THE HOST-PARASITE RELATIONSHIP OF *GRAPHOGNATHUS* SPP. LARVAE AND *NEOAPLECTANA DUTKYI*

by

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A larval population of the whitefringed beetle, *Graphognathus* spp., in a Louisiana grassland field was reduced 38% by *Neoaplectana dutkyi* Jackson applied at 40,000 nematodes/ft^2. In Mississippi an introduced larval population was reduced 50% by the nematode applied at 50,000/ft^2.

Tests were conducted to study some of the environmental factors in the field and to determine how they affected the host and the parasite. There were no significant differences in host survival between the treated and check plots or between watered and nonwatered plots. There were significant differences in the survival of larvae at 3 soil depths, with the survival being greater at the 4 in. than the 2 or 6 in. depth. There was a significant difference in weight after 42 days between larvae from nonwatered and watered plots. There were no significant differences in larval head capsule widths for watered vs. nonwatered

or the 3 soil depths.

Ilex crenata var. hetzi Thunberg potted plants containing feeder and nonfeeder larvae were held for 28 days at 16° or 27°C after treatment with 50,000 nematodes per plant. There were significant differences in larval survival between treatments, temperatures and stages. The higher temperature yielded better control of both feeder and nonfeeder stage larvae by the nematode. The survival of both stages in the checks was lower at the higher temperature. The number of treated feeder stage larvae was significantly lower than the check larvae recovered at 16°C. This was not true for the nonfeeder larvae at this temperature.

Ten whitefringed beetle larvae were injected with nematodes to determine the rate of reproduction of the parasite in this host. The average nematode yield was 6000 per larva, or 162/mg of body weight.

INTRODUCTION

The various species of the genus Graphognathus are individually and collectively called whitefringed beetles. Tissot (1938) classified the whitefringed beetle as: Order Coleoptera; Family Curculionidae; Subfamily Otiorhynchinae; Genus and species Naupactus leucoloma Boheman.

The whitefringed beetle, native to South America, was first found in the continental United States in 1936 devastating crops in northwest Florida and southern Alabama (Watson 1937a, b). Populations of whitefringed beetles now extend from Florida north to Virginia and Missouri and west to eastern Texas (Gross et al. 1972b). In these areas, the use of chlorinated hydrocarbon insecticides has until recently held damage by this insect to a minimum. However, with the restricted usage of these persistent pesticides and the development of resistance to them (Harlan et al. 1972), damage by whitefringed beetle larvae has begun to increase.

Larvae of the whitefringed beetle remain in the soil from 6 months to 3 years (Young et al. 1950). The moisture and temperature conditions vary greatly during this period; thus the potential for use of a nonchemical control such as a biological agent appears promising. Studies were conducted at the Whitefringed Beetle Investigations Laboratory, Gulfport, Miss., from 1966 to 1972 to determine if the nematode Neoaplectana dutkyi Jackson could control larval whitefringed beetles.

LITERATURE REVIEW

HOST

Taxonomy. - The whitefringed beetle was first described by Boheman in 1840 as Naupactus leucoloma. Buchanan (1939) stated that the whitefringed beetle belonged to the Pantomorus - Naupactus complex, which included 240 or more named species, all indigenous to the New World. Of these about 193 were natives of South America and about 45 of Mexico or Central America. In 1939 the majority of the South American species were catalogued in Naupactus, most of the others were in Pantomorus. Buchanan's 1939 publication dealt with the taxonomy of 14 species and varieties then known from the United States; all these were assigned to Pantomorus. Graphognathus was assigned (Buchanan 1939) to subgeneric rank under the genus Pantomorus. Buchanan (1939) identified 2 species of the whitefringed beetle that were discovered in the United States. His identifications included Pantomorus (Graphognathus) leucoloma Boheman and a new species, Pantomorus (Graphognathus) peregrinus Buchanan.

Buchanan (1942) listed 4 new species of whitefringed beetles from the southeastern United States. These were: Pantomorus (Graphognathus) minor Buchanan, Pantomorus (Graphognathus) pilosus Buchanan, Pantomorus (Graphognathus) striatus Buchanan, and Pantomorus (Graphognathus) ambius Buchanan.

After further study, Buchanan (1947) found that the true leucoloma

described by Boheman did not exist in the United States and also raised Graphognathus to generic rank. The 2 species peregrinus and minor were distinguishable enough to remain as species, but the other species (pilosus and dubius) described previously along with 2 additional ones, fecundus and imitator, became races in the leucoloma complex.

Anderson (1938) used a brief key to separate larvae of the white-fringed beetle found in the United States from the larvae of closely related species.

V. H. Owens (personal communication 1973) has prepared a manuscript which I believe will simplify the taxonomy of the whitefringed beetle in the United States, since this paper will redescribe and recognize only 4 species of Graphognathus from the United States, based on characters different from those used by Buchanan.

Biology. - Until the whitefringed beetle was first found in the United States in 1936, very little was known about its biology. The whitefringed beetle Graphognathus spp. is considered to be native to South America, and has been reported from Peru, Chile, Brazil, Argentina and Uruguay (Berry 1939, 1947, Bosq 1934, Buchanan 1939, Bullock 1940, Cortes 1941). Only in recent years has crop damage by whitefringed beetles been reported from South America, where alfalfa, potatoes, lentils, beans, and peppers have been the crops most severely affected, and only in local areas are they considered major pests (Young et al. 1950).

The whitefringed beetle was recorded in Australia in 1933 (Wallace 1940) and in New Zealand on North Island in 1960 (Cumber 1960, Todd 1964) and on South Island in 1965 (Perrott 1966). The whitefringed beetle was first found in the United States severely damaging crops in

Walton and Okaloosa Counties, Florida, in 1935 and 1936 (Watson 1937a). The form originally found in the United States was Graphognathus leucoloma fecundus (Young et al. 1950).

The whitefringed beetle is now distributed from Florida, north to Virginia and Missouri and west to eastern Texas (Gross et al. 1972b).

Watson (1937b) gave one of the first reports on the biology of the whitefringed beetle in the United States. Other descriptions of its biology in the United States were made by Creighton (1939), Roberts (1952), Young and App (1939), and Young et al. (1938).

The larvae and adults of the whitefringed beetle are general feeders upon a wide range of plant species. The combined list of host plants on which larvae or adults have been observed to feed in the field includes 385 species, distributed in 287 genera, 99 families, and 41 orders (Senn 1969). The adults are known to feed on more than 170 species of plants, including most field and garden crops, as well as ornamentals and many weed species (Young et al. 1950). Most of the damage is caused by the larvae which often destroy the root system of plants. Larvae are known to feed on 240 species of plants.

The adult whitefringed beetle is incapable of flight, having the elytra fused together (Young and App 1939). So far as is known, there are no male whitefringed beetles, and all reproduction is parthenogenetic (Tissot 1938). However, the female reproductive system contains a spermatheca and a copulatory pouch.

After emerging from the soil the adults usually crawl onto plants or other objects nearby and remain quiet for a few hours until their body coverings harden. They then begin to search for food. Senn (1969) reported a definite preference by the adults for night feeding. Egg

deposition begins 5 to 25 days after adult emergence. Gross and Bartlett (1972) found that the beetles produced more eggs in the laboratory when held in constant darkness than when they were held in combinations of light and darkness or constant light. The egg-laying period ranges from 30 to 60 days. Adults may live as long as 150 days after emergence, but most of them die within 90 days.

Eggs are deposited in masses of up to 60 or more, but the usual number per mass is from 11 to 14. Adults fed on peanut foliage at Florala, Ala., deposited an average of 1590 eggs, those fed on grasses deposited an average of 3.6 eggs (Young et al. 1950). The freshly laid egg is milky white, but after 4 or 5 days the color changes to a dull light yellow. The individual eggs and masses are covered with a sticky gelatinous substance which hardens after drying and causes the eggs to adhere to one another and to objects such as plant stems, roots, and soil. Eggs deposited in June, July, and August hatch in 11 to 30 days, the average being about 17 days. Those laid in the fall and winter require much longer periods for incubation, more than 100 days being required for those deposited in December. Although larvae complete their embryonic development within the eggs under dry conditions, they require moisture to emerge (Young et al. 1950).

The entire larval stage is spent in the soil. Although the number of larval instars has not been determined, there appears to be either 4 or 5 instars (Harlan unpublished). Most larvae are found in the upper 9 in. of soil, but some have been taken to a depth of 24 in., and a few at even greater depths.

Most larvae pupate from 2 to 6 in. below the soil surface. During the summer months the length of the pupal period is ca. 13 days; in

cooler weather it is longer.

All species of whitefringed beetles discovered in the United States have only 1 generation per year. They usually pass the winter in the larval stage, and some individuals have remained in the egg stage throughout the winter. Most of the larvae that hatch during 1 summer, emerge as adults the following summer, but a few remain in the larval stage as long as 2 years. A few larvae of G. peregrinus required 3 years to reach maturity in the Gulf coastal area of Mississippi (Young et al. 1950).

Other reports on the biology of the whitefringed beetle including laboratory rearing, range of host plants, crop damage and adult attractants have been published by Barnes and Bass (1972), Bartlett et al. (1967), Bass and Barnes (1969a, b), Cherry (1966), Gross and Bartlett (1972), Gross et al. (1972a, b), Harlan et al. (1970, 1971), Jarvis (1968), Senn (1969), and Stone et al. (1971).

Control. - The research on the biological control of this insect includes that of Glaser et al. (1940) who reported on the nematode Neoaplectana glaseri Steiner as a parasite in laboratory tests. Swain (1943) described tests with whitefringed beetle larvae and N. glaseri and also an undescribed species of Neoaplectana which was found in ca. 2% of field collected larvae. Swain (1945) also described the association of nematodes of the genus Diplogaster with whitefringed beetles, but concluded that these nematodes were not important in natural control of this insect. Harlan et al. (1971) reported field tests with N. dutkyi Jackson and an average reduction in whitefringed beetle larval populations of 50% compared with untreated check populations of ca. 34/ft^2.

Young et al. (1950) stated that unfavorable weather and soil

conditions, parasites, predators, and diseases are important factors in keeping whitefringed beetles in check. They also reported that a fungus, Metarrhizium anisopliae (Metchnikoff), was known to attack the soil-inhabiting stages of the whitefringed beetle in the Gulf coastal area, but killed few beetles. Berry (1939) explored throughout the whitefringed beetle infested countries of South America for natural control agents. He found that 2 species of birds feed on the larvae, but probably had little effect in reducing the populations, as they had access to the larvae only during cultivation.

