Population Dynamics, Persistence, and Efficacy of the 
Entomopathogenic Nematode *Heterorhabditis bacteriophora* (Oswego Strain) in Association with the Clover Root Curculio 
(Coleoptera: Curculionidae) in Pennsylvania

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**ABSTRACT** The population dynamics, persistence, and efficacy of *Heterorhabditis bacteriophora* Poinar (Oswego strain) applied to control the clover root curculio, *Sitona hispidulus* (F.), were investigated in a Pennsylvania alfalfa (*Medicago sativa* L.) field. Nematodes established and persisted following application rates of 2.5, 7, and 15 billion infective juveniles per hectare. Significant differences in nematode densities between treatments were not observed beyond 43 d after application, indicating that application rate likely did not affect long-term persistence. In the third field season, plots that received the original 15-billion nematodes per ha treatment were split into clover root curculio-excluded and clover root curculio-present subplots to assess the effect of this insect on nematode persistence. Nematode populations were significantly lower in the curculio-excluded plots by October, suggesting that the nematodes recycled through that host. However, nematode populations were not significantly different by April of the fourth field season. Nematode efficacy throughout the study was inconsistent. In the first field season, emergence of clover root curculio adults was significantly reduced in plots receiving the 15 billion nematode per ha treatment. However, alfalfa taproot ratings for clover root curculio feeding injury indicated that scarring was reduced only in the 7 billion nematodes per ha treatments. No significant reductions in taproot injury were observed in the second field season, but by the third year, significant reductions in root injury were evident in the plots that originally received 7 and 15 billion nematodes per ha.

**KEY WORDS** entomopathogenic nematodes, *Heterorhabditis bacteriophora*, clover root curculio, *Sitona hispidulus*, alfalfa

**ALTHOUGH INUNDATIVE RELEASES** of entomopathogenic nematodes often cause immediate reductions in insect populations, most nematode species and strains persist poorly in the soil environment beyond the season of application (Smits 1996). Thus, there have been few attempts at long-term, classical biological control with these organisms. If entomopathogenic nematodes are to be used more effectively in biological control, their soil ecology must be better understood (Gaugler et al. 1997). Studies that investigate the long-term persistence of entomopathogenic nematode populations, particularly in relationship to the synchronization with insect host densities in the soil, are needed. Because of the high cost associated with multiple nematode applications, understanding persistence is important when developing integrated pest management (IPM) programs that use entomopathogenic nematodes in low-value commodities such as forage crops.

This tactic would likely require the establishment of permanent nematode populations.

There are studies on entomopathogenic nematode ecology that have contributed to our understanding of a long-term approach to biocontrol with these organisms. Klein and Georgis (1992) reported that *Heterorhabditis bacteriophora* Poinar (NC strain) was able to establish in turfgrass and provide >90% control of Japanese beetle grubs, *Popillia japonica* Newman, 386 d after application. Nematodes were able to persist by reproducing in the grubs and did not negatively affect nontarget arthropods. Following inoculative release against mole crickets, the entomopathogenic nematode *Steinernema scapterisci* Nguyen and Smart established in Florida turfgrass (Parkman et al. 1993). Campbell et al. (1995, 1996, 1998) studied the dynamics of endemic entomopathogenic nematode populations in turfgrass with an emphasis on the relationship between the nematodes and potential soil hosts. Endemic populations of *H. bacteriophora* likely suppressed populations of Japanese beetle larvae and re-
cycled through these hosts (Campbell et al. 1995, 1998).

A highly infective and ecologically persistent strain of the entomopathogenic nematode species *H. bacteriophora* was isolated from an alfalfa field in Oswego County, NY, in 1990 (Schroeder et al. 1993). Designated “Oswego,” this nematode strain has been shown to significantly reduce larval populations of the alfalfa snout beetle, *Otiorynchus lugistici* (L.), in New York (Shields et al. 1999), while persisting in alfalfa soil throughout multiple seasons (Ferguson et al. 1995, Shields et al. 1999). The infectivity and field persistence of *H. bacteriophora* Oswego, coupled with its “cruising” host-searching behavior, suggested that this entomopathogenic nematode could also be an effective control agent against larvae of the clover root curculio, *Sitona hispidulus* (F.).

