ENTOMOPATHOGENIC NEMATODES FOR CONTROL OF ROOT WEEVILS OF CITRUS

W. J. SCHROEDER
U.S. Department of Agriculture
Agricultural Research Service
2120 Camden Road
Orlando, Florida 32803

ABSTRACT

The entomopathogenic nematodes, *Steinernema carpocapsae* (All strain), *Heterorhabditis bacteriophora* (HP-88 strain), *H. bacteriophora* (Florida strain), applied to the soil of a citrus grove near Lake Jem, Florida in March 1989, significantly reduced adult emergence of the root weevil, *Diaprepes abbreviatus* (L.). The total number of weevils captured in 160 traps from April 1989 through May 1990 was 833. Weevil emergence was reduced 70% compared with the check in May, June, and September (1989) and March and April 1990. There was no difference between nematode treatments. Efficacy trials conducted in a grove in Fort Pierce, Florida, for control of the citrus root weevil, *Pachnaeus opalus* and *P. litus*, gave similar results. A study to determine seasonal variation of a native heterorhabditid population in a grove adjacent to the Lake Jem grove was inconclusive.

RESUMEN

Los nematodos entomopatogenicos, *Steinernnema carpocapsae* (All strain), *Heterorhabditis bacteriophora* (HP-88) strain, *H. bacteriophora* (Florida strain) al aplicarse al el suelo de un huerto de citricos cerca a el lago Jem, Florida en Marzo de 1989, redujeron significativamente la emergencia de adultos de el picudo de la raiz *Diaprepes abbreviatus* (L.). El numero total de adultos capturado en 160 trampas desde Abril 1989 hasta Mayo 1990 fue 833. La emergencia de picudos disminuyo en un 70% comparada con el testigo en Mayo, Junio y Septiembre (1989) y en Marzo y Abril de 1990. No hubo diferencias significativas entre los diferentes tratamientos de nematodos. Las pruebas de eficacia conducidas en un huerto en Fort Pierce, Florida, para controlar los picudos de los citricos, *Pachneus opalus* y *P. litus*, dieron resultados similares. Un estudio realizado para determinar la variacion estacional de las poblaciones de un heterorhabditido nativo en un huerto adjacente al del Lake Jem dio resultados inconclusos.

Rhabditid nematodes of the families Steinernematidae and Heterorhabditidae are obligate parasites of insects and are characterized by a mutualistic relationship with *Xenorhabdus* bacteria. Interest in the use of entomopathogenic nematodes as biocontrol agents was enhanced through public concern about chemical pesticides and with recent developments in nematode production. They are lethal to a broad range of economically important insect pests (Poinar 1971). Both exotic and native entomopathogenic nematode species are now utilized as biological insecticides in agriculture. The recent introduction of a commercial product consisting of the nematode *Steinernema carpocapsae* (Weiser) has allowed for increased use of this biocontrol agent in citrus for management of the root weevil complex.

Five species of weevils attack Florida citrus: the Fuller's rose beetle, *Asynonychus godmami* (Crotch); the little leaf notcher, *Artipus floridanus* Horn; the citrus root weevils, *Pachnaeus litus* (Germar) and *P. opalus* (Oliver); and the sugarcane rootstalk borer weevil, *Diaprepes abbreviatus* (L.). The life cycles of the five species are similar: Eggs are deposited in the canopy of the host tree. The neonate larvae fall to the ground, enter the soil, and feed on roots. Major injury to citrus results from larval feeding on roots which leads to host decline and subsequent reduced yields.

The adult weevils are foliage feeders and cause a characteristic notching of leaves, usually with little damage to the tree. The little leaf notcher, however, can cause extensive notching resulting in reduced yield. Larvae of the little leaf notcher and the Fuller's rose beetle feed on small roots while the other three species feed on major roots usually on the underside and at the base of the tree. *Diaprepes abbreviatus* is new to Florida (Woodruff 1964) and is potentially the most destructive because it is the largest of the five species. The weevil is a large insect, about 2 cm long. This weevil is one of about 10 species in a complex of root weevils found in the Caribbean. The Fuller's rose beetle is a major problem for growers who send fruit to Japan (Coats & McCoy 1990). The weevil lays eggs under the calyx of the fruit, and entire shipments of U.S. citrus have required fumigation to pass Japanese quarantine (Haney et al. 1987, Morse & Lakin 1987). The citrus root weevils, *P. opalus* and *P. litus*, are found throughout the citrus-producing areas in Florida (Wolfenbarger 1952) and are characterized by their blue-green color.

