Field Evaluation of *Steinernema carpocapsae* (Rhabditida: Steinernematidae) Against Black Cutworm (Lepidoptera: Noctuidae) Larvae In Field Corn[1]

Eli Levine and Hassan Oloumi-Sadeghi[2]

Office of Agricultural Entomology, University of Illinois and Illinois Natural History Survey
607 East Peabody Drive
Champaign, IL 61820

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ABSTRACT An entomopathogenic nematode, *Steinernema carpocapsae* (Weiser) All strain (BioVector®), applied at two rates (1.25 and 2.5 $\times$ 10^9 nematodes/ha) was compared with several registered insecticides for controlling black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae), larvae during the 1991 growing season in Illinois. Fonofos, tefluthrin and chlorpyrifos were applied at planting time; permethrin and the two rates of nematodes were applied as postemergence rescue treatments. The nematodes generally performed as well as or better than the conventional insecticides in controlling black cutworm larval injury to seedling corn. Bioassays with black cutworm larvae showed that nematode efficacy was lost 8 days after application in the field. Entomopathogenic nematodes hold promise for controlling black cutworms in corn, particularly for corn grown under irrigation.

KEY WORDS Biological control, black cutworm, *Agrotis ipsilon*, entomopathogenic nematodes, *Steinernema carpocapsae.*

The black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae), is a major but sporadic pest of field corn in the Corn Belt (Luckmann 1978). Black cutworm larvae are leaf feeders until they become half grown (that is, about fourth instar), after which they can cause serious damage by cutting corn plants above, at or below the soil surface (Levine et al. 1983). Generally, the larval and pupal stages are found in the soil. Scouting and postemergence rescue (emergency) treatments, applied as needed, are generally recommended, however, many farmers apply soil insecticides at planting time with the intention of controlling both corn rootworm, *Diabrotica virgifera virgifera* LeConte, and *D. barberi* Smith and Lawrence (Coleoptera: Chrysomelidae) and black cutworm larvae.

The black cutworm has several natural enemies that can moderate the severity of the pest's damage, but these beneficial organisms are generally not present in sufficient numbers for effective control. These include the parasitoids

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[2] Present address: American Cyanamid Co., Agricultural Research Division, P.O. Box 400, Princeton, NJ 08540.

Meteorus leviventris (Wesmael) (Hymenoptera: Braconidae), *Microplitis kewleyi* Muesebeck (Hymenoptera: Braconidae), and *Bonnetia comta* Fallen (Diptera: Tachinidae) (Schoenbohm and Turpin 1977, Sajap et al. 1978, Levine and Clement 1981). Based on laboratory studies, carabid beetles were reported to be competent predators of black cutworm larvae (Best and Beegle 1977), although their effectiveness in the field is not known. Two nuclear polyhedrosis viruses have shown promise as black cutworm larval control agents when incorporated into baits, but these studies were only conducted on young (that is, pre-cutting stage) larvae (Johnson and Lewis 1982).

