

INTEGRATED CONTROL OF FALL ARMYWORM
(LEPIDOPTERA: NOCTUIDAE) USING RESISTANT PLANTS
AND ENTOMOPATHOGENIC NEMATODES
(RHABDITIDA: STEINERNEMATIDAE)

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ABSTRACT

Laboratory experiments were conducted at Tifton, GA to determine the compatibility of plant resistance with antibiosis and entomopathogenic nematodes, *Steinernema carpocapsae* (Weiser) All strain and *S. riobravis* (Cabanillas, Raulston & Poinar) for controlling prepupae of the fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith). Treatments consisted of 2 nematode species as factor A, 5 diets (the regular pinto bean diet (RPBD) and RPBD diluted at the rate of 3 ml diet/2 ml water (DPBD) with added Celuflil (controls), DPBD + 2.5 g of Zapalote Chico silks (ZC), DPBD + 5.0 g of ZC and DPBD + 7.5 g of ZC, as factor B, and 4 nematode concentrations (0, 2, 6 and 18 nematodes/ml) as factor C. There was a significant interaction between diets and nematode concentration. There was no significant difference in mortality of prepupae on different diets when treated with 0 or with 18 nematodes. However, when treated with 2 nematodes the mortality was significantly higher for prepupae produced on the diets containing resistant silks than for prepupae produced on RPBD or DPBD. When treated with 6 nematodes the mortality was significantly higher for prepupae produced on any of the diets containing resistant silks and the DPBD than for those produced on RPBD. Thus the effects of the resistant silks was masked by the highest concentration of nematodes, whereas, the lower levels of nematodes interacted with the resistant silks to enhance FAW mortality. This study showed that the combination of entomopathogenic nematodes and resistant corn silks could enhance the mortality of FAW prepupae and, therefore, could be useful for integrated management of this insect pest.

Key Words: *Spodoptera frugiperda*, host plant resistance, *Steinernema carpocapsae*, *S. riobravis*, compatibility

RESUMEN

Experimentos de laboratorio fueron realizados en Tifton, Georgia, para determinar la compatibilidad de la resistencia vegetal a través del mecanismo de antibiosis y los nemátodos entomopatógenos, *Steinernema carpocapsae* (Weiser) cepa All y *S. riobravis* (Cabanillas, Raulston y Poinar) para controlar prepupas del gusano cogollero, *Spodoptera frugiperda* (J. E. Smith). Los experimentos se llevaron a cabo usando un diseño completamente al azar con arreglo trifactorial con tres repeticiones, 30 prepupas por repetición. Los tratamientos estuvieron constituidos por la combinación entre tres factores: dos especies de nemátodos, *S. carpocapsae* cepa All y *S. riobravis*, como

factor A; cinco dietas: la dieta regular de frijol pinto (RPBD), RPBD adicionada de celulosa (Celufil) como dietas testigo, RPBD + 2.5 g de estigmas de maíz Zapalote Chico (ZC), RPBD + 5.0 g de ZC y RPBD + 7.5 g de ZC, como factor B; y cuatro concentraciones de nemátodos: 0, 2, 6 y 18 nemátodos/ml como factor C. Los porcentos de mortalidad se registraron diariamente durante un período de 120 horas. Las concentraciones letales promedio CL_{50} se calcularon para ambas especies de nemátodos. Se encontraron diferencias significativas dentro de cada factor en todas las comparaciones. Se observó una tendencia a disminuir el número de nemátodos necesarios en ambas especies, asociados con el incremento de la concentración de estigmas de maíz. Sin embargo, *S. riobravus* mostró CL_{50} más bajas que *S. carpocapsae* cepa All. Por otro lado, las CL_{50} en las dietas portadoras de estigmas fueron más bajas que las testigos. Este estudio mostró que los nemátodos entomopatógenos y los estigmas de maíz resistente podrían incrementar la mortalidad del gusano cogollero y serían útiles en programas de manejo integrado de esta plaga.

