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# Control of Larval Northern Corn Rootworm (*Diabrotica barberi*) with Two Steinernematid Nematode Species[1]

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*Abstract:* The entomogenous nematodes *Steinernema feltiae* and *S. bibionis* did not penetrate the roots of corn, *Zea mays*, to infect larval northern corn rootworm (NCR), *Diabrotica barberi*, feeding within. Laboratory bioassays against first instar NCR indicated that *S. feltiae*, Mexican strain ($LD_{50}$ = 49 nematodes/insect) is more virulent than *S. bibionis* ($LD_{50}$ = 100). Numbers of NCR larvae in a grain corn crop were reduced by both nematode species applied at corn seeding time at the rate of 10,000 infective-stage juveniles per linear meter of corn row. The chemical insecticide fonofos provided significantly better control than either nematode species.

*Key words:* biological control, corn rootworm, *Diabrotica barberi*, entomogenous nematode, fonofos, *Steinernema bibionis*, *Steinernema feltiae*.

The northern corn rootworm (NCR), *Diabrotica barberi* (Coleoptera: Chrysomelidae), is a serious pest of corn in North America. The larvae feed on corn roots, resulting in weakened root systems and reduced nutrient and water uptake which lead to yield reductions. In recent years corn rootworm (*Diabrotica* spp.) infestations have involved 12–16 million hectares in the United States, costing farmers an estimated $1 billion per annum in crop losses and insecticide application expenses (1986 estimate) (13). Problems associated with the use of chemical insecticides (e.g., resistance, environmental contamination, effects on nontarget organisms) require that alternative techniques to control this insect pest be developed.

Steinernematid and heterorhabditid nematodes are effective in reducing pest insect populations in some situations and are most effective against insects found in cryptic habitats such as the soil (1,9,15,16). Because *Diabrotica* spp. spend a considerable portion of their lives in the soil, they may be suitable candidates for control with entomogenous nematodes. Previous studies (10,17) have shown that *Diabrotica* spp. are susceptible to infection by steinernematid nematodes, but field tests have been inconclusive. The possibility that *D. barberi*, a pest of grain corn in Quebec, could be controlled with entomogenous nematodes was tested in a series of experiments with *Steinernema feltiae* and *S. bibionis*.

## Materials and Methods

*Nematodes:* The nematodes used in all experiments were *Steinernema feltiae* All and Mexican strains and *S. bibionis* Sn strain. These nematodes, supplied by Biosys, Palo Alto, California, were stored on open cell foam in plastic bags at 12 C until used (1–2 months). Before use the nematodes were washed from the foam with distilled water and examined microscopically for activity.

*Accessibility:* Previous studies have shown that all stages of *Diabrotica* spp. except the egg are susceptible to attack by steinernematid nematodes under laboratory conditions (10,20). Although all stages of *D. barberi* can be found in the soil, the majority of the larvae are usually found within corn roots and may not be accessible to entomogenous nematodes. Accessibility trials were conducted to determine whether steinernematids could penetrate corn roots to infect NCR larvae tunneling inside.

Grain corn, *Zea mays*, was planted in 2-liter pots maintained in an incubator with a 14-hour photoperiod (25 C day, 18 C

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night). Eleven days later, 50 NCR eggs that had been incubated in distilled water at 23 C for 11 days, and were therefore ready to hatch within 3 days (4), were added to each pot. At 21 days postplanting, 50,000 infective-stage juvenile (IJ) nematodes in an aqueous suspension were added to the surface of the soil in each pot. Treatment with each nematode was replicated four times. The plants were harvested at 35 days postplanting, when the NCR larvae were second instars. The roots were washed and examined for feeding damage and dissected to extract NCR larvae. All recovered larvae were dissected and checked for the presence of nematodes. To determine whether nematodes were present in the soil at the end of the experiment, each pot was baited with two late-instar larvae of the greater wax moth, *Galleria mellonella*, placed in the soil (2). After 5 days these larvae were removed from the soil, dissected, and examined for the presence of nematodes.