English and Graham (1938) tested several types of treatments on whitefringed beetle larvae in balled and burlapped plants including hot water and various chemical treatments. They concluded that potassium cyanide in aqueous solution showed toxicity to whitefringed beetle larvae, but killed the plants. Livingstone et al. (1940), Livingstone and Swank (1942), McClurkin (1953), Swank (1949), and Swank and Latta (1950) reported the use of methyl bromide fumigation on soil as satisfactory for destruction of whitefringed beetle larvae. Harlan et al. (1972) stated that D-D mixture used at 100 gal/acre killed all whitefringed beetle larvae in the soil. Young (1944) and Keiser and Henderson (1951) stated that the use of DDT formulations provided good control of adult whitefringed beetles and Henderson et al. (1952) reported the use of this insecticide for control of the larvae. Bennett (1967) used dieldrin to protect direct-seeded longleaf pines from larval whitefringed beetles. Bartlett et al. (1968) reported the use of disulfoton as a systemic insecticide provided excellent control of adult whitefringed beetles on peanuts in pots of soil. Press et al. (1970) and Vardell et al. (1973) stated that the use of dichlorvos treatments

prevented the spread of whitefringed beetle adults in harvested wheat. Other workers (Anonymous 1962, 1969, Denmark 1957, and Young et al. 1950) recommended using DDT, chlordane or dieldrin for controlling whitefringed beetles in cropland and residential areas. However, Harlan et al. (1972) reported resistance to dieldrin in larval whitefringed beetles. Woodham and Bartlett (1973) compared the use of GLC to biological tests to determine resistance to dieldrin in larval whitefringed beetles. Bowman et al. (1965) listed minimal concentrations of aldrin, dieldrin, and heptachlor for the control of whitefringed beetle larvae as determined by parallel gas chromatographic and biological assays.

PARASITE

Taxonomy. - The nematode is classified as: Phylum Nematoda; Class Secernentea; Order Rhabdita; Superfamily Rhabditoidea; Family Neoaplectanidae and Genus and species, Neoaplectana dutkyi Jackson (Turco et al. 1971).

The nematode N. dutkyi was first found in 1954 in larvae of the codling moth, Carpocapsa pomonella (L.) (Dutky and Hough 1955). This nematode was examined by Dr. G. Steiner who considered it to be a member of the Steinernematidae very close to N. chresima Steiner (Anonymous 1955). However, the nematode was not described and has been known by the code number DD-136 which was applied to it by Dutky. Jackson (1965) made serological and scant morphological comparisons of N. glaseri Steiner, N. carpocapsae Weiser, and DD-136. In this paper Jackson referred to DD-136 as N. dutkii Welch, but a description was never published by Welch. In 1953, Weiser (1955) found and later described N. carpocapsae from diseased codling moth larvae in Czechoslo-

vakia. Poinar (1967) proposed that DD-136 be considered the DD-136 strain of N. carpocapsae and that the original N. carpocapsae be called the Czechoslovakian strain. He described DD-136 and showed that the mating of N. carpocapsae with DD-136 resulted in infective juvenile progeny. He also found no consistent qualitative morphological characters which could be used to separate the 2 nematodes. These facts could be explained if the specimens that Poinar received from Weiser in Czechoslovakia as N. carpocapsae were in reality part of the original DD-136 culture which Dutky had previously sent to Weiser (Dutky personal communication 1969). Since the original specimens of N. carpocapsae were destroyed, it is impossible to substantiate this occurrence (Turco et al. 1971). However, both of the cultures tested by Poinar were probably DD-136. Turco et al. (1971) described the DD-136 nematode and redescribed the other species of Neoaplectana. He contended that N. dutkyi Jackson was a valid species name.

Biology. - Dutky and Hough (1955) reported the DD-136 nematode in larvae of the codling moth. They also reported that a bacterium was associated with the nematode. Poinar and Thomas (1965) described a new bacterium, Achromobacter nematophilus discovered in the intestinal lumen of the DD-136 nematode. Poinar (1966) reported that studies showed that most infective juveniles of DD-136 contained cells of A. nematophilus Poinar and Thomas in the ventricular portion of their intestinal lumen. When the infective stage penetrated into the body cavity of a suitable host, the bacteria were released through the anus and multiplied rapidly in the host's body, resulting in a fatal septicemia. Poinar and Thomas (1966) found that the infective-stage juveniles of the DD-136 nematode were able to penetrate and kill the insect host

without the presence of A. nematophilus or any other bacterium. However, without accompanying bacteria, the nematode was unable to reproduce.

Dutky (1956) described the life cycle of the DD-136 nematode. The infective stage of the nematode is the ensheathed second-stage larva. The sheath is actually the cuticle of the second-stage larva which is retained and the new cuticle for the third-stage larva is formed under this outer cuticle. The nematode seeks out the host insect or is eaten with the food, enters by way of the mouth parts, exsheaths, penetrates the intestinal wall, after which the associated bacterium is released into the body cavity of the host. This induces the septicemia that kills the host. The entire process from exposure to death at 30°C takes less than 24 hours.

After the death of the host, the invading nematodes maturate and become adults. If both males and females are present (the sex ratio is 1:1), they mate and give rise to young.

The young are born matricidally, since fertile ova produce embryos within the ovaries of the gravid female. The eggs hatch, and the young feed on the tissues of the adult female. They escape after her death as second-stage larvae. Some of these (about 80%) are ensheathed and do not develop further. Others maturate, producing adults that mate and again produce young. Several generations may be completed until the host cadaver is filled with ensheathed larvae. These ensheathed (infective-stage) larvae then emerge from the cadaver in search of a new host. At 25-30°C the nematode life cycle is completed in 8 days.

Poinar and Leutenegger (1968) studied the fine structure of the infective and normal third-stage juveniles of DD-136 and found pronounced differences in the structure of the sensory organs, digestive

tract, hypodermal chords, excretory system, and somatic musculature. They found that the digestive tract of the normal juvenile was functional while that of the infective stage was not, and that the amphids and somatic muscles were more highly developed in the infective stage.

The infective stage of DD-136 can survive for long periods under proper conditions of temperature and moisture without loss of infectivity. The infective stage nematode is also resistant to many chemicals including most of the insecticides and fungicides in common use (Dutky 1969).

The nematode is not resistant to drying and is quickly killed by desiccation. It also requires a moist surface in order to migrate in search of a host.

The DD-136 nematode can be propagated in enormous numbers in the laboratory. Dutky et al. (1964) described a technique for mass propagation using larvae of the greater wax moth, Galleria mellonella (L.) as the host. House et al. (1965) described the use of a dog biscuit medium for successfully propagating the DD-136 nematode.

Dutky et al. (1967a, b) determined some of the sterol requirements of the DD-136 nematode.

Laboratory and field tests. - Niklas (1969) listed more than 120 species of insects as hosts for the DD-136 nematode. The DD-136 nematode has been field and laboratory tested as a biological control agent against several soil insects with varying degrees of success. This nematode infected over 30 species of Canadian insects in laboratory tests (Anonymous 1960). Harlan et al. (1971) reported a reduction in the whitefringed beetle larval population of 50% compared with the untreated check of ca. 34 larvae/ft^2. Creighton et al. (1968) reported

variable control in field tests on coleopterous larvae. Poinar and Himsworth (1967) described the parasitism of the greater wax moth by the DD-136 nematode and Poinar and Thomas (1967) reported the nature of the associated bacterium as an insect pathogen. Reed and Carne (1967) stated that DD-136 readily infected the larvae of the pruinose scarab, Sericesthis geminata Boisduval, and the dark soil scarab, Othnonius batesi Olliff, in the laboratory, but low mortality of S. geminata was obtained in the field. Moore (1962, 1965, 1970) reported successful field tests with the DD-136 nematode on some garden insects and the forest insect Dendroctonus frontalis Zimmerman. Drooz (1960) reported its use on the larch sawfly, Pristiphora erichsonii (Hartig), and Webster and Bronskill (1968) used an evaporation retardant material in testing the nematode on this insect. Nash and Fox (1969) also used evaporation retardants and other solutions with the nematode on the Nantucket pine tip moth, Rhyacionia frustrana (Comstock). Schmiege (1963) also discussed the feasibility of using the DD-136 nematode for control of various forest insects. Chamberlain and Dutky (1958) reported an 85% reduction in larval population in field tests on the tobacco budworm, Heliothis virescens (F.) on tobacco. Cheng and Bucher (1972) indicated that the nematode controlled Hylemya spp. on tobacco as well as did the standard insecticide, diazinon. Jaques (1967) reported mortality of 5 apple insects induced by the DD-136 nematode. Welch and Briand (1961a, b) had some success in controlling the Colorado potato beetle, Leptinotarsa decemlineata (Say), and the cabbage maggot, Hylemya brassicae (Bouche). Lam and Webster (1972) used DD-136 and a preparation of Bacillus thuringiensis var. thuringiensis Berliner for controlling Tipula paludosa Meigen larvae. Welch and Bronskill

(1962) reported parasitism of mosquito larvae by the DD-136 nematode, but also found that some of the nematodes were encapsulated by the larvae after penetrating the gut wall. Reviews on the use of the DD-136 nematode in insect control have been written by Dutky (1967), Nickle (1972), Steinhaus (1964), and Welch (1962, 1963, 1965).

MATERIALS AND METHODS

Infective stage DD-136 nematodes used in tests described herein were produced by Dr. S. R. Dutky, USDA, Agr. Res. Serv., Insect Pathology Pioneering Research Laboratory, Beltsville, Md.; Nutrilite Products, Incorporated, Buena Park, Calif.; or USDA, Agr. Res. Serv., Whitefringed Beetle Investigations Laboratory, Gulfport, Miss. Those produced by Dutky or the Gulfport Laboratory were propagated in larvae of the greater wax moth according to the method of Dutky et al. (1964). Those produced by Nutrilite were reared on dog food biscuits by the method of House et al. (1965).

The whitefringed beetle larvae used in these tests were from several locations where different species of this insect are found. The larvae cannot be identified to species. Therefore, in this section the whitefringed beetles are referred to as Graphognathus spp..

Calco dye tests. - Most infection by DD-136 is through entry of the host's mouth with the food. Therefore, a test was set up to determine if whitefringed beetle larvae ingest soil and might consume the nematode also. Calco N-1700 powdered dye was mixed with benzene to make a 1% dye solution. This solution was then mixed with sandy loam soil at a rate of 1 ml/3 g soil. The soil was then air-dried to remove the benzene, and used to fill 40 plastic pots 1.5 inches in diam. Two field collected whitefringed beetle larvae were then placed in each pot and held at ca. 24°C. The larvae were examined for a 10-day period to determine if there was dye in the alimentary canal. The

first group of larvae were examined after 3 days and daily thereafter.

After concluding the first dye test, a second was set up using the same procedures as the first. Fifty larvae were placed 2/pot in 25 pots of dyed soil. The larvae were examined during the next 7 days beginning 1 day after they were placed in the soil.

DD-136 test on adult whitefringed beetles. - To determine the susceptibility of whitefringed beetle adults to DD-136 nematodes, a feeding test was conducted on peanut foliage. A spray treatment of the nematodes (furnished by Dutky) was applied to peanut leaves with a one-quart hand sprayer. A water suspension of the nematodes was sprayed onto the leaves of 5 freshly cut peanut plant branches. The number of active nematodes applied per branch was calculated to be 165,000.

A treatment was also applied by dipping peanut branches having ca. equal numbers of leaves into a water suspension of the nematodes. This method was calculated to give 2000 active nematodes on each of 5 peanut branches. Three peanut branches were dipped in tap water and used as checks.

After treatment each peanut branch was placed in a cage similar to that described by Gross and Bartlett (1972). These consisted of cylindrical scree cages 12 in. high, over 6 in. earthenware flower pots filled to within 1 in. of the top with clay loam soil. A 2 oz bottle of water was imbedded in the center of the pot and supported the peanut foliage. Twenty field collected whitefringed beetle adults were placed in each cage and held in a screened insectary. The branches of peanut foliage were removed after 5 days and replaced with fresh branches. The test was concluded after 20 days.

