

Systemic Action of Neem Seed Extract on Mountain Pine Beetle (Coleoptera: Scolytidae) in Lodgepole Pine

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J. Econ. Entomol. 87(6): 1580–1585 (1994)

ABSTRACT A botanical insecticide derived from seeds of the neem tree, *Azadirachta indica* A. Juss, was tested for the "bait and kill" strategy for control of outbreaks of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins. Application of neem into frills at the base of beetle-attacked lodgepole pine, *Pinus contorta* Douglas var. *latifolia* Engelman, trees reduced numbers of mountain pine beetle larvae per unit area of bark, decreased densities of larvae per unit length of adult gallery, and increased larval mortality when applied at doses >0.25 g (AI) (azadirachtin) per tree. Suggestions for improving the efficacy of neem treatments are discussed.

KEY WORDS *Dendroctonus ponderosae*, neem, translocation

THE MOUNTAIN PINE BEETLE, *Dendroctonus ponderosae* Hopkins, is the most economically important bark beetle in western North America. In British Columbia, it attacks lodgepole pine, *Pinus contorta* Douglas var. *latifolia* Engelman, and ponderosa pine, *P. ponderosa* Lawson, often decimating trees over extensive areas (Furniss & Carolin 1977). Current control measures include harvesting of large, infested areas and control of spot infestations by trapping into pheromone-baited trees for later destruction. Immediate and effective control of spot infestations is essential to prevent the spread of outbreaks and their ascension to outbreak status (Borden 1993). Beetle larvae in baited trees usually are killed by wintertime "fall and burn" treatments or by the injection of a systemic pesticide, monosodium methane arsenate. However, monosodium methane arsenate is potentially toxic to applicators and other organisms, and its mode of action is not fully understood (MacLauchlin et al. 1988).

Extracts of neem tree (*Azadirachta indica* A. Juss) seeds have a number of properties useful for insect pest management. These include repellency, feeding and oviposition deterrence, insect growth regulator activity, low mammalian toxicity and low persistence in the environment (Koul et al. 1990, Schmutterer 1990). Neem is also less toxic to nonphytophagous insect species than many conventional insecticides, including pest natural enemies (Hoelmer et al. 1990, Stark

1992, McCloskey et al. 1993, Lowery & Isman 1994). The most important constituent of neem seed extracts is the tetranortriterpenoid compound azadirachtin.

Neem seed extracts have been tested against various beetle species. Results include mortality and interference with molting in Scarabaeidae (Ladd et al. 1984), Tenebrionidae (Mukherjee & Ramachandran 1989), and Coccinellidae (Schluter 1985, Ascher & Gsell 1981); impaired reproductive capacity (Scarabaeidae: Kaethner 1991); and repellency and feeding deterrence (Chrysomelidae: Karel 1989, Meisner & Mitchell 1982, Reed et al. 1982; Tenebrionidae: Jilani et al. 1988; Bostrychidae: Jilani & Saxena 1990). In phytophagous beetles, as in other taxa, neem-induced developmental impairment and, feeding deterrence, or both, can result in death, and diminished host damage. To date, no studies of neem's effects on scolytid beetles have been reported. Neem seed extracts could provide an alternative to monosodium methane arsenate.

Several studies have demonstrated that neem seed extracts or their components are translocated within plants (Marion et al. 1990, Osman & Port 1990, Larew 1988, Saxena 1987). The purpose of the study reported here was to examine whether neem seed extract can be used systemically to control immature mountain pine beetles in recently attacked lodgepole pine.

Materials and Methods

A proprietary emulsifiable concentrate (neem EC) formulation of neem seed extract, containing 5% AI (azadirachtin) was obtained from Phero Tech (Delta, BC, Canada). Dilutions were made

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with methanol, and rates expressed in terms of ppm of azadirachtin.