Research on the use of the entomopathogenic nematodes for management of the root weevils *D. abbreviatus* and *P.* sp. was initiated following a survey for soil entomopathogens. The survey for natural subterranean enemies of *D. abbreviatus* larvae was conducted from June 1979 through December 1980 in nine citrus groves and one ornamental nursery in Central Florida (Beavers et al. 1983). Two species of entomopathogenic nematodes, *S. carpocapsae* and *Heterorhabditis bacteriophora* (Poinar), were found active in soil and were infectious to *D. abbreviatus* larvae. The nematodes were isolated and evaluated as potential biocontrol agents for the root weevil complex. The nematodes were reared on *Galleria* larvae using the method described by Dutky et al. (1964). Tests were then conducted with the native strains of *Steinernema* and *Heterorhabditis* to ascertain their potential for weevil infection.

Susceptibility of *D. abbreviatus* to entomopathogenic nematodes was reported by Beavers (1984), Roman & Figueroa (1985), and Schroeder (1987). In the laboratory, mortality of 3-month-old larvae ranged from 24 to 88% when nematodes were applied

TABLE 1. CAPTURE OF ADULT *DIAPREPES ABBREVIATUS* IN 160 GROUND EMERGENCE TRAPS FOLLOWING APPLICATION IN MARCH (1989) OF ENTOMOPATHOGENIC NEMATODES AT THE RATE OF 5×10^6 PER TREE.

	Mean Number of Adult Weevils in 10 Traps[1]									
	1989							1990		
Nematode	May	June	July	Aug	Sept	Oct	Nov	Mar	Apr	May
S. carpocapsae (All strain)	7.0b	3.3b	3.5a	3.0a	0.3b	1.8a	0.0a	2.2b	5.1b	4.5a
H. bacteriophora (HP-88)	4.5b	2.5b	2.5a	2.3a	1.3b	0.5a	0.1a	3.2b	3.0b	3.2a
H. bacteriophora (Florida strain)	5.3b	2.8b	3.0a	3.3a	0.7b	1.3a	0.5a	2.4b	3.0b	2.0a
Check	15.3a	9.5a	4.8a	5.3a	3.3a	1.3a	0.6a	7.2a	8.5a	5.6a

[1]In columns, means followed by the same letter are not significantly different ($P = 0.05$; Duncan's [1955] new multiple range test).

to the soil surface at rates of 25, 250, and 2,500 per cm^2. In the greenhouse bioassay (Schroeder 1987), when nematodes were introduced to *D. abbreviatus*-infested citrus seedlings, larval populations were reduced by 90%. Weevil mortality increased when roots were in the bioassay indicating that larval feeding probably enhanced infection by nematodes. In 1988 the USDA-ARS made funds available for a 3-year pilot program to evaluate entomopathogenic nematodes for the management of soil insects under field conditions. The target insects were *D. abbreviatus*, *P. opalus*, and *P. litus* on citrus and *Ligyrus subtropicus* on sugarcane. The research was part of the pest management program in a commercial citrus grove (Schroeder 1990) and the results are reported here.

