Dendroctonus frontalis Infection by the DD-136 Strain of *Neoaplectana carpocapsae* and Its Bacterium Complex

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Abstract: The DD-136 strain of *Neoaplectana carpocapsae* Weiser (Steinernematidae) after spray application to pine bark in 0.1% Formalin plus wetting agent entered pine bark beetle tunnels and killed 44% of the brood and adults of *Dendroctonus frontalis* Zimmermann at 18 and 26 C, 60% relative humidity and at ambient temperatures and humidities. *Key Words: Neoaplectana carpocapsae, Dendroctonus frontalis*, Southern pine bark beetles, Temperature, Humidity, *Achromobacter nematophilus.*

Several authors [Jaques (5), Moore (7), Schmiege (9), and Welch and Briand (11)] mentioned that the DD-136 strain of the nematode, *Neoaplectana* sp., an insect parasite, is best suited to moist environments such as soils. The ensheathed, infective, second-stage nematode larva is somewhat protected from the environment. Dutky (2) ascribed to the nematode an ability to seek out and penetrate hosts, an attribute questioned by Schmiege (9), and negated by Fox and Nash (4). Field tests produced varying results. Tested against forest insects by Drooz (1), Jaques (5), Schmiege (9), Webster and Bronskill (10), and Welch and Briand (11), the nematode was able to enter and readily kill some hosts in the laboratory and field. To destroy populations of insects in the field, large numbers of nematodes are required but they soon die when exposed to moving air. Nash and Fox (8) and Webster and Bronskill (10) tested evaporation retardants to extend nematode viability in the field. When nematodes were applied to foliage in conjunction with drying retardants, Webster and Bronskill (10) demonstrated larch sawfly mortality up to 90%. Using various nematode concentrations and methods of application, Jaques (5) produced nematode infections ranging 2–98% in the winter moth, *Operophtera brumata* (L.).

Little other work has been done with this nematode in moist environments. Therefore, this paper considers: (i) the ability of the DD-136 strain of *N. carpocapsae* to parasitize the southern pine beetle at three different temperature treatment levels, and (ii) the ability of the nematode to enter bark beetle tunnels and live for an extended period under moist conditions.

MATERIALS AND METHODS

The nematodes, *Neoaplectana carpocapsae* Weiser, for this study were furnished by S. R. Dutky and were associated with an entomogenous bacterium *Achromobacter nematophilus* Poinar and Thomas. The test nematodes were reared by inoculating mature larvae of the wax moth, *Galleria mellonella* L., and harvested with 0.1% Formalin in shallow pans (3).

Shortleaf pine bolts, 75-cm long, containing southern pine beetle brood were sprayed with *N. carpocapsae* (DD-136) nematodes. A garden sprayer was used to apply 100 ml of 0.1% Formalin that contained Tween-20®[2] as spreader and 30,000 infective second-stage nematodes (approximately 740 nematodes per square foot of bark). The sprayed bolts were tested at two temperature levels, 18 and 26 C, and 60% relative humidity (RH) for 14 replications. Three replications of the tests at ambient temperature, the third treat-

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[2] Mention of a proprietary product does not imply recommendation of it to the exclusion of other materials that may also be satisfactory.

TABLE 1. Survival of *Neoaplectana carpocapsae* (DD-136) nematodes in inner bark and bark beetle tunnels.

Test	Time to 90% mortality (Days) 18 C	26 C	Ambient	Nematodes in inner bark and beetle tunnels per 7.5 × 16 cm sample (Number)
Nematodes applied with 0.1% Formalin and Tween-20				
1	—	8	—	20– 30
2	28	28	11	200–300
3	14	20	2	10– 30
4	14	4	4	2– 10
5	15	15	—	20– 30
6	14	8	—	0– 10
7	14	14	—	0– 20
8	14	14	—	2– 30
9	11	13	—	0– 10
Avg	15.4	13.7	5.2	
Nematodes applied with 0.1% Formalin, Tween-20, and glycerine to 10%				
10	6	6	—	30– 50
11	13	13	—	10– 30
12	10	12	—	0– 10
13	4	4	—	80–100
14	14	6	—	0– 50
Avg	9.2	8.1		

ment level, were made at variations of 50–80% RH. In Tests 1–9, Formalin and Tween-20 with nematodes were used; in Tests 10–14, the Formalin-nematode suspension was mixed with glycerine at a ratio of 9:1 to retard evaporation.

Bark sections, 7.5 × 16 cm, were cut from sprayed bolts and moribund *D. frontalis* larvae were removed from the tunnels and examined. Successive checks were made until the bark dried out and all living, uninfected beetles had emerged. As a control, adult *D. frontalis* beetles in bark sections from unsprayed bolts were examined for nematode infection.