Plant resistance (HPR) and biological control are generally considered compatible pest management strategies. The antibiotic mechanism of plant resistance offers a biologically, economically, and environmentally sound alternative to conventional pesticides for controlling the fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) in corn, *Zea mays* L. (Hamm & Wiseman 1986). The potential for deleterious effects of plant quality to aid in the control of third trophic level organisms has stimulated research on compatibility of plant resistance and biological control (Bergman & Tingey 1979).

The success of natural enemies of aerial herbivorous insects can often be related to plant variety or plant chemistry (Price et al. 1980). An allelochemical called may-sin, a luteolin-C-glycoside, in Zapalote Chico silks enhanced the mortality of corn earworm (CEW), *Helicoverpa zea* (Boddie) when used with a nuclear polyhedrosis virus (NPV) (Wiseman & Hamm 1993), reduced about 50% the damage by FAW, and enhanced mortality of FAW 2-fold in comparison with the susceptible check (Hamm & Wiseman 1986). From a practical standpoint, HPR is compatible with other pest management tactics where combined effects are either additive or synergistic (Hare 1992).

The FAW spends its prepupa and pupa stages in the soil, and may become infected by soil-inhabiting pathogens. Usually, soil-inhabiting insect pests are managed by the application of pesticides to soil. Interest in biological control to manage crop pests has increased because of concerns about the economic, environmental, and health costs of chemical crop protection practices, and the recognition that production systems should be both environmentally and economically sustainable (Barbercheck 1993). Nematodes in the families Steinernematidae and Heterorhabditidae show considerable potential as biological control agents against a number of soil-inhabiting pests (Kaya & Gaugler 1993). Soil is the natural habitat of a number of entomopathogenic nematodes which possess a durable and motile infective stage that can actively seek out a host insect, are virulent against a broad range of insects, and do not infect mammalian and avian fauna. Because of these attributes, as well as their ease of mass production and exemption from Environmental Protection Agency (EPA) registration, a number of commercial enterprises are producing entomopathogenic nematodes as biological control agents for inundative release (Barbercheck 1993).

Fuxa et al. (1988) reported the effect of host age and nematode strain on susceptibility of the FAW. We recently corroborated these results, but also determined that the *S. carpocapsae* All strain and *S. riobravus* were the most pathogenic against 7-day-old FAW larvae, prepupae and pupae, and that the prepupae was the most susceptible

stage (Molina-Ochoa et al. 1996). On the other hand, we also determined the amounts of resistant silks to mix into meridic diets that allow the FAW to reach the prepupae stage (Molina-Ochoa et al. 1997). It has been hypothesized that the pathogenic activity of the entomopathogenic nematodes against FAW prepupae could be enhanced by increasing the levels of plant antibiotics. Here we report the results of laboratory experiments to determine the compatibility of the HPR with varying levels of antibiotics and the entomopathogenic nematodes to control the FAW prepupae.

MATERIALS AND METHODS

Insect Colony

Laboratory experiments were conducted at Tifton, Ga, in the USDA-ARS, Insect Biology and Population Management Research Laboratory (IBPMRL) during 1995. FAW used in this study were obtained from a laboratory colony at the IBPMRL, Tifton, Ga. Larvae were reared individually in plastic diet cups (30 ml) containing meridic diets.

Diets

The control groups consisted of larvae reared on a regular pinto bean diet (RPBD) (Burton 1969, Burton & Perkins 1989) and a diluted pinto bean diet (DPBD) (3 ml diet: 2 ml water) with added Celufil™. The modified diets contained 2.5, 5.0 and 7.5 g of "Zapalote Chico #2451 P(C3)" (ZC silk diets) silks per 400 ml DPBD (Wiseman & Widstrom 1986). These modified diets were selected in a previous study, because the FAW reached the prepupa stage (Molina-Ochoa et al. 1997). Diets were dispensed into 30 ml plastic cups and allowed to solidify for 2 h. The larvae were placed individually in the cups with a camels-hair brush, and held at $27 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ RH with a photoperiod of 14:10 (L:D) (Wiseman et al. 1992). Zapalote Chico is a Mexican dent corn with silk and ear resistance to feeding by larvae of FAW and CEW (Gueldner et al. 1991, Wiseman & Widstrom 1986). When the FAW reached the prepupal stage, both controls and treatment prepupae were exposed to different concentrations of the two entomopathogenic nematodes.

