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Influence of Application Method and Pest Population Size on the Field Efficacy of Entomopathogenic Nematodes

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Abstract: Application method had an appreciable effect on the efficacy of *Heterorhabditis* sp. (isolate T390) in reducing the numbers of *Otiorhynchus sulcatus* infesting field-grown strawberries. Results were related to nematode placement. In Trial 1, the mean weevil mortality was 36% for trickle-irrigated *Steinernema* sp. (isolate NC513) at a dose of 100,000 nematodes per plant, whereas the same dose of *Heterorhabditis* sp. (isolate T390) resulted in mortality of 65% and 86% for trickle-irrigated and surface-sprayed nematodes, respectively. Mortality rate (y) was inversely related to initial weevil population size (x) by $y = 4.96x^{-0.957}$ and $y = 4.71x^{-0.558}$ for trickle-irrigated *Steinernema* sp. (isolate NC513) and *Heterorhabditis* sp. (isolate T390), respectively. In Trial 2, using 100,000 *Heterorhabditis* sp. (isolate T390) per plant, mean weevil mortalities were 61%, 63%, and 79% for single-injection, irrigation, and multiple-injection techniques, respectively.

Key words: application method, biological control, *Fragaria, Heterorhabditis,* nematode, *Otiorhynchus sulcatus,* pest density, *Steinernema,* strawberry.

The black vine weevil *Otiorhynchus sulcatus* (F.) is a serious and widespread pest of ornamental and soft-fruit crops in Europe, North America, and parts of Asia. Entomopathogenic nematodes, notably *Heterorhabditis* isolates, have been used effectively to control *O. sulcatus* infesting potted plants and in crops grown in greenhouses (2,5,8,13,14,16). This success is reflected in the commercial development and production of *Heterorhabditis* isolates in several countries. However, the need to control *O. sulcatus* is not restricted to the potted plant and greenhouse industry, and an extension of the use of entomopathogenic nematodes to control *O. sulcatus* in field-grown crops and cultivated plants would be desirable, especially for field-grown strawberries.

Several application techniques have been developed that deliver entomopathogenic nematodes close to insects infesting plant roots. These include soil drenching with a nematode suspension in water by either application to the soil surface (13,14) or the dipping of pots into the nematode suspension (9), soil injection techniques (2,5), and the use of trickle irrigation systems to deliver entomopathogenic nematodes (3,12). Differences in experimental designs and conditions in these previous studies have precluded an assessment of the efficacy of different application methods.

The major focus of this study was to compare application techniques for the delivery of entomopathogenic nematodes in a field planting of strawberries with a natural infestation of *O. sulcatus.*

MATERIALS AND METHODS

Site: The strawberry planting was located at the Tasmanian Department of Agriculture's Newtown site in S.E. Tasmania on a loamy sand soil (24.7% coarse sand, 55.9% fine sand, 16.4% silt, 3% clay). The

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experimental area had been established in 1986 for varietal and cultivation trials. Plants were trickle irrigated with one button (pressure compensating) type dripper placed at the level of the crown of each plant. The system was fed by main water pressure with a nominal flow rate of 1–2 liters per hour per outlet under normal working pressures. Soil moisture was monitored with tensiometers and irrigation applied to maintain a moisture level of 30 centibars (pF 2.5) throughout the experiment.

Nematode application: Heterorhabditis sp. (isolate T390) and *Steinernema* sp. (isolate NC513) nematodes cultured *in vitro* (1) were extracted into water and aerated at 15 C for 2 days prior to use. *Heterorhabditis* sp. (isolate T390) was originally isolated from soil from Tasmania, and its DNA profile bears a close resemblance to *Heterorhabditis zealandica* Poinar. *Steinernema* sp. (isolate NC513) was isolated from soil from North Carolina and although morphologically similar to *Steinernema glaseri* (Steiner) Poinar, it is a distinct species (5).

Two separate trials were conducted; in each trial the nematodes were applied during the day, under overcast conditions. For trickle irrigation, the appropriate feeder lines were isolated by clamping the irrigation tubing proximal to the branch line; the nematodes were injected in a total of 100–150 ml of water with a 50-ml syringe into the pressurized line. After 30 minutes of irrigation with the nematode suspension, the clamps were removed from the ends of the feeder lines and water was allowed to flush the system. The number of nematodes injected was adjusted for each line to give an average of 100,000 nematodes per plant. Previous experiments had established that all nematodes passed out of the line within 30 minutes. The delivery volume was measured at 1,340 ± 130 ml per 0.5 hours, based on 23 randomly selected outlets; the number of nematodes passing through individual outlets was not determined.