Root injury by larvae of the clover root curculio reduces the quality, yield, and stand persistence of alfalfa, *Medicago sativa* L., in Pennsylvania (Hower et al. 1995) and other states (Godfrey and Yeargan 1987, Godfrey et al. 1987, Dintenfass and Brown 1988, Kelb et al. 1994). Larvae of this univoltine species are found in the soil from May to July in the northeastern United States (Bigger 1930, Underhill et al. 1955). Early instars damage root nodules and fibrous and lateral roots, and fourth and fifth instars cause injury to taproots (Bigger 1930, Marshall and Wilbur 1934, Byers and Kendall 1982, Dintenfass and Brown 1986, Tan and Hower 1991). Root scarring also increases susceptibility of the alfalfa to soil pathogens and leads to premature stand decline (Hill et al. 1969, 1971; Godfrey and Yeargan 1989; Leath and Hower 1993; Hower et al. 1995). Management of the clover root curculio with soil insecticides is difficult, expensive, and may be destructive to established natural enemies of the alfalfa weevil, *Hypera postica* (Gyllenhal). Consequently, management practices such as the use of entomopathogenic nematodes would be a useful alternative.

Although the research in New York demonstrated multiple-season persistence of *H. bacteriophora* Oswego, ecological information on nematode persistence relative to the clover root curculio is needed. The population dynamics of *H. bacteriophora* Oswego in Pennsylvania alfalfa will influence approaches to long-term management of the clover root curculio with entomopathogenic nematodes. Quantitative descriptions of the decline in the number of applied nematodes, the density at which the nematode population eventually establishes, and the persistence of that population over time are important factors to consider in this system. The rate of nematode application is another important consideration because it is not known whether field efficacy and persistence are attained only at high nematode application densities, or if the nematode can establish and build a population when applied at lower rates.

The impact of the clover root curculio on the population dynamics of *H. bacteriophora* Oswego also remains uninvestigated, but it is likely an important parameter influencing nematode persistence. Although this nematode strain persists for multiple seasons (Ferguson et al. 1995, Shields et al. 1999), it is unclear whether and how this persistence might be achieved in Pennsylvania alfalfa fields. The nematodes exist free-living in the soil as third-stage infective juveniles and complete their development after infecting and killing a host insect (Kaya and Gaugler 1993). After reproduction in the insect cadaver, new infective juveniles are released back to the soil environment, typically within 1 to 2 wk (referred to as “recycling” of the nematode population) (Kaya et al. 1993). In the laboratory, *H. bacteriophora* Oswego readily infects and reproduces in clover root curculio larvae and pupae (3,800–7,000 infective juveniles per cadaver) (Loya 2001), but its ability to recycle in the field has not been investigated. Nematodes might recycle through the clover root curculio or other soil inhabiting insects, or they might persist from the original application.

This study contributes to the knowledge of entomopathogenic nematode ecology by focusing on the following objectives: (1) to monitor the quantitative temporal dynamics of *H. bacteriophora* Oswego populations after inundative application at varying rates; (2) to evaluate the impact of clover root curculio on long-term field persistence of *H. bacteriophora* Oswego; and (3) to assess the multiple-season field efficacy of this nematode against the clover root curculio.

### Materials and Methods

**Establishment of Nematode Populations.** *H. bacteriophora* Oswego nematodes were obtained from infected greater wax moth larvae, *Galleria mellonella* (L.), and were reared in commercially available wax moth larvae following the procedures of Woodring and Kaya (1985) and Flanders et al. (1996). Nematodes were stored in tissue culture flasks at 12°C in 100 ml distilled water (~10,000 nematodes/ml) and were used within 2 to 3 wk of emergence.

Nematode populations were established in plots of alfalfa at the Pennsylvania Agricultural Experiment Station Farm at Rock Springs, PA, on 3 June 1998. The alfalfa (Pioneer variety 5373) had been planted in a Hagerstown loam soil and was in its first full year of production in 1998. The plots were not irrigated before nematode application because 2.5 cm of rain had fallen the previous evening. The alfalfa field had been harvested on 25 May and trimmed with a rotary mower the day of application to remove logged stems. The alfalfa field was divided into 3 × 3 m² plots with 3-m alleys and arranged in a randomized complete block design.

Nematodes were applied using a CO₂-powered backpack sprayer with a 3-m boom equipped with flat fan nozzles (8006) mounted 30 cm apart. The nematodes were mixed with 11.4 liter water and applied by walking the spray boom over the plots until the suspension was completely emptied (approximately eight passes over the plot). To thoroughly saturate the soil, an additional 11.4 liter of water was applied to the
plots in a similar manner after the nematode application. Application of nematodes began at ≈1700 hours EDT and was completed by 2000 hours. Nematode concentrations equivalent to 2.5, 7, and 15 billion infective juveniles per hectare were applied. Control plots received the same quantity of water minus nematodes. The treatments were replicated four times.

**Nematode Temporal Dynamics. 1998–1999.** To determine the temporal dynamics of the nematode population in the first two seasons after application, soil samples were collected in 1998 and 1999. Nematodes in the soil were quantified using the *Galleria* baiting method (Kaya and Stock 1997). This sampling technique was appropriate for this study because no endemic heterorhabditid nematodes were detected in control plots either before application or throughout the study.

