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A FOUR-YEAR FIELD TRIAL OF ENTOMOPATHOGENIC NEMATODES FOR CONTROL OF *DIAPREPES ABBREVIATUS* IN A FLATWOODS CITRUS GROVE

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Abstract. **Attempts to eradicate the West Indian sugarcane rootstalk borer weevil, *Diaprepes abbreviatus* L. with chemical insecticides have all been unsuccessful. The use of entomopathogenic nematodes to control the larval stage of *D. abbreviatus* in Florida citrus is gaining acceptance by growers. No trials have been reported on the performance of these nematodes to protect citrus trees in a *D. abbreviatus* infested grove over a period of time greater than 1 year. This paper attempts to determine some of the potential benefits of multiple applications of entomopathogenic nematodes over 4 years in a 7 acre Red Grapefruit grove infested with *D. abbreviatus* located near Vero Beach, Florida. The emergence of adult weevils was monitored with emergence traps and tree mortality was recorded. A total of 493 adult *D. abbreviatus* were captured over a 3.5 year period. Little difference was observed in the number of trapped adults or in the incidence of tree decline from root damage due to larval feeding between the treated and the untreated areas. Consecutive 45% and 48% annual reductions in captured adult weevils in 1993 and 1994, respectively, was speculated to be due to a high population of endemic parasites found in the soil. Soil bioassays for entomopathogens confirmed a high level of *Heterorhabditis sp.* throughout the grove.**

The West Indian sugarcane rootstock borer weevil, *Diaprepes abbreviatus* L., was first detected in Florida citrus in 1964 (Woodruff 1964). Since then it has spread to 18 counties and has been reported by the Florida Dept. of Plant Industry (1994) to infest 22,000 acres of commercial citrus. Early attempts to eradicate *D. abbreviatus* with chemical insecticides were unsuccessful (Bullock, 1988). Currently there are no chemical pesticides labeled to control the destructive larval stage beyond the first instar of *D. abbreviatus* and the potential for further spread is tremendous (Knapp, 1994, McCoy, 1994). Since it has been reported that adults can be found throughout the year with 2 peak emergence periods in June and September, the use of multiple costly adulticide sprays would be required (Beavers, 1983, Futch, 1994). Unfortunately, multiple pesticide applications would likely disrupt established biological control programs and could lead to resistance (McCoy, 1994). The use of an entomopathogen to control *D. abbreviatus* would address these problems as well as eliminate any environmental concerns.

Beavers (1983) reported that *D. abbreviatus* larvae were parasitized by native soil populations of *Steinernema carpocapsae* (Weiser) and *Heterorhabditis* sp. (Poinar) nematodes in weevil infested citrus groves and that they had the potential to control this pest with minimal undesirable effects on the environment. Since then several studies have reported that the use of applied entomopathogenic nematodes reduced the adult emergence of *D. abbreviatus* (Schroeder, 1987, 1990, 1992, Downing, 1991) and the number of *D. abbreviatus* larvae in the soil (McCoy, personal communication). However, the performance of entomopathogenic nematodes to protect the root system of citrus trees in *D. abbreviatus* infested groves has not been determined (McCoy, 1994). Since citrus trees grown in flatwoods soils have a shallow root system, larval damage is more pronounced (Futch, 1994). Therefore, a long term field trial was conducted in a *D. abbreviatus* infested flatwoods citrus grove to determine if multiple applications of 2 entomopathogenic nematodes can reduce both the number of emerging adult weevils and tree mortality.

Materials and Methods

The trial began in March, 1991 at the Kerr Center groves located west of Vero Beach in Indian River County. *D. abbreviatus* was first detected at the Kerr Center in May, 1990 and in the ensuing year, it reached a level of infestation at the study site were adults could be routinely observed by intense scouting. The 7.2 acre study site consisted of 1,000 vigorously growing 4 year old Ruby Red Grapefruit trees on Swingle citrumelo rootstock planted at 140 trees/acre (12.5 × 25 ft) on double row beds. The soil type is predominately Winder fine sand with a smaller portion of Manatee loamy fine sand. A well maintained irrigation system equipped with microemitters and 2 equally divided irrigation zones allowed for an untreated or control area and a nematode treated area. Each area had 500 trees. Nematode applications were made by injection into the irrigation system with the filter removed via a 12 volt pump. To assure a uniform distribution, nematodes were continually agitated with a circulation pump throughout the injection period. The irrigation system was run for 1 hr prior to injection and for 1 hr after injection. Nematode viability was determined by removing a small sample prior to injection and viewing the nematodes under a microscope for mobility.