Steinernema carpocapsae (Weiser) (Rhabditida: Steinernematidae) (=*Neoaplectana carpocapsae* Weiser = *N. feltiae* Flipjev sensu Stanuszek; taxonomy of Poinar [1990]), an entomopathogenic nematode, has been reported to have good potential for controlling soil-inhabiting insects, (e.g., Gaugler 1981, 1988; Klein 1990). Poinar (1990) listed 24 strains of *S. carpocapsae*. Entomopathogenic nematodes should be useful in the management of soil-inhabiting insects, because the soil provides a good environment for their survival (high humidity, temperature moderation, and protection from ultraviolet radiation; Gaugler 1981, 1988, Kaya 1990a). Morris et al. (1990) showed that black cutworm larvae were highly susceptible to *S. carpocapsae* (All strain) in laboratory bioassays with moist filter paper in petri dishes. In laboratory bioassays with soil, black cutworm larvae were weakly parasitized by the same strain of nematode (Morris and Converse 1991). Capinera et al. (1988), using *S. carpocapsae* (Mexican strain; at a rate equivalent to 5×10^9 nematodes/ha) in field trials with black cutworm larvae, showed some reduction in black cutworm injury to corn compared with an untreated control, but only at a level approaching significance ($0.05 < p < 0.10$) in one year of a two-year study. The *S. carpocapsae* All strain has been reported by Morris et al. (1990) to have significantly lower LD_{50} values in laboratory bioassays (using moist filter paper in petri dishes) than the Mexican strain. Therefore, we wished to compare in a field trial the efficacy of the entomopathogenic nematode *S. carpocapsae* All strain (BioVector®; produced by Biosys, Palo Alto, California) with that of several registered organophosphate and pyrethroid insecticides against black cutworm larvae. The conventional insecticides were applied either as planting-time or postemergence rescue (applied after insect injury appears) treatments. The nematodes were applied only as rescue treatments, because persistence in the soil was anticipated to be short-lived (entomopathogenic nematodes are susceptible to inactivation by solar radiation and even with irrigation, *S. carpocapsae* tends to remain near the soil surface, particularly in heavier soils [Gaugler 1988]). A laboratory bioassay was also conducted to examine the impact of nematode applications on black cutworm mortality and nematode survival. Most entomopathogenic nematodes are not active dispersers, even in the presence of a host. Therefore, irrigation after application is thought to enhance efficacy against soil-inhabiting pests by moving them into the soil environment (Kaya 1990a).

Materials and Methods

Field Studies. Field corn (Pioneer 3417) was planted in 76-cm rows at a plant population of 64,500/ha on 28 May 1991 in a field in Urbana, IL. Soil was of the silt loam class and consisted of 22.5% sand, 62.5% silt, and 15.0% clay (pH of 6.2). The top 5 cm of soil was moist at planting time. Plots were 15×3 m with four border rows of corn between each plot. The experimental design was a randomized complete block with five replications. One untreated check plot was included in each replication. After plants had reached the two-leaf stage (Ritchie and Hanway 1982) on 6 June, a 4-m^2 barrier was placed around a portion of the middle two rows of each plot to create a test arena. The average number of plants enclosed within each barrier arena was 17 (range of 14 to 20 plants). Barriers were made of 14-gauge steel with sides that were 13 cm tall and 2 m long. Barriers were embedded in the ground to a depth of 2.5 cm. Soil was packed around the edges of each barrier to prevent black cutworm larval escape.

Planting-time granular insecticides included tefluthrin (Force 1.5G, ICI Americas, Inc., Wilmington, DE), fonofos (Dyfonate 20G, ICI Americas, Inc., Wilmington, DE), and chlorpyrifos, (Lorsban 15G, DowElanco, Indianapolis, IN) and were metered through Noble units (Precision Machine Co., Lincoln, NE) mounted on each planter unit. Granules were applied in-furrow (tefluthrin, 3.4 g[AI]/305 m of row) or in an 18-cm band ahead of the planter firming wheels (tefluthrin, 3.4 g [AI]/305 m of row; fonofos, 34 g[AI]/305 m of row; and chlorpyrifos, 34 g[AI]/305 m of row). Spring tines mounted behind each planter unit incorporated the insecticides into the soil.

Ten fourth-instar black cutworm larvae, reared on a meridic diet that was modified by Levine et al. (1982), were placed within each barrier (about 7 P.M.) on 6 June. Arenas were kept free of weeds by hand-weeding throughout the duration of the experiments.

The permethrin (Ambush 2E, ICI Americas, Inc., Wilmington, DE; 112 g[AI]/ha) rescue treatment was broadcast one day after black cutworm infestation of plots (on 7 June) with a 3-m boom mounted on the rear of a tractor (boom height of 43 cm). TeeJet XR8004 (Spraying Systems Co., Wheaton, IL) extended range flat fan nozzles were spaced 50 cm apart on the boom. A compressed-air system was calibrated to deliver 340 l/ha at a pressure of 2.8 kg/cm^2 at a speed of 3.2 km/h. To apply rescue treatments without disturbing the barriers, the boom was offset 4 m to the side of the tractor.

