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Field Application of Entomopathogenic Nematodes for Control of *Delia radicum* in Collards[1]

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Abstract: Control of *Delia radicum* (cabbage maggot) in field collards (*Brassica oleracea*) was compared after one or two applications of entomopathogenic nematodes, *Steinernema carpocapsae* (All strain) and *Heterorhabditis bacteriophora* (HP88 strain), a single application of granular chlorpyrifos, and a water-only treatment. Nematodes were applied with a sprayer during the egg stage of first-generation *D. radicum*, and chlorpyrifos was hand placed around collard stems during the same period. A second nematode application was made 10 days later. Chlorpyrifos treatment resulted in fewer puparia per plant, less root damage and higher yield than all other treatments, including the control. Collard yield from nematode-treated beds did not differ from controls. These data indicate that, under these field conditions, the species or strains of entomopathogenic nematodes tested did not reduce the number of active cabbage maggots, nor did they prevent collard root damage.

Key words: biological control, cabbage maggot, chlorpyrifos, collards, *Delia radicum*, entomopathogenic nematode, *Heterorhabditis bacteriophora*, nematode, *Steinernema carpocapsae*.

The cabbage maggot, *Delia radicum* (Diptera: Anthomyiidae), is a cosmopolitan pest of broccoli, cauliflower, collards, and other cole crops. Eggs of the economically important first generation are deposited around and on the stems of early-season (April–May) field plants. Immature *D. radicum* hatch in several days then tunnel into root tissue, where feeding occurs (7).

In the New England region of the United States, standard treatment is a soil drench of diazinon, fonofos, or chlorpyrifos 7–10 days after *D. radicum* eggs are first detected. Soil incorporation of chlorpyrifos 15 (granular formulation) was shown to be efficacious, economical, and labor saving (15), but this technique is not yet practiced by regional growers. Because of widespread concern over continuing application of organophosphate insecticides, particularly in a soil regime, modification of the current *D. radicum* management strategy is being pursued from several directions. Laboratory bioassays demonstrated that growth regulators may constitute a promising alternative control strategy (9,16), whereas other studies have included placement of exclusion barriers around or over plants to prevent oviposition near stems (13).

Entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae are being evaluated as inundative biological controls against a variety of subterranean or otherwise cryptic insect pests that infest vegetables, fruits, turf, and trees (1,10,12). Demonstration of field efficacy by nematodes against particular pest species is required to provide satisfactory evidence for ultimate grower acceptance.

Several trials have provided evidence of the potential efficacy of entomopathogenic nematodes against *Delia* species. First instar *D. radicum* was shown to be susceptible under laboratory conditions (2). Nematodes caused mortality in *D. radicum* and *D. antiqua* (4,5) and reproduced within these hosts (14). Attempts at simulating field conditions were performed, but applications were not conducted under commercial field conditions (2).

In the current study, two species of entomopathogenic nematodes were compared with chlorpyrifos treatment of field collards during 1986 and 1987 (Simser, unpublished). Results were positive, as crop yields were similar, but these investigations were confounded by lack of a control group, at the growers' discretion. In

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1988, a control was included, permitting comparison of nematode, chlorpyrifos, and control treatment. Suppression of first-generation cabbage maggots was interpreted by recording crop yields, root damage, and number of puparia from field samples.

Materials and Methods

Field site: The experimental site was placed 25 m from the field edge, on a mixed vegetable farm in Whately, Massachusetts. Adjacent beds of collards (*Brassica oleracea* cv. Blue Max) were divided into 10-m units spaced 0.5 m apart. Six treatments with six replicates were arranged in a randomized complete block design. Each treatment replicate included three rows of approximately 50 collards, with 20 cm between plants and 45 cm between rows.

Collards were field transplanted on 22 April 1988, and the first *D. radicum* eggs were noted 15 days thereafter. Treatments were made 5 days after eggs were found. The soil type was Windsor loamy fine sand (Typic Udipsamment), with a pH of 7.1 and a temperature of 20 C at a depth of 10 cm. Rainfall (ca. 1 cm) was recorded less than 48 hours before the applications, and soil was moistened by irrigation at ca. 48-hour intervals throughout the trial.