The south half of the grove was treated with 7 applications of *Steinernema carpocapsae* (All strain), (Biovector®) over a 3 year period at either 2 million nematodes per tree (label rate) or 4 million per tree (double label rate). One application was made in March, 1991 and 3 applications (including 1 handgun application directly on and around the trap area) were made in 1992 and all were at the label rate. Three applications (2 at double label rate) were made in 1993. One application of *Heterorhabditis bacteriophora* C1 strain, (Otinem®) nematodes was made in November 1993 at 7.8 million nematodes per tree. Trees in the untreated zone or control area received irrigation water only. Source water was from an artesian well with 1300 ppm Total Dissolved Solids.

Adult weevil emergence traps were used to monitor adult *D. abbreviatus* emergence rates from the ground to determine nematode efficiency (Schroeder, 1990, 1992, Downing,1991). The trap was a 1 m diameter cone constructed from galvanized screen fitted with a clear plastic cup at the top to hold the trapped adult weevils until collected. One hundred emergence traps were installed in each zone next to the tree trunk and within the tree row on the south side of the tree. Traps were examined weekly for each area from June to October, 1991, April to October, 1992, February to August, 1993, and January to April, 1994. The number of captured adult *D. abbreviatus* was recorded and the data were grouped by month.

The performance of applied entomopathogenic nematodes, in terms of their ability to protect the roots of *D. abbreviatus* infested trees, was determined by comparing the number of declining trees in both areas after 3 years of 8 nematode treatments. Trees were described as "declining" if the tree was not producing harvestable fruit or appeared to be dying due to the presence of root damage by the feeding activities of *D. abbreviatus* larvae. Portions of the root system of the declining trees were examined to confirm root scarring from larval feeding by digging with a shovel to expose the roots or entirely by uprooting with a front end loader. The soil was examined with a screen sieve for larvae, pupae, and teneral adults (alive and dead) which were counted and examined with a stereomicroscope for entomopathogenic nematodes. Trees in both sites were evaluated, marked, and recorded on 12 May, 1994.

Soil samples in both areas were assayed by Dr. W. J. Schroeder at the USDA Laboratory in Orlando, Florida, to determine if entomopathogenic nematodes were present in the soil. Nematode presence and identification were determined using the *Galleria* trap method (Bedding, 1975) and confirmed by Koch's postulates. Soil samples were collected 4 May and 29 August 1994 from 8 randomly selected trees in both the treated and the control areas and consisted of four, 1 x 6 inch cores taken from within the dripline of each tree. The number of *Galleria* larvae infected with *Heterorhabditis sp.* nematodes as indicated by a characteristic brick red color were counted. For a complete methodology, see Schroeder, 1992.

Results

The total number of weevils captured during the 4 year trial was 493. After 8 applications of entomopathogenic nematodes over 4 years, a slightly greater number of adult *D. abbreviatus* was captured in the treated area than in the control area (Table 1). When the data for emergence were grouped monthly, no short term suppression of adult emergence is detectable after the indicated applications of *S. carpocapsae* (Fig-

Table 1. Comparison of the number of declining[z] trees and the number of adult *Diaprepes abbreviatus* captured in a Diaprepes infested flatwoods citrus grove treated with entomopathogenic nematodes from March 1991 to November 1993. Vero Beach, Florida.

Treatment	No. of Applications	No. of Declining Trees/500 (%)	No. of Adult Captured/ 100 traps	Percent of Total Adults Captured
Nematodes	8	26 (5.2)	261	53
Control	0	4 (0.8)	232	47

[z]A tree was defined as "declining" if it was dying or not producing harvestable fruit due to root damage by *D. abbreviatus* larvae.

ure 1&2). A single application of *H. bacteriophora* did not indicate suppression of emergence either (Figure 3). The yearly emergence summary presented in Figure 4 shows no effect for nematode treatments when the data are grouped in an annual fashion.

The lack of performance of nematode applications in terms of protecting the root system of infested trees in our trial is evidence by a 6 fold increase in tree decline in the treated area 3 years after the first of 7 applications of *S. carpocapsae* in March, 1991 (Table 1). Three representative dying trees were uprooted from each area to confirm root damage; all exhibited extensive root damage due to *D. abbreviatus* larvae.