RESULTS AND DISCUSSION

After the bolts were sprayed, a close examination revealed that at least 70% of the nematodes were moving into bark crevices and bark beetle galleries. The minimum distance through the bark and into the *D. frontalis* tunnels to a host was 3–4 cm. Moore (6) observed that the infective nematode larvae could move about 2 cm in 15 min over moist media. At this rate and under ideal conditions, it would take the nematodes 30–45 min to enter bark beetle tunnels and encounter hosts. Bolts in the laboratory sprayed with Formalin and Tween-20 dried in approximately 1 hr, whereas those sprayed with a mixture of Formalin, Tween-20, and glycerine remained moist for 4–5 hr. Thus, the Formalin and Tween-20 alone provided minimal time for entry, but with glycerine added the time was increased to a safe limit. No ill effects were observed on the nematodes from the use of any of the additives. Neither Fox and Nash (4) nor Webster and Bronskill (10) found any ill effects with the additives they used.

Neoaplectanid nematodes move very poorly on any surface without a film of water. Thus, providing a moist surface is a requirement for them to penetrate any type of plant tissue to find a host. Jaques (5) and Moore (7) established that most infective, larval nematodes died 90–120 min after the surface water evaporated. Welch and Briand (11) observed that the second-stage larvae died within 15–60 min after application to foliage in the field.

Inner bark from bolts containing maturing *D. frontalis* larvae averaged about 50% moisture at the start of the tests. In Tests 1–9 with Formalin and Tween only, the DD-136 nematodes lived in the beetle tunnels from 4–28 days, averaging 15.4 and 13.7 at 18 and 26 C, respectively (Table 1). At this time, the inner bark was reduced to less than 30% moisture. In Tests 10–14, the nematodes lived 9.2 and 8.1 days in the 18 and 26 C tests. Bolts in Tests 10–14 were a little older at the start of testing, and this may account for the reduced longevity of the nematodes.

TABLE 2. Southern pine beetles killed by parasitic *Neoaplectana carpocapsae* (DD-136) nematodes at 3 temperatures.

Test	Beetles per 7.5 × 16 cm sample				Beetles killed							
					18 C			26 C			Ambient	Check
	18 C (No.)	26 C (No.)	Ambient (No.)	Check (No.)	(No.)	%	± SD	(No.)	%	± SD	(No.)	(No.)
Nematodes applied with 0.1% Formalin and Tween-20												
1	15	15	–	10	0	0	42.8	7	46	3.6	–	2[a]
2	12	8	3	16	4	33	9.8	1	8	41.8	1	2
3	1	5	0	21	1	100	57.2	5	100	50.2	0	3
4	10	5	1	22	3	30	12.8	0	0	49.6	0	0
5	19	7	–	24	7	36	6.8	3	43	6.8	–	6
6	3	11	–	19	2	66	23.2	5	45	4.8	–	2
7	34	22	–	54	13	38	4.8	10	45	4.8	–	4
8	8	4	–	65	4	50	7.2	4	100	50.2	–	6
9	3	10	–	31	1	33	7.8	6	60	10.2	–	0
Avg					3.8	42.8		4.3	49.6			2.7
Nematodes applied with 0.1% Formalin, Tween-20, and glycerine 10% by volume												
10	18	17	–	33	4	78	31.6	8	67	30.9	–	7
11	40	12	–	18	6	22	24.4	1	8	28.1	–	2
12	38	34	–	72	3	8	38.4	3	9	27.1	–	0
13	9	9	–	31	5	55	8.6	2	22	14.0	–	0
14	14	8	–	39	10	71	24.6	6	75	39.8	–	0
Avg					5.3	46.4		4	40.1			1.8

[a] No nematodes in dead beetles.

The nematode infection and mortality of *D. frontalis* larvae and adults averaged 42.8% in 9 tests without glycerine and 46.4% in 5 tests with glycerine at 18 C. At 26 C the infection was 49.6% without glycerine and 40.1% with glycerine; at ambient temperatures the three tests run without glycerine averaged 11% infection. Six tests at 18 and 26 C were lower than 11% and 5 were 75% or above. The range was from 0–100% (Table 2). Although the nematodes may have entered the bark more easily with glycerine added to the spray material, there was little difference in the quantity of infected beetles. These results indicate that the nematodes in the bolts survived long enough to find the beetles. One might assume that the nematodes had difficulty finding the beetle larvae, except that beetles exposed to a large number of nematodes in a petri dish infected about the same number; e.g., from one-third to two-thirds of those present.

One primary difference between methods used by different investigators was where the nematodes were applied. They are frequently applied to needles or leaves and are consumed with the plant material. Less frequently, the sprayed nematodes must penetrate the plant to find the host. In a field test of DD-136 nematodes against Nantucket pine tip moth, Nash and Fox (8) obtained 5–15% moth population decrease in sprayed pine tips.

The DD-136 nematode primarily parasitized mature beetle larvae; few young larvae or adults, and no pupae, were attacked. Live

nematodes were found in most dead beetles taken from the tunnels. No nematodes were found in live beetles in the sprayed bolts or in dead beetles from the control bolts.

This work demonstrates the ability of the nematode DD-136 strain of *N. carpocapsae* to seek out hosts in dense material, and also shows it must have a moist environment to survive. Additional tests with drying retardants may show that it can be established in the field under certain conditions.

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