RESEARCH NOTES

Evaluation of Neoaplectana carpocapsae¹ for Biological Control of the Western Spruce Budworm, Choristoneura occidentalis: Ineffectiveness and Persistence of Tank Mixes²

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In North America, the western spruce budworm (WSBW), Choristoneura occidentalis, is a persistent and destructive defoliator of western fir forests (3). Suppression of WSBW larval populations with viruses, bacteria, and entomogenous nematodes has given inconsistent results. Kaya et al. (7) concluded that a formulation of Neoaplectana carpocapsae, and its mutually associated bacterium, Xenorhabdus nematophilus, which enhances nematode survival on foliage, may provide more effective suppression. We report herein the evaluation of various spray additives to nematode suspensions for suppression of WSBW larvae and pupae, and survival of the nematode after spray application.

The All strain of N. carpocapsae was mass-produced monoxenically on a dog food medium as described by Hara et al. (5). The following additives with aqueous suspensions of the nematode were field tested based on foliar applications conducted by MacVean et al. (8): a methylcellulose polymer, Methocel J75MS (Dow Chemical Co.) at 0.5% (w/v); a water absorbent polymer, Norbak (Dow Chemical Co.) at 0.1% (w/v); and a drift-control agent for herbicides, Nalco-trol (Nalco Chemical Co.) at 0.05% (v/v). Methocel J75MS was adjusted to pH 8.5 by adding 0.001 M Tris buffer. A sticker, Nu-Film-17 (Miller Chemical and Fertilizer Co.), was added to the above nematode suspensions at $0.05\,\%$ (v/v) to enhance contact at the additive-foliage interface. A Nu-Film-17 nematode suspension was also applied. The test formulations were applied to 2-3-m grand fir (Abies grandis) using a Hudson sprayer until runoff. There were 10 trees per treatment. Applications were made against fourth and fifth instars on 30 June and 1 July 1981. Treatments were at 16,000 nematodes/ml of test formulation; each tree received between 8×10^6 and 1.3×10^7 nematodes. As a comparison, the microbial insecticide Bacillus thuringiensis (Dipel 4L) was sprayed against larvae at 1×10^{9} International Units per tree. Control trees were left unsprayed or were sprayed with the additives without nematodes. Immediately after application, the distal 38-cm portion of one branch per tree was covered with a paper bag in an attempt to increase nematode survival and WSBW mortality. Nematodes with Nalco-trol + Nu-Film-17 and with Nu-Film-17 were also applied at 16,000 nematodes/ml against WSBW pupae on 23 July.

Counts of WSBW larvae and pupae were taken 24 h before treatment and 96 h after treatment. The sampling procedure for the prespray counts consisted of removing two 38-cm branch samples from the midcrown level of each sample tree and counting the number of larvae and buds (2). The sampling procedure for the postspray counts was the same, except that one bagged and one unbagged branch sample were taken per sample tree.

To determine the persistence of the nematode with various additives, foliage was sprayed on 1 July 1981 at 2130 h, with two trees per treatment. Three 10-cm terminal branch samples from bagged and unbagged branches of the mid-crown of each tree were removed and placed individually into 200 ml of water for 2 min. The first 100-200 nematodes observed with a dissecting microscope were counted as alive or dead. Samples were taken at 4-h intervals up to 20 h postspray. Climatological data during the test period were also obtained.

Infectivity of the nematode was checked in the laboratory by spraying grand fir foliage containing 25 field-collected fourth-

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or fifth-stage WSBW larvae with the spray mixtures from the field on 30 June and 1 July. There were five larvae in each of five petri dishes per treatment. Mortality was checked 72 h later. Field-collected pupae were treated with 200 nematodes in petri dishes as described by Kaya and Hara (6). Ten days later larvae and pupae were examined for nematode infection.