To illustrate the seasonal abundance, the 1992 to 1994 monthly emergence data were combined from both areas in Figure 5. A broad period of adult emergence from spring to fall is indicated with the greatest emergence activity occurring from April to June. There was also a downward trend in adult emergence evident from 1992 to 1994. This downward trend is more apparent in Table 2 which presents the number of emerging adults from the same 12 week period for each year (15 April - 1 July) during peak emergence. *D. abbreviatus* was detected at the Kerr Center groves in May 1990. In 1991 only 6 adults were captured over a 5 month period from all 200 traps. In 1992 the number of trapped weevils soared to 187 adults. However, in 1993 and 1994, the number of trapped *D. abbreviatus* decreased by 45% and 48% respectively (Table 2). The data in Table 2 does show a slightly greater decrease in adult emergence for the treated area indicating a possible effect of the nematode applications.

The results of 2 bioassays shown in Table 3 revealed a high population of *H. bacteriophora* in the soil at the study site. This is dramatically evident in the May, 1994 soil samples which produced a 70% mortality rate of the *Gallaria* larvae due to *H. bacteriophora*. Similar levels of *H. bacteriophora* were observed in both the control and treated areas as indicated by no significant difference ($P = 0.05$) in *Gallaria* larvae mortality for either sampling date. Furthermore, when the declining trees were uprooted to confirm the presence of *D. abbreviatus* larvae and their characteristic root damage, we observed a high in-