A 12-1 hand sprayer (Hudson model no. 61183 with a hollow cone nozzle but with no screen) was calibrated to deliver two rates of the nematodes (1.25×10^9 and 2.5×10^9 nematodes/ha) on 7 June (between 5 and 6 P.M.), both rates applied in 1400 liters of water/ha (tap water, pH 8.5). We irrigated each of the nematode treatment plots on 6 June, 1 day prior to nematode application, with 19 liters of water. In addition, we applied 8 liters of water to the nematode treated plots on 7, 8 and 12 June (43 liters of water total per barrier over the course of the study, which was the equivalent of 1.3 cm of rainfall).

Plant counts were recorded on 6 June, immediately prior to the release of black cutworm larvae in the arenas and again just before the application of rescue treatments. Plants cut by natural infestations of larvae (only 0.16% of the plant population) and by manual (artificial) infestations prior to the application of the rescue treatments were marked with color-coded stakes. These damaged plants were not included in our damage assessments. Post-treatment assessment of insect damage was determined on 8, 13 and 17 June. These dates corresponded to 11, 16 and 20 days after the application of planting-time treatments or 1, 6 and 10 days after the application of rescue treatments. The cumulative percentage of plants that were cut by black cutworm larvae was recorded for all treatments. The condition of the cut plants was also assessed on the final evaluation date (17 June) for all treatments; that is, cut plants were classified as either "dead" or "recovered" from earlier cutting. The recovered plants were assumed to contribute to the final yield (Showers et al. 1979, Levine et al. 1983, Whitford et al. 1989). Corn seedling stage of development was also recorded on each sampling date (Ritchie and Hanway 1982).

Field Data Analysis. Data were analyzed using the ANOVA procedure of SAS (SAS Institute 1985). Treatment means were separated by Fisher's least significant difference test (P = 0.05).

Nematode Bioassays. Before application of the nematodes, two 30-ml plastic cups each containing a plug of meridic diet (modified by Levine et al. 1982), were placed in each of the five plots to be sprayed with the high rate of nematodes. After application of the spray, these treated diet cups were then taken to the laboratory, and one fourth-instar black cutworm was placed in each cup. Ten cups containing untreated diet, each with one fourth-instar black cutworm, served as controls. The cups were placed in an environmental chamber (25°C, 14L: 10D photoperiod), and the fate of each larva was recorded every 24 hours for 3 days.

The second and third bioassays were conducted by taking samples from the top 5 cm of soil 1 or 8 days after nematode application (high rate only) and placing one fifth-instar black cutworm larva into 30-ml plastic cups containing either moistened nematode-treated (n = 10 for both bioassays) or moistened untreated soil (n = 10 for both bioassays). In both of these assays, the cups were half-filled with soil. In the second bioassay, the larvae were transferred from the soil after 25 hours to cups containing meridic diet and kept at 25°C (14L: 10 D photoperiod). In the third bioassay, larvae were transferred to diet 50 hours after being in the treated or untreated soils. In both of the latter bioassays, the fate of each larva was recorded daily until successful moth emergence or larval or pupal death.

Results and Discussion

Field Studies. The mean percentage of corn seedlings cut per barrier by black cutworm larvae 1 day after application of rescue treatments is presented in Table 1. Corn was at the two to three-leaf stage on this sampling date. Plots treated with nematodes or insecticide had significantly lower percentages of plants cut per barrier than did the control plots. Nematodes applied at the lower rate performed significantly better than fonofos or tefluthrin applied as band treatments at planting or than the higher rate of nematodes (see below). Nematodes applied at the higher rate were associated with significantly less

Table 1. Mean cumulative percentage of plants cut per barrier by black cutworm larvae 1, 6 and 10 days after application of rescue treatments (DAT), Urbana, Illinois, 1991.*,†

