

Potential of Entomopathogenic Nematodes for Biological Control of *Acalymma vittatum* (Coleoptera: Chrysomelidae) in Cucumbers Grown in Conventional and Organic Soil Management Systems

C. D. ELLERS-KIRK,¹ S. J. FLEISCHER,² R. H. SNYDER,³ AND J. P. LYNCH³

The Pennsylvania State University, University Park, PA 16802

J. Econ. Entomol. 93(3): 605-612 (2000)

ABSTRACT *Acalymma vittatum* (F.) is the primary insect pest of fresh-market cucumber and melon crops in much of the eastern United States because of their herbivory and interactions with several diseases, most notably bacterial wilt. A study was conducted to determine how soil management affects viability and infectivity of an entomopathogenic nematode that may be used for the control of *A. vittatum*. Dose-mortality curves under laboratory conditions suggested several *Steinernema* spp. as potential biocontrol agents. Field injections combined with soil bioassays showed that *Steinernema riobravis* Cabanillas, Poinar & Raulston (Rhabditis: Steinernematidae) longevity exceeded *A. vittatum* immature development time in both conventional and organic soil management systems. Mean root length densities of cucumbers increased in both soil management systems with the inclusion of nematodes. Soil management alone also influenced *A. vittatum* larval survivorship, with higher survival rates in the organic compared with the conventional soil management system. A 50% reduction in *A. vittatum* larval survival rates in both soil management systems, as determined by adult *A. vittatum* emergence, demonstrated the potential of incorporation of entomopathogenic nematodes for integrated pest management of diabroticites in commercial cucumber production.

KEY WORDS *Acalymma vittatum*, entomopathogenic nematodes, biological control, cucumber

BLACK PLASTIC MULCH and drip or trickle irrigation are often used for fresh-market cucumber and muskmelon production in Pennsylvania. Combined with an herbicide, mulch can reduce the incidence of disease, insects and weeds, and increase yields (Bhella 1988, Necibi et al. 1992). Drip irrigation, installed simultaneously under the plastic mulch, uses less water than conventional irrigation and helps control weed growth while reducing insect and disease damage because the water is concentrated around the base of the plants (Orzolek et al. 1996). Used in combination, black plastic mulch and drip irrigation systems make microenvironmental conditions less favorable for foliar disease development and exclude weeds from the plant beds, creating a more manageable and homogeneous microenvironment (Lamont 1992).

The striped cucumber beetle, *Acalymma vittatum* (F.), and the spotted cucumber beetle or southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber, are the most serious arthropod pests of cucurbits, especially cucumbers and muskmelons, in Pennsylvania. Larval *A. vittatum* and *D. u. howardi* are economic pests because of their ability to reduce a plant's root density. Adults are capable of reducing leaf area and of vectoring *Erwinia tracheiphila* (E. F.

Smith) Holland, the causal agent of bacterial wilt in cucurbits (Rand and Enlows 1916, Sherf and MacNab 1986). Although serological data suggested weed hosts were involved in the life cycle of *E. tracheiphila* (Bassi 1982, Blua et al. 1994), more thorough studies have shown that positive serological tests in nonsymptomatic weeds were associated with nonviable bacteria (de Mackiewicz et al. 1998). This reinforces the close association of the pathogen with the beetle, and serological estimates suggest that the proportion of beetles that harbor the pathogen is 3-5 times higher than the proportion of individual beetles that cause disease in caged bioassays (Fleischer et al. 1999). Disease progression is a function of the dose of *E. tracheiphila* (Lukezic et al. 1996), interactions between wounding and placement of inoculum (Brust 1997a), and feeding behavior and beetle density (Brust and Rane 1995, Yao et al. 1996, Brust 1997b). Thus, disease management is closely tied to management of the diabroticite vector, and recent studies have developed economic thresholds for the adult vector in cantaloupe (Brust and Foster 1999).

A black plastic mulch/drip irrigation system similar to that already in use in central Pennsylvania reduced diabroticite pressure in muskmelons in Missouri (Necibi et al. 1992). Mulch alone, however, does not provide sufficient control. Currently, bacterial wilt is controlled via management of its vectors through the use of systemic and foliar insecticides (Fleischer et al.

¹ Current address: Department of Entomology, The University of Arizona, 410 Forbes Building, P.O. Box 210036, Tucson, AZ 85721.

² Department of Entomology.

³ Department of Horticulture.