Sampling for *H. bacteriophora* Oswego began 2 wk after application. Samples were taken once every 2 wk until mid-September; after that date, nematodes were sampled once per month in October and November. Sampling dates in 1998 were 18 June; 3, 16, and 29 July; 13 and 28 August; 13 September; 21 October; and 25 November (or, 15, 30, 43, 56, 71, 86, 102, 140, and 175 d after application). Sampling resumed in May 1999, and samples were taken approximately every 10 d until late July. Sampling dates in 1999 were 8 April; 17 and 27 May; 8, 18, and 29 June; and 13 and 22 July (or, 309, 348, 358, 370, 380, 391, 405, and 414 d after application). The plots treated with 2.5 and 15 billion nematodes were also sampled on 22 September 1999 (475 d after application).

Samples consisted of five soil cores randomly collected from each plot (a total of 20 samples per treatment) with an Oakfield soil corer, Oakfield Apparatus, Inc., Oakfield, WI (2 cm diam × 15 cm deep, or 47.1 cm³ soil total). Cores were collected within 25 cm of a plant crown. Cores were placed in 350-ml plastic containers and returned to the laboratory where each core was crumbled and then moistened with distilled water to approximately −10 to −20 kPa water potential (Kaya and Stock 1997). One day later, five wax moth larvae were added to the soil. The containers were inverted and held at room temperature (23°C). After 4 d, infected wax moth larvae were removed and replaced with new larvae. This process was repeated two more times for three rounds of baiting.

Nematodes that had infected the wax moth larvae were quantified following the technique developed by Mauleon et al. (1993) and modified by Caroli et al. (1996). Infected larvae were rinsed, cut in two longitudinally, and placed in a 20-ml vial containing 4.0 ml of a pepsin solution: 0.8 g pepsin (Ward’s, Rochester, NY), 2.3 g NaCl, 2.0 ml HCl, and 94.0 ml water. The tubes were incubated in a hot water bath at 52°C for 1 h, vortexed for 20 s, and returned to the bath. After another 1.5 h, 6.0 ml of a 0.1% Tween 80 solution was added to each tube, and the solution was vortexed again for 20 s. The suspension was poured into a glass petri dish lit from beneath, and the nematodes were counted under a dissecting microscope.

Nematode density data from each sampling date were subjected to analysis of variance (ANOVA), and significant differences between means at *P* ≤ 0.05 were detected using the Tukey honestly significant difference (HSD) test (StatView; SAS Institute 1998). Population densities were converted to nematodes per 100 cm³ of soil. The plots that originally received no nematodes were not included in data analysis and not discussed further because these plots were void of endemic heterorhabditids on all dates sampled. Nematode data for each date are also represented as the percentage of soil cores that contained nematodes (i.e., positive cores). Percentages were transformed to arcsine of the square root for the Tukey-type multiple comparison test (*α* = 0.05) (Zar 1999).

To calibrate the *Galleria*/pepsin sampling technique for *H. bacteriophora* Oswego, known quantities of nematodes were placed in containers of sterilized soil in the laboratory (soil volume reflected that of a typical field sample). The samples were then baited with rounds of five larvae for 4 d until no further nematodes were retrieved. Treatments consisted of 10, 40, 160, and 640 infective juveniles. The nematodes in the infected wax moth larvae were then quantified using the pepsin technique. The percentage of nematodes recovered (number recovered / number added to samples) ranged from ≈5.0 to 30.0% of nematodes in a sample and was more accurate for lower densities of nematodes (Table 1). The percentage of nematodes recovered from three rounds of *Galleria*-baiting (number recovered in three rounds / total number recovered) revealed that 55.0–98.0% of the recovered nematodes were retrieved in the first three rounds, indicating that three rounds were sufficient to recover most of the potentially infective nematodes in a field sample. Nematodes were recovered from all test samples, even those with just 10 nematodes added.