*Virulence bioassays:* Standard petri dish bioassays were conducted to compare virulence of nematodes to first-instar NCR larvae. The nematodes were applied in 2 ml distilled water onto two filter paper discs (9-cm-d) in petri dishes (100 × 15 mm) at doses of 0–200 IJ/NCR larva; then 10 larvae were placed on the filter paper. Each dose was replicated at least four times. The dishes were maintained in darkness at 23 C, and larval mortality was determined at 24 and 48 hours. Mortality data were analyzed by probit analysis (7) to calculate $LD_{50}$ values.

*Field trial:* The most and least virulent nematodes, *S. feltiae* (Mexican strain) and *S. bibionis* (Sn strain), were selected for a field trial. These nematodes were compared with the chemical insecticide fonofos (O-ethyl S-phenylethylphosphorodithioate) and an untreated control for their ability to reduce larval NCR populations in the field.

The experimental site was located in a field at Pike River in southwestern Quebec that had been planted to grain corn for 15 consecutive years. No insecticides had been used the last 9 years and a high NCR population was present the previous year (6). The experiment was established in a randomized complete block design with 10 blocks and six treatments. Each block consisted of an area of field 10 m long by 23 m (30 rows) wide. The treatments, applied 5 May 1986, consisted of applications of two nematode species at two rates each, an insecticide treatment, and an untreated control. Each plot (treatment within a block) consisted of two adjacent 10-m rows. Two rows of corn were left untreated between each plot. The corn was planted using a six-row seeder with the press wheels raised so the seeds were left exposed. The treatments (either nematode or insecticide) were applied in-furrow on top of the seeds which then were covered to a depth of ca. 5 cm with soil. The nematodes were applied by hand as a water suspension, with a 1-liter plastic wash bottle, at rates of 10,000 or 100,000 IJ/m corn row. These rates are equivalent to 130 million and 1.3 billion IJ/ha. Fonofos was applied in a narrow band at the manufacturer's recommended rate of 0.85 kg a.i./ha using a hand dispenser described by Elhag (6).

Plots were sampled for NCR larvae 1 month after treatment. No samples were taken from the final meter of any plot to reduce potential interference from the adjoining block. Three corn plants per plot were selected randomly, cut at ground level and the roots were removed in a block of soil (20 × 20 × 15 cm), as recommended by Matin (12). Soil and root samples were examined by hand for the presence of NCR larvae. On 21 and 27 September, 25 plants per plot were selected at random and measured from the bottom of the tassel to the first node above ground; the ears were removed, dried in a commercial corn drier, shelled, and weighed for yield determination per plot.

All data were analyzed using the analysis of variance (ANOVA) procedure for balanced data (18) and Duncan's multiple-range test (5).

TABLE 1. Virulence of *Steinernema feltiae* and *S. bibionis* to first-instar *Diabrotica barberi* larvae in petri dish bioassays.

| Nematode (strain) | $LD_{50}$ (48 hours) | 95% fiducial limits |
|---|---|---|
| *S. feltiae* (Mexican) | 49 a | 33–71 |
| *S. feltiae* (All) | 67 ab | 50–89 |
| *S. bibionis* (Sn) | 100 b | 77–129 |

Values are number of nematodes per insect; $LD_{50}$ values followed by the same letter are not significantly different based on nonoverlapping 95% fiducial limits.

## RESULTS AND DISCUSSION

*Accessibility:* The number of NCR larvae recovered from the plants was not affected by treatment (control: 3.0 ± 0.8 larvae/plant; treatments: 3.1 ± 0.6 larvae/plant). All recovered larvae were alive and feeding inside the roots; no nematodes were found in the dissected larvae. When the soil was baited with *Galleria* larvae, nematodes were recovered from 11 of the 12 treatment pots, but none were recovered from the control pots. The absence of infected NCR was possibly due to inability of the nematodes to penetrate corn roots or to follow the narrow feeding tunnels of early-instar NCR larvae.

Because *Steinernema* spp. were incapable of infecting NCR larvae feeding inside corn roots, control programs using these nematodes should be directed against first-instar larvae before they enter the roots. Soil inoculation with nematodes at seeding time might achieve this goal.

