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NATURAL ENEMIES OF *DIAPREPES ABBREVIATUS* LARVAE IN FLORIDA[1]

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Abstract. **A survey of the subterranean natural enemies of** *Diaprepes abbreviatus* **(L.) larvae was conducted monthly from June 1979 through December 1980 in 9 citrus groves and 1 ornamental nursery in central Florida. Four entomopathogenic fungi,** *Metarhizium anisopliae, Beauveria bassiana, Paecilomyces lilacinus* **and** *Aspergillus ochraceous,* **and 2 entomogenous nematodes,** *Neoaplectana carpocapsae* **Weiser and** *Heterorhabditis* **sp. Poinar, were found active in the soil and infectious to** *D. abbreviatus* **larvae. Fungus-infected larvae were most prevalent from May through July, while nematode parasitism was most predominant from May through November. A subsequent survey conducted in 55 citrus groves throughout Florida in August 1980 and again in February 1981 showed that 27.0% and 45.0%, respectively, had detectable populations of** *N. carpocapsae* **or** *Heterorhabditis* **sp., or both species.**

Diaprepes abbreviatus (L.), the sugarcane rootstock borer weevil, is a major curculionid pest of citrus and sugarcane in Puerto Rico and the West Indies. A single adult of this weevil was detected on citrus in Orange County, Florida, in 1964. In 1968, a high population was discovered and a quarantine area of ca. 2,500 acres (1,000 ha) was established (15). This weevil threatens the citrus, ornamental, and sugarcane industries in Florida, and its quarantine area has been increased to ca. 79,000 acres (31,000 ha) in Orange, Seminole, Lake, and Broward counties. The adult weevils feed on young, succulent foliage of citrus and other host plants and deposit their eggs in masses between mature leaves, which are held together by an adhesive secretion. The hatchling larvae burrow into the soil, where they remain for 1 to 2 yr (14) and cause serious root injury to the host plant.

Biological control has been investigated as a means to reduce populations of other developmental stages of *D. abbreviatus.* An exotic hymenopterous egg parasite, *Tetrastichus haitiensis* Gahan, has been introduced from Puerto Rico and successfully established in Florida (2). Buren and Whitcomb (5) found first-instar *D. abbreviatus* on the soil surface vulnerable to predation by several species of ants. However, no information was available on the natural enemies of the subterranean larval stage of this pest.

Therefore, we conducted a study from June 1979 through March 1981 to 1) identify the natural enemies present which attack the subterranean larvae of *D. abbreviatus,* 2) determine the relationship of select environmental factors on the natural enemies, and 3) determine the distribution of the predominant soil-inhabiting, natural enemies in selected citrus groves outside of the known *D. abbreviatus*-infested areas of Florida.

Materials and Methods

Two studies were conducted. In the first, within the *D. abbreviatus* regulated area of central Florida, 9 citrus groves and 1 ornamental nursery which have had high weevil populations in the past were selected for the natural enemy survey. Three groves were in Lakeland (thermic, coated typic quartzipsamments) and 6 in Blanton (loamy, siliceous, thermic grossarenic paleudults) soil types, the nursery was planted in Everglades (euic, hyperthermic typic medihemists) mucky, peat soil. All sites had been treated previously with chlorinated hydrocarbon soil insecticides during weevil eradication efforts in the early 1970's, but had no chemical treatment during this study.

Diaprepes abbreviatus larvae (4-6 months old) reared on artificial diet (1) were placed individually in 3- x 5-inch (7.5- x 12.5-cm) wirescreen cages and buried at 6- and 12-inch (15- and 30-cm) depths under the canopy of a single tree at each site on a monthly basis from June 1979 through December 1980. Six cages were buried at each depth (12 cages/tree) at each site. Each cage was filled with soil taken from the depth at which it was buried. The location was marked by an 18-inch (45-cm) wire attached to the cage with a color-coded ribbon attached to the opposite end. After 3 wk, the cages were recovered from the soil and all larvae (alive or dead) were placed individually in 1-oz plastic cups for observation and subsequent diagnosis of pathogens. Soil temperature was recorded in the field at each depth at the time larvae were placed in the soil. Also, pH and gravimetric moisture determinations were made from each soil depth at each site. Rainfall was also recorded at a central location monthly.

