Journal of Nematology 28(1):68–74. 1996.
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Activity and Persistence of *Steinernema carpocapsae* and *Spodoptera exigua* Nuclear Polyhedrosis Virus against *S. exigua* Larvae on Soybean[1]

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Abstract: Experiments were conducted to assess the effects of the entomopathogenic nematode *Steinernema carpocapsae* and *Spodoptera exigua* multinucleocapsid nuclear polyhedrosis virus (SeMNPV), alone and in combinations, on mortality of the beet armyworm, *S. exigua*, larvae on soybean. In 1991 tests, field-grown soybean plants were treated with *S. carpocapsae* at 0.3 and 0.6 nematodes/cm^2 of leaflet, SeMNPV at 20 and 40 polyhedral inclusion bodies (PIB)/cm^2, and all possible combinations. Treated leaflets were collected from plants and bioassayed with 5-day-old larvae. The combination of *S. carpocapsae* at 0.6 nematodes/cm^2 + SeMNPV at 40 PIB/cm^2 produced significantly higher larval mortality (61.7%) compared with either *S. carpocapsae* (24.8–35.1%) or SeMNPV (26.5–33.7%) alone. In 1992, similar tests were repeated using *S. carpocapsae* at 0.2 and 0.5 nematodes/cm^2, and SeMNPV at 14 and 35 PIB/cm^2. The combination of 0.5 nematodes/cm^2 + 35 PIB/cm^2 resulted in significantly higher larval mortality (64.0%) than either pathogen alone (41.5–49.0%). *Steinernema carpocapsae* and SeMNPV produced additive effects on beet armyworm mortality. Persistence of *S. carpocapsae* was 12–24 hours and SeMNPV was 96–120 hours on soybean.

Key words: beet armyworm, biological control, entomopathogenic nematode, interaction, nuclear polyhedrosis virus, *Spodoptera exigua, Steinernema carpocapsae.*

The beet armyworm, *Spodoptera exigua* (Hübner), is an important pest of numerous cultivated plants, including soybean, *Glycine max* (L.) Merrill, in the United States (2,7). On soybean, larvae primarily feed on the foliage; however, they will also feed on bloom buds, blooms, and small pods (2). Insecticide resistance, which has been detected in populations of this species (6), combined with increasing public concern over the health and environmental consequences of chemical insecticide use, indicates a need to develop alternative pest control strategies.

The entomopathogenic nematode, *Steinernema carpocapsae* Weiser (Rhabditida: Steinernematidae), and *S. exigua* multinucleocapsid nuclear polyhedrosis virus (SeMNPV) (Baculoviridae, subgenus A) are biological agents that have been used for beet armyworm control (3,12,19). Kaya (19) reported that all larval instars of beet armyworm were susceptible to *S. feltiae* (*S. carpocapsae*); however, neonate larvae were significantly less susceptible to nematode infection than 3- and 8-day-old larvae. The nematodes usually kill the host insects within 1 to 3 days (14,19). Conversely, Smits and Vlak (24,25) reported that the first and second instar larvae of beet armyworm were more susceptible to SeMNPV infection than older larvae. The NPVs kill the host within 2 to 5 days after exposure with high virulent strains (14,16) and from 14 to 21 days with low virulent strains (23).

In classical biological control, two strategies for the introduction of biological agents are single-species and multiple-species introduction (9). Recent reviews of results obtained from inundative releases of entomopathogenic nematodes reveal that most releases utilized single-species. Laboratory and field trials involving combinations of baculoviruses and other pathogens are few; most have involved combinations of NPVs or granulosis viruses (GVs) with the pathogenic bacterium *Bacillus thuringiensis* Berliner (15). This study reports the combined effects of *S. carpocapsae* and SeMNPV on mortality of beet armyworm larvae, and their persis-

Received for publication 21 December 1994.

[1] Portion of the first author's M.S. thesis, Mississippi State University. Published with the approval of the Director, Mississippi Agricultural and Forestry Experiment Station as Journal Series Paper J-8647.

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The authors thank S. Manweiler for supply of the nematode; D. L. Warkentin for supply of the virus; and P. A. Hedin, G. Sciumbato, and G. L. Windham for critical review of an earlier draft of the manuscript.

tence in simultaneous multiple-species releases on soybean.