In the 30 June and 1 July field tests, differences in host population reduction were not detected between nematode treated and sprayed control trees. Nematode treatments resulted in host population reductions which averaged 49% (Sx 6) for bagged and 48% (Sx 9) for unbagged branches compared with reductions in sprayed controls of 55% (Sx 6) for bagged and 23% (Sx 15) for unbagged branches. This reduction was probably due in part to the older larvae which dropped off the foliage when disturbed during the spray application and bagging the distal 38-cm portion of the branch. This was substantiated when the prespray and postspray counts of unsprayed trees showed no decrease. The prespray count was 30.6 (Sx 4.5) host larvae/100 buds, and the postspray count was 31.4 (Sx 3.8) larvae/100 buds. Seventy-seven percent (n = 26) of the dead larvae found in the nematode treatments showed signs of nematode infections; none of the eight dead larvae found in the control treatments showed signs of infection. Nineteen of the dead larvae in the nematode treatment were on the bagged foliage. The B. thuringiensis treatment gave the best population reduction at 83% for the for the unbagged bagged and 74% branches.

The combined data of bagged and unbagged branches for the field test against pupae showed no mortality in the control or any of the treatments attributable to nematodes.

Laboratory tests showed that the nematodes were not adversely affected by the additives. In all cases, 100% host mortality (n = 25 for each treatment) occurred with 78% of the dead insects containing nematodes. Less than 4% of the control insects died. Field-collected pupae were highly susceptible to the nematode in the laboratory, with 96.4% mortality. These data are in contrast to those obtained by Kaya and Hara (6) who reported 60% pupal mortality after exposure to nematodes. The reasons for these differences are not known but may be related to the physiological condition of the host, increased pathogenicity of the nematode, or both.

The ambient temperatures during the larval spraying period varied from highs of 22-32 C during the day and lows of 4-13 C during the night. Relative humidity ranged from 80 to 95% during the night and from 21 to 26% during the day. Since applications were made at night, the low temperature and high humidity favored nematode survival but reduced infectivity. The only opportunity for nematode infection was during the morning after spray application when temperatures had moderated and before the relative humidity had dropped. The persistence study showed that the nematodes remained alive longer on bagged foliage than on unbagged foliage (Table 1). The most effective tank mix was Norbak + Nu-Film-17 in which nematodes persisted between 16 and 20 h. The majority of the nematodes were dead on unbagged foliage by 12 h after spray application, while the majority were alive on the bagged foliage for all treatments. Thus, the major mortality factor for the nematode was probably desiccation.

Utilization of *N. carpocapsae* against foliage feeding insect pests has been generally discouraging because of nematode desiccation (4). Earlier attempts to increase survival on foliage by incorporating the nematodes in antidesiccant formulations were only partly successful (1,7,8,9,10,11).

Our tests have confirmed those by Mac-Vean et al. (8); Norbak and Methocoel J75MS extended nematode survival the longest. We have also shown that bagged foliage increases nematode survival. However, even under these conditions, we could not obtain significant nematode infections of WSBW larvae and pupae. Because of the low night temperatures, nematode survival of 15 h is not sufficient to infect WSBW larvae and pupae. The most successful use of *N. carpocapsae* has been in moist situations such as in soil or insect galleries where *N. carpocapsae* survival is not a problem (4). Accordingly, the use of foliar applications

Nematode formulation†	Persistence* Sample times (h) posttreatment										
	2130 h‡	4 h		8 h		12 h		16 h		20 h	
		U	В	U	В	U	В	U	В	Ū	В
Methocel J75MS + Nu-Film-17	96.7	91.4	95.3	72.7	97.7	36.7	92.4	0	0	_	-
Nalco-trol + Nu-Film-17	95.6	86.4	92.3	62.2	93.4	0	88.6	-	0	-	
Norbak + Nu-Film-17	95.7	97.9	92.1	57.8	92.4	7.7	82.5	0	83.8	-	0
Nu-Film-17	94.1	87.5	82.3	2.5	88.7	0	85.7	-	0	-	-

Table 1. Persistence of *Neoaplectana carpocapsae* in various formulations on unbagged (U) and bagged (B) grand fir, *Abies grandis*, foliage.

*Mean percent alive of three replicates.

[†]Aqueous nematode suspension containing 16,000 nematodes/ml.

Time of spray application.

of *N. carpocapsae* should be discouraged until a formulation is developed which extends nematode survivability and provides significant insect mortality.

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