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Steinernematid and Heterorhabditid Nematodes for Control of Larval European Chafers and Japanese Beetles (Coleoptera: Scarabaeidae) in Potted Yew

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ABSTRACT Four species of entomogenous nematodes [*Steinernema feltiae* (Filipjev) [=*Neoaplectana carpocapsae* Weiser] ('All' strain), *S. glaseri* (Steiner), *Heterorhabditis heliothidis* (Khan, Brooks & Hirschmann), and *Heterorhabditis* sp. ('Holland' strain)] were compared with two insecticides (chlorpyrifos and isofenphos) for control of third- (last-) instar larval Japanese beetles (*Popillia japonica* Newman) and European chafers [*Rhizotrogus majalis* (Razoumowsky)] in potted Japanese yew (*Taxus cuspidata* Siebold & Zuccarini). Efficacy was evaluated 17–21 d after treatment. *Heterorhabditis* sp. ('Holland' strain) at 92 nematodes per cm^2 of soil surface and *H. heliothidis* at 192 nematodes per cm^2 provided >90% control of Japanese beetles compared with 71% for chlorpyrifos (9.0 kg [AI]/ha) and 84% for isofenphos (4.5 kg [AI]/ha). *S. glaseri* provided 84 and *S. feltiae* 29% control (both at 385 nematodes per cm^2). Both nematodes and insecticides were less effective in controlling European chafer larvae. Control with nematodes ranged from 46 to 59% with *S. glaseri*, *H. heliothidis*, and *Heterorhabditis* sp. at 385 nematodes per cm^2, whereas *S. feltiae* at 385 nematodes per cm^2 did not significantly reduce larval survival compared with the untreated control. Chemical control of European chafer larvae resulted in reductions of 44 and 62% with isofenphos and chlorpyrifos, respectively.

KEY WORDS Insecta, entomogenous nematodes, biological control

THE ORNAMENTALS industry is one of New York State's largest cash crops; nursery production of woody ornamentals in 1984 has been conservatively estimated to have a retail value of about $100 million (Anonymous 1985). Because it is earmarked for export, a significant portion of this production must be certified "pest-free" before shipment (Ladd & Lawrence 1986). With the loss of effective residual insecticides such as chlordane, several species of white grubs (Japanese beetle, *Popillia japonica* Newman, European chafer, *Rhizotrogus* (*Amphimallon*) *majalis* (Razoumowsky), Asiatic garden beetle, *Maladera castanea* (Arrow), and oriental beetle, *Anomala orientalis* Waterhouse) have become important soil insect pests of nursery crops. These insects are also pests of turf in New York and surrounding areas (Tashiro 1987).

Several insecticides are labelled for control of white grubs on turf in New York (bendiocarb, chlorpyrifos, diazinon, ethoprop, isofenphos, and trichlorfon); only bendiocarb and trichlorifon are labelled for white-grub control in ornamentals (Smith & Wilson 1986). However, all labelled insecticides have only limited effectiveness in turf, and studies have shown that these insecticides are ineffective for grub control in balled and container-grown ornamentals using existing control procedures (M.G.V., unpublished data).

Entomogenous nematodes offer a potential alternative to insecticides for control of white grubs. Cost of nematode production is declining due to improved mass-rearing techniques (Bedding 1981, 1984), and their low toxicity to vertebrates has been documented (Gaugler & Boush 1979, Poinar et al. 1982, Obendorf et al. 1983). The potential of several species of nematodes to control soil insects attacking ornamentals (e.g., black vine weevil, *Otiorhynchus sulcatus* (F.), see Rutherford et al. [1987] and references cited therein) and numerous other crops (Kaya 1985) has been demonstrated. Previous research has demonstrated the susceptibility of the Japanese beetle to the nematode *Steinernema glaseri* (Steiner) (Fleming 1968), but there is little information concerning the susceptibility of the white-grub species common in the northeastern United States to various species of entomogenous nematodes.

This study was conducted to provide information on the potential of several species of entomogenous nematodes to control Japanese beetle and European chafer larvae (two common grub species in New York) in potted yews (*Taxus* L.).