Applications: Nematodes (Biosys, Palo Alto, CA) were held in refrigeration (ca. 4.4 C) for 2 weeks before field application. One or two applications of *Steinernema carpocapsae* (All strain), and *Heterorhabditis bacteriophora* (HP88 strain), a single soil incorporation of chlorpyrifos 15 (granular formulation), and a single spray application of water (control) were compared. The control treatment was made with a piston pump (Hypro, St. Paul, MN) powered by a 5-hp gasoline engine (Briggs and Stratton, Milwaukee, WI). Thirty liters of water per control block was sprayed at a pressure of 5.2 kg/cm^2 with a #6 flat nozzle that directed the spray in a swath 10 cm wide.

Nematodes were removed from refrigeration, mixed in water, and applied at the same pressure and spray pattern at a rate of 250 nematodes/cm^2. Chlorpyrifos was incorporated into the soil, ca. 2 cm deep, at a rate of 28 mg a.i./plant. The second nematode treatment was conducted 10 days after the initial treatment. Nematode viability was verified by host bioassay. Late instar larvae of the greater wax moth, *Galleria mellonella,* were buried 2.5 cm deep between plants before nematode application (four larvae per replicate), collected after 7 days, placed in petri dishes (9 cm diameter) and held in darkness at ca. 25 C. Insect mortality (>90%) and subsequent nematode propagation with cadavers demonstrated infectivity of the nematodes. Additional grower input to the collards included the herbicide dacthal in early April and a foliar treatment with carbaryl on 17 May for flea beetle control.

Data collection: Collards were evaluated 25 days after the initial application. Five plants per replicate were chosen randomly and harvested. Leaves that were considered marketable (more than 20 cm across) were removed from the main stem and weighed. After this harvest, a root–soil core was taken by centering a golf course putting hole tool over each stem remnant, then removing a core at least 25 cm deep and within a 5-cm radius of the stem. This soil was placed into containers and the roots washed and examined.

Roots were rated for damage and assigned a numerical damage value, with i equaling one of these values: 1 = no noticeable maggot tunneling; 2 = slight tunneling with 1–5 tunnels; 3 = moderate damage with 5–10 tunnels; and 4 = severe damage with more than 10 tunnels. Root damage ratings were transformed to a mean root damage index (RDI), used previously as a critical assessment of maggot activity and feeding (6). This index was calculated for the numerical damage values by the following formula:

$$\text{RDI} = \left[\sum_{i=1}^{4}\left(\frac{\text{no. of plants at rating } i}{\text{no. of plants sampled}}\right) \times 100\,(\text{rating } i)\right] \Big/ 4.$$

Soil was sieved, and all maggots and pu-

paria collected from each sample. These specimens were placed in 9-cm-d petri dishes lined with moistened filter paper, and the dishes were held in continual darkness in a laboratory closet at ca. 25 C. All *D. radicum* larvae pupated and were considered as puparial samples during data analysis. Fresh collard leaf weights were submitted to analysis of variance, and treatment means were separated by Duncan's multiple-range test. Root damage indices were calculated as described, and the number of puparia per treatment group were transformed to $\sqrt{\times} + 0.5$ prior to analysis. Treatment means were then separated by Duncan's multiple-range test.

Results and Discussion

There was no difference in fresh collard leaf weight between any of the four nematode treatments and the control group, as this weight was between 242 and 279 g. The fresh collard leaf weight of chlorpyrifos-treated plants (ca. 320 g) was greater ($P < 0.05$) than the other collards (Table 1). Similarly, damage to roots within the nematode applications and the control group was not different, with mean root indices ranging from 63.3 to 71.6. However, the chlorpyrifos-treated plants, with a lower root damage index of 39.2, had less damage ($P < 0.05$) than all the other treatments (Table 1). Any relationship between leaf weight and root damage was not evaluated in this study.

Evidence for lack of maggot suppression by any nematode application was further demonstrated by comparison of the number of *D. radicum* puparia collected from the root zones of treated plants (Table 1). Whereas the nematode or control treatments averaged between 2.6 to 5.2 puparia per plant, the chlorpyrifos treatment averaged 0.2 puparia per plant ($P < 0.05$).

