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# Entomogenous Nematodes as Biological Control Agents of European Chafer and Japanese Beetle (Coleoptera: Scarabaeidae) Larvae Infesting Turfgrass

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**ABSTRACT** In laboratory studies, infective juveniles of *Steinernema feltiae* Filipjev (= *Neoplectana carpocapsae* Weiser) (All strain) and *Heterorhabditis heliothidis* (Khan, Brooks & Hirschmann) were tested for their ability to control third-instar European chafer (EC), *Rhizotrogus majalis* (Razoumowsky), in soil at rates from 19.4-310.0 nematodes per cm<sup>2</sup> of soil surface. *H. heliothidis* provided better control of EC larvae than *S. feltiae* over the range of rates tested. After 25 d, treatment with *H. heliothidis* provided 94% control of larvae at the lowest rate tested (19.4 per cm<sup>2</sup>). In a field test in turf against a mixed population of Japanese beetle, *Popillia japonica* Newman, and EC larvae, *H. heliothidis* (310 per cm<sup>2</sup>) provided >60% control (47 d after treatment), which was equivalent to the control achieved with the labeled rates of chlorpyrifos, trichlorfon, and isofenphos against this combination of white grubs.

**KEY WORDS** Insecta, Nematoda, entomogenous nematodes, biological control

SEVERAL GRUB SPECIES are pests of turf and ornamental crops in the northeastern United States (Tashiro 1987). In New York State these include the Japanese beetle, *Popillia japonica* Newman, the European chafer, *Rhizotrogus majalis* (Razoumowsky), and the oriental beetle, *Anomala orientalis* Waterhouse. Many of the plants damaged by these soil insects are grown in close proximity to homes, office buildings, and recreational sites in urban and suburban areas.

Difficulties in achieving consistent and effective control of white grubs with current insecticides (Harris 1982, Baker 1986), as well as public concern about pesticide use, point to the importance of developing biological control strategies for these pests. Klein (1982), in reviewing the status of several potential tactics for the biological suppression of turf insects (diseases, attractants, parasitoids, and predators), suggests promise for many biological control tactics when they are carefully integrated with conventional pesticide treatments. Previous work has demonstrated the potential of milky disease, caused by *Bacillus popilliae* and *B. lentimorbus*, and the entomogenous nematode *Steinernema glaseri* (Steiner) for control of the Japanese beetle (Fleming 1968). Work summarized by Kaya (1985) has demonstrated the potential of several species of entomogenous nematodes to control soil insects on various crops. We recently evaluated several species of steinernematid and heterorhabditid

nematodes against Japanese beetle and European chafer larvae in potted yews (Wright et al. in press). Results indicated that Japanese beetles were much more susceptible to several species of nematodes than were European chafers. This paper describes studies conducted to examine more closely the efficacy of nematodes against European chafer grubs in the laboratory and a field trial to evaluate application of nematodes in turf against both grub species.

## Materials and Methods

Nematodes used in all studies were obtained from Biosis (Palo Alto, Calif.). Nematodes were reared using in vitro procedures, then infective juveniles were shipped to Geneva, N.Y., on moist sponge pads where they were stored at 8°C until use. Experiments discussed in this manuscript were conducted with nematodes <24 hours after arrival in Geneva.

**Laboratory Studies.** Third-instar European chafer (EC) used in these studies were collected in turf at the New York State Agricultural Experiment Station, Geneva, in October 1985 and held overwinter at 4°C in boxes filled with soil. These larvae were supplemented with larvae collected from the same location in April 1986. Larvae from the two collection dates were mixed together before the studies began. Containers (15 by 10 by 8 cm) were filled with a loamy sand soil mixed with grass seed (Agway Shady Green Mixture) and infested with five EC larvae per container during 24-28 June and held at 4°C until the studies began. Soil moisture was maintained at 10-12% during