Gross analyses of all larvae recovered were made with a dissecting microscope. Larvae containing nematodes were held for emergence, and the nematodes were either 1) preserved in a 3.0% formalin and a 3.0% glycerin solution for later identification, or 2) used to inoculate fresh *D. ab-*

[1]This paper reports the results of research only. Mention of a pesticide does not constitute a recommendation for use by the USDA, nor does it imply registration under FIFRA. Mention of a commercial or proprietary product does not constitute an endorsement by the USDA and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

breviatus larvae to confirm parasitism. Larvae containing fungi were surface sterilized in a 3.0% sodium hypochlorite solution, and then placed on Sabouraud maltose agar with yeast extract at room temperature. When a fungus sporulated on the weevil cadaver, slides of the fungus were prepared for identification of the organism. Pathogenicity was confirmed by exposing fresh *D. abbreviatus* larvae to a high concentration of conidia of the isolated fungi.

In the second test, soil samples from 55 selected citrus groves throughout Florida were taken in the summer (August 1980) and again in the winter (February 1981). Three 2-lb. (0.8 kg) soil samples from each site were taken 6-12 inches deep from under the tree canopies. In the laboratory, the soil samples were transferred to 1-qt (0.9-liter) containers, and 3 *D. abbreviatus* larvae were placed in the soil in each container. The larvae were held in the soil for 3 wk and then recovered and placed in 1-oz cups for observation as previously described. These larvae were monitored primarily for nematode infection, and recovered nematodes were preserved as before.

Results

In the first study, the entomopathogenic fungi *Metarhizium anisopliae* Metch. Sorokin, *Beauveria bassiana* Bals. Vuillemin, *Paecilomyces lilacinus* (Thom) Samson, and *Aspergillus ochraceous* Wilhelm were recovered from *D. abbreviatus* larvae at the initial sampling period from June through August 1979, and appeared again January through December 1980, except for April and September (Fig. 1).

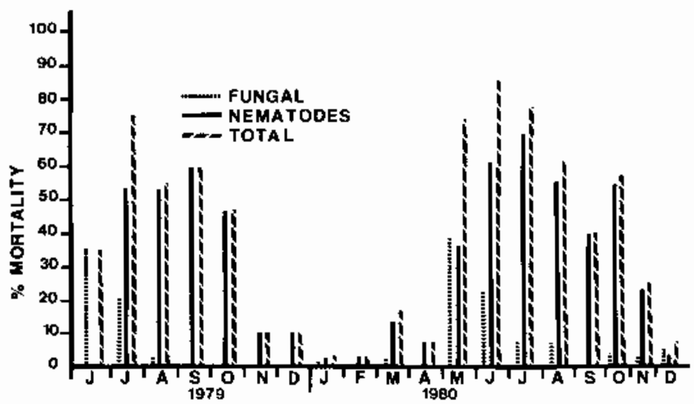

Fig. 1. Monthly recovery of pathogenic fungi and entomogenous nematodes attacking *Diaprepes abbreviatus* larvae in subterranean cages from 10 trees (12 larvae/tree) during 1979-1980. Apopka, Florida.

The peak fungal period of infection was May through July. Maximum fungal infection (38% of *D. abbreviatus* larvae) was observed in May 1980. The entomogenous nematodes *Neoaplectana carpocapsae* Weiser and *Heterorhabditis* sp. Poinar were also recovered from dead *D. abbreviatus* larvae and appeared to be more efficacious than the fungi. Nematodes occurring in the genera *Cephalobus, Rhabditis,* and an unidentified diplogasterid were also recovered but were not found to be pathogenic to *D. abbreviatus* larvae or *Galleria mellonella* (L.) larvae in subsequent laboratory tests. Larval mortality in the field associated with nematode parasitism occurred throughout the year (Fig. 1), but the greatest parasitism occurred from May through November. Maximum larval mortality attributed to nematode parasitism (70.0%) was observed in July 1980.