Materials and Methods

Source of pathogens: The nematode *S. carpocapsae* (All strain) was provided by biosys, Palo Alto, California, as infective juveniles formulated in a gel medium on a mesh screen, at approximately 1 to 2 × 10^5 nematodes/ml of gel. The virus SeMNPV variant (Spod-X) was supplied by Espro Inc., Alva, Florida, as a wettable powder with a concentration of 2.9 × 10^{10} polyhedral inclusion bodies (PIB)/g. Stocks of *S. carpocapsae* suspension of 2 × 10^3 nematodes/ml and SeMNPV suspension of 10^7 PIB/ml were prepared, stored at 7 C, and used within 2 weeks.

Source of larvae: Laboratory reared 5-day-old (early third instar) larvae of beet armyworm were used in all tests. The initial stock culture was supplied by the Southern Insect Management Laboratory, USDA ARS, Stoneville, Mississippi. The larvae were maintained on wheat germ-soybean flour diet (20) and incubated at 27 ± 2 C and a 14:10 hour (L:D) photoperiod.

1991 tests: Two tests were conducted on the North Plant Science Research Farm at Mississippi State University. Soybeans (cv. Forrest, determinate, maturity group V) were planted with 96.5 cm between rows and 26 plants per meter of row in a plot size of 6.0 × 5.5 m. The plants were at the V8–V12 stages of development (10) and approximately 0.8–1.0 m tall when the tests were initiated. Treatments were applied on 19 July in test 1 and 22 August in test 2.

The experimental design for both tests was a randomized complete block in a factorial arrangement. Treatment combinations consisted of nine pathogen treatments and two leaflet collection sites. Pathogen treatments consisted of two doses of *S. carpocapsae* (ca. 0.3 and 0.6 nematodes/cm^2 of leaflet), two doses of SeMNPV (ca. 20 and 40 PIB/cm^2), the four possible combinations of *S. carpocapsae* + SeMNPV, and one control. The doses of *S. carpocapsae* and SeMNPV were close to the LD_{25} and LD_{50} of the pathogens, based on preliminary laboratory tests. The two leaflets collection sites included the upper half and lower half of the soybean plant canopy. Control plots were treated similarly except the inoculum was omitted. Each treatment was replicated four times, with 25 larvae per replicate.

Steinernema carpocapsae and SeMNPV inocula were suspended in sterile water containing 0.01% Triton X-100 surfactant. The pathogen suspensions were applied in the late afternoon (6:00–8:30 p.m.), using a CO_2-charged backpack sprayer with three TeeJets 80015E flat fan nozzles without screens. A total spray volume of 187.3 liters/ha was applied to the two middle rows of each plot.

Soybean leaflets were collected at random from each canopy level in each plot at 0, 12, 24, 48, 72, 96, and 120 hours after application. Fifteen leaflets from each canopy level were placed in a 400-ml glass jar lined with a wet filter paper, and bioassayed with 25 larvae for 24 hours. Larvae were then transferred singly to 30-ml plastic cups containing diet. To reduce microbial contamination, the diet was overlaid with sterile corncob grits containing 0.04% (w/w) Phaltan and 0.03% (w/w) tetracycline hydrochloride using a hand-operated inoculator (8). Larvae were incubated at 27 ± 2 C under a 14:10 (L:D) cycle.

Beet armyworm larvae remained on the diet until death or pupation; mortality was recorded daily. Tissue smears of dead larvae collected within 2 days after treatment were examined for the presence of *S. carpocapsae*. For larvae that died between 3 and 4 days after treatment, tissue smears were examined for the presence of *S. carpocapsae* and SeMNPV. Larvae that died 5 days after treatment and beyond were examined for the presence of SeMNPV. Smears were examined using stereo microscopy for *S. carpocapsae* and phase-contrast microscopy for SeMNPV.