			Mean % cut plants§		
Treatment	Rate‡	Placement	1 DAT	6 DAT	10 DAT
Check	–	–	28.8 a	46.6 a	48.9 a
BioVector®	2.5	rescue	13.8 b	23.9 b	25.0 b
Tefluthrin	3.4	band	13.6 b	18.4 bc	18.4 bc
Fonofos	34.0	band	12.5 bc	19.9 bc	21.2 bc
Tefluthrin	3.4	in-furrow	9.2 bcd	14.9 bc	14.9 bc
Permethrin	112.2	rescue	7.5 bcd	10.0 c	10.0 c
Chlorpyrifos	34.0	band	5.9 cd	9.4 c	9.4 c
BioVector®	1.25	rescue	4.8 d	11.1 c	11.1 c
P > F			0.0001	0.0001	0.0001
LSD			7.0	12.5	12.0

* Planting-time treatments were applied on 28 May. Barriers were infested with black cutworm larvae on 6 June. Rescue treatments were applied on 7 June.

† Corn seedlings were at leaf stages two to three, four, and five on 1, 6 and 10 days after application of rescue treatments, respectively.

‡ Rate for tefluthrin, fonofos, and chlorpyrifos is expressed as grams of active ingredient (AI)/305 m of row; rate for permethrin is expressed as grams (AI)/ha; rate for nematodes is expressed as number of nematodes (x 10^9) /ha.

§ Means in a column followed by the same letter are not significantly different (P = 0.05; Fisher's least significant difference test).

damage than the control treatment but performed similarly to tefluthrin (band and in-furrow treatments), fonofos, and permethrin (Table 1). These results confirm earlier reports (Kaya 1990b, Gaugler 1988) that *S. carpocapsae* can act very quickly (within 24-48 hours).

Six days after the application of rescue treatments, when corn was in the four-leaf stage, the cumulative percentage of cut plants for all treatments was significantly lower than that for the untreated check. Regardless of insecticide formulation or time of application, all insecticide treatments performed statistically the same. Nematodes at the lower rate were again statistically superior to nematodes at the high rate and to the untreated check in reducing black cutworm larval damage to corn (Table 1).

Table 1 also shows mean cumulative percentages of cut plants on the final sampling date (10 days after rescue treatments were applied when corn was at the five-leaf stage). Black cutworm larvae seldom inflict serious injury to a corn stand after this point (Showers et al. 1983, Whitford et al. 1989), simply because they cannot cut as many of these larger plants. Plots treated with all products had significantly lower percentages of cut plants per barrier than did untreated plots. Application method did not affect the performance of the insecticides (Table 1). Again, the lower rate of nematodes performed significantly better than the higher rate. This could be due to increased aggregation of nematodes at the higher rate. Although this can increase nematode survival, it can also reduce colonization of hosts (Hominick and Reid 1990).

Throughout this study we observed that some injured plants began to regrow and recover. These plants presumably were cut by black cutworms above the growing point; such plants probably contributed to grain yield (Showers et al. 1979, Levine et al. 1983, Whitford et al. 1989). The final percentages of plants that did not recover from black cutworm injury are shown in Table 2. Performance of both rates of nematodes was statistically similar to the performance of the planting-time or rescue treatments with respect to the percentages of plants that were cut by black cutworm larvae and finally died. To our knowledge, this is the first report of entomopathogenic nematodes controlling black cutworm larvae on corn in the field at a level comparable to that of registered soil insecticides. Nematode strain (we used the All strain) and irrigation probably played a large part in our success, particularly since the rates used were two to four times less than that used by Capinera et al. (Mexican strain with no additional irrigation; 1988). Differences in soil types were of questionable significance. In the Capinera et al. (1988) study, soil consisted of 40% sand, 26% silt, and 34% clay (60% in the heavier silt-clay portion). In our study, 77.5% of the soil consisted of silt or clay. Nematodes have greater difficulty dispersing as the percentage of silt and clay increases in a soil (Georgis and Poinar 1983).