Soil surface spraying was accomplished by spraying a nematode suspension from a back-pack sprayer, the nozzle being placed under the plastic mulch and moved in a circle around the plant crown to wet the soil surface surrounding each strawberry plant. The nematode suspension had been adjusted so that each plant received 100,000 nematodes in a timed discharge (delivery volume 200 ml). The back-pack spray was continuously shaken to ensure that the nematodes remained in suspension. To emulate soil injection techniques, an 8-mm-diameter steel lance was pushed through the plastic mulch into the soil to a depth of 10 cm to make the "injection" hole. Two methods were employed to deliver 100,000 nematodes per plant into the preformed holes: (i) the nematode suspension was applied via the trickle irrigation system such that the water from the button outlet dripped down into a single hole (irrigation for 0.5 hrs); (ii) after six holes were made equidistant from each other on the circumference of a 15-cm-diameter circle surrounding the plant crown, the nematode suspension was applied by back-pack sprayer with the nozzle placed in each of the six holes in turn in a timed discharge (total volume 200 ml).

Experimental design and measurements: The nematode treatments were applied during mid-March 1988, after the peak weevil egg laying period in January–February. Trial 1 was established 1 week prior to Trial 2. The experimental design for both trials was a randomized block, replicated four times.

Trial 1 took place on an established strawberry cultivar trial of eight rows, each row with 24 plants. This area was planted with eight strawberry varieties, each replicated twice in randomly located blocks of 12 plants (the varieties were 'Benton', 'Ostara', 'Red Gauntlet', 'Shuksan', 'Tioga', 'Totem', 'Tyee', and 72/14). This area was divided into four blocks each of four rows by 12 plants to compare four treatments: (i) *Heterorhabditis* sp. (isolate T390) delivered by trickle irrigation, (ii) *Heterorhabditis* sp. (isolate T390) delivered by spraying on the soil surface, (iii) *Steinernema* sp. (isolate NC513) delivered by trickle irrigation, and

(iv) a control with water only, delivered by trickle irrigation.

Trial 2 took place on 16 rows of an established pruning and cloching trial. Each row contained 20 Red Gauntlet plants, and the area was divided into four blocks of four rows each. Trial 2 compared trickle irrigation, single-, and multiple-injection application methods for *Heterorhabditis* sp. T390.

Three weeks postinoculation for Trial 1 and 4 weeks postinoculation for Trial 2, the soil from a 20-cm-long × 50-cm-wide section of the row was removed at each plant, and the dead and live weevil larvae and pupae were counted. All dead larvae and pupae were retained. In the *Heterohabditis*-treated plots, all developed the orange or red coloration associated with infection by *Heterorahbditis* sp. (isolate T390); dead individuals from a subsample were dissected and 15/15 contained nematodes. All plants in both trials were examined: 48 plants per treatment in Trial 1 and 80 plants per treatment in Trial 2. Soil temperature was measured with a soil thermometer inserted to a depth of 10 cm; the lowest daytime temperature recorded was 17.2 C. Soil moisture was measured with tensiometers.

Statistical analyses: Analysis of deviance scaled according to mean chi-square was used to test for the influence of strawberry plant variety, and differences in cultivation, row, and other position effects on weevil counts. Because no influence was found, analysis of variance was used to test for significant ($P \leq 0.05$) treatment effects on transformed data ($\log_{10}(y + 1)$) for weevil counts (15). Where significant ($P \leq 0.05$) effects existed, regression analysis (15) was used to relate weevil mortality to number of weevils per plant.

Results

In both trials, there was significant reduction in the number of live *O. sulcatus* larvae per plant on strawberry plants treated with nematodes compared to the control plants (Tables 1 and 2). In Trial 1, *Heterorhabditis* sp. (isolate T390) was more effective than *Steinernema* sp. (isolate NC513) in reducing the number of weevils. Soil surface spraying was a more effective method of application for *Heterorhabditis* sp. (isolate T390) than trickle irrigation. In Trial 2 there was a significantly greater reduction in the number of live weevils following application of entomopathogenic nematodes by the multiple-injection technique than by the single-injection or trickle-irrigation methods; there was no significant difference between the last two techniques.

