Effects of a neem seed extract against the white pine weevil, *Pissodes strobi* (Coleoptera: Curculionidae), in Sitka and white-Engelmann spruce.

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ABSTRACT

A botanical insecticide based on extracts of seeds from the neem tree, *Azadirachta indica* A. Juss, was applied systemically to Sitka spruce and white-Engelmann spruce hybrids and sprayed directly onto the leaders of white-Engelmann spruce to test its efficacy in controlling the white pine weevil, *Pissodes strobi* Peck. Neither treatment had any effect on weevils or damage in the white-Engelmann spruce, and control was not economical.

Key words: neem, weevil, azadirachtin

INTRODUCTION

The white pine weevil, *Pissodes strobi* Peck, is a transcontinental pest of spruce, *Picea* species, attacking such important timber species as Sitka spruce, *P. sitchensis* (Bong.) Carr, Engelmann spruce, *P. engelmannii* Parry, and white spruce, *P. glauca* (Moench) Voss. Larval feeding in the phloem can girdle the leader causing the terminal growth to wither. This in turn leads to stunted trees, stem deformities, decreased lumber volume, and repeatedly attacked trees are short and overtopped by competing vegetation to the point where a severely attacked plantation may be unmerchantable (Alfaro 1989).

In British Columbia, planting of Sitka spruce has been greatly reduced and is limited to areas of low beetle attack (Heppner and Wood 1984). This is despite the fact that it outgrows all other conifer species on alluvial and rich, low-elevation coastal sites and that log values for second growth Sitka spruce timber are higher than for other species on those sites (B.C. Min. of Forests 1990). Thousands of hectares of young spruce plantations also are in danger of becoming unmerchantable unless a suitable method can be found to control *P. strobi*. Spruce is also being affected in the interior of B.C. where salvage of bark beetle infestations of naturally-occurring white-Engelmann hybrids has produced large spruce plantations. Intensive silvicultural practices, such as the creation of open-grown stands through spacing and clearing brush, have produced particularly susceptible trees (McLean 1989, Taylor *et al.* 1994).

At present, there is no economically viable control method for *P. strobi*. Clipping of attacked leaders is expensive and time consuming and augmentation of natural enemies has not proven successful (Rankin & Lewis 1994). Foliar sprays of insecticides such as oxydemeton-methyl and acephate will give complete control of white pine weevil in the year of application and some control the following year (Gara *et al.* 1980), however widespread aerial sprays of insecticides are increasingly out of favor. Fraser and Heppner (1993) demonstrated that systemic applications of oxydemeton-methyl and acephate decreased leader attack of Sitka spruce.

Seed extracts of the neem tree, *Azadirachta indica* A. Juss (Meliaceae), have a number of desirable properties for managing insect pests including repellency, feeding and oviposition deterrence, insect growth regulation, low mammalian toxicity, and rapid degradation (Mordue & Blackwell 1994, Schmutterer 1990). Because the anti-feedant and IGR effects are seen mostly against immature insect stages that have ingested neem, it may also be safer to non-target insects than most conventional insecticides (Hoelmer *et al.* 1990, Lowery and Isman 1994, McCloskey *et al.* 1993, Stark 1992). The most important constituent of neem seed extracts (NSE) is the limonoid compound, azadirachtin.

The translocation of azadirachtin has been demonstrated in agricultural crops (Larew 1988, Osman & Port 1990, Saxena 1987) and trees (Marion *et al.* 1990). Naumann *et al.* (1994) found that systemic applications of NSE could decrease the numbers of mountain pine beetle, *Dendroctonus ponderosae* Hopkins, larvae surviving within lodgepole pine trees, *Pinus contorta* Douglas var. *latifolia*.

In this study, we examined whether systemic applications or sprays of NSE directly onto the leaders can be used to protect against loss of leaders from weevil attack and to destroy white pine weevil larvae within Sitka spruce and hybrid trees of the white-Engelmann spruce complex.

MATERIALS AND METHODS

A solution of NSE in methanol, containing 2.5% azadirachtin (25,000 ppm), was obtained from Phero Tech Inc. (Delta, B.C. Canada).

Systemic treatments were applied to Sitka spruce in the Benson River area, near Port McNeill, on Vancouver Island, B.C., and to white-Engelmann spruce at two sites near Williams Lake B.C. : Gavin Lake and Quesnel Lake. Leader sprays were tested only on white-Engelmann spruce at Gavin Lake.

Systemic Treatments. Two treatments were used: NSE at 25,000 ppm azadirachtin, and a control of methanol only. The treatments were administered by drilling holes, 6 mm in diameter, 1.5 cm deep, and sloping downwards, into the trees at breast height and then filling the holes with one or other of the liquids. One hole was drilled for approximately every 2.5 cm of trunk diameter at breast height (dbh). Each hole received approximately 0.4 ml of fluid, i.e., 0.01 g of azadirachtin.