The variables measured included daily and total mortality percentages of beet ar-

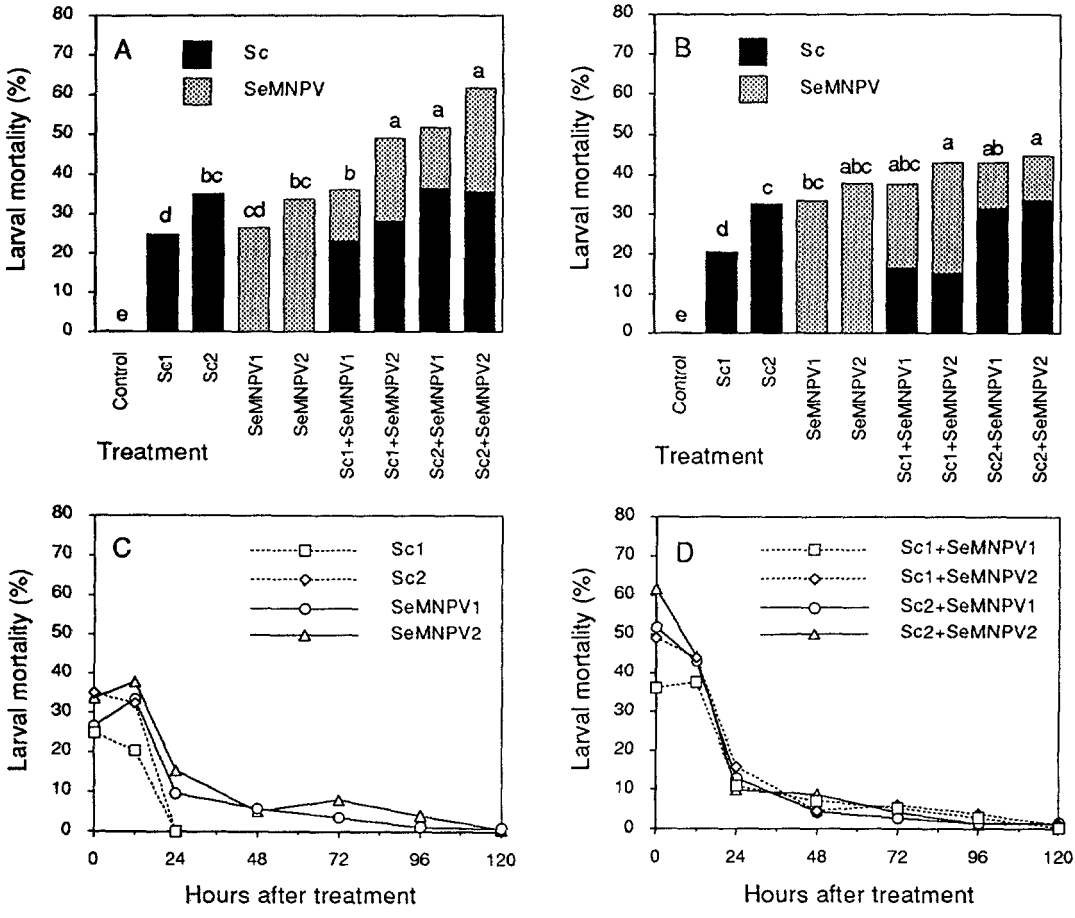

FIG. 1. Mortality of *Spodoptera exigua* larvae at 0 (A) and 12 hours (B) after treatment, and persistence of single (C) and combination (D) treatments of *Steinernema carpocapsae* (Sc) and *S. exigua* multinucleocapsid nuclear polyhedrosis virus (SeMNPV) on soybean leaflets in 1991 tests. Means of eight replications with 50 larvae/replicate. Bars with the same letter are not significantly different (FLSD, $P = 0.05$). 1 = Sc 0.3 nematodes/cm^2 of leaflet, SeMNPV 20 polyhedral inclusion bodies (PIB)/cm^2; 2 = Sc 0.6 nematodes/cm^2, SeMNPV 40 PIB/cm^2.

myworm larvae (at 0, 12, 24, 48, 72, 96, and 120 hours after treatment). Mortality data were corrected using Abbott's formula (1) and analyzed using the Statistical Analysis System (SAS) general linear model (GLM) procedure (22). Means were separated using Fisher's Protected Least Significant Difference (FLSD) test at $P = 0.05$. Percentage data were transformed to arcsine before GLM, but actual percentages are presented. A two-tailed paired *t*-test was used to analyze differences in larval mortality in treatments between the July and August tests. Significant interaction between *S. carpocapsae* and SeMNPV in each combination treatment was further analyzed for *Z* parameter at $P = 0.05$, where the observed combined effect was compared with the expected effect of the corresponding combination treatment.