<table>
<thead>
<tr>
<th>No. nematodes applied</th>
<th>No.</th>
<th>Mean nematodes recovered (± SE)</th>
<th>% Recoverya (± SE)</th>
<th>Recovery over first three rounds +/- recovery in all rounds (± SE)</th>
<th>% positive samples</th>
<th>Mean no. infected <em>Galleria</em> Oswego (± SE)</th>
</tr>
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<tbody>
<tr>
<td>10</td>
<td>10</td>
<td>2.9 (0.5)</td>
<td>29.0 (4.6)</td>
<td>0.975 (0.025)</td>
<td>100.0</td>
<td>2.7 (0.5)</td>
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<tr>
<td>40</td>
<td>10</td>
<td>7.5 (0.8)</td>
<td>18.8 (1.9)</td>
<td>0.967 (0.033)</td>
<td>100.0</td>
<td>6.4 (0.6)</td>
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<tr>
<td>160</td>
<td>10</td>
<td>21.8 (1.3)</td>
<td>13.6 (0.8)</td>
<td>0.937 (0.017)</td>
<td>100.0</td>
<td>13.0 (1.0)</td>
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<tr>
<td>640</td>
<td>7</td>
<td>38.1 (7.5)</td>
<td>6.0 (1.2)</td>
<td>0.646 (0.030)</td>
<td>100.0</td>
<td>18.4 (0.5)</td>
</tr>
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*a* Recovery ranged from three to six rounds.

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demonstrating that nematodes in field samples were likely to be detected by this sampling method. The laboratory calibration of the Galleria baiting method did not recover all H. bacteriophora Oswego added to the soil samples. Therefore the field data did not measure absolute nematode density but were a measure of active, infective nematodes able to establish in a host at a given time. This parameter may be considered more ecologically informative than absolute nematode density in a biological control study (Curran 1993).

**Impact of Clover Root Curculio on Nematode Persistence.** The experimental design from the first two field seasons was modified to determine the impact of clover root curculio on nematode persistence into a third field season. The plots that had originally received the 15-billion nematodes per ha treatment in 1998 were split into two subplots in the fall of 1999. To provide two treatments (i.e., nematode plots with and without clover root curculio), a cage that excluded curculio adults was placed over one of the two subplots. Cages were 3.7 m long × 1.8 m high × 1.8 m wide. The 52-mesh netting of the cages allowed sunlight and rain to enter the plots. The second subplot remained unmodified. A piece of sheet metal was placed 25 cm deep between the two subplots to prevent interplot movement of nematodes. Because clover root curculio oviposition occurs in the fall and again in the spring, and because adults can become active in the winter during warm weather (Bigger 1930), cages were secured over the plots from mid-September 1999 until late June 2000. Some adults that had been in summer diapause were already present in the plots when the cages were placed in the field, therefore the alfalfa inside the cages was vacuumed five times with a leaf blower/vacuum in late September and early October 1999 to remove curculio adults. All other captured insects were returned to the cages. Vacuuming continued until no further clover root curculio adults were collected from the caged subplots.

Nematodes in the curculio-excluded (caged) and curculio-present (nuncaged) subplots were sampled on 22 September 1999; 19 April, 25 May, 26 June, 11 July, 11 August, and 16 October 2000; and 30 April 2001 (or, 475, 684, 720, 752, 767, 798, 864, and 1,060 d after application). On each sampling date, 10 soil cores were taken (2 cm diam × 15 cm deep) from each plot (40 samples per treatment). Nematodes in the samples were quantified using the Galleria baiting method previously described. At each sampling date, significant differences (P = 0.05) between mean numbers of nematodes in the curculio-excluded and curculio-free subplots were detected using t-tests (StatView; SAS Institute 1998).

To verify that the cages effectively excluded clover root curculio, soil samples for curculio eggs were taken on 12 May 2000. Five soil cores were taken from each plot with a golf cup cutter (10.8 cm diam) to a depth of 2 cm for a total of 20 samples per treatment. Samples were stored at 4°C for 6 d until processed. Egg extraction was modified from the procedures of Danthanarayana (1966) and Ng et al. (1977). The soil samples were first washed through three sieves (No. 10, 40, and 60, USA Standard Testing Sieves). The contents of the No. 60 sieve were placed in a beaker of water saturated with NaCl (354 g NaCl/liter). After 1 h, floating objects were suctioned off with a pipette and placed in a petri dish containing filter paper. The eggs were counted under a dissecting microscope. Significant differences between mean egg densities in the subplots were detected using a t-test (StatView).

The plots also were sampled on 26 June 2000 to verify that the cages did not exclude other soil macroinvertebrates that could serve as nematode hosts during the time that the clover root curculio inhabits the soil, thus affecting interpretations about the impact of this insect on nematode persistence. Three soil cores (10.8 cm diam × 10.2 cm deep) were taken with a cup cutter from each nematode-applied plot and control plots (12 cores per treatment). To collect the insects from each core, the soil was crumbled by hand and visually inspected. Particular care was taken to detect sedentary larvae of Coleoptera that are ideal hosts for a "cruiser" nematode such as H. bacteriophora. The cores were stored at 4°C for ≈2 wk until processed.