DD-136 + Aqua-Gro wetting agent on whitefringed beetle larvae in balled and burlapped, and potted nursery plants. - This test was conducted to determine if more parasitism of whitefringed beetle larvae by the DD-136 nematodes could be attained when a wetting agent was used on the soil. The theory was that one reason the nematode was not infecting a greater number of the whitefringed beetle larvae in the soil was because the nematode was unable to move freely through the soil due to the high surface tension of the water on the soil particles. The nematode requires a free film of moisture on which to move when seeking out a host (Dutky 1956). If a wetting agent was used to lower the surface tension of the water on the soil particles more water would be available for nematode movement and therefore possibly greater infectivity of the host.

Aqua-Gro, a wetting agent which is used on golf courses to make more water available to the grass roots by lowering the surface tension of the water, was tested with the nematode on whitefringed beetle larvae in nursery plants.

Two year old azalea nursery plants for the test were bought from a wholesale nursery near Mobile, Ala. The plants consisted of 21 balled and burlapped, and 21 potted in 1 gal containers. One ounce of Aqua-Gro granular formulation per gal of water was used to make the solution for dipping 8 of the balled and burlapped plants. The plant ball was soaked in this solution for 30 sec. Eight potted plants were treated by placing 0.5 tsp of Aqua-Gro in each pot and watering it into the soil.

One week later 20 field collected whitefringed beetle larvae were placed in each container. The nematode treatment was made 2 days after

introducing the larvae. Five hundred milliliters of nematode solution was used to treat each of 16 balled and burlapped plants, 8 of which had been treated with Aqua-Gro. Five balled and burlapped and 5 potted plants were treated with 500 ml of distilled water and used as checks. The nematodes were in a solution of 0.1% formalin and each 500 ml contained ca. 200,000 active infective stage DD-136 nematodes. These nematodes were also furnished by Dutky.

After treatment the plants were maintained outside for 60 days at which time the soil was washed and the number of larvae remaining was recorded. During this holding period the plants were kept watered.

DD-136 from Nutrilite Products, Inc., on whitefringed beetle larvae in balled and burlapped plants. - In order to test DD-136 nematodes produced by Nutrilite Products, Inc., Lakeview, Calif., the nematodes were used to treat whitefringed beetle larvae in balled and burlapped azaleas. Fifteen 2 year old azalea plants were bought from a nursery near Mobile, Ala. and taken to Gulfport, Miss. where the tests were conducted. Twenty-five field collected whitefringed beetle larvae were placed in each soil ball. The soil moisture content of the plant balls was determined by the gravimetric method (Gardner 1965) on 100 ml of soil from each plant. A soil sample was also taken to determine if insecticides were in the soil by GLC (Woodham and Bartlett 1973). One-hundred milliliters of nematode solution and 50 ml of water (used to rinse the container) was poured on each of 10 plant balls. This produced about 1 million active infective stage nematodes per plant. Five check plants were treated with 50 ml of water. The nematodes were used in the test the same day they were received from the supplier. The nematodes were shipped via air freight in an insulated jug containing

a solution of 0.09% sodium chloride, 1% Gelgard and $1:4x10^5$ Thimerosol. After treatment each soil ball was placed in a plastic bag to keep the soil moisture at a high level. The bag was taped at the top with the above-soil part of the plant out of the bag. The plants were then placed in a room at 27°C, 90% RH, and 8 hr of light per day. The soil moisture of 4 of the plants was determined 2 wk after treatment and that of 4 of the others was determined 4 wk after treatment. Surviving whitefringed beetle larvae were retrieved 4 wk after treatment by washing the soil from the plant balls through 8, 16, and 24 mesh/in. screen and floating the larvae from the screens into a water-filled pan (Harlan et al. 1971).

Whitefringed beetle larvae in pots of rye treated with DD-136 nematodes from Nutrilite Products, Inc. - In an effort to determine if a high concentration of insecticides found in the soil had adversely affected the results in the recovery of larvae in the previous test, another test was conducted with insecticide-free sandy loam soil, which had been tested and found to be free from detectable amounts of insecticide. The soil was used to fill twenty 6 in. diam clay pots, and then were watered and planted to 'Elbon' rye, Secale cereal L.. Fourteen days later, 20 field collected whitefringed beetle larvae were placed in the soil in each pot. Then after 8 days 12 of the pots were treated with 1 million active infective stage DD-136 nematodes contained in 40 ml of solution. These nematodes were also from Nutrilite Products, Inc., and the carrying solution was the same as described for the previous test. Eight of the pots were held as untreated checks. The pots were maintained outside on a wooden bench where they were watered as needed. Thirty days after treatment the soil from the pots was washed and the

larvae retrieved.

DD-136 treatment of whitefringed beetle larvae in potted nursery plants held at different temperatures. - A test was conducted to determine the effects of temperature and host developmental stage on control of whitefringed beetle larvae by DD-136 nematodes. Twenty-four 2 year old Ilex crenata var. hetzi Thunberg plants in 1 gal plastic pots were purchased from a nursery near Mobile, Ala. Whitefringed beetle larvae were collected near Dothan, Ala. and taken to Gulfport, Miss. The larvae were divided into 2 groups according to feeder and nonfeeder stage. The feeder stage was determined by the gut content which was easily observed through the integument and appeared very dark in color. The nonfeeder or prepupal stage was determined by the white appearance of the gut. Six days after the larvae were collected they were placed 10/pot in the soil of the 24 Ilex hetzi plants. Twelve pots were infested with feeder stage and 12 with nonfeeder stage larvae. Then 6 pots containing feeder and 6 containing nonfeeder larvae were placed in a chamber with a temp of 16°C, 80% RH, and 10 hr of light per day. The procedure was then duplicated with the remaining 12 pots at 27°C and the same relative humidity and light conditions. Three days later 3 of the pots from each temp and larval stage were treated with 100 ml of distilled water containing 50,000 active infective stage DD-136 nematodes. Duplicate pots were treated with 100 ml of distilled water without the nematodes and held as checks. After treatment, the pots were returned to their respective environments. The nematodes for this test were produced in the laboratory at Gulfport, Miss. They were used in the test the same day they were harvested from the culture pans. The pots were watered as needed to keep the soil moist during the following

28 days after which the soil was washed as previously described and the larvae were recovered.

Rate of reproduction of the DD-136 nematode in whitefringed beetle larvae. - Before conducting field tests with the DD-136 nematode on whitefringed beetle larvae, it was necessary to know the potential rate of increase of the nematode in this host. Ten field collected larvae were each weighed and placed individually in petri dishes containing a disc of no. 2 moistened filter paper 9 cm in diam. Each larva was then injected with 3 ul of nematode suspension containing 5 nematodes. The injection was performed with an ISCO Model M micro-applicator with a calibrated 0.25 ml syringe and a 30 gauge needle. The larvae were returned to the petri dish after injection and held for 24 hr at 29°C. One day after treatment the dead larvae were removed from the petri dishes and placed on a nematode-trapping container (White 1927). The trap was made by using a 4 cm x 4 cm x 2 cm high watch glass which was wrapped with no. 42 Watman filter paper and placed inverted in a 9 cm diam x 5 cm high dish. A 1:1000 formalin to water solution was added to the dish to a depth ca. 1 cm. The whitefringed beetle larva was placed on the filter paper on the inverted bottom of the watch glass, so when the infective stage nematodes began emerging from the insect larva they could move down the moistened filter paper, and into the formalin solution where they could be harvested and counted. The larvae were held in these dishes at 27°C for ca. 24 days or until the nematodes ceased emerging. Beginning 14 days after injection, the nematodes were collected and counted by determining the numbers in ten 0.1 ml samples from each collection. These numbers were then used to estimate the number of nematodes produced per mg of host tissue.

DD-136 nematodes against whitefringed beetle larvae in small field plots, 1966. - Tests were conducted near Gulfport, Miss., in 1966-67 to study the effects of various treatments of DD-136 nematodes on whitefringed beetle larvae, and the ability of the nematode to become established in the soil. This test was conducted with an introduced population of whitefringed beetle larvae in a field that was planted to 'Elbon' rye on November 17, 1966. The field, Ruston sandy loam soil type, was divided into sixteen 10x10 ft plots, with 10 ft borders between plots. A Latin square design with 4 replicates of each of 4 treatments was used to determine differences in treatments. A 17th plot was also set up for sampling purposes. Newly hatched whitefringed beetle larvae, from eggs collected in the laboratory, were introduced into each plot at the rate of 80/ft^2 during Nov. 11-21. Just prior to introducing the larvae the plots were watered with a garden hose to runoff to insure adequate moisture while the larvae were becoming established. On Dec. 13, the plots were treated with 0; 10,000; 40,000; or 100,000 active infective stage DD-136 nematodes/ft^2, in 2 gal of water per plot with a 2 gal watering can. One-half of the 17th plot was also treated with the highest rate while the other one-half was left untreated. At a depth of 3 in., the soil was 13°C, and the air temp was 5.5°C at the time of treatment. The nematodes for the test were supplied by Dr. S. R. Dutky. The nematodes were personally delivered to Gulfport by Dr. Dutky, who assisted in applying them to the test plots. After treatment, the whitefringed beetle larval populations were determined at monthly intervals (except for Apr.) through May by taking six 4 in. diam soil cores per plot to a depth of 8 in. The cores were placed in plastic bags and taken to the laboratory where they were washed to re-

cover and count the larvae as previously described.

At ca. weekly intervals throughout the test period, the treated and untreated halves of the 17th plot were sampled to study the larval whitefringed beetle and nematode populations. On Dec. 5, four random 4 in. diam soil cores 4 in. deep were taken from this plot since it had not been treated at this time. However, on the dates following treatment, the samples were taken by collecting two 4 in. diam soil cores 8 in. deep from the treated and untreated halves of the plot. The cores were divided into a top one-half (0-4 in.) and a bottom one-half (5-8 in.). The 2 halves from the corresponding depths and treatments were mixed together and enough soil removed to fill one 4 in. diam petri dish, to which was added 5 large feeder-stage whitefringed beetle larvae. The dishes were then held at 27°C for a 2 wk period after which the larval mortality was determined and the dead larvae were examined for DD-136 nematodes. The remainder of the soil not used for the petri dishes was washed through screens and the larvae floated out in a pan of water and their numbers recorded.

On May 12, after most of the test reported here was concluded, the nematode population from 2 replications of each of the 4 treatments was also checked by bioassay with whitefringed beetle larvae. Two 4 in. diam soil cores 6 in. deep were taken from each of 8 plots. These soil cores were washed through a 60 and a 325 mesh/in. sieve. Material from the 325 mesh sieve was washed into 4 centrifuge tubes and centrifuged at 300 g for 4 min. After centrifuging, the water was decanted and a sucrose solution was added to the mud and nematodes in the bottom. This sucrose solution was made by dissolving 1 lb of sugar with enough water to total 1 qt. The tubes were shaken well to mix the mud with

the sugar solution and then were centrifuged for 3 min. The supernatant sugar solution was then poured through a 325 mesh sieve. The nematodes caught on the sieve were gently washed with water to remove all sugar and then washed into a beaker. The material in the beaker was allowed to settle. The supernatant was decanted leaving 3 ml volume which was poured onto a no. 3 Watman filter paper disc in the bottom of a 4 in. petri dish. Five feeder-stage whitefringed beetle larvae were placed in each dish which was then held at 27°C for a 2 wk period after which the larval mortality and per cent nematode infectivity was determined.