1992 tests: Tests in 1992 were similar to 1991 tests except that the soybean canopy was considered as one unit. The LD_{25} and LD_{50} of *S. carpocapsae* were ca. 0.2 and 0.5 nematodes/cm^2, and SeMNPV were ca. 14 and 35 PIB/cm^2 of leaflet, respectively. Tests were initiated on 30 July and 5 September. In both tests nine treatments were arranged in a randomized complete block design, with four replications. The air temperature and RH within the soybean plant

TABLE 1. Interactions between *Steinernema carpocapsae* (Sc) and *Spodoptera exigua* multinucleocapsid nuclear polyhedrosis virus (SeMNPV) on *S. exigua* larvae on soybean leaflets at 0 and 12 hours after treatment.

	1991				1992			
	0 Hour		12 Hours		0 Hour		12 Hours	
Treatment	Z	Interaction[a]	Z	Interaction	Z	Interaction	Z	Interaction
Sc1 + SeMNPV1[b]	−0.79	A	−1.29	A	−0.41	A	0.70	A
Sc1 + SeMNPV2	−0.05	A	−0.74	A	−1.13	A	−0.10	A
Sc2 + SeMNPV1	0.01	A	−1.91	A	−0.57	A	0.14	A
Sc2 + SeMNPV2	0.44	A	−2.22	Ant	−0.86	A	−0.67	A

[a] A = Additive, Ant = Antagonistic.
[b] In 1991 tests: 1 = Sc 0.3 nematodes/cm^2 of leaflet, SeMNPV 20 polyhedral inclusion bodies (PIB)/cm^2; 2 = Sc 0.6 nematodes/cm^2, SeMNPV 40 PIB/cm^2. In 1992 tests: 1 = Sc 0.2 nematodes/cm^2, SeMNPV 14 PIB/cm^2; 2 = Sc 0.5 nematodes/cm^2, SeMNPV 35 PIB/cm^2.

canopy during application (6:00–8:30 p.m.) ranged from 26.7–34.4 C and 68–95%, respectively, in July, and from 27.2–35.0 C and 64–92%, respectively, in September.

RESULTS

1991 tests: Larval mortalities on July and August, and soybean canopy levels were not significant; therefore, data from the two tests and the two canopy levels were combined. On leaflets collected at 0 hour after application, pathogen treatments significantly affected beet armyworm mortality. In general, larval mortality increased with doses of the pathogens within the combination treatments. Combinations of *S. carpocapsae* + SeMNPV at 0.3 nematodes/cm^2 + 40 PIB/cm^2, 0.6 nematodes/cm^2 + 20 PIB/cm^2, and 0.6 nematodes/cm^2 + 40 PIB/cm^2 produced 49.2, 51.9, and 61.7% mortality, respectively (Fig. 1A). These mortality percentages were higher than those produced by either *S. carpocapsae* (24.8–35.1%) or SeMNPV (26.5–33.7%) alone, at corresponding dose levels. Similar trends were observed at 12 hours after treatment. Larval mortality in those three combination treatments were higher (44.0–44.2%) than *S. carpocapsae* alone (20.3–32.4%) (Fig. 1B); two of those also produced higher mortality than SeMNPV alone (33.3%) at 20 PIB/cm^2.

Persistence of *S. carpocapsae* on soybean was 12 hours. At 12 hours after treatment the nematodes applied alone at 0.3 and 0.6 nematodes/cm^2 produced 20.3 and 32.4% beet armyworm mortality, respectively (Fig. 1C). Activity of SeMNPV was recorded until 120 hours after treatment; however, virus activity was greatly reduced 24 hours after treatment. When SeMNPV was applied alone, larval mortality from both doses decreased from 26.5–33.7% at 0 hour to 9.4–15.2% at 24 hours after treatment (Fig. 1C). The mortality percentages continued to decrease to 0.5–0.8% by 120 hours after treatment. The same trend was apparent in all combination treatments, which produced larval mortality of 36.2–61.7% at 0 hour and 0.3–1.5% at 120 hours after treatment (Fig. 1D).