**Assessment of Nematode Efficacy.** Nematode efficacy was assessed in the first field season (1998) using cages that collected emerging curculio adults. Treatments consisted of the three nematode concentrations stated previously and a nontreated control. The cages were 25 cm diam × 76 cm high cylinders consisting of a 5.1 × 10.2 cm wire frame covered with 18-mesh fiberglass screen. Two cages were positioned randomly in each plot, for a total of eight cages per treatment, and secured by wooden stakes. Soil was placed around the base of the cage to prevent curculio escape and to prevent contamination from other curculios outside the cages. The adult clover root curculios inside the cages were collected on 14 July 1999 by cutting the alfalfa in each cage and then vacuuming the caged area. This material was bagged and returned to the lab where the adults were counted. Adult emergence data were subjected to ANOVA, and significant differences between means were detected using the Tukey HSD test (StatView). In 1998, nematode efficacy also was assessed by rating alfalfa taproots for clover root curculio feeding scars. Because of time constraints, this was the only method used to assess nematode efficacy in 1999 and 2000. In 1998, five roots were dug randomly from each plot for a total of 20 roots per treatment. In 1999 and 2000, 10 roots were collected per plot for a total of 40 roots per treatment. Treatments included the three original nematode application rates plus a nontreated control. The taproots were rated on a visual scale of 1–8 (1 = little damage; 8 = severe damage) to assess feeding injury. This visual scale was developed previously (AAH, unpublished data) and is based upon the percentage of taproot area damaged. Photographs representing each category were used to standardize classification of the alfalfa taproots. Root data were subjected to ANOVA, and significant differences between means were detected using the Tukey HSD test, as before.
**Results**

**Nematode Temporal Dynamics. 1998–1999.** *H. bacteriophora* Oswego established and persisted in the alfalfa plots through 1998 following application at all three concentrations (Fig. 1). Mean nematode populations ranged from a high of 26.7 nematodes/100 cm³ soil in the 15 bil/ha plots on 16 July (43 d after application) to a low of 2.5 nematodes/100 cm³ soil in the 2.5 bil/ha plots on 18 June (15 d after application). Nematode densities were significantly different only on 18 June and 16 July (15 and 43 d after application, respectively). On 18 June, significantly more nematodes were detected in the 15 bil/ha and 7 bil/ha plots than in those receiving the 2.5 bil/ha treatment ($F_{11005} = 8.627; P = 0.0005; df = 2, 57$). On 16 July, significantly more nematodes were collected from the 15 bil/ha treatment than from the 2.5 bil/ha treatment ($F = 4.493; P = 0.0154; df = 2, 57$).

The percentage of soil cores containing *H. bacteriophora* Oswego did not drop below 60.0% in the 1998 field season (Fig. 2). Those plots that received 7 and 15 bil/ha had significantly more positive cores than the 2.5 bil/ha plots ($\chi^2 = 10.91; P = 0.0043; df = 2$) on 18 June (the first sampling date). The percentages of positive cores were not significantly different between treatments on any other date.

*H. bacteriophora* Oswego populations persisted into and throughout 1999 after establishment at all three application rates in 1998 (Fig. 3). Mean nematode populations ranged from a high of 13.9 nematodes/100 cm³ soil in the 2.5 bil/ha plots on 8 April (296 d after application) to a low of 1.9 nematodes/100 cm³ soil in the 7 bil/ha plots on 13 July (394 d after application).

Significant differences in the percentage of positive cores among treatments were observed on 8, 18, and 29 June 1999 ($359–380$ d after application) (Fig. 4). On 8 June, the 7 and 15 bil/ha plots had significantly more nematode-positive cores than the 2.5 bil/ha plots ($\chi^2 = 8.4; P = 0.0150; df = 2$). On 18 and 29 June, all three concentrations had significantly different percentages of positive cores ($\chi^2 = 16.35; P = 0.0003; df = 2$ and $\chi^2 = 11.9; P = 0.0025; df = 2$, respectively). The 15 bil/ha plots contained the highest percentage of positive cores on these dates; the 2.5 bil/ha plots had the lowest.

*H. bacteriophora* Oswego population trends within and between 1998 and 1999 were compared. Regardless of application rate, densities in 1998 stabilized at 5–10 nematodes/100 cm³ soil by August after a period of variability earlier in the summer. Mean nematode densities after August 1998 and throughout 1999 rarely approached the levels observed in the two months following application in 1998. Nematode populations were stable from the end of 1998 to the first sampling date in April 1999.