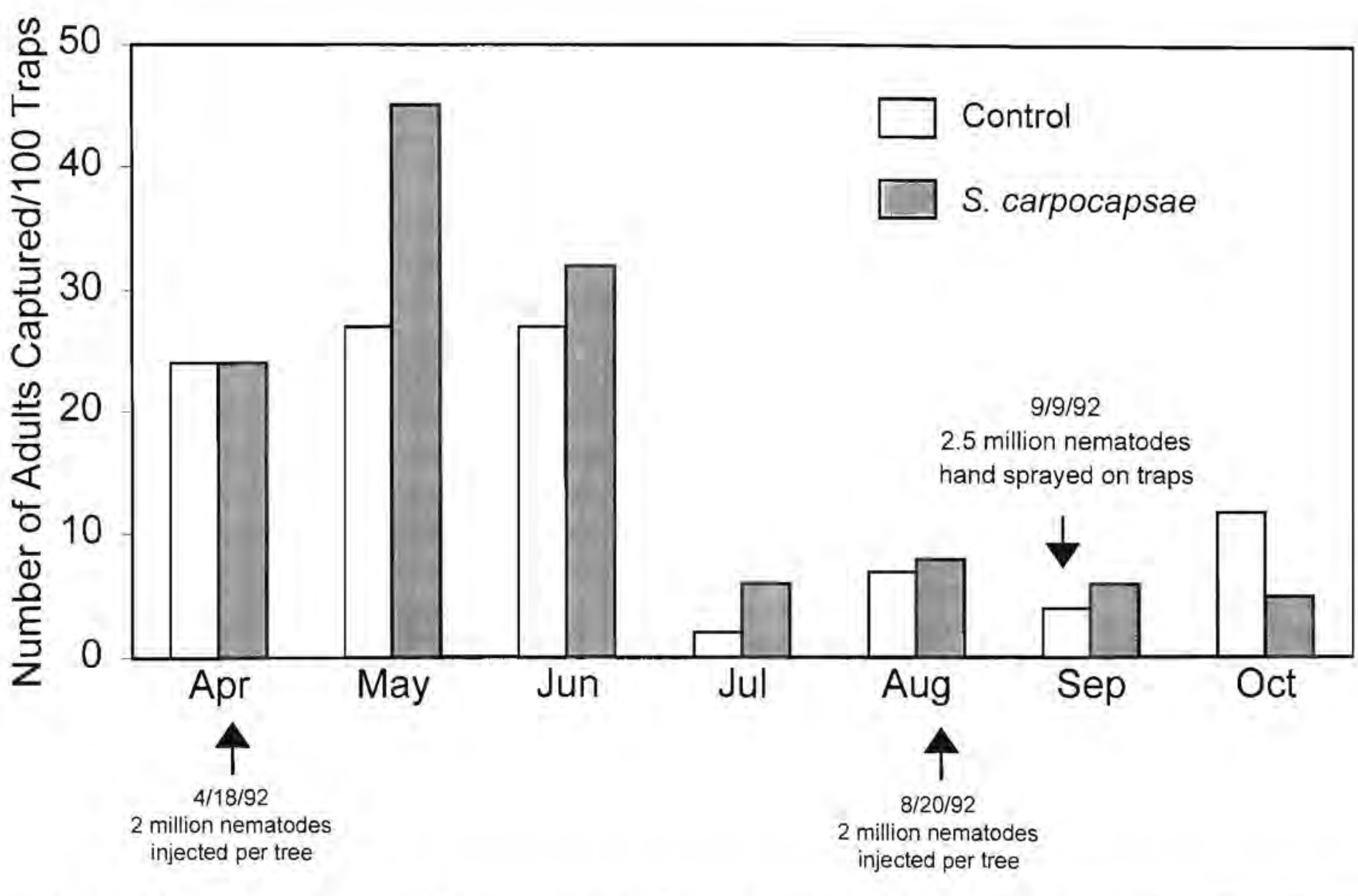

Figure 1. The effect of multiple applications of *Steinernema carpocapsae* on the monthly emergence of adult *Diaprepes abbreviatus* from April to October 1992, Vero Beach, Florida.

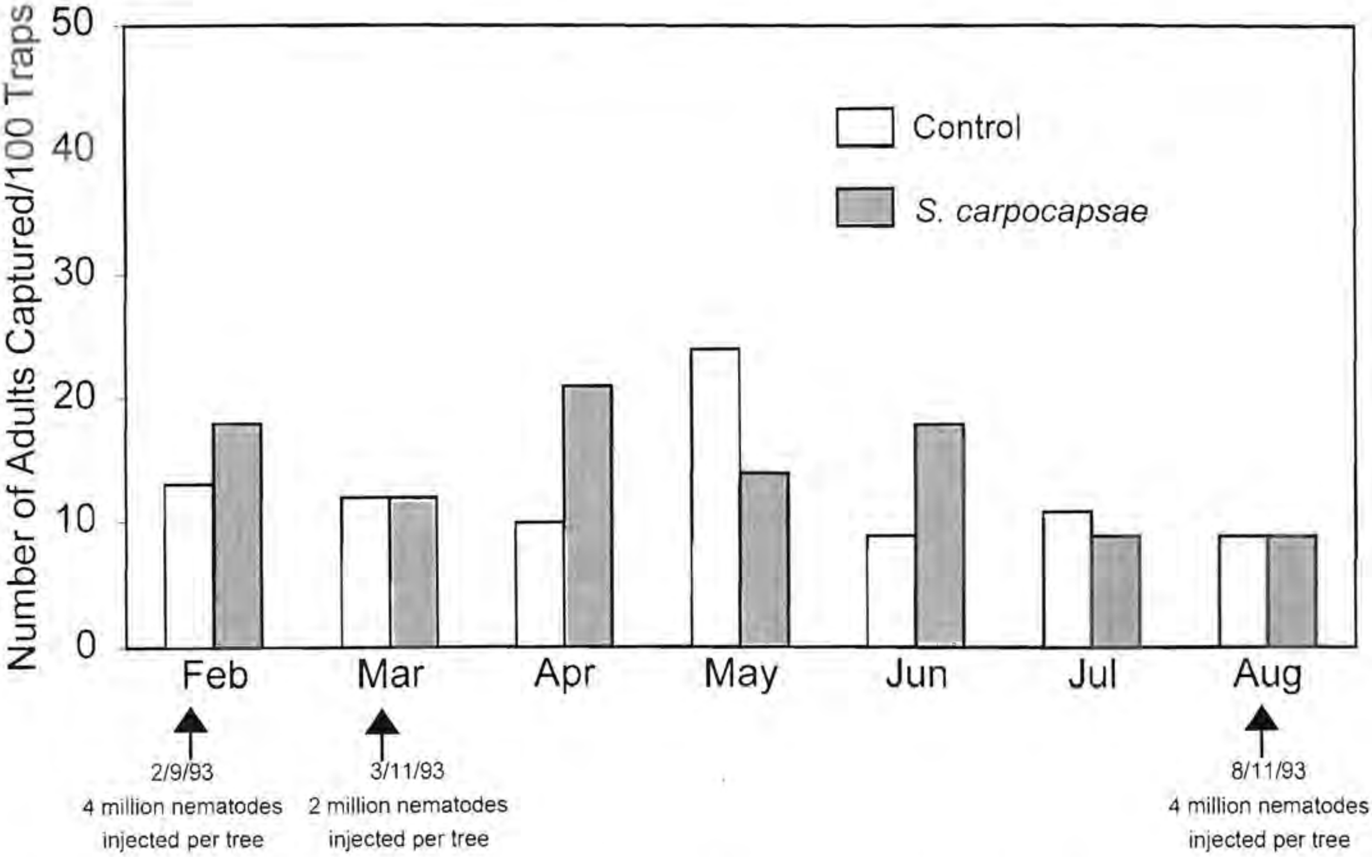

Figure 2. The effect of multiple applications of *Steinernema carpocapsae* on the monthly emergence of adult *Diaprepes abbreviatus* from February to August 1993, Vero Beach, Florida.