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Materials and Methods

Bare-rooted Japanese yews (*Taxus cuspidata* Siebold & Zuccarini 'Densiformis') were planted individually in pots (3.8-liter) containing a mixture of sand and peat moss (1:1, by volume) on 24 April 1986. Plants were infested with either third- (last-) instar Japanese beetle or European chafer larvae on 25 April at a density of 10 larvae per pot. European chafer larvae were collected from turf at the New York State Agricultural Experiment Station, Geneva, on 8–9 April 1986, and Japanese beetle larvae were collected from turf at Sleepy Hollow, N.Y., on 29–30 October 1985 and stored at 4°C.

A total of 19 treatments was applied to infested plants in a randomized complete block design with five replications. The treatments included a control (water alone), chlorpyrifos (Dursban 50W) at 9.0 kg (AI)/ha, isofenphos (Oftanol 2I) at 4.5 kg (AI)/ha, and infective juveniles of the entomogenous nematodes *Steinernema feltiae* (Filipjev) [=*Neoaplectana carpocapsae* Weiser] ('All' strain), *S. glaseri*, *Heterorhabditis heliothidis* (Khan, Brooks & Hirschmann), and *Heterorhabditis* sp. ('Holland' strain), each applied at rates of 46, 92, 192, and 385 nematodes per cm^2 of soil surface. Nematodes were reared by Biosis, A Biological Pest Control Company (Palo Alto, Calif.) using in vitro procedures (F.A.-S., unpublished data), and shipped to Geneva on moist sponge pads. They were stored at 8°C until used.

All treatments were applied in 300 ml water by pouring them evenly over the soil surface of each pot as a drench application. The water control, and the chlorpyrifos and isofenphos treatments, were applied on 1 May. The insecticide rates applied were twice the maximum labelled rates for grub control on turf and were chosen based on previous studies with these grub species (M.G.V., unpublished data). The nematode treatments were applied on 2 May. Concentrated nematode suspensions were made and the concentration of live nematodes was determined by microscopic examination of subsamples of the suspensions. The appropriate nematode treatments were then made by dilution.

Pots were held in a greenhouse from 1 to 5 May, due to severe weather, then moved outdoors. Pots were watered as needed to maintain plant health. Soil temperature in the pots was continuously recorded at the 7.5-cm depth. Additional untreated pots were monitored throughout the experiment to determine soil moisture. A soil sample (ca. 100 g wet weight) was taken from each of two pots on 1, 8, and 15 May. At the end of the study (19 May), soil samples were taken from one pot in each treatment in one replicate. Soil samples were oven-dried and percent soil moisture was determined by weight.

Treatment efficacy was evaluated on 19–22 May by destructively sampling all pots. The number and stage of living and dead insects were recorded. The location of insects in the pot was also recorded (as either in the top 10 cm, bottom 5 cm, or in the roots).

Dead insects and soil samples from each treatment were collected at this time. Dead grubs were dissected and examined microscopically to determine the presence of entomogenous nematodes. Nematodes were identified to family based on morphological characteristics described in Poinar (1985). Residual activity of nematodes in the soil was assessed using a *Galleria* bioassay for determination of nematode presence. In this bioassay, soil samples from each replicate were combined by treatment. A 35-g subsample of soil from each treatment was placed in a Petri dish and 10 last-instar *Galleria mellonela* (L.) (Lepidoptera: Pyralidae) larvae were added. Dishes were covered and held at 25°C for 5 d and then *Galleria* mortality was assessed. Dead *Galleria* were dissected and examined microscopically for presence or absence of entomogenous nematodes based on morphological characteristics as described above.

Data on number of surviving grubs were analyzed by analysis of variance. Means were separated by the least significant difference (LSD) test, at $P = 0.05$ (Little & Hills 1978). Analyses were done on the raw data, as the variances were not dependent on the means based on correlation analyses ($P < 0.05$). Data from the nematode treatments were also analyzed by use of linear regression (Ryan et al. 1985) to characterize the density-dependent response of the treatments. The data on the location of insects were analyzed by the χ^2 statistic. For each grub species, χ^2 tests were made of the number of surviving insects in the three areas (top, bottom, and in the roots) in the untreated controls compared with those of each of the nematode species (all rates combined) and with the insecticide treatments (both compounds combined).