**Impact of Clover Root Curculio on Nematode Persistence.** The number of clover root curculio adults vacuumed from the caged subplots in the fall of 1999 ranged from 42 to 92; the mean ($\pm$SE) was 61.0 $\pm$ 11.0 adults per cage. Samples of clover root curculio eggs indicated that the presence and absence of exclusion cages exposed the nematode populations to two significantly different densities of clover root curculio ($t = 7.64, P < 0.0001, df = 38$). The mean number of eggs recovered per 100 cm³ was 18.1 ($\pm$0.6) in the noncaged plots and 4.4 ($\pm$1.7) in the caged plots.

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Fig. 1. Persistence of *Heterorhabditis bacteriophora* Oswego in 1998 following application on 3 June 1998 at three concentrations.

Therefore, the cages did not completely exclude clover root curculio.

In September 1999, when cages were placed over the subplots, there were no significant differences between nematode populations in the curculio-excluded (4.4 ± 1.4 nematodes/100 cm³) and curculio-present subplots (3.9 ± 1.2 nematodes/100 cm³) (Fig. 5). By July of the following year, after the clover root curculio had finished the soil-associated portion of its life cycle, mean nematode density in the curculio-present plots was consistently greater than in the curculio-excluded plots. Differences were significant by the end of the field season on 16 October 2000 (t = 2.117; P = 0.0375; df = 78). Although nematode levels

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**Fig. 2.** Percentage of soil cores containing *H. bacteriophora* Oswego in 1998 following application on 3 June at three concentrations.

**Fig. 3.** Continued persistence of *H. bacteriophora* Oswego in 1999 following application in 1998 at three concentrations.
remained higher in the curculio-present plots on 30 April 2001, this difference was not statistically significant.

There were no significant differences in the percentage of cores positive for nematodes in 2000, except on 16 October, when percentage of soil cores that contained nematodes was significantly higher in the curculio-present subplots ($\chi^2 = 6.65; P = 0.0099; \text{df} = 1$) (Fig. 6). By April 2001, however, the percentage of positive cores was not significantly different between the two treatments.

Soil samples for macroinvertebrates that could potentially serve as hosts for *H. bacteriophora* Oswego indi-

![Graph showing percentage of soil cores containing *H. bacteriophora* Oswego in 1999 following application in 1998 at three concentrations.](image)

**Fig. 4.** Percentage of soil cores containing *H. bacteriophora* Oswego in 1999 following application in 1998 at three concentrations.

![Bar graph showing persistence of *H. bacteriophora* Oswego in 2000-2001 in response to presence or absence of clover root curculio.](image)

**Fig. 5.** Persistence of *H. bacteriophora* Oswego in 2000-2001 in response to presence or absence of clover root curculio. Nematodes were originally applied at a concentration of 15 bil/ha in 1998. Within a sampling date, means followed by the same letter were not significantly different at $P < 0.05$ (t-test).
cated the presence of Japanese beetle larvae in the clover root curculio-excluded subplots only (0.025 ± 0.015 larvae/100 cm³). The Japanese beetle was the only other insect commonly detected in the plots on 26 June 2000.

Assessment of Nematode Efficacy. Emergence of adult clover root curculio in 1998 was significantly reduced in plots receiving 15 bil nematodes/ha compared with that in control plots (\( F = 3.06; P = 0.0447; df = 3, 28 \)) (Table 2). Root ratings in 1998 indicated that scarring by clover root curculio was reduced in plots receiving the 7 bil nematodes/ha treatment compared with control plots, but no other treatments significantly reduced injury (Table 3). No significant differences in root injury among plots were detected in 1999. In 2000, however, after three field seasons of nematode persistence, significant reductions in root injury were again evident in the plots that originally received 7 and 15 bil nematodes/ha.

Discussion

Stabilization of the nematode population by the end of the first and throughout the second field season, as indicated by the lack of significant differences between treatments on a given date, suggests that the original application rate did not influence the level of long-term persistence. It is possible that the nematode population levels observed after the first season in this study approached that of natural populations. If so, then the natural carrying capacity for \( H. \) bacteriophora in Pennsylvania alfalfa would be ≈ 10 nematodes/100 cm³ soil. Quantitative data on the carrying capacity of natural populations of entomopathogenic nematodes in different crops/commodities and environmental conditions are rare. Campbell et al. (1998) observed endemic \( H. \) bacteriophora populations in New Jersey turfgrass ranging from 0.2 to 432.7 nematodes/0.228 m², or 0.0012–1.26 nematodes/100 cm³ to a depth of 15 cm. The nematode populations were estimated from the percentage of infected wax moth larvae in that study.