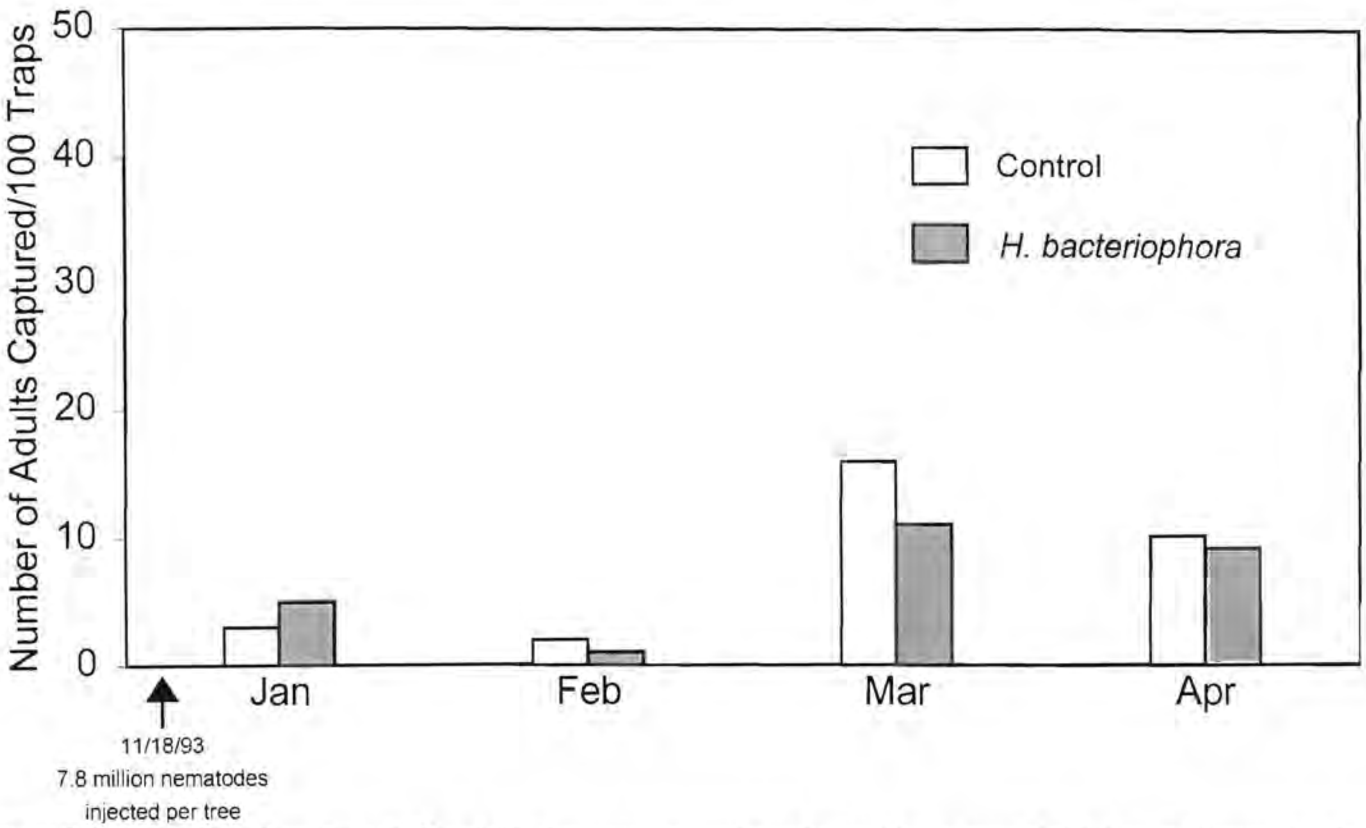

Figure 3. The effect of a single application of *Heterorhabditis bacteriophora* nematodes on the monthly emergence of adult *Diaprepes abbreviatus* from January to April 1994, Vero Beach, Florida.