1998), but many of the commonly used insecticides are detrimental to pollinators, which are required for cucurbit production. Entomopathogenic nematodes have shown potential as biological control agents against *Diabrotica* and related beetle larvae (Creighton and Fassuliotis 1983, 1985; Reed et al. 1986; Jackson and Brooks 1989, 1995; Brust 1991; Warshaw 1992; Wright et al. 1993; Jackson 1996), and may reduce in-field development of a second generation of *A. vittatum* adults. These nematodes are exempt from federal and state registration requirements, are considered safe to vertebrates and vegetation, and may be applied through several methods, including drip irrigation. Nematodes applied for control of *A. vittatum* and *D. u. howardi* in fresh market cucurbits may be exposed to a hot environment (under black plastic mulch), therefore, a heat tolerant species of nematode may be desirable. *Steinernema riobravisi* Cabanillas, Poinar & Raulston, a nematode isolated from the Rio Grand Valley of Texas (Cabanillas et al. 1994), is capable of infecting and establishing in the last instars of *Galleria mellonella* (L.) at temperatures of 37°C and of reproducing at 35°C (Grewal et al. 1994). Several nematode species are readily mass produced, stored, and shipped (Gaugler and Kaya 1990), and are commercially available. Entomopathogenic nematodes may also be used in conjunction with many pesticides currently in use (Kaya 1990), making them favorable for integration into a pest management system.

We investigated the use of entomopathogenic nematodes to control *A. vittatum* in two soil management systems. Specific objectives were to compare nematode species under laboratory conditions, determine the viability and infectivity over time of *S. riobravisi* introduced via drip irrigation under field conditions, and determine the impact of an inundative release of *S. riobravisi* on cucumber root length density and immature *A. vittatum* survivorship under field conditions.

Materials and Methods

Laboratory Comparison. Standard filter paper bioassays (Woodring and Kaya 1988) were conducted to compare infectivity of *Steinernema feltiae* Filipjev, *S. riobravisi*, *S. carpocapsae* Weiser, and *Heterorhabditis bacteriophora* Poinar to diabroticite larvae. Nematode species were chosen based on a literature review, commercial availability, and heat tolerance. In vitro-produced *S. feltiae*, *S. riobravisi*, and *S. carpocapsae* were acquired as an aqueous suspension from Biosys (Columbia, MD). In vivo-produced *H. bacteriophora* were acquired from an in-house culture reared on *G. mellonella* larvae (Department of Entomology, The Pennsylvania State University). All were stored in an aqueous suspension in plasma flasks without light at 19°C before use.

Trials were conducted using five late instar *D. u. howardi* obtained from DuPont Agrochemicals (Newark, DE) placed in 50 by 9-mm self-closing petri plates with varying concentrations of nematodes. Concen-

trations were 0, 1×10^2 , 1×10^3 , 1×10^4 , or 1×10^5 nematodes per 400 ml distilled water in a first trial. In a second trial, the highest concentration was not repeated, and half of a *Cucurbita maxima* 'Blue Hubbard' cotyledon was added as a food source. In the first trial there were 10 replicates per concentration for *S. riobravisi* and *H. bacteriophora*, 20 each for *S. feltiae* and *S. carpocapsae*, and 50 control replicates. In the second trial there were 15 replicates per concentration of *S. carpocapsae* and *S. riobravisi*, and 50 controls. Plates were shielded from UV light and placed at 23°C. Mortality was assessed daily over a 5-d period by gently probing with a soft brush. Abbott's correction (Abbott 1925) was used to correct for mortality in the controls, and logit mortality was regressed against log dose using probit analysis to determine the concentration-mortality relationships (SAS Institute 1989).

Infectivity of *Steinernema riobravisi* Under Two Commercial Soil Management Systems. This study was conducted over two field seasons, 1995 and 1996, in a Hagerstown silt loam at the Russell E. Larson Agricultural Experiment Station, Rock Springs, PA, under two soil management systems using horticultural practices as in Orzolek et al. (1996). One of these systems, termed organic, used composted dairy manure and a perennial rye cover crop that was grown the previous winter and tilled under in the spring for fertility and maintenance of soil structure. The other system used commercial fertilizer and limestone (quantity determined by soil tests), and is termed conventional. There were four plots each of the conventional and organic soil management systems. Each plot contained four 12-m rows of cucumber, *Cucumis sativus* L. 'Speedway' (Petoseed), spaced 30 cm apart for an approximate total of 40 plants per row. There was 1.5 m between rows. The plots were established using \approx 6 to 8-wk-old transplants grown from seed.

In 1995, a completely randomized plot design was used. Of the four plots for each soil management system, two were randomly designated for inundative release of *S. riobravisi*, and two plots for control (i.e., no nematodes). All plots in the conventional soil management system were covered with one mil black embossed plastic mulch (Climgro, Montreal, Quebec, Canada). In the organic soil management system, the inundative release plots were covered with black plastic mulch. Thus, the inundative release plots had black plastic mulch in both soil management systems.

In 1996, a replicated split plot design was used. As in 1995, there were four plots of each soil management system. In 1996, each plot was split into two nematode subplots: one row was designated for the inundative release of *S. riobravisi* and one as the control. All rows in both soil management systems were covered with the one mil black embossed plastic mulch and a spunbonded plastic row cover that draped over the plants and secured along the edges.