Nematodes

Nematodes were obtained from Harry K. Kaya, University of California, Davis, and Grover C. Smart, Jr., University of Florida, Gainesville. Cultures of the two species of entomopathogenic nematodes used for the bioassays, *S. carpocapsae* All strain and *S. riobravisi*, were reared *in vivo* in sixth instar *S. frugiperda* modifying the standard rearing procedure described by Dutky et al. (1964). Nematodes were stored in tissue culture flasks and maintained in aqueous suspension at low temperature (7-10°C) and used within 1 month of collection from the White traps (White 1927).

Pathogenicity Bioassays

The Petri plate bioassay procedure was used for evaluating the virulence of both nematode species against FAW prepupae from the control and treatment diets. One prepupa was placed into each Petri dish (60 × 15 mm) containing different numbers (0, 2, 6 and 18) of infective juvenile (IJ) nematodes. The IJ of each species were suspended in 1 ml of sterile distilled water, and distributed evenly onto two pieces of 5.5

cm-diameter filter paper (Whatman No. 1) in the bottom of the Petri dish 60 min before placing the prepupa on the filter paper (Epsky & Capinera 1993). Only sterile distilled water (1 ml) was added to the controls.

The treatments were distributed in a completely randomized design with a trifactorial arrangement of treatments. The treatments were the combination of two species of nematodes, *S. carpocapsae* All strain and *S. riobravo*, as factor A; 5 diets with the 2 controls RPBD and DPBD + Celufil, and three levels of resistant silks incorporated into the DPBD (2.5, 5.0 and 7.5) as factor B; and 4 concentrations of nematodes (0, 2, 6, and 18/ml) as factor C. Each treatment had three replications, and 30 prepupae per replicate in most of the bioassays.

The Petri dishes of each replication were subsequently placed in a double plastic bag and incubated in the dark at room temperature ($23 \pm 2^\circ\text{C}$) to avoid desiccation. Prepupal mortality was recorded every 24 h for 96 h (Epsky & Capinera 1994). The percent of cumulative mortality data was used for analysis.

Data were normalized by angular transformation prior to analysis by the General Linear Models Procedure. Significant means were separated by Least Square Means (SAS Institute 1989). Median lethal concentrations (LC_{50}) and associated statistics were estimated by the Probit analysis (Finney 1971) using POLO-PC Probit program (LeOra Software 1987).

RESULTS

There were significant differences among treatments for percent mortality ($F = 42.06$; $df = 39, 80$; $P < 0.0001$) of prepupae. There was no significant interaction between species of nematodes and concentration of nematodes or diets, and no significant effect due to the nematode species. A significant interaction of diets x nematode concentration was found (Table 1). The mean separation of mortalities of FAW prepupae show a positive association between all diets with the increase of nematode concentration. However, there was no significant difference in mortality of prepupae between 2 and 6 nematodes for prepupae produced on the diet containing the highest level of ZC silks (DPBD = ZC 7.5). There was no significant difference in mortality of prepupae produced on the different diets when treated with 0 or with 18 nematodes. However, when treated with 2 nematodes the mortality was significantly higher for prepupae produced on any of the diets containing resistant silks than for prepupae produced on RPBD or the DPBD + Celufil. When treated with 6 nematodes the mor-

TABLE 1. EFFECTS OF THE INTERACTION OF ZC DIETS x NEMATODE CONCENTRATIONS ON THE MEAN PERCENT OF MORTALITY OF FAW PREPUPAE.