Interactions between *S. carpocapsae* and SeMNPV in most combination treatments were additive (Table 1). Additive effects between the two pathogens were observed at 0 hour ($Z = -0.79$ to 0.44; $P = 0.05$) and 12 hours after treatment ($Z = -1.91$ to -0.74; $P = 0.05$) except in combination of the pathogens at 0.6 nematodes/cm^2 + 40 PIB/cm^2 ($Z = -2.22$; $P = 0.05$), in which an antagonistic effect was observed.

1992 tests: Similar trends were apparent in 1992 tests. Beet armyworm mortality was not significantly different when the two pathogens, alone and in combinations, were applied on July and September. At 0 hour after treatment, beet armyworm mortality in combination of *S. carpocapsae* + SeMNPV at 0.5 nematodes/cm^2 + 35 PIB/cm^2 was higher (64.0%) than those in

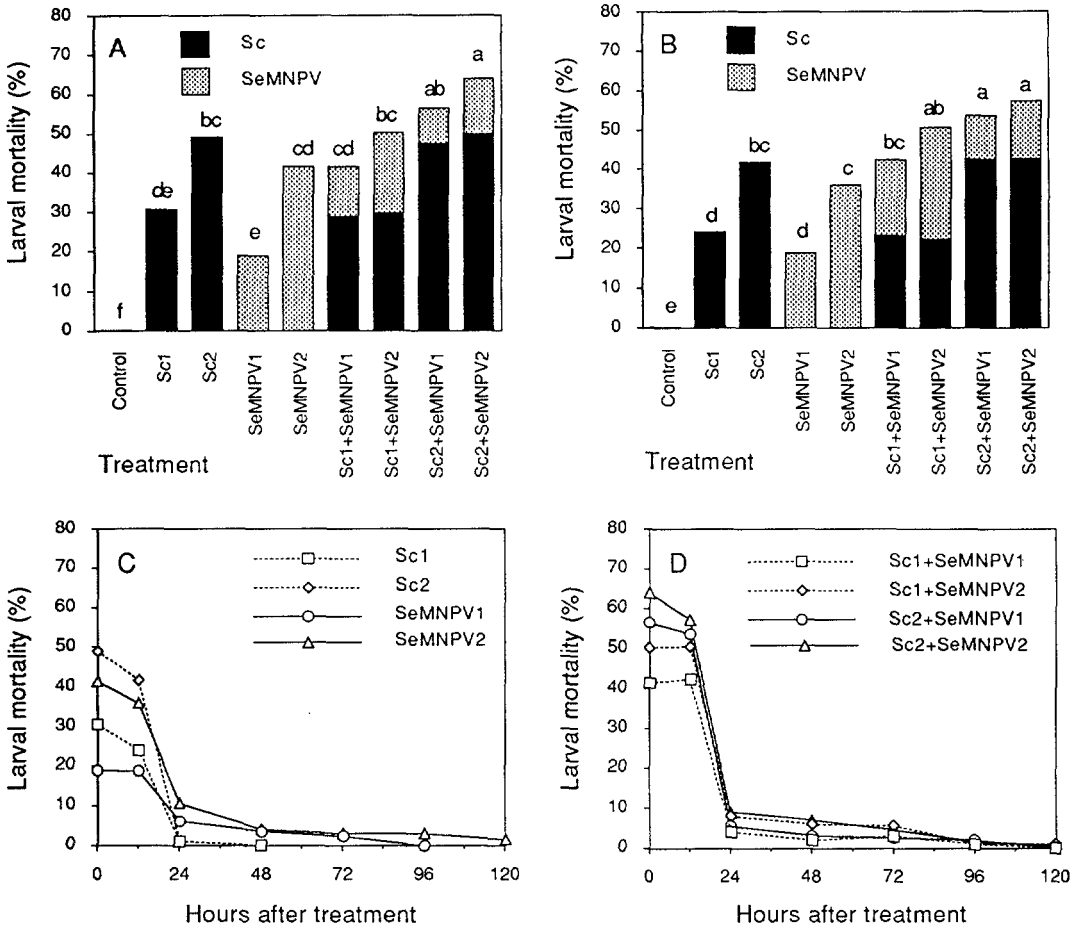

FIG. 2. Mortality of *Spodoptera exigua* larvae at 0 (A) and 12 hours (B) after treatment, and persistence of single (C) and combination (D) treatments of *Steinernema carpocapsae* (Sc) and *S. exigua* multinucleocapsid nuclear polyhedrosis virus (SeMNPV) on soybean leaflets in 1992 tests. Mean of eight replications with 25 larvae/replicate. Bars with the same letter are not significantly different (FLSD, $P = 0.05$). 1 = Sc 0.2 nematodes/cm^2 of leaflet, SeMNPV 14 polyhedral inclusion bodies (PIB)/cm^2; 2 = Sc 0.5 nematodes/cm^2, SeMNPV 35 PIB/cm^2.

either pathogen alone (41.5–49.0%) (Fig. 2A). In other combinations, larval mortality was higher than at least one of the pathogens alone, at corresponding dose levels. At 12 hours after treatment, all combinations produced significantly higher mortality (42.4–57.2%) than either *S. carpocapsae* (23.9–41.7%) or SeMNPV (18.8–35.9%) alone (Fig. 2B). The highest mortality (57.2%) was produced by combination of the higher doses of the pathogens.

Persistence of *S. carpocapsae* was 24 hours (Fig. 2C and D). However, at 24 hours the nematode produced only 1.0% larval mortality. Persistence of SeMNPV was recorded up to 96–120 hours after treatment. Additive effects were observed in all combinations of *S. carpocapsae* + SeMNPV at 0 hour ($Z = -1.13$ to -0.41; $P = 0.05$) and 12 hours ($Z = -0.67$ to 0.14; $P = 0.05$) after treatment (Table 1).

DISCUSSION

This is the first report involving *S. carpocapsae* and SeMNPV in a simultaneous multiple-species field release. According to Benz (5), Harper (15), and Kreig (21), interactions between two pathogens in one