In this study, nematode densities during the 2-yr study in the 2.5 bil/ha treatment were lowest on the

![Fig. 6. Percentage of soil cores with \( H. \) bacteriophora Oswego in 2000–2001 in response to presence or absence of clover root curculio. Nematodes were originally applied at a concentration of 15 bil/ha in 1998. Within a date, percentages followed by the same letter were not significantly different at \( P < 0.05 \) (chi-square test).](https://academic.oup.com/article-abstract/31/6/1240/463088)

![Table 2. Field efficacy of \( H. \) bacteriophora Oswego against clover root curculio in 1998 as assessed by mean emergence of adult clover root curculio.](https://academic.oup.com/article-abstract/31/6/1240/463088)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No.</th>
<th>Mean no. adults emerged (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 bil/ha</td>
<td>8</td>
<td>5.5 (1.6)bc</td>
</tr>
<tr>
<td>7 bil/ha</td>
<td>8</td>
<td>3.5 (1.3)bc</td>
</tr>
<tr>
<td>15 bil/ha</td>
<td>8</td>
<td>1.6 (0.6)ab</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>6.6 (1.0)c</td>
</tr>
</tbody>
</table>

Means followed by the same letter were not significantly different at \( P \leq 0.05 \) (Tukey HSD).

![Table 3. Field efficacy of \( H. \) bacteriophora Oswego against clover root curculio as assessed by injured alfalfa taproots after initial nematode application on 3 June 1998.](https://academic.oup.com/article-abstract/31/6/1240/463088)

<table>
<thead>
<tr>
<th>Nematode treatment</th>
<th>1998(^a)</th>
<th>1999(^b)</th>
<th>2000(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean root injury (± SE)</td>
<td>3.08 (0.23)bc</td>
<td>3.44 (0.21)a</td>
<td>3.78 (0.22)bc</td>
</tr>
<tr>
<td>2.5 bil/ha</td>
<td>3.35 (0.24)ab</td>
<td>3.53 (0.21)a</td>
<td>3.05 (0.14)a</td>
</tr>
<tr>
<td>7 bil/ha</td>
<td>3.38 (0.34)bc</td>
<td>3.03 (0.19)a</td>
<td>3.06 (0.19)ab</td>
</tr>
<tr>
<td>15 bil/ha</td>
<td>3.88 (0.22)c</td>
<td>3.64 (0.22)a</td>
<td>3.88 (0.22)c</td>
</tr>
<tr>
<td>Control</td>
<td>3.88 (0.22)c</td>
<td>3.64 (0.22)a</td>
<td>3.88 (0.22)c</td>
</tr>
</tbody>
</table>

Means within a column followed by the same letter were not significantly different at \( P \leq 0.05 \) (Tukey HSD); taproot rating based on range of injury from 1 to 8 with 1 = no scarring.

\(^a\) \( n = 20 \) roots per treatment

\(^b\) \( n = 40 \) roots per treatment
first sampling date in 1998 (15 d after application). By 73 d after application, mean nematode densities in these plots were ≈10 times higher than at 15 d, indicating probable recycling of the nematode population through a host such as clover root curculio. Recycling also might have occurred in the 7 and 15 bil/ha treatments, because nematode levels observed later in these plots also exceeded the levels observed at 15 d.

In 1999, the percentage of soil cores that contained nematodes remained >60.0% and did not differ significantly among treatments after the first sampling date. However, percentages ranged from 25.0 to 100.0% in 1999 and differed significantly among treatments on three sampling dates. These sampling dates were in June when clover root curculio larvae were present in the field. Nematodes persisting from the preceding season could have infected the clover root curculio and recycled during this time.

Observations on the percentage of positive cores in this study are similar to those of Shields et al. (1999). In their study, H. bacteriophora Oswego was detected in 50.0% of soil cores 705 d after broadcast application at the rate of 15 bil nematodes/ha. In our study, H. bacteriophora Oswego was detected in 70.0 and 55.0% of cores after 684 and 720 d, respectively, after similar application. In both studies, the percentage of cores containing entomopathogenic nematodes generally decreased over time. By the 2001 field season, the percentage of positive cores in our study had fallen to 22.5% overall, the lowest observed throughout the study. This reduction in positive cores may have reflected a declining nematode population or possibly that the nematode population became more aggregated. Campbell et al. (1998) noted that the distribution of inundatively released heterorhabditids in turfgrass became contiguous and resembled that of endemic nematodes. It has been hypothesized that nematode populations become more aggregated as a result of the distribution of their hosts in the soil (Stuart and Gaugler 1994). Considering that an aggregated dispersion of the clover root curculio in alfalfa has been observed previously (Quinn and Hower 1985), it is likely that as the nematodes recycle over multiple seasons, their presence within a given soil core will depend upon the nearness of that core to an infected clover root curculio larva.