Diet	0	2	6	18
RPBD	2.47 Aa	22.77 Ab	35.55 Ac	72.77 Ad
DPBD + Celufil	1.52 Aa	17.14 Ab	50.55 Bc	71.34 Ad
DPBD + ZC 2.5	0.50 Aa	29.33 Ab	48.00 Bc	78.00 Ad
DPBD + ZC 5.0	5.16 Aa	32.50 Bb	49.16 Bc	72.50 Ad
DPBD + ZC 7.5	0.50 Aa	42.00 Bb	49.50 Bb	76.00 Ac

Means within a column followed by the same capital letter are not significantly different. Means within a row followed by the same small letter are not significantly different ($P < 0.05$, Least Squares, SAS 1989).

tality was significantly higher for prepupae produced on any of the diets containing resistant silks and the DPBD + Celuflil than for those produced on RPBD.

The results of the interaction between resistant silks and nematodes showed that fewer nematodes were required to produce 50% mortality of prepupae from the diets containing resistant silks. The LC_{50} estimated for the *S. carpocapsae* All strain ranged from 8.06 to 4.42 nematodes/ml for the control diet and the DPBD+ ZC 7.5 diet, respectively. Also, there was a reduction in the LC_{50} with the addition of resistant silks and Celuflil although the 95% fiducial limits overlapped (Table 2).

The results with *S. riobravis* on FAW prepupal mortality showed a reduction in the LC_{50} from 9.39 to 4.11 nematodes, for RPBD and DPBD + ZC 2.5, respectively (Table 3). While the LC_{50s} for prepupae produced on all diets with ZC silks added were lower than LC_{50s} for prepupae produced on RPBD or DPBD plus Celuflil, the 95% fiducial limits for all diets overlapped.

DISCUSSION

This is the first report on the integration of resistant maize and entomopathogenic nematodes as a strategy to control the FAW. We selected the resistant silks of corn with meridic diets in our tests because of the well-described antibiotic relationship between FAW and CEW immature stages and maysin content in silks from the exotic variety of corn "Zapalote Chico" (Wiseman & Widstrom 1986, Gueldner et al. 1991, Wiseman et al. 1992). Maysin is an excellent antibiotic compound which inhibits the growth and development processes in both mentioned pests (Gueldner et al. 1992, Snook et al. 1994). Nematodes in the families Steinernematidae and Heterorhabditi- dae, along with their symbiotic bacteria, are pathogenic to insects (Poinar 1979). These entomopathogenic nematodes occur naturally in the soil and show considerable promise as biological control agents of insects (Timper et al. 1988). FAW larvae mi- grate from the plant to the soil and spend the pupal stage there, it is during this pe- riod that it is feasible to infect them with nematodes.

Host plants can mediate interactions between insects and biocontrol agents, in- creasing or decreasing the impact of the insect on the plant (Bergman & Tingey 1979, Schultz 1983). The effects of insect pathogens on their herbivorous hosts have been in- vestigated without regard to effects of host plant on pathogen virulence (Barbercheck

TABLE 2. MEAN LETHAL CONCENTRATIONS FOR *S. CARPOCAPSAE* ALL STRAIN ON FAW PREPUPAE FED ON DIETS CONTAINING DIFFERENT CONCENTRATIONS OF ZC SILKS.

<i>Steinernema carpocapsae</i> All Strain Diets					
	RPBD	DPBD + Celuflil	DPBD + ZC 2.5	DPBD + ZC 5.0	DPBD + ZC 7.5
Sample Size	270	270	225	180	180
LC_{50}	8.06	7.50	7.17	7.18	4.42
95% Fiducial Limits					
Lower	5.47	5.11	4.78	4.36	2.75
Higher	12.13	11.17	10.92	11.92	6.96
Slope ± SD	1.77 ± 0.24	1.42 ± 0.22	1.14 ± 0.22	1.19 ± 0.28	1.12 ± 0.25

TABLE 3. MEAN LETHAL CONCENTRATIONS FOR *S. RIOBRAVIS* ON FAW PREPUPAE FED ON DIETS CONTAINING DIFFERENT CONCENTRATIONS OF ZC SILKS.