In both years, irrigation was run for 1 h before inoculation (36.7 liter/12 m of row per h) to enhance survivorship and facilitate movement of the nematodes. In vitro-produced *S. riobravisi* acquired from

Biosys (Columbia, MD) in an aqueous suspension (3 billion nematodes in 6,400 ml of water) were used immediately upon arrival. In 1995, *S. riobravus* were transferred using 60-cc syringes into the already running drip irrigation tape near the header tube for distribution into the row, for a total of 1.82×10^8 nematodes per 12 m of row. Holes created by the injections were patched with duct tape, and the drip irrigation was allowed to run for an additional 2 h (73.4 liter/12 m of row per 2 h) to facilitate survivorship and nematode movement into the soil. In 1996, *S. riobravus* were transferred into the already running drip irrigation tape near the header tube through a Dosatron DII6P2 injector (Dosatron, Clearwater, FL) at the same application rate as in 1995. As the soil moisture content was already high in 1996 because of rain, the drip irrigation was not allowed to run for another 2 h as in 1995.

Nematode Infectivity. Nematode infectivity over time was measured using a bioassay technique modified from Gaugler and Boush (1978). A bulb planter that collects from 500 to 1,000 ml was used to collect soil samples at three points along each 12-m row. These three samples were mixed, and one 50-ml sample was removed and placed in a petri plate for bioassay with larval *G. mellonella*, a sensitive indicator species. In 1995, soil was collected at 5, 8, 22, 34, and 41 d after inoculation. A total of 32 rows (16 with nematodes added and 16 control) were sampled on each date. In 1996, samples were collected from 16 rows (eight with nematodes added and eight control) on a weekly basis starting 20 d after inoculation and ending 72 d after inoculation. Five *G. mellonella* larvae were added to each petri plate, and plates were stored in the dark at 23°C. In 1995, plates were checked at 24 h and any dead *G. mellonella* larvae were recorded, removed, and replaced. At 48 h, the dead *G. mellonella* larvae were again counted and recorded. In 1996, plates were checked at 48 h, as it was determined the previous year that this was the average time period required for mortality, and *G. mellonella* larval mortality was recorded. For each sampling date, soil management system, and nematode treatment, the total number of dead *G. mellonella* larvae was divided by the total number introduced into the plate to determine proportion mortality. Proportion mortality of *G. mellonella* was compared between soil management systems and with and without inundative release of *S. riobravus* using an analysis of covariance, with days as the covariant (Minitab 1991).

Cucumber Root Length Density. The effects of entomopathogenic nematodes and soil management on cucumber roots were assessed during the 1995 and 1996 field studies described above. Root length density was measured in 40 cm deep root cores taken ≈ 5 –10 cm from the tap root through a slit in the plastic mulch with a manually operated Giddings soil tube (Giddings Machine, Fort Collins, CO). Two replications of each soil management system (conventional and organic), with and without inundative release of nematodes,

were sampled for a total of eight rows. Data for each soil \times nematode replication were obtained from four subsamples (4 cores per plot), which were pooled. The cores were divided into three depths (0–10, 10–20, and 20–30 cm). Root fragments were extracted by washing, and roots were stained by placing them in 0.169 g/liter of Neutral Red (Sigma, St. Louis, MO) for 20–30 min, and rinsing in distilled water until no more stain leached from the roots. Root fragments were scanned with a computer and analyzed by image analysis software for root length. Mean root length densities (cm root length per milliliter of soil) were analyzed using repeated measures, with depth as the repeated measure (Minitab 1991).

Immature Survivorship of *Acalymma vittatum*. Effects of entomopathogenic nematodes and soil management upon *A. vittatum* were assessed during the 1996 experiments described above. Egg-to-adult survivorship rates of *A. vittatum*, estimated by introducing a known number of *A. vittatum* eggs into the field plots and measuring adult beetle emergence, were compared between soil management systems and with and without augmentative release of nematodes. Eggs were collected from a laboratory colony (Ellers-Kirk 1996) and stored in water at 19°C before use. The day before introduction, the eggs were separated into sets of 100 and stored in water in 50 by 9-mm self-sealing petri plates for transport to the field. Viability of these eggs was confirmed using a subset of eggs which was reared in the laboratory at 27°C, 60% RH, and a photoperiod of 16:8 (L:D) h. One hundred eggs were introduced into each of three locations in both the *S. riobravus* and control rows (subplots) of each plot (i.e., 300 eggs per row per plot).

Floating row covers were placed over all rows at transplanting to exclude the eggs of immigrating beetles. Approximately 2 wk after transplanting (plants were 8–10 wk old), row covers were removed for 1 d and known numbers of *A. vittatum* eggs were introduced. A hand trowel was used to create a 5-cm slit in the soil at the base of each plant, a single petri dish of 100 eggs was rinsed into this slit with water, and the soil was gently pushed to seal the slit. Twelve days after introduction, 60 by 60 by 12.5-cm emergence cages were placed over the plants where eggs had been introduced. Beginning 33 d after egg introduction, all cages were checked three times per week for emerging *A. vittatum* adults until the fields were prepared for winter (72 d after seeding with eggs).

Cumulative percentage of emergence was calculated by dividing the cumulative number of beetles that had emerged by the total number of eggs introduced and multiplying by 100. The percentage cumulative emergence was logit-transformed and regressed against days after introduction of nematodes. The regression lines for the two nematode treatments were compared in each soil management system using a test for the heterogeneity of slopes (Littell et al. 1992). Also, the regression lines for the untreated controls (plots without nematodes) were compared between soil management systems using the same test for the heterogeneity of slopes.