DD-136 nematodes applied in fall or spring to control whitefringed beetle larvae. - The test was begun in Sept. 1967 to determine if a nematode treatment was more effective when applied in the fall or spring on an introduced field population of whitefringed beetle larvae. The test was conducted in the same general field area described in the previous test. A different portion of the field was used. The soil in this area was fumigated with methyl bromide at 1 lb/100 ft^2 on Aug. 14. A soil sample was taken Sept. 1 for chemical analysis to determine if there was any bromine residue. Thirty-two 10x10 ft plots were set up with 10 ft borders between plots. They were arranged as 4 plots across and 8 plots deep, and divided into 2 groups of 16 plots each, with 1 group to be used for the fall treatment and the other for the spring treatment. Each set of treatments was arranged in a Latin square design with 4 replicates of each of 4 treatments including a check. The plots were sown with 'Elbon' rye Sept. 1. Newly hatched whitefringed beetle larvae, from eggs collected in the laboratory, were introduced at the rate of 80/ft^2 into each plot to be treated in the fall during Sept. 1 - Oct. 11. Larvae were introduced into the plots to be treated in the

spring, during Oct. 13-20. On Sept. 12, the fall treatments were made with 0; 10,000; 40,000; or 100,000 active infective stage DD-136 nematodes/ft^2 in 2 gal of water per plot with a 2 gal watering can. The air temp at the time of treatment was 27°C. The nematodes for this test were also produced by Dr. S. R. Dutky and were shipped from Beltsville, Md. to Gulfport via air express the day before they were distributed onto the plots. Sampling of the larval population in these plots was begun Nov. 13 by taking six 4 in. diam soil cores 6 in. deep from each plot. Another sample was taken in Dec. However, only 3 soil cores were taken per plot from Jan. through June. The soil cores were taken to the laboratory and washed to recover larvae as previously described. A portion of the samples from each plot was placed in a petri dish to which 4 last-instar wax moth larvae were added to determine nematode infectivity. Wax moth larvae were used because they have been reported to be one of the preferred host of the nematode (Dutky et al. 1964). The dishes were held at 27°C for a 2 wk period after which time larval mortality and nematode infectivity was determined.

The spring treatment plots were treated Mar. 15, 1968 at the same rates used on the fall treated plots. These plots were sampled in Apr., May and June by taking three 4 in. diam by 6 in. deep soil cores from each plot and washing the cores to recover the larvae. The cores were also used to check for nematodes by exposing 4 wax moth larvae to soil in a petri dish.

DD-136 nematodes against a natural population of whitefringed beetle larvae. - In order to test the nematodes in natural field conditions, a test was conducted against a natural population of whitefringed beetle larvae in a 12 acre grassland field on a Stough fine

sandy loam soil type located near Franklinton, La. Twelve 100x100 ft plots separated by 20 ft borders were used for the test. Larval populations were checked before the treatment by counting the number present in 25 samples (1x1x1 ft) from each plot (5 rows 20 ft apart).

Four treatments of 0; 10,000; 20,000; or 40,000 active infective-stage DD-136 nematodes/ft^2 were applied to the 12 plots (3 replicates in a row) on Apr. 24, 1968, with a piston-type pump sprayer mounted on a jeep. The sprayer had 11 cone-type nozzles spaced 1 ft apart which covered a swath 10 ft wide. The nematodes used were supplied by Dr. S. R. Dutky. Nematodes were stored in insulated jugs at 50,000/ml, and 7.1°C, and transported at a concentration of 100,000/ml. They were brought to Gulfport by airplane by Dr. S. R. Dutky (who also assisted in applying them) and transported by automobile to La. The insulated containers were always protected from heat. The nematodes were applied at the rate of 12 gal of water per plot at an operating pressure of 12 - 14 psi. The soil temp was 19°C at the time of application, and 1 in. of rain had fallen 4 hr before the treatments were applied.

The plots were sampled monthly for nematodes by taking twenty-five 2 in. diam soil cores per plot to a depth of 6 in. (taken in 5 rows, 20 ft apart, cores within a row 20 ft apart), avoiding resampling the exact location of previous months. Aliquots of a composite sample of moist soil, composed of the cores from each plot, were portioned out in 3 petri dishes, 5 last-instar wax moth larvae were placed in each dish. The dishes were then held for 2 wk at 29°C and any dead wax moth larvae were examined each wk for DD-136 nematodes. One month post-treatment the plots were sampled for whitefringed beetle larvae by the same method described for the pretreatment samples. In addition, on

March 26, 1969, 11 months after the nematodes were applied, the larval population was again checked by collecting seventy-five 2 in. diam soil cores 6 in. deep from each plot (5 rows of 15 cores each), placing them in plastic bags, and taking them to Gulfport where they were washed and the larvae counted.

DD-136 nematodes against whitefringed beetle larvae in small field plots, 1968. - A test was conducted with an introduced population of whitefringed beetle larvae in a field near Gulfport that had been planted to rye on Sept. 15, 1968. The area which was in the same field described in 2 of the previous tests was arranged as thirty-two 10x10 ft plots set up in a randomized complete block design with 8 replicates of each of 4 treatments. Newly hatched whitefringed beetle larvae, from eggs collected in the laboratory, were introduced into each plot at the rate of 100/ft^2 during Oct. 2 - 6. Just prior to introducing the larvae, the plots were watered with a garden hose to runoff to insure adequate moisture while the larvae were becoming established. On November 15, the plots were treated with 0; 5000; 20,000; or 40,000 active infective-stage DD-136 nematodes/ft^2, in 2 gal of water per plot with a 2 gal watering can. The area had received a 2 in. rain 4 days before application of the nematodes and the moisture level was high. The 2 in. soil temp was 18°C, and the air temp was 22°C at the time of treatment. The soil pH was 5.2. After treatment, the average moisture for the top 10 in. of soil was determined weekly by taking two 2 in. diam cores 10 in. deep from 2 plots and using the gravimetric method to determine percentage of moisture. Rainfall was also recorded from a rain gauge. Two 2 in. cores were taken each month to a depth of 10 in. in each plot to determine nematode infectivity. This soil was put into petri dishes, and 5 wax

moth larvae were held in each dish at 29°C for 1 wk after which the dead larvae were removed and examined for nematodes. On Feb. 25, 1969, about 3 months posttreatment, populations of whitefringed beetle larvae were sampled by taking six 2 in. diam soil cores 10 in. deep in each plot (2 rows of 3 cores each) placing the cores in plastic bags, and taking them to the laboratory where they were washed and the larvae collected.

Effects of environmental factors on the host and the parasite. - In the spring of 1972 effects of environmental factors in the field on whitefringed beetle larvae and the DD-136 nematode were determined. Initially an attempt was made to establish an introduced population with newly hatched whitefringed beetle larvae as described for previous tests. However, the newly hatched larvae which were introduced in Dec. 1971 were present in the plots in very low numbers in Feb. 1972. This was apparently the result of poor condition of the larvae due to adverse storage conditions of the eggs, since larvae used in other tests in the laboratory and field at this time also died. To overcome this problem, field collected larvae were used in the test.

Larvae were collected Mar. 15, 1972 from a field near Dothan, Ala. and taken to Gulfport. One wk later the larvae were divided into groups of 21 and placed in petri dishes. Each group of larvae was weighed and the average weight per larva calculated. After weighing, the larvae were used to infest 6 plots 5x5 ft with 5 ft borders between the plots. These plots were located near Gulfport in the same area described for some of the previous tests. Each plot was divided into 7 rows spaced 8 in. apart. The 21 larvae in each petri dish were used to infest a row. The larvae were placed 2 in. apart alternately at 2, 4 and 6 in.

deep in each row. A total of 147 larvae was placed in each plot. On Apr. 12, the plots were treated with 0; 50,000; and 100,000 active infective-stage DD-136 nematodes/ft^2 of soil surface with 2 replications per treatment. The nematodes were reared in the Gulfport laboratory. They were applied in the field the same day they were harvested. The plots were watered to runoff and the nematodes were applied in 1 gal of water with a 2 gal watering can. One gal of water was also applied to the check plots. After watering, the plots avg 22% soil moisture. The air temp was 30°C. Small rye plants were growing on the plots.

One of the plots receiving each treatment was watered daily on wk days throughout the test. The higher soil moisture level was maintained to determine if this would increase the infectivity of the nematode on the beetle larvae. Beginning the day the nematodes were applied, the soil moisture, soil temp and rainfall were recorded from the plots until the test was terminated. The temp was recorded from one watered plot throughout the test at the levels of 2, 4 and 6 in. deep in the soil. The last 2 wk of the test, these same levels were also recorded from a nonwatered plot. A YSI Model 47 automatic scanning telethermometer with Type 401 probes was used in conjunction with a YSI Model 80 strip chart recorder. The temp was monitored at each depth at hourly intervals. The Trapezoidal Rule (Fisher and Ziebur 1965) was used on this data to determine the area under the curve for the temp data for each day. The formula for this rule is: $A_T=h[\frac{1}{2}(Y_o+Y_n)+Y_1+Y_2+\ldots+Y_{n-1}]$. The soil moisture was determined from each plot in the pm Monday through Friday of each wk. The moisture was measured with a Troxler Model 217 surface moisture gauge. This gauge employs a neutron source and was

used with a Troxler Model 1651 scaler-ratemeter. The gauge yields a soil moisture determination based on the top 8 - 12 in. of soil, but biased toward the more moist zone. After determining the moisture content for each plot, 3 of the plots were watered to runoff. A small U. S. Forest Service size rain gauge was used to collect rainfall data.

Two wk after the plots were treated with nematodes, 1 row in each plot was dug with a shovel and the whitefringed beetle larvae remaining in that row were recovered. Later, at 2 wk intervals, the larvae were recovered from 2 rows in each plot. The depth of the larva in the soil was noted and the larvae were returned to the laboratory where on some dates they were weighed and/or the head capsule width was measured. On May 5 and June 1, two 2 in. diam soil cores were taken from each plot at depths of: 0-2 in.; 2-4 in and 4-6 in. The 2 cores from each plot at each depth were used to fill 3 petri dishes into which were placed 5 wax moth larvae each. These were held at 27°C for 2 wk before they were examined for dead wax moth larvae containing DD-136 nematodes.

RESULTS

Calco dye tests. - The results of this test showed that whitefringed beetle larvae definitely ingest soil. Only 1 day or less was required for the dye to be found in the alimentary canal. This indicates that the whitefringed beetle larvae probably ingest enough soil in 1 or 2 days to consume challenging concentrations of a pathogenic organism which might be in the soil.