<i>Steinernema robravis</i> Diets					
	RPBD	DPBD + Celufil	DPBD + ZC 2.5	DPBD + ZC 5.0	DPBD + ZC 7.5
Sample Size	269	252	225	180	225
LC ₅₀	9.39	7.37	4.11	5.67	4.55
95% Fiducial Limits					
Lower	5.68	4.42	2.29	3.00	2.60
Higher	16.40	12.59	7.03	10.61	7.60
Slope ± SD	1.236 ± 0.22	1.765 ± 0.23	1.668 ± 0.24	1.147 ± 0.26	0.761 ± 0.22

et al. 1995). Synergistic effects between resistant plants and pathogens to control lepidopterous pests were reported by Hamm & Wiseman (1986), Wiseman & Hamm (1993) and Meade & Hare (1995). The influence of the host plant or diet on the insect susceptibility to nematode pathogenesis were previously reported by Barbercheck (1993), Epsky & Capinera (1994) and Barbercheck et al. (1995).

Our experiments were designed to determine the effects of host diet on the susceptibility of FAW prepupae to infection by entomopathogenic nematodes. The effect of host age and nematode strain or species on susceptibility of *S. frugiperda* to steinernematid and heterorhabditid nematodes was reported previously by Fuxa et al. (1988) and Molina-Ochoa et al. (1996). FAW prepupae were more susceptible (lower LC₅₀s) to the *S. carpocapsae* All strain and *S. riobravis* when fed on ZC silk diets. In the *S. carpocapsae* All strain experiment, the LC₅₀s in the least susceptible FAW prepupae, i.e., larvae which were fed RPBD and DPBD + Celufil were, 1.82-fold and 1.69-fold higher than the LC₅₀ for the most susceptible treatment, i.e., DPBD + ZC 7.5.

Similar responses were observed for *S. riobravis* where the LC₅₀s were lower than those from the *S. carpocapsae* All strain. The LC₅₀s for the least susceptible FAW prepupae, those from larvae fed on RPBD and DPBD + Celufil, were 2.28-fold and 1.79-fold higher than the most susceptible, those from larvae fed on DPBD + ZC 2.5.

Cabanillas et al. (1994) recently described the nematode *S. riobravis* from soil samples in corn fields at harvest from the Lower Rio Grande Valley of Texas. Observations made in that area and the northeastern of the state of Tamaulipas, Mexico indicated that prepupae and pupae of corn earworm, *Helicoverpa zea* (Boddie) (CEW) and FAW were naturally infected by *Steinernema* sp. nematodes in about 34% and 24% of the corn fields, respectively (Raulston et al. 1992). Since then, successful results have been obtained with this species for control of CEW (Cabanillas & Raulston 1994, 1995), root weevil *Diaprepes abbreviatus* L. (Schroeder 1994), and the pink bollworm *Pectinophora gossypiella* Saunders (Henneberry et al. 1995).

If the FAW larvae fed on foliage of resistant varieties of corn during the growing season, their growth would be reduced, their development time increased, and they would be exposed to parasites and predators for a longer period of time. Our data suggest that smaller prepupae entering the soil from larvae that developed on resistant plants would result in enhanced mortality by entomopathogenic nematodes and/or other biocontrol agents, preventing adults from emerging and subsequently migrating to infest other crops. Cabanillas & Raulston (1996) mentioned that delivering nema-

todes through irrigation could be a potential system for suppressing CEW populations. This proposal could also be implemented for FAW that have fed on resistant corn.

High levels of nematodes masked the effects of plant resistance on the insect. However, minimal levels of nematodes (such as 2 nematodes per insect) resulted in enhanced mortality using the combination of plant resistance and nematodes. These results support the proposal of Wiseman (1994) that the resistant cultivar is the base from which integrated pest management decisions should be made and that the integration of plant resistance with biocontrol agents, specifically pathogens and/or nematodes, should be compatible strategies for suppressing insect pest populations. Thus, the combination of resistant plants and entomopathogenic nematodes could provide crop protection that would be biologically, ecologically, economically, and socially feasible.

ENDNOTE

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