Parasitism of *Neoaplectana dutkyi* in White-fringed Beetle Larvae

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Abstract: A larval population of the white-fringed beetle, *Graphognathus peregrinus* (Buchanan), in a Louisiana grassland field was reduced 38% by *Neoaplectana dutkyi* Jackson ('DD-136' nematode) applied at 430,000 nematodes per m^2. In Mississippi an artificial population was reduced 50% by the nematode applied at 538,000 per m^2. *Key Words:* Biological control, Insect-parasitic nematodes.

The 'DD-136' nematode which was first found in larvae of the codling moth, *Carpocapsa pomonella* (L.) (2), is quite similar to *Neoaplectana carpocapsae* Weiser. Poinar (5) proposed that it be considered the 'DD-136' strain of *N. carpocapsae* and that the original *N. carpocapsae* be called the 'Czechoslovakian' strain. Turco (7) described the 'DD-136' nematode, and contended that *N. dutkyi* Jackson (4) was its valid species name. This nematode has been field-tested as a biological control agent against several soil insects with varying degrees of success (1, 6), and laboratory evaluations proved it to be effective against larvae of the white-fringed beetle, *Graphognathus peregrinus* (Buchanan), which is known to feed on the roots of 240 plant species (8). The purpose of the two tests reported here was to evaluate the pathogenic effectiveness of this nematode in the field.

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MATERIALS AND METHODS

TEST 1: The first test was conducted against a natural population of white-fringed beetle larvae in a 48.6-hectare grassland field on a Stough-Myatt fine sandy loam (association) soil type located near Franklinton, Louisiana. Twelve 30.5 × 30.5 m plots separated by 6.1-m borders were used for the test. Populations of larvae were checked before the treatment by counting the number of larvae in 25 samples (30.5 × 30.5 × 30.5-cm) from each plot (five rows 6 m apart).

Four treatments (430,000; 215,000; 107,500; or 0 active infective-stage nematodes/m^2) were applied to the 12 plots (three replicates in a row) on April 24, 1968, with a piston-type pump sprayer mounted on a jeep. The sprayer had 11 cone-type nozzles spaced 0.3 m apart which covered a swath 3 m wide. The nematodes were reared at the Insect Pathology Pioneering Research Laboratory, Beltsville, Maryland with larvae of the greater wax moth, *Galleria mellonella* (L.) the propagation host (3). Nematodes were stored in insulated jugs at 50,000/ml, and 7.1 C. For transporting, the concentration was adjusted to 100,000/ml. They were brought to Gulfport, Mississippi, by airplane and transported by automobile to Louisiana. The insulated containers were always protected from heat. The nematodes were ap-

plied in 45.4 liters of water per plot at an operating pressure of 0.84–0.98 kg/cm^2. The soil temperature was 19 C at the time of application, and 2.5 mm of rain had fallen 4 hr before the treatments were applied.

The plots were sampled monthly for nematodes by taking twenty-five 5-cm-diameter soil cores per plot to a depth of 15-cm (taken in 5 rows, 6 m apart, cores within a row 6 m apart), avoiding re-sampling 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 three petri dishes, five last-instar wax moth larvae were placed in each dish (wax moth larvae were used because they are a preferred host of the nematode). The dishes were then held for 2 weeks at 29 C, any dead wax moth larvae were examined each week for nematodes. The nematodes present were identified. One month posttreatment the plots were sampled for white-fringed 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 beetle larval population was again checked by collecting 75 5-cm diameter soil cores 15-cm deep from each plot (five rows of 15 cores each), placing them in plastic bags, and taking them to Gulfport where they were washed through four screens (3.2, 6.4, 9.6, and 15 mesh/cm). The screens were then placed in water so floating beetle larvae could be collected and counted.

