive species should include a combination of high humidity (97–99%) and the use of antidesiccant compounds or enclosing tree seedlings in storage bags to reduce water loss. Coating stems with an antidesiccant

compound before transplanting also greatly reduces water stress and improves growth. While growers should take precautions to protect the roots of all bare-root stock from desiccating conditions during and after storage, sensitive species require both root and shoot protection to minimize water loss and increase survival.

Introduction

Shoot surfaces of woody plants have specialized protective layers that minimize water loss to the atmosphere. Primary organs such as leaves and young stems are covered by a cuticle whereas older secondary organs develop a periderm (7) which consists of layered phellogen, phellem and phelloderm. Cells within these layers vary in the degree of suberization (19). Suberin is a lipid polymer, and water permeability of suberized cells is low (9). Cuticles provide an effective barrier to water movement and enable plants to withstand conditions of water stress (18). Cuticles are layered lipid membranes composed of soluble waxes and an insoluble polymer matrix, primarily containing pectin, cellulose and lipids (13). Schonerr (16) found that the cuticular resistance to water transport was attributable to the soluble lipids embedded in the polymer matrix. Cuticle thickness and composition vary between plant species and are influenced by environmental conditions (2). Seiler (20) reported increased epicuticular wax content on *Alnus glutinosa* leaves exposed to twelve weeks of sublethal water stress.

Plant lenticels, loose arrangements of cells in the periderm, are assumed to function in gas exchange (7). The stems

¹Received for publication September 5, 1995; in revised form March 18, 1996. We wish to recognize and thank the Virginia Nurserymen's Association for funding this research.

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