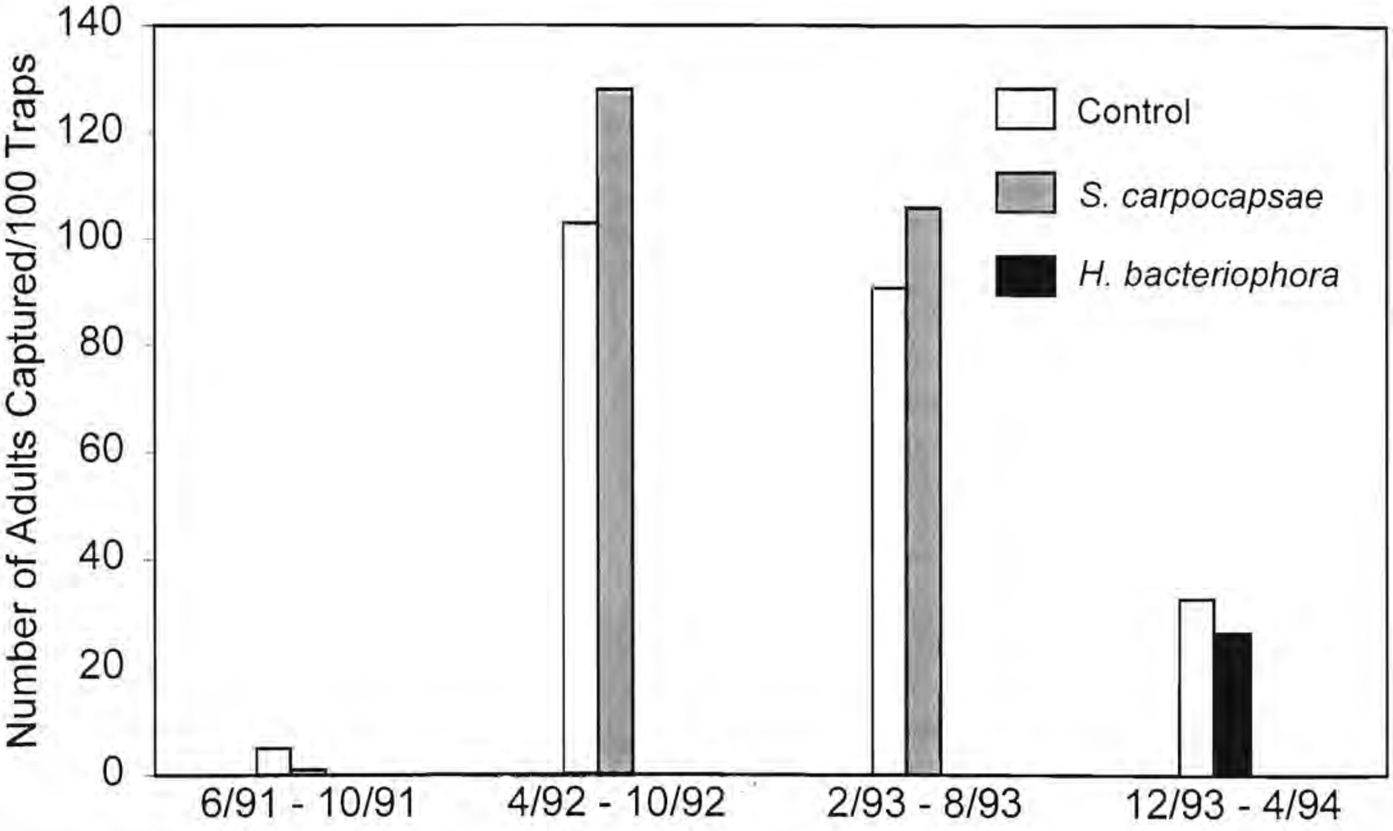

Figure 4. Yearly summary of the effects of multiple applications of entomopathogenic nematodes on the emergence of adult *Diaprepes abbreviatus* for 1991 to 1994, Vero Beach, Florida.