Results

Generally, all treatments provided better control of Japanese beetles than European chafers (Fig. 1). The two *Heterorhabditis* spp. at the lowest nematode rate applied (46/cm^2) provided control of Japanese beetles equivalent to the best insecticide treatment. *S. glaseri* at ≥192 nematodes per cm^2 provided control equivalent to the best insecticide treatment. *S. feltiae* ('All' strain) at 385 nematodes per cm^2 provided significantly less control than either of the two insecticide treatments. Except for *S. feltiae*, all nematode species significantly reduced survival of European chafers compared with the control, at least at the higher nematode rates. There were few significant differences between *S. feltiae*, the lower rates of the other three nematode species, and the insecticide treatments (Fig. 1).

The number of surviving Japanese beetle grubs exhibited a linear response to nematode application rates for all four nematodes as determined by

regression analysis.[4] With the European chafer, there were significant linear responses to nematode rates for all of the nematodes except *S. feltiae*.[5]

Low levels of entomogenous nematode activity were detected in soil samples taken 17–21 d after treatment based on the bioassay with *Galleria* larvae (Table 1). No nematode activity was detectable in soil from the controls or in that treated with either *S. feltiae*, chlorpyrifos, or isofenphos in either Japanese beetle- or European chafer-infested pots. There was an apparent trend for greater residual activity of nematodes in soil treated with higher nematode rates and in pots infested with Japanese beetles.

The majority of dead grubs removed from the nematode-treated pots 17–21 d after treatment contained entomogenous nematodes, as determined by dissection (Table 2). All nematodes found in the dead grubs were entomogenous and taxonomically corresponded to the families Steinernematidae and Heterorhabditidae. However, some dead white grubs of both species containing entomogenous nematodes were found in both the untreated and insecticide-treated pots.

For the European chafers but not for the Japanese beetles, some of the treatments were significantly different from the untreated controls in the number of surviving insects in the top, bottom, or in the roots. For both *Steinernema* spp. treatments, there were significantly fewer surviving grubs in the top layer and in the roots and more in the bottom layer than expected (*S. feltiae*, $\chi^2 = 24.34$, $P < 0.001$; *S. glaseri*, $\chi^2 = 23.06$, $P < 0.001$; both with 2 df), whereas for the insecticide treatments, significantly more grubs survived in the top layer and fewer in the roots and bottom layer than expected compared with the untreated controls ($\chi^2 = 6.25$, $P < 0.05$, 2 df). The distribution of surviving grubs in untreated pots was similar for both grub species (Japanese beetle, 63, 12, and 25%; European chafer, 51, 18, and 31% in top, in roots, or in bottom, respectively).

Soil moisture averaged 22.4% (range, 18–26%) during 1–19 May. Daily soil temperatures (7.5-cm depth) averaged 26.7°C (max) (range, 24–31°C), and 13.5°C (min) (range, 11–17°C) for the period 2–5 May while the pots were in the greenhouse and 29.9°C (max) (range, 18–36°C) and 12.5°C (min) (range, 7–18°C) for the period 6–19 May while the pots were outdoors.