Two tests were conducted at the white-Engelmann spruce sites. The first (pre-attack treatment) tested the effectiveness of an NSE application in saving leaders from destruction, the second (post-attack treatment) more closely examined the potential lethal effects on weevil larvae within already attacked leaders. In the pre-attack test, treatments were applied to a total of 60 trees at each of the two white-Engelmann spruce sites immediately before oviposition, during the first week of May, 1994. Post-attack injections were applied to different trees on July 4. Twelve and 14 trees per treatment group were used at Gavin Lake and Quesnel Lake, respectively. By that time, weevil-attacked trees could be identified by their drooping leaders. Only a pre-attack trial was conducted in Sitka spruce; 30 trees per treatment group were injected in the third week of April.

Pre-attack test trees at all sites were chosen for healthy appearance, tall leaders and relatively open locations, i.e., high susceptibility to weevil attack. Transects were run through the test stands, and suitable trees within 10 m of the transect lines assigned alternately to the neem and control treatments. Trees within the Sitka spruce plantation averaged approximately 10 cm dbh. Trees at the Gavin Lake site averaged 7.6 cm dbh and those at Quesnel Lake 8 cm dbh. There were no significant differences in tree diameters between treatments.

Leaders were cut from the white-Engelmann spruce trees in the second week of August, before the most mature weevils had completed pupation. The parameters measured were overall frequency of attack and leader destruction (for the pre-attack treatment only), and numbers of feeding punctures, oviposition punctures, larvae, pupae, and natural enemies per leader. Leaders from the Sitka spruce trees were collected in mid-October and compared for overall frequency of leader destruction, and numbers of larvae, pupal cocoons, adult exit holes, and natural enemies per leader. At all three locations, the great majority of natural enemies collected were larvae of the Dipteran genus *Lonchea*.

Data were analyzed by Chi-square test (frequency of attack and leader destruction), t-tests (numbers of different events per leader), and linear regression (to determine the strength of the relationship between numbers of natural enemies and hosts within the leaders)(Zar 1984). All analyses were run using Statistix statistical software (Anonymous 1991).

Leader Sprays. NSE (diluted with water to 100 ppm AI) was applied to tree leaders until runoff. Leaders were sprayed from the tip to the penultimate whorl of branches. Four treatments were used: a neem spray applied approximately 1 week after the main period of weevil oviposition (June 20, 1995), neem applied 2 weeks later, neem applied on both dates, and an unsprayed control. There were 30 trees in each treatment group. Leaders were cut from the trees in August and dissected to determine numbers of adult exit holes, pupal (chip) cocoons, surviving larvae, and surviving natural enemies. The sum of larvae and pupal cocoons per leader was used as a measure of total weevil survival. Data were analyzed by one way analysis of variance (Anonymous 1991).

RESULTS

Systemic NSE applications had no observed effects on *P. strobi* survival in white-Engelmann spruce (Tables 1, 2). The frequency of leader destruction was also not diminished by a pre-attack treatment of NSE. Conversely, a pre-ovipositional application to Sitka spruce caused significant reductions in the frequency of leader destruction, numbers of pupae/leader, number of successfully emerging adults, and number of natural enemies per leader (Table 3). There was no correlation between the number of individual natural enemies and number of hosts in the Sitka spruce leaders (r^2 = 0.04). Leader spraying to white-Engelmann spruce had no significant effects on any of the parameters measured (Table 4).

Table 1
Effects of pre-oviposition systemic application of NSE on white pine weevils in white-Engelmann spruce near Williams Lake. Means ± SE. Significant differences ($p \leq 0.05$) marked by *.

	control	NSE	
% trees with attacked leaders[1]			
Gavin Lake	46%	63%	$X^2 = 1.78, p = 0.19$
Quesnel Lake	29%	23%	$X^2 = 0.05, p = 0.82$
% trees with killed leaders			
Gavin Lake	23%	13%	$X^2 = 1.08, p = 0.30$
Quesnel Lake	23%	23%	$X^2 = 0$
# feeding punctures/leader			
Gavin Lake	28.3 ± 9.5	16.5 ± 5.6	$t = 1.07, p = 0.29$
Quesnel Lake	65.2 ± 9.9	76.4 ± 15.0	$t = 0.62, p = 0.53$
# oviposition holes/leader[2]			
Gavin Lake	16.4 ± 2.5	13.3 ± 3.9	$t = 0.67, p = 0.51$
Quesnel Lake	13.3 ± 2.7	32.0 ± 7.4	$t = 2.63, p = 0.05$*
Total surviving weevils/leader[3]			
Gavin Lake	7.0 ± 2.7	2.0 ± 1.1	$t = 1.72, p = 0.10$
Quesnel Lake	4.3 ± 2.0	4.1 ± 1.4	$t = 0.07, p = 0.94$
# natural enemies/leader			
Gavin Lake	11.9 ± 8.2	6.7 ± 3.6	$t = 1.41, p = 0.13$
Quesnel Lake	11.6 ± 4.5	8.1±10.9	$t = 0.56, p = 0.59$

[1] Leaders with two or more oviposition holes were considered to have been attacked.
[2] Values for numbers of egg holes and survivors are attacked leaders only.
[3] Value includes all stages alive when leader dissected plus number of exit holes.

Table 2
Effects of post-oviposition systemic applications of NSE on white pine weevils in white-Engelmann spruce near Williams Lake. Means ± SE.