Materials and Methods

Efficacy of Introduced Entomopathogenic Nematodes

From 1988 to 1990, a test was conducted in a weevil-infested grove, Lake Jem, Florida, to evaluate efficacy of two species of entomopathogenic nematodes against late instar *D. abbreviatus* larvae (Schroeder 1990). The nematodes used were *S. carpocapsae* (All strain) and *H. bacteriophora* (HP-88), both obtained from Biosys, and *H. bacteriophora* that was isolated from a citrus grove located near Winter Garden, Florida. The nematodes were reared on wax moth larvae, *Galleria mellonella* (L.) (Dutky et al. 1964) and sprayed on the soil surface in March at rates of 0 and 100 nematodes per cm^2 (5×10^6 per tree) applied in 1 liter of water. Adult emergence traps placed adjacent to the trees were used to evaluate treatment effects. The trap was a cone constructed of wire cloth 45 cm high, 1 m in diameter, with a 1-liter plastic container on top to retain adult weevils.

The field plot design was a randomized complete block. There were 10 trees per replicate, 1 trap per tree, and 4 replicates per treatment. Traps were examined twice a week from April through October in 1989, weekly from November 1989 to February 1990, and twice a week from March to May 1990. The grove was abandoned in June 1990 because of cold damage. Data were grouped by month. Significant differences in treatment means were identified ($P < 0.05$) by Duncan's (1955) multiple range test. Similar field efficacy trials using the same methods and materials were conducted in Fort Pierce, Florida, by Dr. Bullock. The target root weevil species were *P. opalus*, *P. litus*, and *D. abbreviatus*. The rate was 0 and 5×10^6 nematodes per tree and adult emergence traps were used to determine treatment effects. The nematodes were applied in April 1989.

Seasonal Variation of Endemic Heterorhabditis

The presence of an endemic population of *Heterorhabditis* nematodes in the Lake Jem area was confirmed when soil was sampled prior to the efficacy trials. Presence was detected by the *Galleria* trap method (Bedding & Akhurst 1975) and confirmed by Koch's postulates. To determine possible seasonal variation of this native population, soil samples were collected monthly from an adjacent grove. The sampling period was from January 1989 through December 1990. A soil sample consisted of a 10 cm diam. petri dish of moist soil. There were 10 sites with 10 samples from each site and each sample was bioassayed with 5 *Galleria* larvae (Hominick & Briscoe 1990). Only *Galleria* larvae infected with *Heterorhabditis* nematodes were counted. Nematodes emerging from each cadaver were collected (Dutky 1964) then used to infect *Galleria* larvae to confirm pathogenicity. Data were by month and subjected to ANOVA.

Results and Discussion

Efficacy of Introduced Nematodes

The total number of weevils captured in the 160 traps from April 1989 through May 1990 was 833. There were 142, 141, 183, and 367 weevils collected from 40 traps for each treatment: *H. bacteriophora* (HP-88), *H. bacteriophora* (Florida strain), *S. carpocapsae* (All strain), and control, respectively. The percent reduction for all treatments compared with the check was 58%. Weevil emergence from trees treated with nematodes was significantly different ($P < 0.05$) compared with the check in May, June, September (1989), and March and April (1990) (Table 1). The greatest effect of treatment was in May to June 1989 and March to April 1990 when adult emergence was reduced by 70%. There was no significant difference in weevil control between *H. bacteriophora* (HP-88), *H. bacteriophora* (Florida strain), or *S. carpocapsae* (All strain) at any time in the field comparison study.

Results of the efficacy trials conducted by Dr. Bullock with nematodes applied for control of *P. opalus*, *P. litus*, and *D. abbreviatus* were similar to the Lake Jem study. He reported 70% reduction of adult emergence for the three weevil species (Bullock per comm.).

Seasonal Variation of Endemic *Heterorhabditis*

With the method described we could not demonstrate seasonal variation for the 2-year period (Table 2). In 1989 the number of *Galleria* infected with *Heterorhabditis* in February and March were below average for the year (13%), and in 1990, above the yearly average of 17% mortality. *Heterorhabditis* nematodes were recovered on all sample dates indicating activity by nematodes throughout the year.

Entomopathogenic nematodes, when applied to the surface of the soil, provided a significant reduction in emergence of adult *D. abbreviatus*, *P. opalus*, and *P. litus*. The use of entomopathogenic nematodes as part of the citrus root weevil IPM program that includes oil sprays to reduce egg attachment (Schroeder et al. 1977) and an adulticide during periods of peak adult populations should significantly reduce damage caused by weevil feeding. Use of the nematode, *S. carpocapsae*, in the program offers an environmentally safe alternative to the use of chemical insecticides.