Table 1. Logit regressions expressing the mortality rate of *D. undecimpunctata howardi* as a function of the log concentration (nematodes/400 ml) of four species of entomopathogenic nematodes

Nematode spp.	N ^a	Pearson χ^{2b}	Slope (SE) ^c	Intercept (SE)	LC ₅₀ (95% CI)
<i>H. bacteriophora</i>	250	1.61	1.76 (0.84)	5.94 (2.78)	3.38 (2.26, 6.61)
<i>S. carpocapsae</i>	800	0.17	1.10 (0.33)	2.75 (1.04)	2.51 (1.40, 3.05)
<i>S. feltiae</i>	500	3.53	1.95 (0.44)	6.14 (1.47)	3.15 (2.74, 3.53)
<i>S. riobravisi</i>	550	0.39	2.37 (0.86)	5.55 (2.23)	2.34 (1.49, 2.85)

^a Total number of insects tested.

^b No chi-square values were significant at $P = 0.05$.

^c All slopes significant at $P < 0.03$.

Results

Laboratory Comparison. All four nematode species were capable of causing concentration-dependent mortality of *D. u. howardi* in petri plates under laboratory conditions (Table 1). Among the three *Steinernema* species bioassayed against *D. u. howardi*, *S. riobravisi* and *S. feltiae* displayed a greater response, as indicated by a slope of 2.37 (± 0.86) and 1.95 (± 0.44), respectively, compared with 1.10 (± 0.33) for *S. carpocapsae* (Table 1). The *Heterorhabditis* species tested was also capable of causing concentration-dependent mortality, but mortality rates were low until higher doses were reached. Although the logit model was a significant fit to the data, the confidence interval for the LC₅₀ was wider (2.26–6.61, Table 1) with *H. bacteriophora* than for any of the *Steinernema* species tested.

Infectivity of *Steinernema riobravisi* under Two Commercial Soil Management Systems. *Nematode Infectivity.* In 1995, the proportion mortality of *G. mellonella* was greater in plots treated with nematodes ($F = 47.5$; $df = 1, 35$; $P < 0.01$) but soil management system had no effect ($F = 0.00$; $df = 1, 35$; $P = 0.95$) (Fig. 1, top). There was no interaction of soil management system and nematode infectivity ($F = 0.00$; $df = 1, 35$; $P = 0.97$). Days, as a linear covariant, had a significant effect on *G. mellonella* mortality ($F = 18.9$; $df = 1, 35$; $P < 0.01$). In the inundative release plots, the proportion mortality decreased from ≈ 0.80 at 5 d after inoculation to < 0.25 ≈ 3 wk later. During this same period, the proportion mortality in the control plots varied from 0.06 to 0.00. *S. riobravisi* was no longer a significant mortality factor 22 d after inoculation ($F = 4.16$; $df = 1, 4$; $P = 0.11$), although an increase was seen in mortality on day 34, which again resulted in a significant effect caused by nematodes ($F = 18.00$; $df = 1, 4$; $P = 0.01$).

In the 1996 season, nematodes again had a significant effect on the proportion mortality of *G. mellonella* ($F = 34.1$; $df = 1, 27$; $P < 0.01$) but the type of soil management system had no effect ($F = 0.70$; $df = 1, 27$; $P = 0.41$) (Fig. 1, bottom). The soil management system did not interact with nematode infectivity ($F = 1.09$; $df = 1, 27$; $P = 0.31$). As a linear covariant, days did not have a significant effect on *G. mellonella* mortality ($F = 1.24$; $df = 1, 27$; $P = 0.28$). However, there was noticeable variation in infectivity over time. Infectivity in the conventional soil declined with time

until day 44, and then increased. A mortality rate of ≈ 0.50 was still being observed in the nematode-treated plots of both soil management systems when this experiment was terminated 72 d after the inundative release of *S. riobravisi*.

Cucumber Root Length Density. There was no significant interaction of depth with nematode treatment in either year ($F = 0.14$; $df = 2, 65$, $P = 0.87$ in 1995; $F = 0.71$; $df = 2, 36$, $P = 0.50$ in 1996), therefore data were pooled over depth. Mean root length density was increased in nematode-treated plots in both 1995 and 1996 (Table 2). The beneficial effect of nematodes was independent of type of management (organic versus conventional) ($F = 0.23$; $df = 1, 65$, $P = 0.64$ in 1995; $F = 0.41$; $df = 1, 36$; $P = 0.52$ in 1996) but root length density was greater in conventional plots with nematodes than in organic plots with nematodes. The percentage increase from the addition of nematodes was 57% in the conventional soil in 1996, and ranged from 64 to 84% among all other comparisons.