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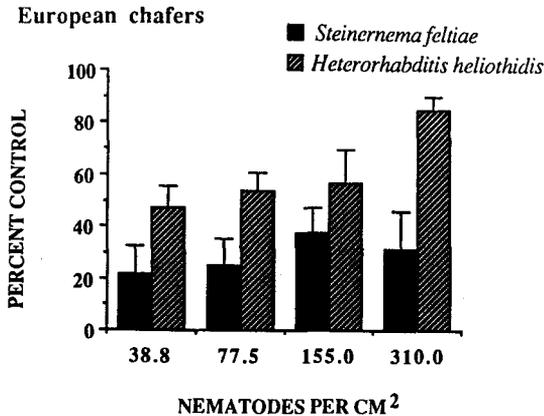


Fig. 1. Efficacy of *Heterorhabditis heliothidis* and *Steinernema feltiae* (All strain) against third-instar European chafer in a laboratory soil bioassay.

the studies. In both studies, soil containers were held at 20°C and a photoperiod of 12:12 (L:D) after treatment with nematodes.

One study was conducted to compare *H. heliothidis* and *S. feltiae* (All strain). Ten containers (50 grubs per treatment) were infected on 10 July with each nematode species at 38.8, 77.5, 155.0, and 310.0 per cm<sup>2</sup>, and with an untreated control. Nematode efficacy was assessed 20 d after treatment. Larvae were categorized as either alive (healthy and moribund individuals were considered alive for statistical analysis) or dead. Dead larvae were dissected, and the presence or absence of nematodes was noted; observations were also made on the color of the larvae (i.e., whether they were reddish in color, characteristic of infection with the symbiotic bacteria associated with *H. heliothidis* [Khan et al. 1976]).

A second study was conducted to determine the dosage-mortality relationship of *H. heliothidis* and European chafer. *H. heliothidis* were applied in 9.5 ml of water at rates of 0, 19.4, 38.8, 77.5, 155.0, and 310.0 infective juveniles per cm<sup>2</sup> on 3 July. Thirty containers (150 per treatment) were treated with each rate of nematode; 10 containers from each treatment were destructively sampled 12, 18, and 25 d after treatment. Nematode efficacy was assessed as described above. Data from both studies were analyzed by analysis of variance (ANOVA) for a completely randomized design and by regression analysis (Ryan et al. 1985).

**Field Test.** A study was conducted to compare the efficacy of *H. heliothidis* with that of several insecticides labeled in New York for control of the white grub complex in turf. The experimental design was a randomized complete block with four replications. Individual plots were 3 by 3 m. The insecticide treatments were chlorpyrifos (Dow Chemical, Midland, Mich.) (Dursban 2.3 Granular [G]), 4.48 kg (AI)/ha; bendiocarb (Nor-Am Chemical, Wilmington, Del.) (Turcam 2.5G), 4.70 kg

(AI)/ha; trichlorfon (Mobay Chemical, Kansas City, Mo.) (Dylox 5G), 8.96 kg (AI)/ha; carbaryl (Union Carbide, Research Triangle Park, N.C.) (Sevin SL [flowable]), 8.96 kg (AI)/ha; ethoprop (Rhone-Poulenc, Monmouth Junction, N.J.) (Mocap 5G), 11.20 kg (AI)/ha; and isofenphos (Mobay Chemical, Kansas City, Mo.) (Oftanol 5G), 2.24 kg (AI)/ha. These treatments were applied to a fairway at Drumlin Country Club, Syracuse, N.Y., on 21 August 1986 against a mixed population of Japanese beetle and European chafer grubs. Additional details concerning the study site are reported elsewhere (Villani & Wright in press). Granular materials were applied with a precalibrated Gandy 2.5 spreader. Flowable materials were premeasured in the laboratory and applied in two directions within each plot with 11.4 liter water through a watering can. The nematodes (*H. heliothidis*) were applied a day later (22 August) at a rate of 310 per cm<sup>2</sup> in 11.4 liter water through a watering can to the center 3.7 m<sup>2</sup> of each plot. Following treatment, the plots were irrigated (equivalent 0.6 cm rain) by sprinkler.