DD-136 test on adult whitefringed beetles. - The results of this test are shown in Table 1. The nematode treatment did not appear to have any effect on the whitefringed beetle mortality when compared to the untreated checks. Dead adults examined from the treated cages showed no evidence of nematode activity. These results differ from those of Turco et al. (1970), who reported parasitism of adults of the rice water weevil, Lissorhoptrus oryzophilus Kuschel, and the spotted cucumber beetle, Diabrotica undecimpunctata howardi Barber, by Neoaplectana glaseri.

DD-136 + Aqua-Gro wetting agent on whitefringed beetle larvae in balled and burlapped, and potted nursery plants. - Thirty days after treatment with 200,000 active infective stage DD-136 nematodes, the soil from 1 balled and burlapped and 1 potted plant was washed to recover the whitefringed beetle larvae; the remaining plants were washed after 60 days. The recovery of live larvae was very low from the balled and burlapped, and potted plants (Table 2). There were no significant differences between treatments and checks. There was, however, a much

Table 1. - Mortality of adult whitefringed beetles 12 days after feeding on peanut foliage treated with DD-136 nematodes.

	Spray		Dip		Check	
Replicate	No. alive	No. exposed	No. alive	No. exposed	No. alive	No. exposed
1	13	20	17	20	5	20
2	13	20	4	20	15	20
3	13	20	0	20	4	20
4	14	20	9	20	-	-
5	7	20	13	20	-	-
Total	60	100	43	100	24	60
% alive	60		43		40	

Table 2. - Whitefringed beetle stages recovered 60 days after treatment with 200,000 DD-136 nematodes per balled and burlapped or potted plant.

Treatment	No. live larvae	No. dead larvae	No. live pupae	No. dead pupae	No. live adults	No. dead adults	% recovered alive
			Balled and burlapped plants				
Aqua-Gro + DD-136	28	8(3)[a]	2	2	2	0	20[b,c]
DD-136	9	5(2)	9	7(5)	5	2(2)	14
Check	17	2	3	3	0	0	20
			Potted plants				
Aqua-Gro + DD-136	4	4					3
DD-136	13	3(3)					8
Check	6	4					6

a Numbers in parenthesis designate no. dead which contained DD-136 nematodes.

b A total of 160 larvae each was introduced into the plants used in the 2 treatments and 100 were used in the checks.

c Recovery from potted plants significantly (0.01) lower than balled and burlapped plants.

lower recovery from the potted plants than from the balled and burlapped plants. This recovery was different at the 0.01 level of significance by analysis of variance.

DD-136 from Nutrilite Products, Inc. on whitefringed beetle larvae in balled and burlapped plants. - Soil moisture of the test plants avg 33.5% before treatment and 37 and 26.6% at 2 and 4 wk, respectively, after treatment. A soil analysis by GLC yielded the results shown in Table 3. Surviving whitefringed beetle larvae were recovered from the soil balls 4 wk after treatment. The results (Table 4) indicate an avg of 7.2 and 24.8% recovery of larvae from the treated and check plants respectively.

Whitefringed beetle larvae in pots of rye treated with DD-136 nematodes from Nutrilite Products, Inc. - Thirty days after treatment with 1 million active infective stage nematodes per 6 in. pot, the soil was washed and the remaining whitefringed beetle larvae were retrieved. The results (Table 5) indicate a 27 and 38% live recovery rate from the treated and check pots respectively.

DD-136 treatment of whitefringed beetle larvae in potted nursery plants held at different temperatures. - The Ilex hetzi plants containing feeder or nonfeeder larvae were held 28 days at 16 or 27°C after treatment with 50,000 DD-136 nematodes/plant. They were then washed and the larvae were recovered. As shown in Table 6, there were significant differences at the 0.01 level by analysis of variance between treatments, temp and stages. Only 1 feeder stage larva was recovered alive of the 30 treated with nematodes and held at 27°C. However, 9 feeder stage larvae were recovered alive in the checks for this temp. Seven treated feeder stage larvae were recovered alive at 16°C compared with

Table 3. - Insecticides found by GLC of soil from plants used in test of DD-136 nematodes from Nutrilite Products, Inc. on whitefringed beetle larvae.

Insecticide	PPM in soil
p,p' - DDT	3.84
o,p' - DDT	1.24
p,p' - DDE	2.62
p,p' - TDE	0.92
Chlordane	17.49
Heptachlor epoxide	0.85

Table 4. - Whitefringed beetle larvae recovered from balled and burlapped plants 4 weeks after treatment with 1 million DD-136 nematodes from Nutrilite Products, Inc.

Treatment	No. larvae recovered	
	Alive	Dead
Treated	18	92
% of 250	7.2	
Check	31	8
% of 125	24.8	

Table 5. - Whitefringed beetle larvae recovered from pots of rye 30 days after treatment with 1 million DD-136 nematodes from Nutrilite Products, Inc.

Treatment	No. larvae recovered	
	Alive	Dead
Treated	65	39
% of 240	27	
Check	61	16
% of 160	38	

Table 6. - Whitefringed beetle larvae recovered from Ilex crenata var. hetzi plants 28 days after treatment with 50,000 active infective stage DD-136 nematodes per plant[a].

Treatment	Temp	No. larvae recovered			
		Stage			
		Feeder		Nonfeeder	
		Alive	Dead	Alive	Dead
Treated	16°C	7[b]	23	20[b]	10
Check	16°C	17	13	19	11
Treated	27°C	1	29	7	23
Check	27°C	9	21	17	13

a There were significant differences at the 0.01 level by analysis of variance between treatments, temperatures, and stages.

b Total for 3 pots of 10 larvae each.

17 in the checks. From the nonfeeder stage larvae 7 and 17 were found alive from the treated and checks respectively at 27°C. From the 16°C nonfeeder stage larvae, 20 and 19 were recovered alive in the treated and checks respectively.

These results indicate that the higher temperature is better for control of both stages of the whitefringed beetle larvae by the DD-136 nematode. However, the survival of both stages in the checks was lower at the higher temperature. The optimum temperature for both stages of the host appears to be below 27°C. The number of treated feeder stage larvae was significantly lower than the check larvae recovered at 16°C. However, this was not true for the nonfeeder larvae at this temperature. This is probably due to the nonfeeder larvae being very inactive at the lower temperature and, therefore, no nematodes were ingested to kill the larvae.

Rate of reproduction of DD-136 nematodes in whitefringed beetle larvae. - Ten field collected larvae were injected with nematodes to insure an infection in the host. As the infective stage nematodes emerged from the host they were collected and counted. The average nematode yield was 6000/larva, or 162/mg of body weight. This compares with an average yield of 1370 DD-136 nematodes/mg of wax moth larva reported by Dutky et al. (1964).

DD-136 nematodes against whitefringed beetle larvae in small field plots. - The whitefringed beetle larval populations were determined after treatment at monthly intervals (except for Apr.) through May by taking six 4 in. diam soil cores per plot to a depth of 8 in. The results of these samples are shown in Table 7. A statistical analysis of the data collected May 10 showed no significant differences. The highest

Table 7. - Average numbers of whitefringed beetle larvae/ft^2 in small plots treated with DD-136 nematodes Dec. 13, 1966.

Treatment nematodes/ft^2	Avg no. larvae/ft^2[a] Date sampled (1967)			
	Jan. 18	Feb. 15	Mar. 22	May 10[b]
10,000	11.0	7.7	29.6	16.2
40,000	42.5	20.1	25.3	8.2
100,000	7.7	13.9	12.9	6.7
Check	15.7	19.6	26.8	18.7

a Avg of 4 plots determined from 6 - 4 in. diam 8 in. deep soil cores from each plot.

b Statistical analysis performed on data collected May 10.

treatment of 100,000 active infective stage DD-136 nematodes/ft^2 evidently had a reducing effect on the whitefringed beetle larval population when compared to the untreated check plots. The lower treatment of 40,000 nematodes/ft^2 also appeared to be effective.

Plot 17 was used to monitor the larval whitefringed beetle and DD-136 nematode population throughout the test period. This was also accomplished by removing soil cores. These results are presented in Table 8. The whitefringed beetle larvae were seldom found below the 4 in. soil depth in the treated or untreated halves of this plot. The results from these samples also indicated a lower average larval population in the treated portion of the plot than in the untreated portion. The larger number of samples from which no larvae were recovered can also be seen. This method also showed that the nematode was present and infective on whitefringed beetle larvae throughout the test period in plot 17.

On May 12, after most of the test was concluded, the nematode population from 2 replicates of each of the 4 treatments was checked by the centrifuge method. These results are presented in Table 9. It is evident from these results that the DD-136 nematode was still present in the plots and infective on whitefringed beetle larvae.

DD-136 nematodes applied in fall or spring to control whitefringed beetle larvae. - Tests were begun in Sept. 1967 to compare a fall and a spring treatment of the nematodes on an introduced population of whitefringed beetle larvae. The results of this test are presented in Table 10. Neither the fall nor spring nematode treatments caused a significant reduction in the whitefringed beetle larval population in the plots. The nematode was not recovered from the fall treatments by

Table 8. - Soil cores taken from plot no. 17 which was used to monitor the nematode and larval whitefringed beetle population. 1966.

Date cores taken	Depth of cores taken (in.)	No. larvae/ft^2		Nematode infectivity
		Check	Treated	
12-5	4[a]	86	-	
12-22	4	0	6	+
12-30	8	92	23	+
1-5	8	63	23	+
1-12	8	12	17	+
2-2	8	12	6	-
2-9	8	17	0	+
2-27	8	69	0	
3-8	8	6	98	+
3-28	8	12	0	-
4-3	8	12	12	
4-11	8	12	0	-
4-17	6	12	12	-
4-24	6	63	0	+
5-1	6	0	0	+
$\bar{X}$		31	14	

a Two 4 in. diam cores were taken from the treated ½ and the check ½ of the plot except on 12-5 when 4 cores were taken from the check ½ and the other ½ was not sampled.

Table 9. - Mortality of whitefringed beetle larvae exposed to nematodes extracted from field plot soil samples May 12, 1967.

Plot treatment[a]	No. larvae[b] dead	No. larvae infected with DD-136
10,000	4	4
10,000	4	2
40,000	3	3
40,000	3	3
100,000	5	5
100,000	3	1
Check	1	-
Check	0	-

a Infective DD-136 nematodes/ft^2

b Counts and numbers infected determined on 5-24-67.

Table 10. - Whitefringed beetle larvae recovered from plots treated with DD-136 nematodes. 1967-68.

Treatment (nematodes/ft^2)	Date sampled No. larvae/ft^2						
	11-13	12-12	1-17	2-12	4-16	5-16	6-21
			Fall treatment				
10,000	46.1	95.0	83.6	80.6	40.3	7.7	0
40,000	63.4	119.0	91.2	35.5	53.7	9.6	0
100,000	75.8	50.4	83.6	65.3	50.9	8.7	.9
Check	31.2	53.3	70.0	49.0	37.4	8.6	1.9
			Spring treatment				
10,000					60.5	26.9	4.8
40,000					49.9	53.7	4.8
100,000					72.9	24.0	3.8
Check					104.6	25.0	8.6

a Avg no. larvae/ft^2 determined by taking three 4 in. diam soil cores 6 in. deep from each of 4 plots per treatment except on 11-13 when 6 cores/plot were taken.

the wax moth larva bioassay at any time after the treatment was applied. However, this same technique showed that the nematode was present in the spring treatment plots for at least 90 days after the treatments were applied. One of the factors which may have contributed to our inability to find the nematodes after the fall treatment was the presence of 20 ppm Br in the soil on Sept. 1. This fact was not known at the time the nematodes were applied, since the analysis was not completed until October. Even though the nematode is not effected by many pesticides (Dutky 1969) low dosages of Br are lethal (Dutky personal communication).