Immature Survivorship of *Acalymma vittatum*. The introduction of *S. riobravisi* significantly reduced survivorship of *A. vittatum* in both soil management systems. For all soil management systems and nematode treatments, the regression of the logit of the percent cumulative emergence as a linear function of days after nematode introduction was significant ($P < 0.01$ for each line, R^2 ranged from 0.86 to 0.96). These regression lines were significantly different between the *S. riobravisi* and control treatments for the conventional ($F = 31.2$; $df = 1, 43$; $P < 0.01$) and organic ($F = 20.5$; $df = 1, 43$; $P < 0.01$) soil management systems.

Adult *A. vittatum* began to emerge from the organic soil management system ≈ 50 d after egg introduction and continued for ≈ 35 d (Fig. 2, top). The majority of adults (70%) emerged in a 13-d interval starting 60 d after egg introduction. The proportion of 1,200 eggs introduced in the organic soil that emerged as adults was 0.09 from the *S. riobravisi* inoculated soil and 0.21 in the controls (Fig. 2, bottom). This is a 57% reduction in *A. vittatum* emergence rate in the organic soil management system because of the inundative release of *S. riobravisi*.

Acalymma vittatum emergence had already begun 30 d after egg introduction in the conventional soil management system (Fig. 2, top). There did not appear to be any peak emergence period. The proportion of 1,200 *A. vittatum* eggs introduced into the conven-

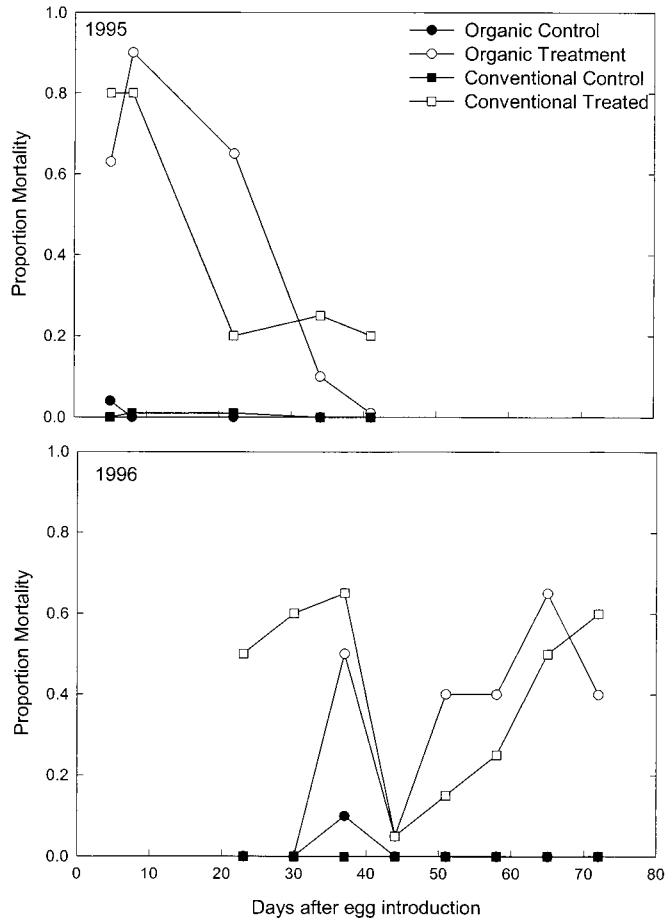


Fig. 1. Proportion mortality of *G. mellonella* at 48 h after introduction to 50 ml of soil from field plots treated with two soil management systems and with or without *S. riobravis* for the 1995 (top) and 1996 (bottom) field seasons. Soil samples were taken up to 41 d (1995) and 72 d (1996) after plots were inoculated with *A. vittatum* eggs.

tional soil that emerged as adults was 0.05 from the *S. riobravis* inoculated soil and 0.09 in the controls (Fig. 2, bottom). This is a 44% reduction in *A. vittatum* emergence rate in the conventional soil management system because of the inundative release of *S. riobravis*.

Soil management system itself, in the absence of any nematode introduction, also significantly influenced survivorship and phenology of emergence of *A. vitta-*

tum. In plots without *S. riobravis*, 57% more *A. vittatum* emerged from the organic than the conventional soil management system (Fig. 2, bottom). The analysis for heterogeneity of slopes comparing the organic and conventional soil management system in the absence of nematodes was also significant ($F = 31.2$; $df = 1, 43$; $P < 0.01$). Cumulative emergence over time was distinctly sigmoidal in the organic system, reflecting the distinct peak in daily emergence, and more linear in

Table 2. Mean root length density (cm root/cc soil) (\pm SE) in cucumbers treated with *S. riobravis* in two soil management systems

Year	Soil Management System	Nematode Treatment ^b		<i>F</i> ^a	df	<i>P</i>
		Control	<i>S. riobravis</i>			
1995	Organic	1.67 (0.80) a	3.08 (0.75) b	4.8	1, 23	0.03
	Conventional	2.53 (0.74) ab	4.45 (0.78) c			
1996	Organic	3.30 (0.49) a	5.42 (0.77) b	11.4	1, 47	<0.01
	Conventional	3.80 (0.61) a	5.96 (0.78) b			

^a *F* test for the effect of nematodes.

^b Different letters denote significant differences among all 4 treatment combinations for a given year, using an LSD means separation test ($P = 0.05$).