Rainfall, soil moisture content, soil type, and soil pH (Fig. 2) were not correlated with the seasonal abundance of the pathogenic fungi or parasitic nematodes recovered. The greatest correlation was with soil temperature (r = 0.5). No

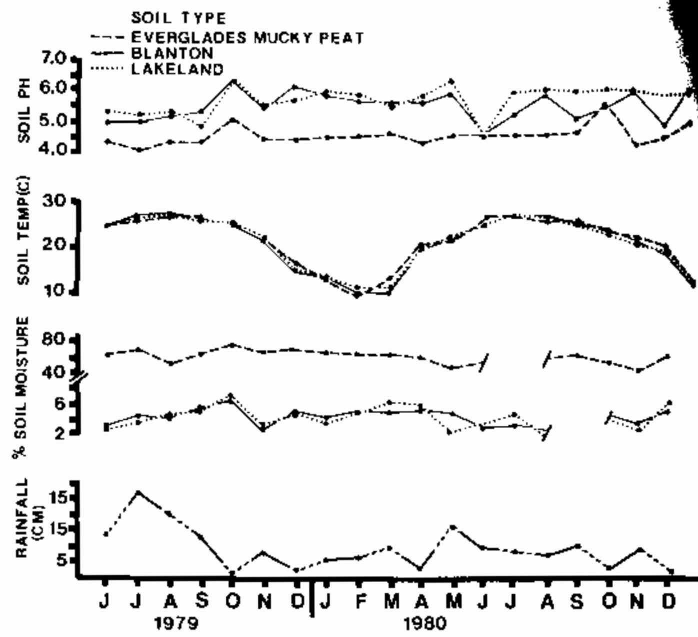

Fig. 2. Monthly variation of some environmental factors in the study of natural enemies of *Diaprepes abbreviatus*, Apopka, Florida.

significant difference in infectivity (P = 0.05) was obtained between the 6- or 12-inch depth for fungi or nematodes.

In the second test, *N. carpocapsae* and *Heterorhabditis* sp. were recovered during both sampling periods. In the summer (August 1980), *N. carpocapsae* was recovered from 9 (16.0%) sites (Fig. 3), and *Heterorhabditis* sp. from 6 (11.0%) sites (Fig. 4). Both species were recovered at 2

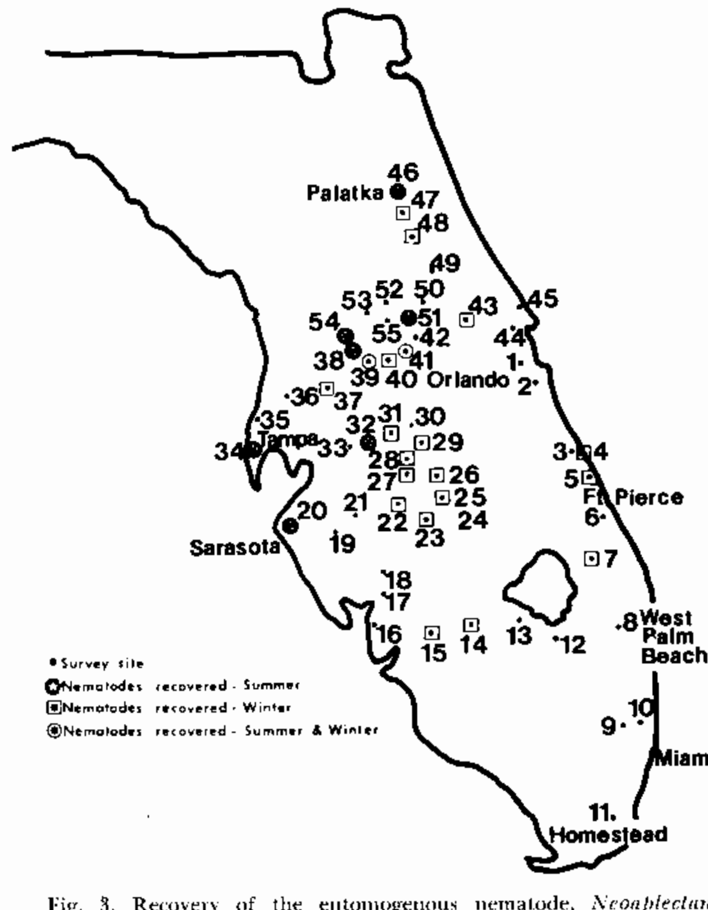

Fig. 3. Recovery of the entomogenous nematode, *Neoaplectana carpocapsae* during a summer (August 1980) and winter (February 1981) from soil samples of 55 citrus groves in Florida.