DD-136 nematodes against a natural population of whitefringed beetle larvae. - A test was conducted against a natural population of whitefringed beetle larvae in a 12 acre field near Franklinton, La. The pretreatment populations avg 9.3, 7.3, 8.8 and 7.8 whitefringed beetle larvae/ft^2 in the plots that were treated with the 40,000; 20,000; 10,000 nematodes/ft^2 and for the check, respectively. One month posttreatment, there were no apparent differences in the populations of beetle larvae among plots. However, soil moisture had been very low, which may have reduced the infectivity of the nematodes. When the larval populations were checked again at 11 months posttreatment, the results were those shown in Table 11. Plots treated with the greatest number of nematodes had 38% fewer larvae than the check plots. However, the differences between treatments and checks were not statistically significant. The monthly sampling for nematodes showed that infective nematodes were present in the treated plots the last month of the test.

DD-136 nematodes against whitefringed beetle larvae in small field plots, 1968. - After introducing newly hatched whitefringed beetle

Table 11. - Effect of DD-136 nematodes upon a natural population of whitefringed beetle larvae 11 months after nematodes were introduced by surface spraying.

Replicates	No. larvae/ft^2			
	Treatment[a]			
	0 (Check)	10,000	20,000	40,000
1	25.2	28.2	16.0	19.6
2	35.6	36.2	21.5	18.4
3	29.4	9.8	20.9	17.8
Total	90.2	74.2	58.4	55.8
% of check	100	82	65	62

a Number of DD-136 nematodes introduced/ft^2.

larvae into the plots, rainfall records were maintained as shown in Table 12. A total of 20.58 in. of rain was recorded during the test period. The effect of this rainfall on the soil moisture in the top 10 in. of soil is shown in Fig. 1. The avg moisture ranged from 14.7 to 22% throughout the test period. About 3 months after the nematodes were applied, the populations of whitefringed beetle larvae were determined by taking six 2 in. diam soil cores 10 in. deep in each plot and washing the cores to remove the larvae. The results of these samples are presented in Table 13. The beetle larval populations were 50% lower in the most heavily treated plots than in the check plots. The differences were not significant at the 0.05 level by analysis of variance. Infective nematodes were present in the treated plots at the end of the test.

Effects of environmental factors on the host and the parasite. - After the nematodes were distributed on the plots, the soil moisture, soil temperature and rainfall were recorded. The soil moisture for the watered and nonwatered plots is shown in Fig. 2. The soil moisture avg 16.29 and 13.05% for the watered and nonwatered plots, respectively. The average for the watered plots is biased toward the minimum since the plots were watered each time after the moisture was determined. A total of 8.52 in. of rainfall during the test period contributed to the average moisture for the unwatered plots.

The soil temperature was recorded hourly for the 2, 4 and 6 in. depths throughout the test in one of the watered plots. However, the same was recorded for a nonwatered plot only during the last 2 wk of the test. A comparison of the soil temp at these 3 depths for the watered and nonwatered plots is shown in Fig. 3, 4 and 5. The Trape-

Table 12. - Rainfall recorded for small field plots treated with DD-136 nematodes 1968.

Date	In. of rain	
	Amount	Cumulative
11-4-68	0.3	0.3
11-12-68	2.0	2.3
11-25-68	1.16	3.46
12-2-68	2.65	6.11
12-9-68	0.84	6.95
12-16-68	0.90	7.85
12-23-68	0.68	8.53
1-6-69	4.09	12.62
1-13-69	0.03	12.65
1-20-69	2.51	15.16
1-27-69	0.15	15.31
2-3-69	2.68	17.99
2-10-69	0.27	18.27
2-17-69	1.45	19.71
2-24-69	0.87	20.58

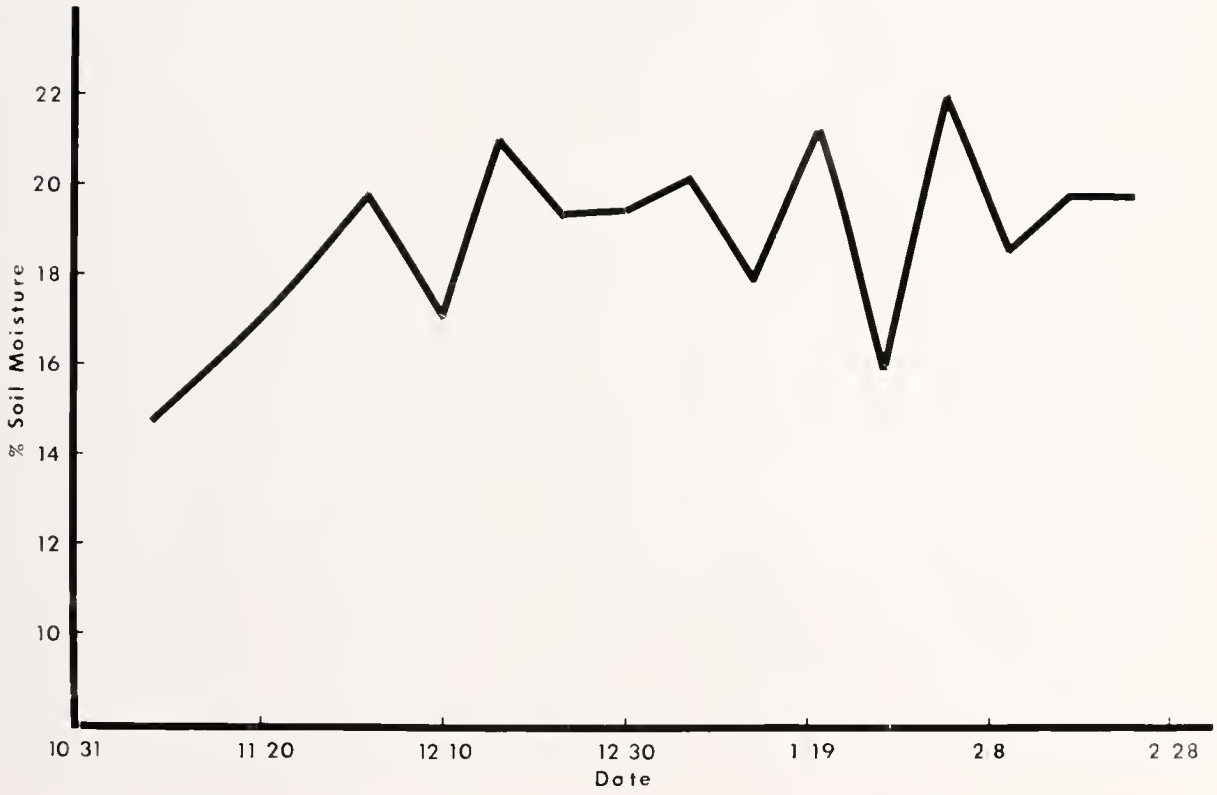

Fig. 1. - Avg percentage moisture in the top 10 in. of soil in small field plots, 1968.

Table 13. - Effect of DD-136 nematodes upon the whitefringed beetle larval population 3 months after the nematodes were introduced onto the surface of small plots.

Replicate	No. larvae/ft^2 Treatment[a]			
	0 (Check)	5000	20,000	50,000
1	15	31	15	23
2	46	8	8	31
3	31	8	31	0
4	15	115	54	8
5	15	15	15	31
6	108	92	31	0
7	46	31	31	15
8	0	15	54	31
Total	276	315	239	139
% of check	100	114	87	50

a Number of DD-136 nematodes introduced/ft^2.

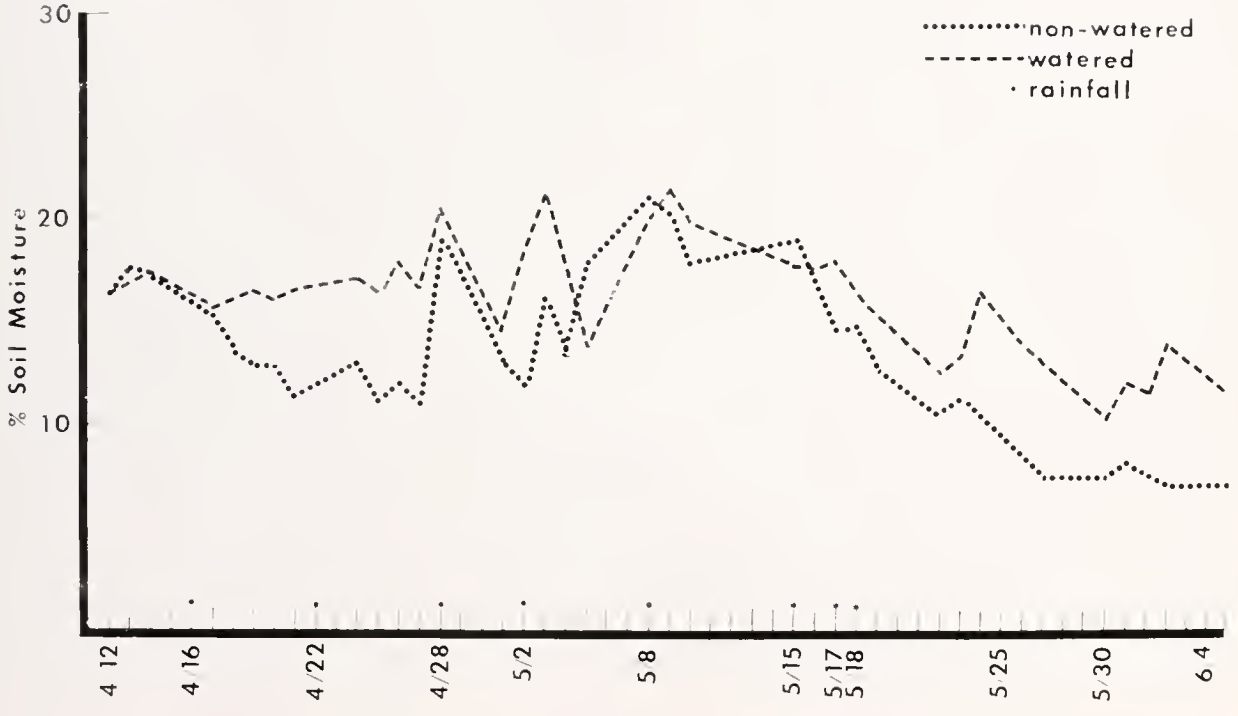

Fig. 2. - Avg percentage soil moisture for watered vs. nonwatered plots, 1972.