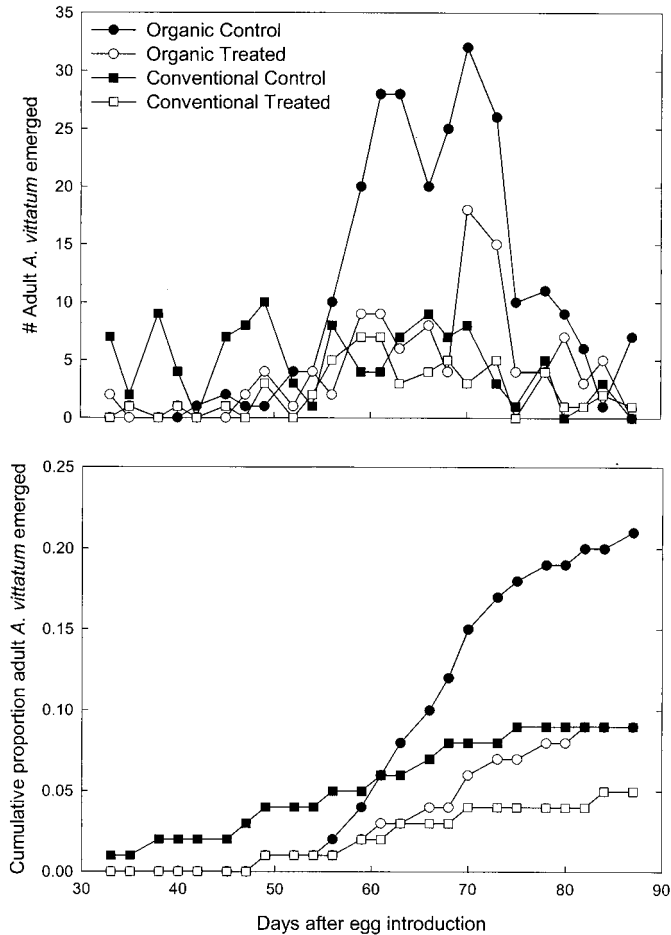


Fig. 2. Daily (top) and cumulative proportion (bottom) emergence over time of *A. vittatum* from caged plots seeded with eggs in the organic (circles) and conventional (squares) soil management system with (open symbol) and without (closed symbol) the addition of *S. riobravis*.

the conventional system, reflecting the absence of any distinct time of peak emergence.

Discussion

The laboratory concentration-dependent mortality study using *D. u. howardi* shows that several *Steinernema* spp. are candidates for use in cucurbit integrated pest management (Table 1). The ability of *S. riobravis* to cause mortality at higher temperatures suggests that it is one appropriate choice for use under black plastic mulch (Grewal et al. 1994). Development of entomopathogenic nematodes for use against di-broticite larvae may be different for cucurbit systems than for corn or other crops because of interactions of the symbiotic bacteria in the nematodes with cucurbitacins (Barbercheck and Wang 1996). We tested *S. riobravis* in the field, but further laboratory assays using *A. vittatum*—such as dose-mortality studies on individuals as opposed to groups, and assays with more realistic environments (i.e., soil or sand arenas)—

need to be conducted and may suggest trials with other species and strains.

Inoculation by drip irrigation of entomopathogenic nematodes is a rapid method of introduction feasible for commercial farming, and injectors make it easy to add the required dose directly into the header line of the irrigation system. Mobile and viable nematodes were collected from all emitters examined, including the most distal site (12 m), and clumping at emitters was not observed. Similar studies using trickle irrigation found uniform nematode distribution along the length of tape (Reed et al. 1986), with increased uniformity as the nematode dose increased (Curran and Patel 1988).

Steinernema riobravis had a strong effect on *G. melonella* mortality following inoculation in three of four tests, and nematode infectivity then decreased over time in 1995 and decreased for the first 40 d following inoculation in 1996 (Fig. 1). It was interesting to note a subsequent increased rate of infectivity after 40 d in the 1996 season. This may reflect a second nematode

generation developing in the field. A higher mortality rate of *G. mellonella* at the end of the season than soon after the inundative release of nematodes in the organic system may be caused by the availability of hosts necessary for reproduction, or an environment conducive to longevity and fecundity in entomopathogenic nematodes. Together, the *G. mellonella* bioassays suggest that *S. riobravis* remain infective for 20–40 d or longer in both soil management systems. The larval stages of *A. vittatum* last \approx 23 d at 27°C (Ellers-Kirk 1996); thus, a single inundative release of entomopathogenic nematodes may influence an entire larval cohort.

Steinernema riobravis had no negative impact on cucumber plant growth and actually yielded a significant increase of >50% in the mean root length density (Table 2). We conclude that the reduction in larval survivorship caused by nematode application (Fig. 2) resulted in superior root growth regardless of the soil management system. Because cucumber has a relatively poor root system, substantial increases in root length density should have favorable impact on water and nutrient acquisition. Further work is needed to test for plant (i.e., root) growth effects associated with entomopathogenic nematodes beyond that caused by mortality of *A. vittatum* larvae.