Posttreatment counts were taken 47–48 d later (8 October) by cutting (3 m by 30 cm by ca. 3 cm deep) lengthwise through each plot with a mechanical sod cutter and examining the sod and underlying soil for grubs. Three 0.9-m<sup>2</sup> samples of sod from the center of each plot were examined in each plot and the surviving grubs were counted. In the nematode-treated plots, separate sets of samples (four 0.9-m<sup>2</sup> samples) were taken from the center 3.7 m<sup>2</sup>, where nematodes had been applied, and from 30–60 cm away from the edge of the center 3.7 m<sup>2</sup> in each of the four compass directions. Grub species identifications were made in the field with a hand lens using characteristics described in Tashiro (1987). At this time Japanese beetle grubs were predominantly third instars with some second instars; all European chafer grubs were third instars. Data (plot totals) were transformed by log<sub>10</sub>(x + 1), as there was a significant correlation ( $P = 0.05$ ) between treatment means and variances. Data were analyzed by ANOVA for a randomized complete block design (Ryan et al. 1985) and mean separation was by Duncan's (1955) multiple range test ( $P = 0.05$ ).

## Results

**Laboratory Studies.** In studies comparing *S. feltiae* (All strain) and *H. heliothidis*, there was a significant difference between the two nematodes in the number of surviving grubs ( $F = 14.59$ ;  $df = 1, 63$ ;  $P < 0.01$ ) as determined by ANOVA. While there was a significant linear relation between the number of surviving grubs 20 d after treatment and the initial application rate of *H. heliothidis* as determined by regression analysis ( $y = 1.90 - 0.004X$ ,  $F = 10.30$ ,  $P < 0.01$ ), there was no significant regression for *S. feltiae* ( $F = 0.52$ ,  $P >$

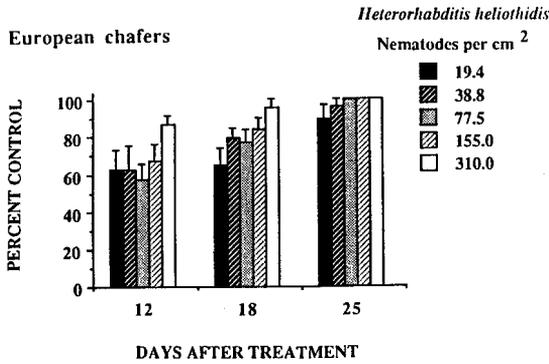


Fig. 2 Influence of *Heterorhabditis heliothidis* application rate and days after treatment on percent control of third-instar European chafer in a laboratory soil bioassay.

0.10; both with  $df = 1, 38$ ). *Heterorhabditis heliothidis* produced a higher percent control of EC grubs than *S. feltiae* over all rates tested (Fig. 1). None of the dead grubs in the untreated controls had evidence of nematode infection, while 43–85% of dead grubs from *S. feltiae*-treated containers and 38–77% of dead grubs from *H. heliothidis*-treated containers had evidence of nematode infection.

On each of the three observation dates, there was a significant linear relation between the number of surviving EC grubs and the initial application rate of *H. heliothidis*, as determined by regression analysis (12 d after treatment,  $y = 2.45 - 0.007X$ ,  $F = 17.59$ ; 18 d after treatment,  $y = 2.23 - 0.008X$ ,  $F = 25.94$ ; 25 d after treatment,  $y = 1.02 - 0.005X$ ,  $F = 12.98$ ; all with  $df = 1, 58$  and  $P < 0.01$ , where  $y$  is the number of surviving grubs and  $X$  is the nematode rate (19.4–310.0). Percent control increased with time after treatment, so that by 25 d after treatment, application of nematodes provided 94% control even at the lowest nematode rate (19.4 per  $cm^2$ ) (Fig. 2). None of the dead grubs in the untreated controls had evidence of nematode infection (presence of nematodes or reddish coloration), but a high percentage of dead grubs in nematode-treated containers showed evidence of nematode infection (92–97% 12 d after treatment, 61–97% 18 d after treatment, 69–83% 25 d after treatment). In the two later observation periods, some dead grubs had decomposed to such a degree that it was impossible to determine whether they had been infected with nematodes; this may be partly responsible for the lower percentages at the later two dates. In this trial and the above study, an increasing percentage of dead grubs gave evidence of nematode infection as nematode application rates increased.

**Field Test.** All insecticides except for chlorpyrifos provided statistically significant control of the white grub complex at this site (Table 1). The area treated with nematodes showed control of white grubs equivalent to the three less effective insecticide treatments (chlorpyrifos, trichlorfon, and isofenphos). There were statistically similar levels of control inside and outside of the central plot area where nematodes were initially applied; however, grub densities outside the central plot area were not significantly different than the untreated control plots.