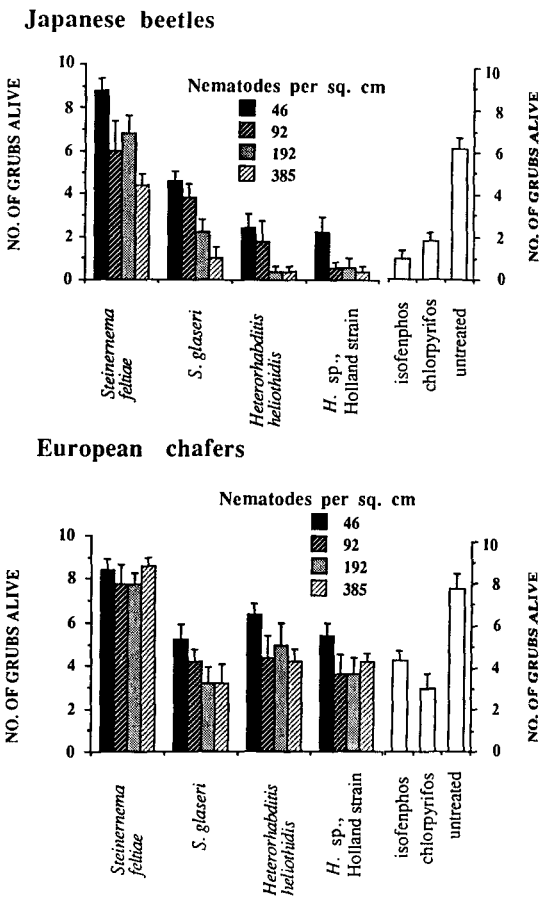

Fig. 1. Efficacy of chlorpyrifos (9.0 kg [AI]/ha), isofenphos (4.5 kg [AI]/ha), and entomogenous nematodes in controlling Japanese beetle and European chafer larvae in potted Japanese yews, evaluated 17–21 d after treatment, Geneva, N.Y. Visible portion of error bar equal to one-half of the standard error of the mean. $LSD_{0.05} = 2.0$ (Japanese beetle); 1.8 (European chafer).

Discussion

These studies have demonstrated that entomogenous nematodes significantly reduced white-grub densities in potted yews and provided control of white grubs equal to or exceeding that provided by twice the labelled rates of two commonly used turf insecticides. Both *Heterorhabditis* spp. provided very good control (>90% reduction) of Japanese beetle grubs at rates from 92–192 nematodes per cm², while the best treatments (*S. glaseri* and *H.* sp.) provided control of European chafers equivalent to that achieved with the two insecticides (Fig. 1).

The soil bioassay procedure provided a qualitative assessment of nematode persistence under the test conditions. Nematodes active at the time of sampling (17–21 d after treatment) could represent either the original application or their progeny. The presence of infective nematodes ca. 3 wk after application suggests the potential for long-term control by nematodes. Further studies are needed to determine the level of long-term control achieved with commercial use. The low residual

[4] *S. feltiae:* $y = 7.43 - 0.007X$, $F = 5.29$, $P < 0.05$. *S. glaseri:* $y = 5.36 - 0.013X$, $F = 42.57$, $P < 0.01$. *H. heliothidis:* $y = 3.92 - 0.012X$, $F = 17.9$, $P < 0.01$. *H.* sp.: $y = 3.54 - 0.011X$, $F = 14.11$, $P < 0.01$. All with 1 and 23 df.

[5] *S. glaseri:* $y = 6.10 - 0.01X$, $F = 12.32$, $P < 0.01$. *H. heliothidis:* $y = 6.63 - 0.007X$, $F = 7.34$, $P < 0.05$. *H.* sp.: $y = 5.88 - 0.007X$, $F = 5.39$, $P < 0.05$. All with 1 and 23 df.

Table 1. Mortality of *G. mellonella* larvae and percentage of dead *Galleria* infested with entomogenous nematodes in bioassay of soil, collected 17–21 d after indicated treatments, from pots infested with either Japanese beetle or European chafer larvae

Treatment	Application rate	No. of dead *Galleria* larvae (*n*) and percent containing entomogenous nematodes (%)			
		Japanese beetle		European chafer	
		n	%	*n*	%
Untreated	—	0	0	0	0
Chlorpyrifos	9.0 kg (AI)/ha	2	0	1	0
Isofenphos	4.5 kg (AI)/ha	1	0	1	0
Steinernema feltiae	46/cm^2	0	0	1	0
'All' strain	92/cm^2	0	0	0	0
'All' strain	192/cm^2	2	0	0	0
'All' strain	385/cm^2	0	0	0	0
S. glaseri	46/cm^2	1	0	1	0
S. glaseri	92/cm^2	1	100	1	0
S. glaseri	192/cm^2	3	33	0	0
S. glaseri	385/cm^2	2	100	0	0
Heterorhabditis heliothidis	46/cm^2	3	0	2	50
H. heliothidis	92/cm^2	4	25	1	0
H. heliothidis	192/cm^2	1	0	1	0
H. heliothidis	385/cm^2	2	100	4	100
Heterorhabditis sp.					
'Holland' strain	46/cm^2	1	0	0	0
'Holland' strain	92/cm^2	1	0	0	0
'Holland' strain	192/cm^2	3	100	0	0
'Holland' strain	385/cm^2	2	100	3	33

activity which was seen in *S. feltiae*-treated pots (Table 1) could be related to the fact that this nematode killed relatively few white grubs of either species (Fig. 1) and probably produced fewer nematode progeny, rather than because of any differences among the species of nematodes in their ability to persist in the soil over this time period. Similarly, the lower residual activity of soil from European chafer-infested pots could be due to the lower number of dead, infected European chafer grubs.