There was a difference in *A. vittatum* survivorship and emergence pattern between the two soil management systems in the absence of nematodes (Fig. 2). Although both management systems were applied to the same soil taxon (Hagerstown silt loam), the systems have been managed differently for 4 yr, presumably leading to differences in biotic and abiotic soil factors (e.g., temperature, microbial activity, pH, nutrient concentrations, water retention, and soil physical characteristics). These and other factors are all capable of affecting *A. vittatum*'s rate of growth and ability to survive over time as soil dwelling eggs, larvae, and pupae. Further research to determine the causal factors of soil management upon *A. vittatum* survivorship is warranted.

Our research showed that *S. riobravis* was capable of causing an \approx 50% decrease in survival of larval *A. vittatum* in both the organic and conventional soil management systems under commercial field conditions. With drip irrigation providing an easy means of introduction (Reed et al. 1986, Curran and Patel 1988) and black plastic mulch providing an environment conducive to nematode but not diabroticite survival (Necibi et al. 1992), it is feasible that entomopathogenic nematodes have potential as a biological control agent of diabroticite larvae in cucurbits.

Acknowledgments

We thank M. Saunders and M. Orzolek, K. Fager, M. Chyb, D. de Mackiewicz, T. Juba, and K. Lotts for technical assistance, and Biosys and DuPont for materials. This work was funded, in part, by grants from USDA SARE (LNE92-3) and the USDA Pesticides Alternatives Program (96-4363-2707).

References Cited

- Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265–267.
- Barbercheck, M. E., and J. Wang. 1996. Effects of cucurbitacin D on *in vitro* growth of *Xenorhabdus* and *Photobacterium* spp., symbiotic bacteria of entomopathogenic nematodes. *J. Invertebr. Pathol.* 68: 141–145.
- Bassi, A., Jr. 1982. The overwintering nature of *E. tracheiphila* (Smith) and resistance to bacterial wilt in cucumber. Ph.D. dissertation, University of Arkansas, Fayetteville.
- Bhella, H. S. 1988. Effect of trickle irrigation and black mulch on growth, yield, and mineral composition of watermelons. *Hortic. Sci.* 23: 123–125.
- Blua, M. J., F. E. Gildow, F. L. Lukezic, S. J. Fleischer, and D. de Mackiewicz. 1994. Characterization and detection of *Erwinia tracheiphila* isolates. *Phytopathology* 84: 1370.
- Brust, G. E. 1991. Augmentation of an endemic entomogenous nematode by agroecosystem manipulation for the control of a soil pest. *Agric. Ecosyst. Environ.* 36: 175–184.
- Brust, G. E. 1997a. Interaction of *Erwinia tracheiphila* and muskmelon plants. *Environ. Entomol.* 26: 849–854.
- Brust, G. E. 1997b. Seasonal variation in percentage of striped cucumber beetles (Coleoptera: Chrysomelidae) that vector *Erwinia tracheiphila*. *Environ. Entomol.* 26: 580–584.
- Brust, G. E., and R. E. Foster. 1999. New economic threshold for striped cucumber beetles (Coleoptera: Chrysomelidae) in the midwest. *J. Econ. Entomol.* 92: 936–940.
- Brust, G. E., and K. K. Rane. 1995. Differential occurrence of bacterial wilt in muskmelon due to preferential striped cucumber beetle feeding. *Hortic. Sci.* 30: 1043–1045.
- Cabanillas, H. R., G. O. Poinar, Jr., and J. R. Raulston. 1994. *Steinernema riobravis* n. sp. (Rhabditida: Steinernematidae) from Texas. *Fundam. Appl. Nematol.* 17: 123–131.
- Creighton, C. S., and G. Fassuliotis. 1983. Infectivity and suppression of the banded cucumber beetle by the merithida nematode *Filipjevimermis leipsandra* (Merithida: Merithidae). *J. Econ. Entomol.* 76: 615–618.
- Creighton, C. S., and G. Fassuliotis. 1985. *Heterorhabditis* spp. (Nematoda: Heterorhabditidae): a nematode parasite isolated from the banded cucumber beetle *Diabrotica balteata*. *J. Nematol.* 17: 150–153.
- Curran, J., and V. Patel. 1988. Use of a trickle irrigation system to distribute entomopathogenic nematodes (Nematoda: Heterorhabditidae) for the control of weevil pests (Coleoptera: Curculionidae) of strawberries. *Aust. J. Exp. Agric.* 28: 639–643.
- de Mackiewicz, D., F. E. Gildow, M. J. Blua, S. J. Fleischer, and F. L. Lukezic. 1998. Herbaceous weeds are not ecologically important reservoirs of *Erwinia tracheiphila*. *Plant Dis.* 82: 521–529.
- Ellers-Kirk, C. D. 1996. Population dynamics of *Acalymma vittata* Fab. (Coleoptera: Chrysomelidae) and incorporation of entomopathogenic nematodes for integrated pest management of diabroticites in fresh-market cucurbits. M.S. thesis, The Pennsylvania State University, University Park.
- Fleischer, S. J., M. D. Orzolek, D. de Mackiewicz, and L. Otjen. 1998. Imidacloprid effects on *Acalymma vittata* (Coleoptera: Chrysomelidae) and bacterial wilt in cantaloupe. *J. Econ. Entomol.* 91: 940–949.
- Fleischer, S. J., D. de Mackiewicz, F. E. Gildow, and F. L. Lukezic. 1999. Serological estimates of the seasonal dynamics of *Erwinia tracheiphila* in *Acalymma vittata* (Coleoptera: Chrysomelidae). *Environ. Entomol.* 28: 470–476.