One or two applications of *S. carpocapsae* or *H. bacteriophora* did not prevent collard root damage, nor did these nematodes decrease the number of *D. radicum* in soil and root samples. However, the chlorpyrifos treatment reduced root damage and also reduced the number of puparia per sample, resulting in significantly higher crop yield.

The apparent lack of control efficacy by nematodes in this study is contrary to other results. An earlier field study (3) provided initial evidence that nematodes may protect plants from anthomyiid pest species. Crop yield (cabbage) was greater than the untreated group but less than diazinon-treated plants. Similar results were obtained from tobacco plants infested by *D. platura;* nematode treatment, as well as diazinon application, provided plant protection (3). Satisfactory yield results were

Table 1. Effect of nematodes or chlorpyrifos on damage to collards by the cabbage maggot, *Delia radicum.*

Treatment	Mean leaf weight (g)	Mean root damage index†	*D. radicum* puparia/plant‡
S. carpocapsae (single)	248.5 ± 27.1 a	68.3 ± 5.9 a	3.6 ± 0.74 a
S. carpocapsae (two)	258.9 ± 14.8 a	65.8 ± 7.0 a	2.8 ± 0.93 a
H. bacteriophora (single)	279.4 ± 23.3 a	74.2 ± 7.5 a	5.2 ± 1.29 a
H. bacteriophora (two)	267.4 ± 18.0 a	63.3 ± 7.0 a	2.6 ± 0.73 a
Chlorpyrifos	319.4 ± 19.6 b	39.2 ± 3.6 b	0.2 ± 0.01 b
Control	241.9 ± 13.5 a	71.7 ± 10.1 a	3.9 ± 1.21 a

Means in columns followed by the same letter are not different ($P < 0.05$) by Duncan's multiple-range test.

† Root damage index (RDI) calculated by the rating i equaling one of these values: 1 = no noticeable maggot tunneling; 2 = slight tunneling with 1–5 tunnels; 3 = moderate damage with 5–10 tunnels, and 4 = severe damage with more than 10 tunnels. Each treatment RDI was calculated by the following formula:

$$\text{RDI} = \left[\sum_{i=1}^{4} \left(\frac{\text{no. of plants at rating } i}{\text{no. of plants samples}} \right) \times 100 \text{ (rating } i) \right] / 4.$$

‡ Number of purpina per plant transformed to $\sqrt{x} + 0.5$ for analysis of variance; raw data are shown.

obtained against *D. radicum* in Brussels sprouts (8). However, insect mortality was not noted in these results, nor were plants evaluated for feeding damage to roots. Apparently, the nematode species applied during the current study did not infect sufficient *D. radicum* larvae to prevent plant damage.

In a recent review by Kaya (11), the principal soil factors affecting nematode movement and subsequent survival were noted as pore size, moisture, aeration, temperature, and soil chemistry, with moisture being the central factor. Thus, soil conditions could have modified nematode longevity, mobility, or infectivity to their target hosts; however, these factors were seemingly within tolerable ranges for nematode activity and survival. As such, soil conditions were not considered to be lethal or otherwise obstructive to nematode movement.

The particle size of sandy loam (<0.05–2.0 mm d) would not have impeded the movement of the nematodes. Similarly, the soil pH (7.1) and temperature during application (ca. 20 C) were acceptable for nematode viability and activity (11). Moisture content or water potential were not calculated during this study; again, however, precipitation prior to the trial generated substantial soil moisture, and the field was irrigated by the grower at regular intervals thereafter.

Furthermore, the positive *G. mellonella* bioassay results obtained during and after the applications showed that viable, infective-stage nematodes were placed around plant stems during each treatment. Whether these infectives actually reached and entered *D. radicum* larvae, within collard roots, is unknown. Mortality or quiescence of these nematodes could have contributed to poor insect control; however, such factors were not determined during this field trial. Identifying and eliminating confounding factors, including use of optimal strains or species, timing of applications, environmental modifications, and delivery method could improve field efficacy.

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