Some of the dead grubs in the untreated and insecticide-treated pots were infested with entomogenous nematodes. We do not know if this was due to contamination between pots, or to preexisting nematode populations in the unsterilized soil and field-collected white grubs used in this study. Nematodes are known to travel over the soil surface (Reed & Wallace 1969) and could have moved between pots, which were arranged randomly with respect to treatment. Alternately, water splashed by rain or during watering could have moved some

Table 2. Incidence of entomogenous nematodes, as determined by dissection, in dead white grubs collected from pots 17–21 d after indicated treatments

Treatment	Application rate	No. of dead grubs dissected (*n*) and percent containing entomogenous nematodes (%)			
		Japanese beetle		European chafer	
		n	%	*n*	%
Untreated	—	2	100	1	0
Chlorpyrifos	9.0 kg (AI)/ha	2	50	13	69
Isofenphos	4.5 kg (AI)/ha	6	67	10	40
Steinernema feltiae	46/cm^2	2	100	4	25
'All' strain	92/cm^2	10	90	2	0
'All' strain	192/cm^2	5	80	4	50
'All' strain	385/cm^2	6	100	2	50
S. glaseri	46/cm^2	13	100	9	56
S. glaseri	92/cm^2	15	93	4	75
S. glaseri	192/cm^2	11	100	12	83
S. glaseri	385/cm^2	19	100	7	100
Heterorhabditis heliothidis	46/cm^2	19	100	10	90
H. heliothidis	92/cm^2	27	96	12	75
H. heliothidis	192/cm^2	28	100	11	82
H. heliothidis	385/cm^2	13	100	2	100
Heterorhabditis sp.					
'Holland' strain	46/cm^2	16	81	5	20
'Holland' strain	92/cm^2	24	79	5	80
'Holland' strain	192/cm^2	21	90	10	70
'Holland' strain	385/cm^2	20	90	8	75

nematodes between pots. Whatever the reason, the relatively low number of dead grubs infested with entomogenous nematodes in pots not treated with nematodes does not diminish the fact that some of the entomogenous nematodes studied significantly increased the mortality of white grubs over that of the insecticide-treated and untreated pots (Fig. 1).

The distribution of surviving European chafer grubs in the different treatments could be due to differences in the responses of the nematode species to gravity, temperature, moisture, host insects, or other factors. Some work has been done on the movement of entomogenous nematodes in the soil. In a sandy loam soil, *H. heliothidis* applied to the soil surface tended to stay at or near the soil surface (0–2 cm) over a 5-d period of observation; the presence of wax moth pupae 10 cm below the soil surface tended to slightly increase the downward movement of the nematodes (Georgis & Poinar 1983c). Similar results were seen with *S. feltiae* (Moyle & Kaya 1981, Georgis & Poinar 1983a). Studies with *S. glaseri* indicated that after application to the soil surface, this nematode tended to disperse downward to a greater degree, again with greater downward movement in the presence of wax moth pupae below the soil surface (Georgis & Poinar 1983b). Recent laboratory studies by Schroeder & Beavers (1987) with these species also demonstrated that *S. glaseri* dispersed downward to the greatest degree over a 30-d period.

The above studies were all conducted in uniform soil columns in the laboratory and (except for Schroeder & Beavers [1987]) over relatively short periods, whereas our studies were conducted in containers with plants growing in a sand-peat mix over a period of ca. 3 wk. Some of our results are different from previous studies with these nematodes. In our study there were differences between grub species in the location of surviving larvae in different nematode treatments; i.e., *Steinernema* spp. appeared to produce relatively more mortality near the soil surface when European chafer larvae were present, whereas no such trend was observed when Japanese beetle larvae were present. No such differences were seen with *Heterorhabditis* spp. Assuming that physical conditions (temperature, moisture, soil texture) were similar in pots infested with different grub species, this differential effect between *Steinernema* and *Heterorhabditis* spp. might be due to differences between grub species in attracting entomogenous nematodes. Increased knowledge of the impact of such factors on nematode efficacy, as well as differences in host susceptibility to different nematodes, will be important in determining what situations are most suitable for use of each nematode.

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