Table 1. Field test comparing *H. heliothidis* and six labeled insecticides for control of Japanese beetle and European chafer larvae in turf, Syracuse, N.Y., August–October 1986

Treatment	Kg (AI)/ha	No. grubs per 0.9 m <sup>2</sup>		% control
		3rd instars	Total	
Japanese beetle				
Chlorpyrifos	4.48	6.2c	9.2cd	30
Bendiocarb	4.70	1.2a	1.4a	89
Trichlorfon	8.96	2.2ab	3.5ab	74
Carbaryl	8.96	0.8a	1.7a	87
Ethoprop	11.20	1.5ab	1.8a	87
Isofenphos	2.24	3.4bc	4.6bc	65
<i>H. heliothidis</i> (310 per $cm^2$ )				
Interior plot		3.6bc	4.8bc	64
Surrounding plot		6.2c	8.6cd	35
Check		8.2c	13.2d	—
All grubs <sup>a</sup>				
Chlorpyrifos	4.48		9.3cd	30
Bendiocarb	4.70		1.4a	89
Trichlorfon	8.96		3.6ab	73
Carbaryl	8.96		1.7a	88
Ethoprop	11.20		1.8a	87
Isofenphos	2.24		4.6bc	66
<i>H. heliothidis</i> (310 per $cm^2$ )				
Interior plot			5.2bc	61
Surrounding plot			9.4cd	30
Check			13.4d	—

Means in the same column not followed by a letter in common differ significantly ( $P = 0.05$ ; Duncan's [1955] multiple range test).

<sup>a</sup> Includes Japanese beetles and European chafers.

## Discussion

These studies demonstrate the ability of *H. heliothidis* to control both European chafer and Japanese beetle larvae, two of the most economically important white grub species in New York. The level of control of the white grub complex in the field trial in turf (Table 1) was equal to that achieved with the labeled rates of several turf insecticides (isofenphos, chlorpyrifos, and trichlorfon). Previous studies (Wright et al. in press) have demonstrated the ability of entomogenous nematodes to control these two grub species in potted yews. The use of entomogenous nematodes in urban and suburban sites should reduce insecticide use against soil insects, lessening the potential of human exposure in these densely populated areas.

Data from our laboratory studies (Fig. 2) suggest that nematodes, like other biological control agents, can be used in two ways. High application rates of nematodes (up to 310.0 per  $cm^2$ ) produced high

rates of mortality quickly (after 12 d), whereas lower rates (19.4 per cm<sup>2</sup>) produced lower initial mortality but produced high levels of control over longer time periods (25 d). This result is most likely caused by the ability of nematodes to reproduce in the body of the dead insect, producing additional infective juveniles which disperse and can attack other insects. Thus, high rates of nematodes (i.e., inundative releases) are needed to produce high levels of control quickly, but lower rates of nematodes can be used as an inoculative release. Assuming that conditions are suitable for nematode survival and reproduction, even with lower nematode rates, a high percent of control can be achieved over a longer time period. Therefore, nematode application rate will be determined not only by economics (control costs versus value of potential crop loss) but also by pest density and speed of control required.

The field trial results (Table 1) illustrate another useful property of entomogenous nematodes—their ability to move from the application site. The level of grub control observed 30–60 cm away from the site of nematode application was not significantly different from that in the central area where nematodes were applied. Field studies by Poinar & Hom (1986) with *S. feltiae* reported a dispersal rate as high as 4.35 cm/d over a 7-d period. Thus in some situations, such as in turf, it may be possible to treat high-value sites only and still achieve some control in adjacent low-value sites not requiring as quick or as high a level of control. The ability of nematodes to move actively through the soil profile, especially if soil insects are present (Moyle & Kaya 1981, Georgis & Poinar 1983, Schroeder & Beavers 1987), is a distinct advantage over traditional synthetic and biological insecticides which cannot be incorporated into turf and must depend upon passive movement into the root zone to be effective. Conversely, environmental conditions that evoke different responses in the parasitic nematode and the grub host will tend to lessen nematode effectiveness and must be addressed.

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