	Control	Neem	t	p
# feeding punctures/leader				
Gavin Lake	88.6 ± 18.5	102.5 ± 21.5	0.49	0.62
Quesnel Lake	149.3 ± 22.3	88.7 ± 20.3	1.98	0.06
# egg holes/leader[1]				
Gavin Lake	63.8 ± 18.5	38.9 ± 11.0	1.16	0.26
Quesnel Lake	29.4 ± 5.4	34.5 ± 7.5	0.56	0.26
# living weevils/leader[2]				
Gavin Lake	21.3 ± 5.1	20.2 ± 5.4	0.16	0.88
Quesnel Lake	11.9 ± 3.1	7.5 ± 1.4	1.29	0.22
# natural enemies/leader				
Gavin Lake	7.3 ± 2.2	26.2 ± 10.1	1.82	0.09
Quesnel Lake	6.6 ± 1.6	5.5 ± 1.1	0.29	0.77

[1] Values for numbers of egg holes and survivors for attacked leaders only.
[2] Value includes all stages alive when leaders dissected plus number of exit holes.

Table 3
Effects of pre-oviposition systemic application of NSE on white pine weevils in Sitka spruce near Port McNeill. Means ± SE. Significant differences ($p \leq 0.05$) marked by *.

	control	neem	
% trees with weevil-killed leaders			
	87%	50%	$X^2 = 11.60, p < 0.001$*
# pupal cocoons/leader[1]			
	102.0 ± 7.7	65.9 ± 9.7	$t = 2.84$, $p < 0.001$*
# adult emergence holes/leader			
	16.1 ± 3.2	0.8 ± 0.6	$t = 4.91$, $p < 0.001$*
# live larvae/leader			
	3.2 ± 0.7	6.1 ± 2.3	$t = 1.19$, $p = 0.25$
# natural enemies/leader			
	27.7 ± 5.6	8.9 ± 2.5	$t = 3.07$, $p = 0.006$*

[1] Values for this category and those below are for killed leaders only.

Table 4
Effects of post-oviposition sprays of NSE on white-Engelmann spruce near Williams Lake. Means ± SE. Sample size = 30. No significant differences (critical value of $p \leq 0.05$).

Spray 1 ~ week after oviposition	Spray ~ 3 weeks after oviposition	Spray on both dates	Control	F	p
# pupal cocoons/leader					
13 ± 2	7 ± 2	8 ± 2	12 ± 3	2.13	0.10
# adult exit holes/leader					
32 ± 6	18 ± 4	22 ± 4	28 ± 5	1.95	0.12
# larvae + pupal cocoons					
33 ± 6	20 ± 4	22 ± 4	30 ± 6	1.52	0.21
# natural enemy larvae per leader					
8 ± 2	4 ± 1	6 ± 2	5 ±1	1.08	0.36

DISCUSSION

In our study, systemic applications of NSE to spruce did not control white pine weevil to the same degree as has been reported for mountain pine beetle in lodgepole pine (Naumann *et al* 1994). However, pre-attack systemic applications of NSE did reduce the frequency with which white pine weevils destroyed Sitka spruce leaders and caused significant decreases in the numbers of weevils surviving to pupae and adults. The NSE also decreased the numbers of natural enemies within the leaders. This reduction was not due solely to a decreased availability of hosts because there was no correlation between the two values. The insecticidal effects of NSE applications have been reported to extend to a higher trophic level (McCloskey *et al*. 1993), although NSE has often been reported to be safe to non-target species (Stark 1992, Lowery & Isman 1994).

The absence of any neem-induced effects in the interior spruce may have been due to inherent differences in translocation or metabolism of azadirachtin as compared to Sitka spruce, or to temperature-related differences in translocation between the warmer, interior and cooler, coastal sites. Differences in tree size was unlikely to have been a factor because mean diameters at breast height differed by approximately one cm, and larger trees received relatively greater volumes of neem formulation.

Direct sprays of neem onto the leaders of the white-Engelmann spruce hybrids also had no effect on the survival of white pine weevils or the frequency of leader destruction. The dose used was approximately twice that shown to be effective for controlling phytophagous pests in agricultural ecosystems (Isman 1995). Lack of efficacy could have been due to insufficient absorption of azadirachtin into the phloem, i.e., to where the larvae were feeding.

The results of our study suggest that NSEs are unlikely to be an important option for protecting large areas of spruce from the white pine weevil. Testing higher doses may be worthwhile but difficulty in obtaining NSE formulations with high enough concentrations of azadirachtin, especially for systemic applications, is an obstacle. Greater numbers of applications are unlikely to be cost effective. The Sitka spruce results are encouraging enough to suggest that systemic NSE applications may be of value for protecting individual, high-value trees from some phytophagous insects. Further evidence for this comes from the success of systemic NSE in killing the mountain pine beetle in lodgepole pine (Naumann *et al.* 1994). Efforts should be directed towards understanding the mechanism by which the NSE affects phytophagous insects within trees, i.e., repellency or larvicidal action, the dose-response relationship for azadirachtin and various forest pests, and the rate and nature of azadirachtin translocation in trees of different species.

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