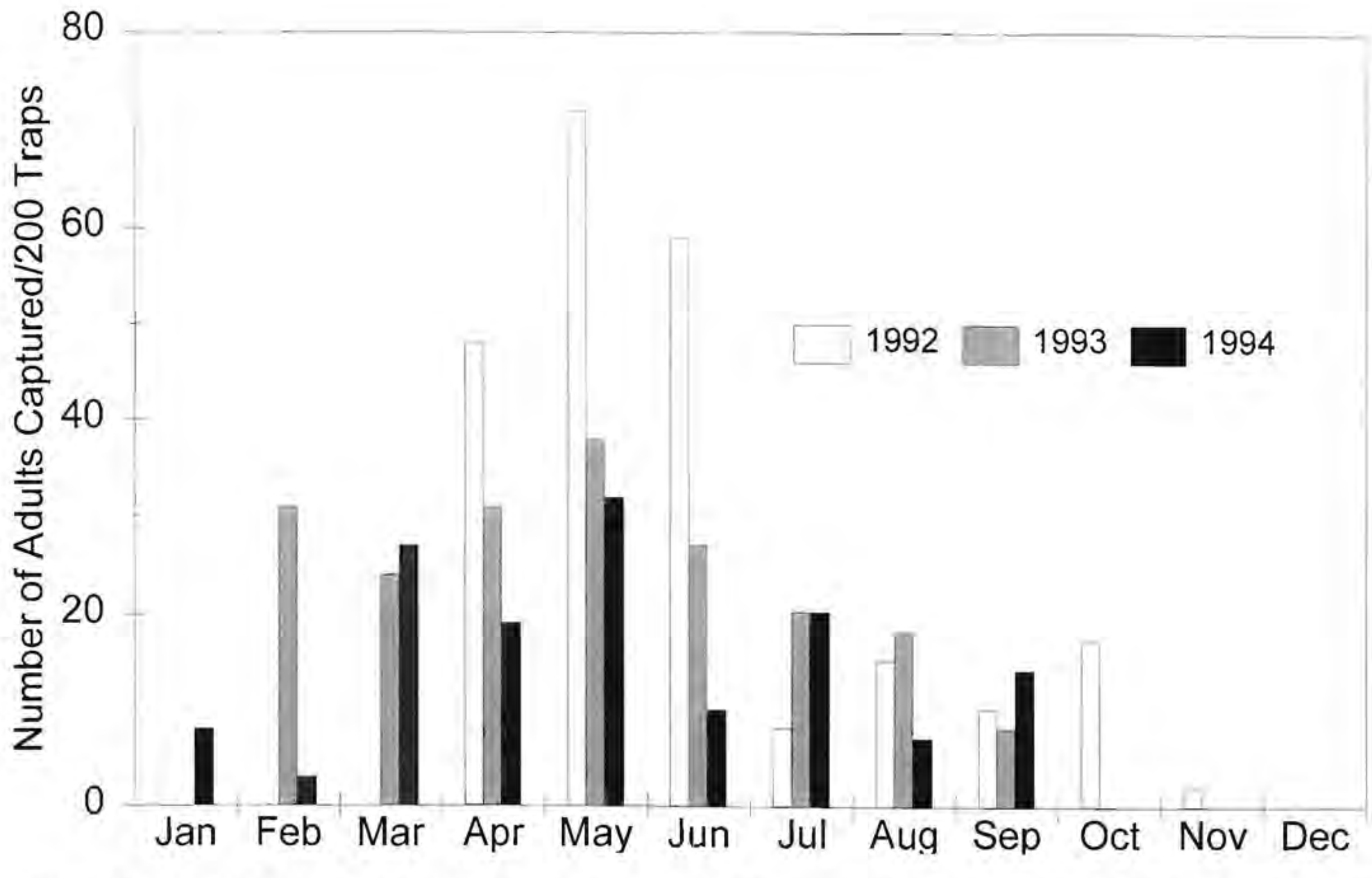

Figure 5. A three-year comparison of the monthly emergence of adult *Diaprepes abbreviatus* collected from all 200 traps from 1993 to 1994, Vero Beach, Florida.

Table 2. Yearly variations in populations of captured adult *Diaprepes abbreviatus* based on twelve weekly (15 April - 1 July) collections from 100 emergence traps located in each area at Vero Beach, Florida.

	Number of Adults Captured			% Decrease in Population by Year		
Date	Control	Treated	Total	Control	Treated	Total
1991[z]	5	1	6	-	-	-
1992	80	107	187	-	-	-
1993	48	55	103	40	49	45
1994	27	27	54	44	49	48

[z]1991 collections were from 19 June to 30 October (20 weeks).

Table 3. Relative levels of *Heterorhabditis bacteriophora* in test site soil as determined by *Galleria* bioassay.

	% Mortality of *Galleria*	
Date Sampled	Control	Treated
4 May 1994	69 a[z]	71 a
29 Aug 1994	42 a	29 a

[z]Mean separation in rows by Student's t-Test at $P = 0.05$

cidence of mortality for teneral adults from both the control and treated areas. Upon examination under a stereomicroscope all the *D. abbreviatus* corpses contained unidentified nematodes.