TABLE 2. PERCENT OF MORTALITY OF *GALLERIA* BY *HETERORHABDITIS* SP. IN SOIL FOR A TWO YEAR PERIOD.

Month	% Mortality		Avg. % Mortality
	1989	1990	
Jan	7.6	15.0	11.3
Feb	5.2	29.4	17.4
Mar	5.0	37.2	21.2
April	8.4	15.4	11.9
May	29.5	15.2	22.3
June	12.6	20.4	16.5
July	5.2	11.0	8.1
Aug	20.0	11.4	15.7
Sept	14.8	3.2	9.0
Oct	17.2	12.6	14.9
Nov	16.0	11.4	13.7
Dec	15.6	22.4	19.0

REFERENCES CITED

BEAVERS, J. B. 1984. Susceptibility of *Diaprepes abbreviatus* to the parasitic nematode *Steinernema glaseri*. Int. Res. Comm. Sys. Med. Sci. 12: 480.

BEAVERS, J. B., C. W. MCCOY, AND D. T. KAPLAN. 1983. Natural enemies of subterranean *Diaprepes abbreviatus* (Coleoptera: Curculionidae) larvae in Florida. Environ. Entomol. 12: 840-843.

BEDDING, R. A., AND R. J. AKHURST. 1975. A simple technique for the detection of insect-parasitic rhabditid nematodes in soil. Nematologica 21: 109.

COATS, S. A., AND C. W. MCCOY. 1990. Fuller's rose beetle (Coleoptera: Curculionidae) ovipositional preference on Florida citrus. J. Econ. Entomol. 83: 860-865.

DUTKY, S. R., J. V. THOMPSON, AND G. E. CANTWELL. 1964. A technique for the mass propagation of the DD-136 nematode. J. Insect Pathol. 6: 471-422.

HANEY, P., J. MORSE, G. CARMAN, A. URENA, O. BRAWNER, H. GRIFFITHS, A. GJERDE, P. PHILLIPS, AND N. SAKOVICH. 1987. The Fuller's rose weevil: Progress report on a potential disaster. Citrograph 72: 147-150.

HOMINICK, W. M., AND B. R. BRISCOE. 1990. Survey of 15 sites over 28 months for entomopathogenic nematodes (Rhabditida: Steinernematidae). Parasitology 100: 289-294.

MORSE, J. G., AND K. R. LAKIN. 1987. A degree-day model for Fuller's rose beetle. Citrograph 72: O-P.

POINAR, G. O., JR. 1971. Use of nematodes for control of insects, pp. 181-203, *in* H. D. Burges and N. W. Hussey [eds.], Microbial control of insects and mites. Academic, London.

ROMAN, J., AND W. FIGUEROA. 1985. Control of the sugarcane rootstalk borer weevil, *Diaprepes abbreviatus* (L.) with entomogenous nematode *Neoaplectana carpocapsae* Weiser. J. Agric. Univ. Puerto Rico 69: 153-158.

SCHROEDER, W. J. 1987. Laboratory bioassays and field trials of entomogenous nematodes for control of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in citrus. Environ. Entomol. 16: 987-989.

SCHROEDER, W. J. 1990. Suppression of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) adult emergence with soil application of entomopathogenic nematodes (Nematoda: Rhabditida). Florida Entomol. 73: 680-683.

SCHROEDER, W. J. 1990a. Water-absorbent starch polymer as a survival aid to entomogenous nematodes for control of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in citrus. Florida Entomol. 73: 129-132.

WOLFENBARGER, D. O. 1952. Some notes on the citrus root weevil. Florida Entomol. 35: 139-142.

WOODRUFF, R. E. 1964. A Puerto Rican weevil new to the United States (Coleoptera: Curculionidae). Florida Dep. Agric., Plant Ind., Entomol. Cir. No. 77: 1-4.