Field observations suggested that where there were large numbers of weevils per plant, the percentage of larvae killed by the nematodes was considerably reduced.

Table 1. Mean number of live *Otiorhynchus sulcatus* larvae per strawberry plant 3 weeks after application of 100,000 nematodes per plant.

Treatment	Larvae per plant† (mean ± s.e.)	% mortality‡
Control	11.7 ± 1.5	
Steinernema sp. (isolate NC513)§	7.5 ± 1.2	36
Heterorhabditis sp. (isolate T390)§	4.1 ± 0.9	65
Heterorhabditis sp. (isolate T390)‖	1.6 ± 0.6	86

† All means are significantly different from one another at $P = 0.05$ according to an L.S.D. test.

‡ Compared to control, calculated as (1 − [mean number alive in treated plot/mean number alive in control plot]) × 100.

§ Nematodes delivered by trickle irrigation.

‖ Nematodes delivered by spraying on soil surface.

Table 2. Mean number of live *Otiorhynchus sulcatus* larvae per strawberry plant 3 weeks after application of 100,000 per plant nematodes *Heterorhabditis* sp. (isolate T390) by three different methods.

Treatment	Larvae per plant† (mean ± s.e.)	% mortality‡
Control	10.4 ± 1.0a	
Single injection	3.6 ± 0.5b	63
Irrigation	4.0 ± 0.7b	61
Multiple injection	2.2 ± 0.4c	79

† All means followed by different letters are significantly different from one another at $P = 0.05$ according to an L.S.D. test.

‡ Compared to control, calculated as (1 − [mean number alive in treated plot/mean number alive in control plot]) × 100.

TABLE 3. Regression coefficients a and b from the equation $y = ax^b$ for mortality rate (%) against initial numbers of weevils per plant.

Treatment	a (mean ± s.e.)	b (mean ± s.e.)	R^2
	Trial 1		
Steinernema sp. (isolate NC513)†	4.96 ± 0.24	−0.957 ± 0.1	0.79
Heterorhabditis sp. (isolate T390)†	4.59 ± 0.1	−0.197 ± 0.04	0.35
Heterorhabditis sp. (isolate T390)‡	4.55 ± 0.08	−0.085 ± 0.04	0.10
	Trial 2		
Irrigation§	4.71 ± 0.13	−0.558 ± 0.07	0.59
Single injection§	4.52 ± 0.11	−0.217 ± 0.06	0.26
Multiple injection§	4.41 ± 0.14	−0.179 ± 0.06	0.12

† Nematodes delivered by trickle irrigation.
‡ Nematodes delivered by spraying on soil surface.
§ *Heterorhabditis* sp. (isolate T390).

This was borne out by subsequent regression analysis of data for all treatments, indicating negative relationships ($P \leq 0.05$) between weevil mortality and initial numbers of weevils per plant (Table 3, Fig. 1).

DISCUSSION

Choice of application method had an appreciable effect on the efficacy of *Heterorhabditis* sp. (isolate T390) in reducing the numbers of *O. sulcatus* infesting field-grown strawberries. Previous studies have noted the need to apply nematodes in a sufficient quantity of water to ensure that nematodes are able to enter the soil protected from desiccation (6). In this experiment, soil moisture levels were kept constant throughout by the use of trickle irrigation in conjunction with tensiometers. This ensured that adequate water was present at all times during the experiment. Thus, any differences in the volume of water used to deliver the nematodes at the time of application were insignificant compared to the volume applied prior to and after application by the irrigation system and are not considered to have influenced the results.