*Virulence bioassays:* Using the criterion of nonoverlapping 95% fiducial limits of the 48-hour $LD_{50}$ values, *S. feltiae* Mexican strain is significantly more virulent to NCR larvae than *S. bibionis* Sn strain but not more virulent than *S. feltiae* All strain (Table 1). These results generally agree with virulence reports by other authors. *Steinernema feltiae* was more virulent than *S. bibionis* to *Spodoptera frugiperda* (8), *S. litura* (11), *G. mellonella* (11; Thurston, unpubl. data), *Lucilia cuprina* (14), and *Listronotus oregonensis* (3). Additionally, no differences between the Mexican and All strains of *Steinernema feltiae* were noted in *Oryctes rhinoceros* and *Tirathaba rufivena* (21), and *L. oregonensis* (Thurston, unpubl. data). However, the Mexican strain was more virulent than the All strain to *L. subtropicus* (19), *Spodoptera frugiperda* (8), *D. virgifera virgifera* (10), and *G. mellonella* (Thurston, unpubl. data), indicating that host susceptibility varies with nematode species and strain.

*Field trial:* The number of larvae recorded per plant was low in all treatments (Table 2). The Quebec field populations of NCR in the summer of 1986 were unusually small. The very early but wet spring that allowed larvae to hatch but delayed farmers from planting corn for several weeks may have resulted in death by starvation of newly hatched NCR larvae. The mean number of larvae per plant in the control plots was 4.3 (Table 2); this compares with a mean of 13.8 larvae per plant the previous year (6).

Although larval numbers were low, the treatments significantly reduced the num-

TABLE 2. Effect of field applications of *Steinernema feltiae, S. bibionis* and the chemical insecticide fonofos on larval *Diabrotica barberi* numbers and *Zea mays* plant height and grain yield (Mean ± SE).

| Treatment | Larvae (no./plant) | Plant height (cm) | Grain yield (g/25 plants) |
|---|---|---|---|
| Control | 4.3 ± 0.5 a | 217 ± 4 a | 2,874 ± 45 a |
| *S. bibionis* (10,000/m) | 2.5 ± 0.6 b | 214 ± 3 a | 2,785 ± 48 a |
| *S. feltiae* (10,000/m) | 2.3 ± 0.5 b | 214 ± 3 a | 2,790 ± 67 a |
| *S. bibionis* (100,000/m) | 2.3 ± 0.4 b | 219 ± 3 a | 2,978 ± 40 a |
| *S. feltiae* (100,000/m) | 2.3 ± 0.4 b | 220 ± 4 a | 2,925 ± 40 a |
| Fonofos (0.85 kg/ha) | 0.9 ± 0.3 c | 216 ± 3 a | 2,945 ± 34 a |

Means in the same column followed by the same letter are not significantly different according to Duncan's (5) multiple-range test ($P = 0.05$).

ber of larvae per plant (Table 2). Both nematode species applied at rates of 10,000 and 100,000 IJ per linear meter of corn row gave significant larval reductions relative to the untreated control. No significant differences were noted between the two nematode species. The results indicate that, although differences between strains and species may be detected in laboratory bioassays, these differences may not apply in the more complex natural field situation. Fonofos caused the greatest decline in larval numbers, reducing them significantly relative to the control and all nematode treatments.

No differences between treatments for plant height or grain yield measurements were noted, which may reflect the very low numbers of NCR larvae attacking the roots in all treatments. Greater differences might be expected when NCR populations are larger.

Both *Steinernema feltiae* and *S. bibionis* can significantly reduce larval NCR populations; however, the control level achieved by these nematodes at the rates used is not as high as control by fonofos. Nevertheless, the level of control obtained with nematodes might be sufficient to reduce larval NCR populations below economic injury levels in years with high NCR numbers (6).

The two nematode species used in our experiment produced virtually identical results in the field but performed quite differently in the laboratory bioassay. These results indicate that screening for virulence in laboratory experiments only is not sufficient when selecting a nematode for field use. Many other factors may be important in the effectiveness of entomogenous nematodes in the field. Accordingly, more basic studies on the ecology of these nematodes are needed to understand better the limitations on their practical use.

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