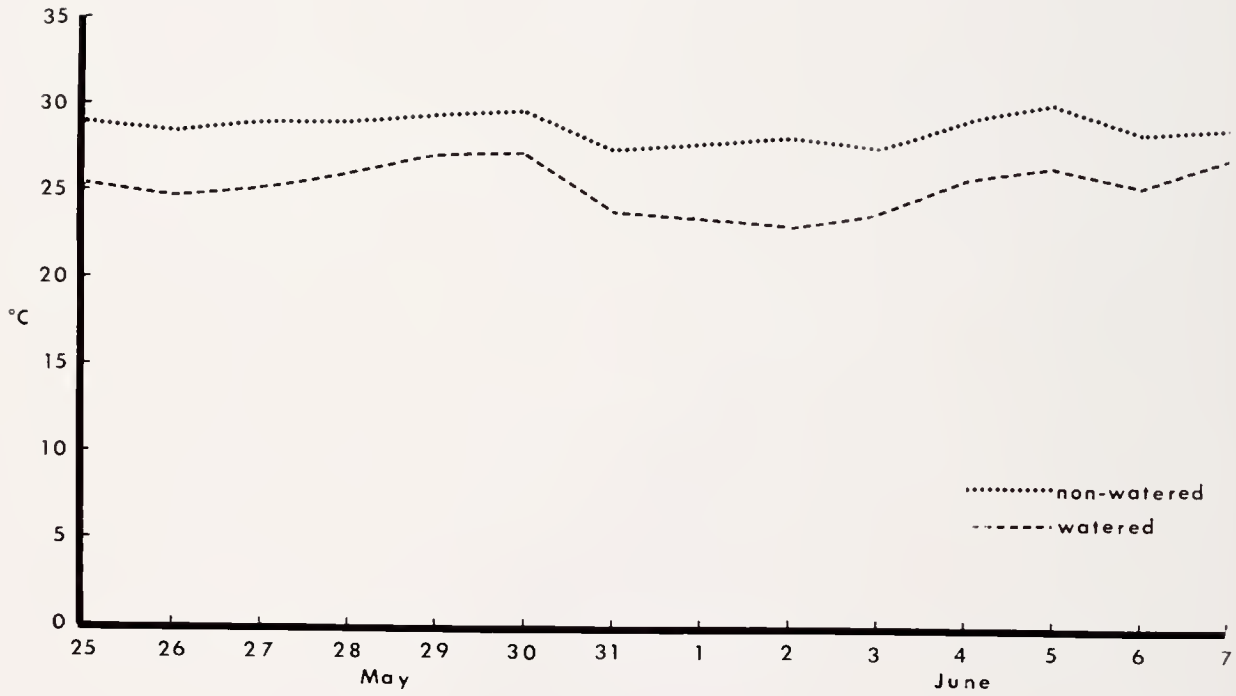

Fig. 3. - Soil temperatures at 2 in. depth in watered vs. nonwatered plots, 1972.

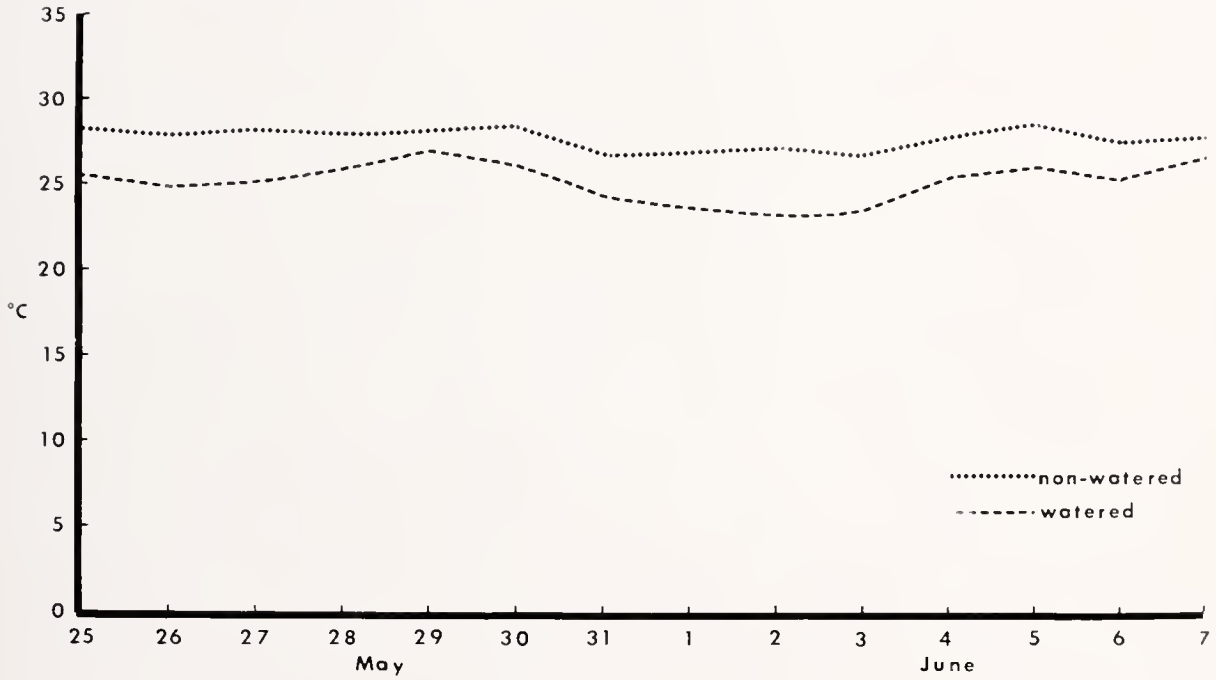

Fig. 4. - Soil temperatures at 4 in. depth in watered vs. nonwatered plots, 1972.

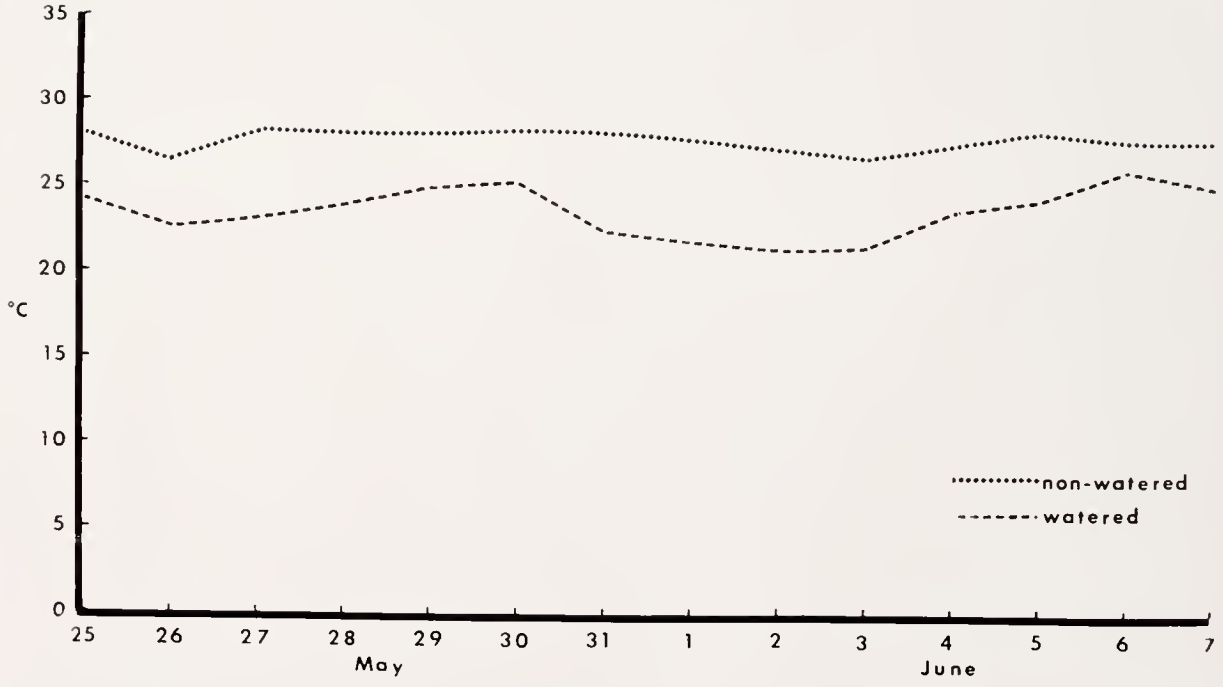

Fig. 5. - Soil temperatures at 6 in. depth in watered vs. nonwatered plots, 1972.

zoidal Rule was used on this data to determine the area under the curve. There was a significant difference (0.01) between the nonwatered and watered plots with mean areas being 619.5 and 544.2, respectively. Therefore the nonwatered plot was significantly warmer than the watered. There were no significant differences in temperature for the 3 depths measured.

The larvae were recovered from the plots at 2 wk intervals. The numbers of larvae recovered are shown in Table 14. There were no significant differences between the treated and nontreated (check) plots. There also were no significant differences in larval survival between the watered and nonwatered plots. However, there were significant differences (0.05) in the survival of larvae at the 3 soil depths, with the survival being greater at the 4 in. than the 2 or 6 in. depth. The larvae were weighed before they were introduced into the plots, and after they were recovered 42 days later. The weights are given in Table 15. There was a significant (0.01) difference in weight after 42 days between the larvae from the nonwatered and watered plots with the mean weights being 43.5 and 22.9 mg, respectively. The higher soil moisture seems to have had a selecting effect towards the smaller larvae. There were no significant differences in weight for the 3 soil depths. The width of the larval head capsule was also measured in an attempt to determine differences in growth. These measurements are given in Table 16. There were no significant differences in head capsule width for the watered vs. nonwatered or the 3 soil depths. The lack of differences in head capsule width is in contrast to the highly significant differences in larval weights.

The use of the wax moth larvae as a bioassay for the DD-136

Table 14. - Whitefringed beetle larvae recovered from plots treated with DD-136 nematodes Apr. 12, 1972.

Date Depth	No. introduced/ plot area	No. recovered alive				
		Check	LT[a]	HT[b]	Watered	Nonwatered
4-26						
2 in.	7	6	3	5	11	3
4 in.	7	2	9	4	9	6
6 in.	7	2	3	3	5	3
5-16						
2 in.	14	9	5	5	10	9
4 in.	14	12	10	9	14	17
6 in.	14	5	3	7	7	8
5-24						
2 in.	14	8	4	6	12	6
4 in.	14	16	10	9	21	14
6 in.	14	12	5	7	12	12

Table 14. - (Continued)

Date / Depth	No. introduced/ plot area	No. recovered alive				
		Check	LT[a]	HT[b]	Watered	Nonwatered
6-8						
2 in.	14	3	2	3	4	4
4 in.	14	10(1)[c]	13(1)	5	15	12
6 in.	14	10	6	4	12	8
$\bar{X}$						
2 in.		6.5	3.5	4.8	9.3	5.5
4 in.*[d]		10.0	10.8	6.8	14.8	12.3
6 in.		7.3	4.3	5.3	9.0	7.8

a 50,000 nematodes/ft^2

b 100,000 nematodes/ft^2

c No. pupae shown in parenthesis

d Overall no. larvae recovered from 4 in. depth significantly greater (0.05) than 2 in. or 6 in.

Table 15. - Weight of whitefringed beetle larvae before introduction into and after recovery from field plots. 1972.

	Mean wt (mg)	
	Before introduction	After introduction
Nonwatered	27.8[a]	43.5[b**]
Watered	26.1	22.0
Treated	27.9	32.0
Check	24.8	34.1
2 in. depth	-	23.5
4 in. depth	-	35.5
6 in. depth	-	39.2

a Weighed 147 larvae/plot.

b Weighed no. of larvae shown in Table 16, May 24. Difference significant (0.01) between nonwatered and watered plots.