Excluding irrigation, a total of 0.58 cm of rainfall was received between planting (28 May) and black cutworm larval infestation (6 June); an additional 1.35 cm of rainfall fell between larval infestation and the time when corn was in the five-leaf stage of development (on the final date of sampling, 17 June). The mean maximum and minimum air temperatures during this latter period were 30.3 and 17.1°C, respectively (23.7°C mean daily temperature).

Bioassay Results. Three bioassays were conducted with black cutworm larvae. In the first bioassay, black cutworm larvae were released in 30-ml cups containing diet that had been treated with the high rate of nematodes in the field. All (n = 10) larvae died within 2 days after being kept at 25°C, again confirming the earlier studies (Gaugler 1988, Kaya 1990b) that *S. carpocapsae* can act very quickly. All larvae (n = 10) reared on untreated diet remained healthy during this same time period. In the second bioassay, all (n = 10) larvae that were kept for 25 hours in the untreated soil and then transferred to diet survived, pupated, and emerged as moths. However, only 40% of the larvae (n = 10) that were kept for 25 hours in the nematode-treated soil (1 day after nematode application) and then transferred to diet emerged as adults; the remaining 60% died as larvae (n = 3) or prepupae (n = 3). In the third bioassay, soil samples were taken 8 days after nematode application. Regardless of the treatment (soil from nematode-treated plots or control plots), 80-90% (n = 10 for each treatment) of the black cutworm larvae pupated and emerged as moths. This suggests that nematode efficacy was lost between 1 and 8 days after field

Table 2. Mean total percentage of plants cut per barrier (recovered versus dead) by black cutworm larvae 10 days (five-leaf stage) after application of rescue treatments, Urbana, Illinois, 1991.*

Treatment	Rate†	Placement	Mean % cut plants‡	
			Recovered	Dead
Check	–	–	6.3	42.6 a
Fonofos	34.0	band	9.9	11.3 b
Tefluthrin	3.4	band	7.3	11.1 b
BioVector®	2.5	rescue	17.4	7.6 b
Permethrin	112.2	rescue	2.4	7.6 b
BioVector®	1.25	rescue	5.0	6.1 b
Chlorpyrifos	34.0	band	3.6	5.8 b
Tefluthrin	3.4	in-furrow	10.3	4.6 b
P > F				0.0001
LSD				7.76

* Planting-time treatments were applied on 28 May. Barriers were infested with black cutworm larvae on 6 June. Rescue treatments were applied on 7 June.

† Rate for tefluthrin, fonofos, and chlorpyrifos is expressed as grams of active ingredient (AI)/305 m of row; rate for permethrin is expressed as grams (AI)/ha; rate for nematodes is expressed as number of nematodes (x 10^9) /ha.

‡ Means in a column followed by the same letter are not significantly different (P = 0.05; Fisher's least significant difference test).

application (probably due to nematode death or dispersal). Three days of maximum air temperatures in the 33-35°C range on 12 through 14 June may have contributed to this decline in efficacy (Kaya 1990a, Kung et al. 1991), although maximum temperatures at the 10 cm depth in nearby plots did not exceed 28°C during this period (soil temperatures at other depths were not measured, but were probably higher at shallower depths). Gray and Johnson (1983) and Kung et al. (1991) reported that survival of *S. carpocapsae* decreased significantly as temperature exceeded 30 or 25°C, respectively. Soil 1 cm below the surface in the nematode-treated plots always remained moist during the course of the study (between nematode application and 17 June). It is also likely that the nematodes were inactivated by solar radiation with time. Even with irrigation, *S. carpocapsae* tends to remain near the soil surface, particularly in heavier soils (Gaugler 1988).

In summary, entomopathogenic nematodes hold promise in controlling black cutworms in field corn, particularly corn grown under irrigation. Although steinernematid nematodes can survive at relatively low soil moisture (provided the soil dries slowly), nematodes exposed on the soil surface probably succumb to the harmful effects of high temperatures and ultraviolet radiation (Kaya 1990a, Georgis and Gaugler 1991, Gaugler et al. 1992).

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