- Gaugler, R., and G. M. Boush. 1978. Effects of ultraviolet radiation and sunlight on the entomopathogenic nematode, *Neoaplectana carpcapsae*. *J. Invertebr. Pathol.* 32: 291.
- Gaugler, R., and H. K. Kaya. 1990. Entomopathogenic nematodes in biological control. CRC, Boca Raton, FL.
- Grewal, P. S., S. Selvan, and R. Gaugler. 1994. Thermal adaptation of entomopathogenic nematodes: Niche breadth for infection, establishment, and reproduction. *J. Therm. Biol.* 19: 245–253.
- Jackson, J. J. 1996. Field performance of entomopathogenic nematodes for suppression of western corn rootworm (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* 89: 366–372.
- Jackson, J. J., and M. A. Brooks. 1989. Susceptibility and immune response of western corn rootworm larvae (Coleoptera: Chrysomelidae) to the entomogenous nematodes, *Steinernema feltiae* (Rhabditida: Steinernematidae). *J. Econ. Entomol.* 82: 1073–1077.
- Jackson, J. J., and M. A. Brooks. 1995. Parasitism of western corn rootworm larvae and pupae by *Steinernema carpcapsae*. *J. Nematol.* 27: 15–20.
- Kaya, H. K. 1990. Soil ecology, pp. 93–111. In R. Gaugler and H. K. Kaya [eds.], *Entomopathogenic nematodes in biological control*. CRC, Boca Raton, FL.
- Lamont, Jr., W. J. 1992. Introduction to drip irrigation of vegetable crops and the short course. *HortTechnology* 2: 24–25.
- Littell, R. C., R. J. Freund, and P. C. Spector. 1992. SAS system for linear models, 3rd ed. SAS Institute, Cary, NC.
- Lukezic, F. L., W. M. Sackett, S. J. Fleischer, M. D. Orzolek, and F. E. Gildow. 1996. Influence of the concentration of *Erwinia tracheiphila* cells on the development of wilt symptoms in field grown cucumbers and cantaloupe plants. *Phytopathology* 86: 123.
- Minitab. 1991. Minitab reference manual, version 8.2. Minitab, State College, PA.
- Necibi, S., B. A. Barrett, and J. W. Johnson. 1992. Effects of a black plastic mulch on the soil and plant dispersal of cucumber beetles, *Acalymma vittatum* (F.) and *Diabrotica undecimpunctata howardi* Barber (Coleoptera: Chrysomelidae), on melons. *J. Agric. Entomol.* 9: 129–135.
- Orzolek, M. D., P. A. Ferretti, K. Arnoldson, A. A. MacNab, J. M. Halbrendt, S. J. Fleischer, Z. Smilowitz, and W. K. Hock. 1996. Commercial vegetable production recommendations. Pennsylvania State Cooperative Extension, University Park, PA.
- Rand, F. V., and E.M.A. Enlows. 1916. Transmission and control of bacterial wilt of cucurbits. *J. Agric. Res.* 6: 417–438.
- Reed, D. K., G. L. Reed, and C. S. Creighton. 1986. Introduction of entomogenous nematodes into trickle irrigation systems to control striped cucumber beetle (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* 79: 1330–1333.
- SAS Institute. 1989. SAS/STAT user's guide, 4th ed., vol. 2. SAS Institute, Cary, NC.
- Sherf, A. F., and A. A. MacNab. 1986. Vegetable diseases and their control. Wiley, New York.
- Warsaw, A. R. 1992. Control of the western spotted cucumber beetle using a predaceous nematode. *J. Nat. Resour. Life Sci. Educ.* 21: 101–103.
- Woodring, J. L., and H. K. Kaya. 1988. Steinernematid and Heterorhabditid nematodes: a handbook of biology and techniques. Southern Cooperative Series Bulletin 331. Arkansas Agricultural Experiment Station, Fayetteville, AR.
- Wright, R. J., J. F. Witkowski, G. Echtenkamp, and R. Georgis. 1993. Efficacy and persistence of *Steinernema carpcapsae* (Rhabditida: Steinernematidae) applied through a center-pivot irrigation system against larval corn rootworms (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* 86: 1348–1354.
- Yao, C., G. Zehnder, E. Bauske, and J. Kloepper. 1996. Relationship between cucumber beetle (Coleoptera: Chrysomelidae) density and incidence of bacterial wilt of cucurbits. *J. Econ. Entomol.* 89: 510–514.

Received for publication 15 September 1999; accepted 29 January 2000.