Table 16. - Head capsule width of whitefringed beetle larvae recovered from plots. 1972.

Date / Depth	Mean head capsule width (mm)				
	Check	LT[a]	HT[b]	Watered	Nonwatered
4-26[c]	1.47	1.61	1.30	1.52	1.40
5-16					
2 in.	1.39	1.55	1.58	1.52	1.49
4 in.	1.54	1.48	1.54	1.57	1.47
6 in.	1.51	1.50	1.55	1.58	1.45
5-24					
2 in.	1.37	1.37	1.41	1.42	1.34
4 in.	1.51	1.55	1.52	1.51	1.54
6 in.	1.62	1.46	1.59	1.54	1.57
6-8					
2 in.	1.55	1.57	1.54	1.44	1.62
4 in.	1.55	1.46	1.56	1.54	1.50
6 in.	1.48	1.38	1.27	1.44	1.37

a 50,000 nematodes/ft^2.

b 100,000 nematodes/ft^2.

c 4-26 larvae from different depths measured together.

nematodes in the treated plots showed some nematodes present. However, this method did not show any differences in numbers or infectivity between the watered and nonwatered plots.

SUMMARY

The purpose of this research was to study the whitefringed beetle and the DD-136 nematode in the laboratory and field. Tests were conducted to try to better understand the host-parasite relationship between these 2 organisms. Laboratory and field tests were conducted over a 7 year period under various temperature, moisture, plant, soil, and population conditions.

In laboratory and field tests, the whitefringed beetle larva does not appear to be as attractive a host for the DD-136 nematode as some of the insects of the order Lepidoptera, e.g. the greater wax moth larva. A nematode treatment applied to peanut leaves did not appear to have any effect on adult whitefringed beetles.

A wetting agent was added to the nematode solution used to treat balled and burlapped, and potted plants containing whitefringed beetle larvae. There were no significant differences between the treatments and checks. However, there was a significantly higher number of larvae recovered from the balled and burlapped than the potted plants.

Under controlled environmental conditions the feeder-stage whitefringed beetle larvae were shown to be more susceptible to attack by the nematode than were the nonfeeder stage larvae. In the checks, both stages survived better at 16°C than at 27°C. Better control of both stages of the larvae was achieved at the higher temperature.

DD-136 nematodes produced by Nutrilite Products Corp., Dr. S. R. Dutky, and our laboratory at Gulfport, were used in field tests with

varying degrees of success. Overall, the nematodes produced by Dutky yielded the best results.

With the different conditions used in the field tests over the 7 year period, the reduction in larval population of 50% compared to the check population of ca. 34 larvae/ft^2 and achieved with a nematode treatment of 40,000/ft^2, was the best control documented.

Much more information was obtained from these tests on the effects of the environment on the insect than could be substantiated for the nematode.

The nematode was shown to be present in some of the field plots for over a year after it was applied. However, the insects were not economically controlled in any tests. This may be partly due to the nematode being less attacted to this insect than to others. It is also hard to determine the number of active infective stage nematodes which must be present in the soil at any one time for the insect to become infected.

DISCUSSION

This study, conducted over a 7 year period, is one of the most extensive investigations yet reported on testing of DD-136 nematodes for possible control of one host insect group. The results of this study indicate that the whitefringed beetle larval population can often be reduced by applying DD-136 nematodes. This reduction in host population might be sufficient for practical control of pests of certain row crops such as potatoes, peanuts, and cotton. Creighton et al. (1968) found that applications of this nematode reduced larval injury to Centennial sweet potatoes by Diabrotica and Systena spp. and to Nugget sweet potatoes by Conoderus falli Lane and Chaetocnema confinis Crotch; however, the protection provided was not adequate when the potatoes were graded for insect injury. The nematode was tested against whitefringed beetle larvae in nursery plant containers to determine if it could be used on plants which are under whitefringed beetle quarantine regulations. The results obtained indicate that this nematode cannot be considered for this use, since complete control must be certain.

Better control of both feeder and nonfeeder-stage beetle larvae was achieved at 27°C than 16°C. The higher temperature may have caused the larvae to feed more actively and consequently caused them to ingest more nematodes than at the lower temperature. Also, the nematodes were probably more active at the higher temperature. Reed and Carne (1967) found that at 30°C the infective juveniles were very active, but at temperatures near 16°C the generation time was doubled, adult

size and fecundity were reduced, and dispersal activity of the nematode could be virtually eliminated. The feeder-stage whitefringed beetle larvae were controlled better than the nonfeeder-stage larvae at both 16°C and 27°C. This data was presumably influenced by the feeder-stage ingesting more food and nematodes than the nonfeeder-stage which would be expected to feed very little. Most nematodes of this type are accidentally ingested by the host in the course of feeding (Reed and Carne 1967).

The DD-136 nematode was found in field plots for more than a year after it was introduced. However, the beetle larval populations which were treated were not reduced below ca. 50% of the nontreated. This occurrence may be due to the low reproductive rate of the nematode in this host. The average nematode yield was 6000/larva or 162/mg of body weight. This compares with an average yield of 1370 DD-136 nematodes/mg of body weight in wax moth larva as reported by Dutky et al. (1964). The low reproductive rate may hinder the parasite from being present in a population dense enough for individuals of a sparse beetle population to ingest the nematode. This may be true only if one assumes that no other host insects are present in the soil of the treated field. Since Niklas (1969) listed more than 120 species of insects as hosts of DD-136, there could possibly be other hosts present. The low reproductive rate of the nematode in the whitefringed beetle larva may be due to a lack of adequate quantities of certain sterols in this host which are necessary for nematode reproduction and growth. Dutky (1967) and Dutky et al. (1967a, b) reported that sterols derived from insect tissue are essential for growth, development, and reproduction of the nematode. The associated bacterium, Achromobacter nematophilus, must also be present

for nematode reproduction (Poinar and Thomas 1966). If environmental conditions such as temperature are not optimum for replication of the bacterium, some of the infective stage nematodes might not carry the bacterium. Soil moisture is also important for nematode movement and survival. Dutky (personal communication) found that a soil moisture of ca. 80% of the ball point of the soil (this was determined by adding water to dry soil until the soil would form a ball) was optimum for nematode movement. I found that with most of the sandy loam soils used in the nematode tests, the 80% of ball point level was ca. equal to 16-20% moisture. This is the moisture level that was sought in the soil moisture studies. The tests did not demonstrate significant differences in nematode infectivity caused by moisture alone.

The infectivity of the nematode varies greatly with different propagation hosts, temperatures, and storage conditions. Some of the problems encountered in storing the nematodes may be avoided if the propagation host is distributed in the field. W. W. Neel and P. P. Sikorowski (personal communication) are distributing bollworm larvae containing DD-136 nematodes onto test plots for controlling pecan weevil larvae. This method also furnishes protection for the nematodes under dry or hot soil conditions until they can become established in the soil. When the nematodes are in a storage solution, the percent activity (no. moving) is usually determined before they are distributed in the field. Even though the activity is low, if adequate numbers of nematodes are used the resulting insect mortality can be significant. W. L. Tedders and E. J. Wehunt (personal communication) obtained 67% mortality of pecan weevil larvae in small field tests when they used nematodes with an average activity of only ca. 19%. However, they applied ca. 1 - 1.5

million active nematodes/ft^2 of soil surface.

In the past the nematode like many other biological control agents (e.g. bacteria, fungi and viruses) has been applied by techniques developed for testing insecticides. The nematodes were sprayed onto the plants or soil and insect mortality was later determined. Investigators are now realizing that environmental factors such as soil moisture, temperature and other factors must be studied along with nematode application rates.

I have attempted to study the effects of soil moisture, temperature, nematode application rates, and host developmental stage on the host-parasite relationship of the DD-136 nematode and the whitefringed beetle larva. Under optimum temperatures (apparently ca. 27°C) good control of the host was achieved. However, control in the laboratory and the field was not consistent. I do not know whether these erratic results were caused by the condition of the nematodes when they were introduced or by conditions present after they were introduced. However, both presumably contributed to the results.

Proper environmental conditions are also important for survival of whitefringed beetle larvae. In 1 experiment, the results indicated a significantly lower survival of larvae in potted than balled and burlapped nursery plants. The potted plants contained a potting mixture which consisted of pine bark, peat moss, sand and soil. The balled and burlapped plants contained only sandy loam soil. Harlan et al. (1971) found that larvae survived better in potting material consisting of sandy loam soil alone than in mixtures with other materials.

The beetle larval survival in a field test was significantly higher at the 4 in. than the 2 or 6 in. soil depth. The higher survival rate

may have been influenced by a combination of differences in moisture and temperature, even though these conditions individually did not cause significant differences in survival. Gross et al. (1972b) stated that declines in whitefringed beetle larval populations during April and May are an annual occurrence, the causes of which are not understood. This decline in larval populations in the Gulf coastal states is usually preceded by an increase in soil temperature and an increase in soil moisture caused by precipitation of ca. 1 in. or more. During this period many of the larvae which die are infected by fungi e.g. Metarrhizium anisopliae and Beauveria bassiana (Balsamo), unidentified bacteria, or nematodes. Young et al. (1950) stated that unfavorable weather and soil conditions, parasites, predators, and diseases are important factors in keeping whitefringed beetles in check. They also reported that a fungus M. anisopliae is known to attack the soil inhabiting stages of the beetle in the Gulf coastal area, but killed few beetles. Swain (1943) was the first to report a parasite of the whitefringed beetle. He stated that a nematode (which appeared to be a species of Neoaplectana) was found in more than 2% of the soil stages of whitefringed beetles collected from Harrison County, Miss.

Nematodes and other naturally occurring parasites and pathogens of whitefringed beetle larvae have been found. I believe the DD-136 nematode would fit into a pest management program for this insect and many other soil insects. The major need now is for a commercial source of large numbers of the nematode which are propagated and stored properly so that they maintain their activity and infectivity.

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BIOGRAPHY

Donald Paris Harlan was born near Kosciusko, Mississippi, on February 9, 1941. He graduated from West Memphis High School, West Memphis, Arkansas, in 1959. He received his B. S. degree from Mississippi State University in 1963, with a major in General Agriculture. After graduation he accepted the position of agricultural field representative with Swift and Company. He held this position for one year while living in Columbia, Missouri. He then entered graduate school at the University of Missouri where he received his M. S. degree in Entomology in January, 1966. In February, 1966, he accepted the position of research entomologist at the United States Department of Agriculture, Agricultural Research Service, Whitefringed Beetle Investigations Laboratory, Gulfport, Mississippi. He held this position until June, 1972, when he transferred to the Bioenvironmental Insect Control Laboratory, Stoneville, Mississippi, where he is now conducting research on the biology and control of livestock insects. He attended the University of Florida from September, 1967, through August, 1968, as part of the USDA educational program.

He was married to the former Miss Hazel Jean Little in December, 1960. They have two daughters, Cathy, aged 11, and Donna, aged 10. They live at 105 Peninsula Drive, Leland, Mississippi.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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Professor of Entomology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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