host may produce antagonism, no interaction, or synergism. In this study, combination treatments of *S. carpocapsae* and SeMNPV resulted in additive effects on beet armyworm mortality except one case in 1991 test, where combination of 0.6 nematodes/cm^2 + 40 PIB/cm^2 produced antagonistic effect at 12 hours after treatment. These additive effects indicate that *S. carpocapsae* and SeMNPV acted independently in two different periods of time, and competitive interaction between the two pathogens may not occur. *Steinernema carpocapsae* and SeMNPV may infect the same hosts at the same time; however, those larvae may become unavailable for virus development since the nematode will kill the larvae within 1 to 3 days (14,19). These results are consistent with previous tests using a computerized pesticide spray chamber under laboratory conditions (14). The antagonistic effect between the two pathogens was unexpected, and the reason for this exception is unknown.

Persistence of *S. carpocapsae* and SeMNPV in the field was short. The present studies show that foliage persistence of *S. carpocapsae* was 12 to 24 hours, a result consistent with Georgis and Poinar (13) and Begley (4). According to Begley (4), the foliar environment exposes the nematodes to unfavorable moisture conditions (<90% RH), which results in their rapid desiccation and death. Moreover, high temperatures (18) and sunlight (11) are detrimental to nematodes exposed on the leaflet surface. In July 1991, the maximum air temperature during the test ranged from 32.1 to 35.1 C, and in August from 24.0 to 31.8 C. In 1992, the maximum air temperature during the tests ranged from 28.9 to 32.3 C in July and from 28.7 to 32.4 C in September. High temperature and sunlight also inactivate NPV (17,26).

Even though the combined effects of *S. carpocapsae* and SeMNPV were not examined using field population of beet armyworm, we have demonstrated the potential use of the two pathogen combinations in beet armyworm management on soybean. Considering the nature of beet armyworm and other insect pest populations in the field, *S. carpocapsae* and SeMNPV combinations can be applied simultaneously in two different situations: first, where several or all larval stages of beet armyworm are present, and secondly, where insect pests other than beet armyworm are also present and those pests are susceptible to *S. carpocapsae*. The attributes that show *S. carpocapsae* has potential in this application are its effectiveness against large beet armyworm larvae (14,19) and its wide host range (26). On the other hand, SeMNPV is more effective against small beet armyworm larvae (24,25) and specific to beet armyworm. These two possible scenarios, however, need further investigation.

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