The long-term establishment of nematodes has been hypothesized to depend on the availability of suitable hosts and the ability of nematodes to recycle through these hosts (Smits 1996). Shields et al. (1999) reported that H. bacteriophora Oswego populations declined in the absence of alfalfa snout beetle in the second season after application, suggesting that recycling is necessary for nematode persistence. Data from this study suggest that, within a growing season, presence of the clover root curculio positively affected populations of H. bacteriophora Oswego. In curculio-excluded plots, nematodes were rarely detected late in the 2000 field season. The population of H. bacteriophora Oswego in these curculio-excluded plots had fallen to 0.2 nematodes/100 cm³ soil by October 2000, or ≈40 times lower than the population level in those plots on 16 April. In contrast, the nematode population in the curculio-present plots in October (2.6 nematodes/100 cm³) was only 1.5 times lower than that observed on 16 April 2000.

Further research is needed to examine the effect of the host beginning soon after application and at higher host densities on nematode persistence. We did not investigate persistence in subplots where clover root curculio was removed until the third field season. Therefore nematode populations were not as high and as uniform as they were early in the study, making detection of differences between treatments more difficult. Also, intense root sampling in these subplots by the third year reduced alfalfa populations, thus potentially straining nematode persistence by limiting clover root curculio density. This study should perhaps be repeated in a crop such as white clover, Trifolium repens L., where clover root curculio populations typically are more dense.

Long-term persistence of H. bacteriophora Oswego in alfalfa was previously observed in New York following application to control alfalfa snout beetle. Ferguson et al. (1995) reported survival and infectivity of the nematodes for 24 mo after initial application. Continued sampling of the plots revealed persistence for >60 mo (Elson Shields, personal communication). Shields et al. (1999) also noted persistence of this nematode in alfalfa soil throughout a 3-yr field trial against alfalfa snout beetle. In the same study, another strain, H. bacteriophora NC, did not persist well beyond the season of application. The authors suggested that H. bacteriophora Oswego persists better because fewer individuals search for hosts at a given time. Data from the laboratory calibration of H. bacteriophora made for the current study also support this “phased-infectivity” hypothesis, in which proportions of heterorhabditid populations remain viable but noninfective, even if conditions for infection are ideal (Campbell et al. 1999). It is also possible that the Oswego strain is more tolerant of the soil temperatures in New York and Pennsylvania.

Studies of persistence of H. bacteriophora Oswego in Pennsylvania and New York alfalfa involved different pest complexes. The alfalfa snout beetle, the target of the New York biocontrol efforts, does not infest Pennsylvania alfalfa. It has a 2-yr life cycle, providing the nematodes with hosts longer than in Pennsylvania where immature clover root curculio inhabit the soil for only 1–3 mo each year. Although this might favor persistence by providing more opportunities to recycle, persistence of the nematode in Pennsylvania was nevertheless similar to that observed in New York. This may be because the period of recycling through the clover root curculio each May through early July in Pennsylvania is sufficient for nematode persistence or that the nematode may recycle through other hosts such as Japanese beetle larvae. Japanese beetles are a possible alternative host for entomopathogenic nematodes in the alfalfa ecosystem (Schroeder et al. 1993,1994), and larvae were collected from field plots.

As alfalfa stands age and subsequently decline, grasses and other weedy plants become established and serve...
as food sources for Japanese beetle larvae. Japanese beetle larvae mature in the fall and overwinter as such (Shetlar et al. 1990), whereas clover root curculio larvae and pupae are found in the soil only in spring and early summer.

Interpretations of nematode efficacy data in the first field season should be made with caution because nematodes were not applied until 3 June; there could have been root injury before this date. Although laboratory experiments indicated that *H. bacteriophora* Oswego provided >70% control at concentrations corresponding to the 2.5 bil/ha application rate used in this study (Loya 2001), this treatment did not provide significant reduction of the curculio population or of root injury. Based on samples of the nematode population from these plots in June 1998, it is likely that the nematodes did not become established at densities high enough to provide control in the first season. However, the significant reduction in root injury observed after three seasons of nematode persistence in the 7 and 15 bil nematodes/ha plots demonstrates the potential effectiveness of *H. bacteriophora* Oswego against the clover root curculio. It is evident that further research is needed to assess the ability of the nematodes to prevent premature alfalfa stand decline.

In conclusion, populations of the entomopathogenic nematode *H. bacteriophora* Oswego established and persisted for multiple seasons in Pennsylvania alfalfa. Kaya (1990) suggested the criteria for systems suitable for long-term biological control with nematodes should include the following: a pest or complex of pests that are present throughout most of the year, a pest that is moderately susceptible to the nematodes and that has a high economic threshold level, and soil conditions that are favorable for nematode survival. In light of these criteria, it is evident that the clover root curculio–alfalfa system holds potential for using entomopathogenic nematodes in a more classical approach to biological control.

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