Discussion

Using the methods described, we could not demonstrate any significant suppression in weevil emergence with 7 successive applications of *S. carpocapsae* or 1 one application of *H. bacteriophora* nematodes. The reasons for most test failures accordingto Georgis (1991) are the use of unsuitable nematode strains or environmental conditions. The possibilities for the failure of *S. carpocapsae* in this trial could be its reported tendency to remain near its point of application and its low host finding ability (Georgis, 1991). In view of the successive failures to suppress weevil emergence presented here, it is unlikely that this species holds any great degree of promise for the control of *D. abbreviatus* in citrus.

Since several studies have demonstrated suppression of adult *D. abbreviatus* emergence with *H. bacteriophora* nematodes, (Schroeder, 1990, 1992, Downing, 1991, McCoy, personal communication) a possible explanation for their failure in this trial is thought to be the presence of a more effective endemic pathogen. Our results indicated a high level of *H. bacteriophora* present in the soil of the study area. Beavers (1983) observed that 45% of 55 groves surveyed in 1981 had detectable populations of entomopathogenic nematodes capable of producing 70% *Galleria* larvae mortality. This is the same degree of *Galleria* larvae mortality attributable to *H. bacteriophora* observed in this trial. The presence of nematode infested teneral adults was found during the examination of the root systems in the control area. Most convincing, is the back to back annual emergence reductions of 40% and 44% in the control area. Since an endemic population of *H. bacteriophora* has been shown to exist at the study site, it could be responsible for the reduction in the population of *D. abbreviatus* larvae in the soil to such an extent that it is masking any possible effect of the applied entomopathogenic nematodes. While all the data in this study indicates that *H. bacteriophora* is the most likely endemic entomopathogen reducing the population of *D. abbreviatus* as indicated by our annual trapping results, further examination of the soil at the study location is required to determine conclusively what organism is responsible for the reductions in *D. abbreviatus* emergence reported here.

Perhaps the most important finding in this trial is the presence of an endemic nematode population of *H. bacteriophora*, that is a known entomopathogen of *D. abbreviatus*. Efforts are underway to determine what citrus management practices could enhance or diminish the *H. bacteriophora* population in the soil. The concept of management practices compatible for citrus crop production and endemic biological control agents represents a possible component of a control strategy for this devastating insect. This new concept warrants investigation.

LITERATURE CITED

Beavers, J. B., D. T. Kaplan, and C. W. McCoy. 1983. Natural enemies of *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 rhabdiud nematodes in soil. Nematologica 21: 109.

Bullock, R. C., C. W. McCoy, and J. Fojtik. 1988. Foliar sprays to control adults of the citrus root weevil complex in Florida. Proc. Fla. State Hort. Soc. 101: 1-5.

Downing, A. S., C. G. Erickson, and M. J. Kraus. 1991. Field evaluation of Entomopathogenic Nematodes against citrus root weevils (Coleoptera: Curculionidae) in Florida citrus. Fla. Entomol. 74(4): 584-586.

Futch, S. and C. W. McCoy. 1994. Citrus Root Weevils. Fla. Coop. Ext. Ser., Inst. Food Agr. Sci., Univ. of Fla., Gainesville. 1-7.

Georgis, R., and R. Gaugler. 1991. Predictability in biological control using Entomopathogenic Nematodes. J. Econ. Entomol. 84(3): 713-720.

Knapp, J. L. 1994. Effective and safe use of agricultural chemicals in citrus production. Florida Citrus Pest Management Guide: 1994. Fla. Coop. Ext. Serv. Cir. SP-43.

McCoy, C. W. 1994. Past and Current IPM Strategies to Combat the Spread of *Diaprepes abbreviatus* (L.) in Florida Citrus. Proc. Carib. Food Crops Soc. (in press).

Rovesti, L. and K. V. Deseö. 1990. Compatibility of chemical pesticides with the Entomopathogenic Nematodes, *Steinernema Carpocapsae* Weiser and *S. Feltiae* Filipjev (Nematoda: Steinernematidae). Nematol. 36: 237-245.

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: Curculiidae) adult emergence with soil application of entomopathogenic nematodes (Nematoda: Rhabditida). Fla. Entomol. 73(4): 680-683.

Schroeder, W. J. 1992. Entomopathogenic Nematodes for control of root weevils of citrus. Fla. Entomol. 75(4): 563-567.

Woodruff, R. E. 1964. A Puerto Rican weevil new to the United States (Coleoptera: Curculionidae). Fla. Dept. Agr., Plant Ind., Entomol. Cir. No. 77: 1-4.