TEST 2: Test 2 was conducted with an artificial population of white-fringed beetle larvae in a field near Gulfport that had been planted to rye on September 15, 1968. The field, Ruston sandy loam soil type, was arranged as 32 3.05 × 3.05-m plots set up in a randomized complete block design with 8 replicates of each of four treatments. First, newly hatched white-fringed beetle larvae, from eggs collected in the laboratory, were introduced into each plot at the rate of 1075/m^2 during October 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; 54,000; 215,000; or 538,000 active infective-stage 'DD-136' nematodes/m^2, in 7.6 liters of water per plot with a 7.6 liter watering can. The area had received a 5-cm rain four days before application of the nematodes so the moisture level was high. The soil temperature was 18 C, and the air temperature was 22 C at the time of treatment. After treatment, the average moisture for the top 25.4 cm of soil was determined weekly by taking two 5-cm diameter cores 25.4-cm deep from two plots and using the gravimetric method to determine percentage of moisture. Also, two 5-cm cores were taken each month to a depth of 25.4 cm in each plot to determine nematode infectivity. This soil was again put into petri dishes, and five wax moth larvae were held in each dish at 29 C for one week. The dead larvae were then removed and examined for 'DD-136' nematodes. On February 25, 1969, about 3 months posttreatment, populations of white-fringed beetle larvae were sampled by taking six 5-cm diameter soil cores 25.4 cm deep in each plot (two rows of three cores each) placing the cores in plastic bags, and taking them to the laboratory where they were washed and the larvae collected, as described for Test 1.

RESULTS

TEST 1: The pretreatment populations averaged 100, 79, 95, and 84 beetle larvae/m^2 in the plots that were to be treated with the 430,000; 215,000; 107,500 nematodes/m^2 and for the check, respectively. One month posttreatment, we found no apparent differences in the populations of beetle larvae among plots; however, soil moisture had been

TABLE 1. Effect of *Neoaplectana dutkyi* upon a natural population of white-fringed beetle larvae 11 months after nematodes were introduced by surface spraying.

	No. of beetle larvae/m²			
	Treatment†			
Rep.	0 (check)	107,500	215,000	430,000
1	270	303	184	211
2	382	388	230	197
3	316	105	224	191
Total	968	796	638	599*
	(100% of check)	(82% of check)	(66% of check)	(62% of check)

* Significantly ($P = 0.05$) different from check by analysis of variance.
† Approximate number of *N. dutkyi* introduced/m².

TABLE 2. Effect of *Neoaplectana dutkyi* upon an artificial population of white-fringed beetle larvae 3 months after nematodes were surface applied with a watering can.

	No. of beetle larvae/m²			
	Treatment†			
Rep.	0 (check)	54,000	215,000	538,000
1	161	333	161	247
2	495	86	86	333
3	333	86	333	0
4	161	1237	580	86
5	161	161	161	333
6	1161	989	333	0
7	495	333	333	161
8	0	161	580	333
Total	2967	3386	2567	1493‡
	(100% of check)	(114% of check)	(87% of check)	(50% of check)

† Approximate number of *N. dutkyi* introduced/m².
‡ Difference not significant at 0.05 level by analysis of variance.

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 1: plots treated with the greatest number of nematodes had 38% fewer larvae than the check plots. This difference was significant at the 0.05 level by analysis of variance. Infective nematodes were present in all treated plots each month of the test.

TEST 2: About 3 months after the nematodes were applied, the populations of beetle larvae in the most heavily inoculated plots were 50% lower than in the check plots (Table 2). This difference was not significant at the 0.05 level by analysis of variance. Infective nematodes were present in all treated plots each month of the test. Since soil moisture ranged between 14.5 and 22% throughout the test period, it should have been adequate for nematode movement.

DISCUSSION

Further tests may show that the nematode will effectively control white-fringed beetle larvae when higher concentrations of nematodes are applied to the soil. We recovered 'DD-136' nematodes from the Louisiana plots 16 months after they were applied. In 1970 the white-fringed beetle larval populations were high in all plots so the nematode may not have been present in great enough numbers to control the beetle larval population. We consider the white-fringed beetle, a soil insect with a one year life cycle, to be ideally adapted for control with a parasite such as a nematode. Already some companies have expressed interest in commercial production of the 'DD-136' nematode if further tests show an adequate market for the nematode. We are continuing with tests to study the host parasite relationship of white-fringed beetle larvae and *N. dutkyi*.

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