A major difference between the application methods tested was the initial placement of the nematodes around the strawberry plant. Soil surface spraying would have delivered a more uniform nematode inoculum around the crown of the strawberry plant, while the button type of trickle irrigation outlet delivered the inoculum to a small area on one side of the plant. Similarly, the multiple-injection method would

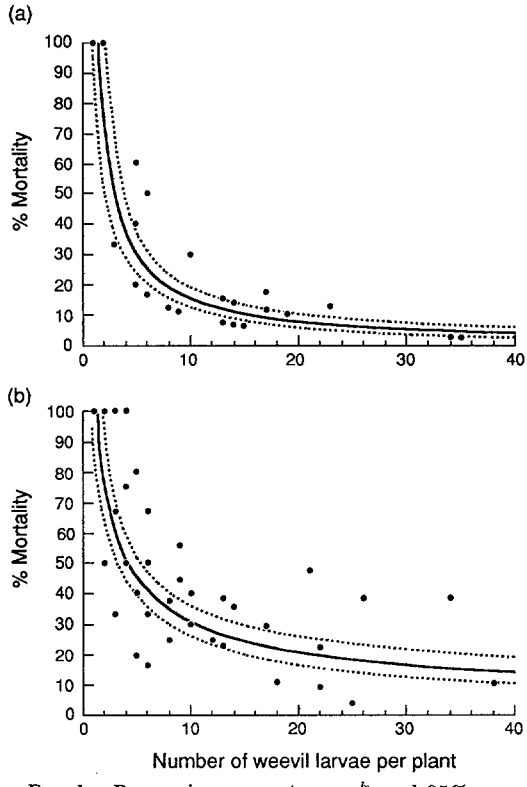

FIG. 1. Regression curve ($y = ax^b$) and 95% confidence limits of weevil mortality (%) against initial weevil population size (live + dead) following application by trickle irrigation of (a) 100,000 *Steinernema* sp. (isolate NC513), regression coefficients: $a = 4.96$, $b = -0.957$, $R^2 = 0.79$, n = 24; (b) 100,000 *Heterorhabditis* sp. (isolate T390), regression coefficients: $a = 4.71$, $b = -0.558$, $R^2 = 0.59$, n = 51.

have provided a more uniform inoculum around the plant than either single injection or trickle irrigation. However, all application methods delivered the nematodes within a 7-cm, circular radius centered on the strawberry crown, an area covering most of the root mass of the strawberry plant. A dispersal rate of up to 4–7 cm/day in sandy soil has been recorded for entomopathogenic nematodes (11), but most studies indicate that entomopathogenic nematodes tend to stay close to the point of application (7). The correlation between differences in weevil mortality and initial distribution of nematodes shows that in this soil (even over the limited distances involved), dispersal was not sufficient to achieve uniform insect mortality between treatments.

This lack of dispersal may be simply due to poor mobility in the soil. However, in both trials there was a negative relationship between initial pest population size and mortality in all treatments. One possible explanation for this relationship is that nematodes dispersing through the soil would tend to be attracted to and accumulate around the first encountered insect, leaving more distant insects uninfected. An expected result of this behavior would be that as the number of available hosts increases, the proportion of uninfected insects would increase. As noted above, trickle irrigation tends to place the nematodes to one side of the plant (and therefore next to the weevil population on that side). An expected consequence of attraction to the nearest host and one-sided placement by irrigation would be an observable increase in the influence of population size on mortality rate. This occurred in both trials, as indicated by the steeper slopes for mortality rate versus population size for irrigation compared to surface spraying or multiple-injection methods of application. Thus, the observed differences in efficacy may be due to the preferential attraction and accumulation of nematodes around the closest insects.

The mortalities achieved in both trials are comparable to previously published field trials. Using a multiple soil injection method and a dose of 250,000 nematodes per plant, Miller et al. (10) reported 79% parasitism of *O. sulcatus* 3 weeks after treatment with *Heterorhabditis* sp. (isolate T327). In Trial 2, a mortality of 79% after 4 weeks with *Heterorhabditis* sp. (isolate T390) was achieved at a dose of 100,000 nematodes per plant. Curran and Patel (4) recorded mortalities of 59% and 25% after application of *H. bacteriophora* (= *heliothidis*) (isolate C1) by trickle irrigation at a dose of 48,000 and 80,000 nematodes per plant, compared to 65% and 63% mortalities at a dose of 100,000 nematodes per plant in Trial 1 and Trial 2.

In this study, delivery of entomopathogenic nematodes by trickle irrigation resulted in lower nematode efficacy compared to either multiple injection or soil surface spraying. However, in selecting the best application method, this lower efficacy must be balanced against the considerable reduction in labor costs in delivering entomopathogenic nematodes by trickle irrigation. Further investigations are warranted to determine if increasing nematode dosage could economically compensate for this effect.

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