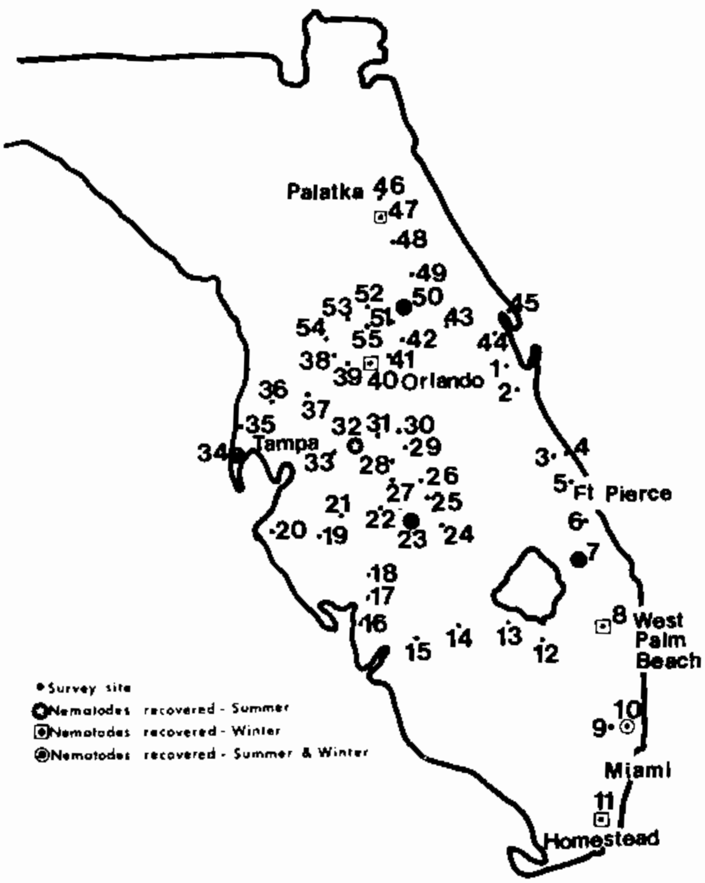

Fig. 4. Recovery of the entomogenous nematode, *Heterorhabditis* sp., during summer (August 1980) and winter (February 1981) from soil samples of 55 citrus groves in Florida.

(4.0%) sites (No. 32 and 34). In the winter (February 1981), *N. carpocapsae* was recovered from 20 (36.0%) sites (Fig. 3) and *Heterorhabditis* sp. from 5 (9.0%) sites (Fig. 4). Both species were again recovered at 2 (4.0%) sites (No. 40 and 42). Overall, during the summer and winter sampling periods, 27.0% and 45.0% of the sites, respectively, had detectable nematode populations. Although mortality due to entomopathogenic fungi was not recorded in this study, the occurrence of *M. anisopliae* and *B. bassiana* was observed on some specimens.

Discussion

The entomopathogenic fungi and entomogenous nematodes show greatest infectivity during the period from April through November, which corresponds to the emergence period of *D. abbreviatus* in Florida (4).

The ability of *N. carpocapsae* and *Heterorhabditis* sp. to parasitize *D. abbreviatus* larvae in the soil may be attributed to their ability to disperse through the soil (12) in response to host karimones (9, 13) or to faint temperature elevations caused by the host (7). Although *M. anisopliae, B. bassiana, P. lilacinus,* and *A. ochraceous* were detected at all 10 sampling sites, only *M. anisopliae* and *B. bassiana* appear to have potential as microbial control agents, since they are able to persist in the soil for long periods.

Reduction of *D. abbreviatus* population levels by *N. carpocapsae* and *Heterorhabditis* sp. would not be surprising since entomogenous nematodes have been reported to reduce field populations of several root weevil species (6, 10). *Diaprepes abbreviatus* adults were host of *N. carpocapsae* in a laboratory host range study (11) and *Neoaplectana carpocapsae* has been reported to control the citrus root weevil *Pachnaeus litus* (Germar) in bagged trees (8). *Beauveria bassiana* and *M. anisopliae* have been associated with *D. abbreviatus* larval and adult mortality (3, 14).

The findings of this study suggest that these entomopathogenic fungi and entomogenous nematodes have contributed to the relatively limited spread of *D. abbreviatus* in Florida since its discovery in 1964. The introduction or augmentation of these organisms into a weevil-infested grove may provide an alternative means of economic control of this pest in citrus groves as well as in ornamental nurseries, with minimal undesirable effects on the environment and public health.

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