Azadirachtin Translocation in Conifers. One ml of neem EC (50,000 ppm azadirachtin) was introduced into each of nine 2-m-tall Douglas firs, *Pseudotsuga menziesii* Douglas, at Mission, BC, Canada. Treatments were applied by cutting a frill with a scalpel, just above the soil. The frills were made at a sharp downward angle, so as to just sever the cambium and the outermost growth ring(s). Neem EC was introduced into the length of the frill with a syringe. Three other trees were similarly treated with a formulation containing no neem (methanol only). After 2 d, the top 20 cm of the terminal leader, ≈ 50 cm² of bark from the midpoint of the stem were collected from each of three neem-treated trees. The remaining neem-treated and control trees were sampled after 7 d.

The samples were oven-dried at 50°C, pulverized in a Waring blender, and 10–30 g of each were soaked in 200 ml of methanol for 3 d. The methanol extracts were filtered, and reduced to oily solids using a rotary evaporator. These residues were redissolved in 2 ml methanol and analyzed for azadirachtin content by high-performance liquid chromatography (HPLC) (Isman et al. 1990).

Field Test of Neem for Mountain Pine Beetle Control. In July 1993, baits containing the aggregation pheromone of mountain pine beetle (Phero Tech, Delta, BC, Canada) were placed on 50 lodgepole pine trees at an infested site near Lyne Creek, near Williams Lake, BC. The baits were distributed in a grid, with baited trees ≈ 50 m apart. Neem treatments were applied in the first week of August, 1993, 1–2 wk after mass beetle flight and attack. Only trees showing signs of heavy, successful beetle attack were used. Most baited trees were surrounded by other heavily attacked trees, some of which were also utilized in the test. Test tree diameters at breast height varied from 17.5 to 41.3 cm, with a mean of 27.2 ± 4.2 (SD) cm, and did not differ between treatments (analysis of variance; $F = 0.42$; $df = 6, 47$; $P = 0.86$). Treatments were applied by forming a frill with an axe, near the base of a tree. Test solutions were applied into the frill. Seven treatments were used: frill only, methanol only, monosodium methane arsenate (Glowon Liquid Tree Killer, Later Chemicals, Richmond, BC Canada) at ≈ 2 ml/cm circumference, and neem at 1,000, 5,000, 12,500, and $3 \times 12,500$ ppm. The three lowest neem doses and methanol were applied at 50 ml per tree; 150 ml of solution was used for the highest dose. The neem treatments thus corresponded to 0.05, 0.25, 0.63, and 1.9 g azadirachtin per tree. Each treatment was applied to eight trees. Monosodium methane arsenate is a highly viscous liquid, the methanol-based neem solutions were prone to spreading rapidly and running out of the frills.

In the second week of October, two 175-cm² bark samples were removed at breast height from the west- and east-facing sides of each tree. The bark was cut with a gas-powered hole-cutting saw into the first layers of xylem and pried loose using chisels so that the bark remained attached to a base of wood.

Bark samples were stored in the dark at $\approx 10^\circ\text{C}$ and 80% RH until they could be dissected. At dissection, the numbers of surviving and dead larvae, the total lengths of adult galleries, and the weights of surviving larvae were recorded for each sample. Sample values for each pair of bark disks were pooled to achieve a single value per tree. In an attempt to correct for different attack rates on different trees, the numbers of surviving larvae were also expressed in terms of number of larvae per 10 cm of adult gallery.

In the second week of November, single 175 cm² disks of bark were removed at 10 m height from five trees each of the highest neem dose, the methanol control, and the monosodium methane arsenate treatments. The discs were dissected as previously described. Meter-long sections of the bole were also collected from five trees each of the methanol control and the highest neem treatments. The sections were cut from directly above the frills into which the treatments had been applied and were stored indoors in individual cages at 22°C and a photoperiod of 12:12 (L:D) h. After adult beetles had begun emerging from the caged log sections (January, 1994), 625-cm² sections of the logs were peeled and the numbers of emergence holes, live larvae, pupae, and adults were determined. Emerged adults within the cages were examined for developmental irregularities.

Data were analyzed by one-way ANOVA (Anonymous 1991) and subsequent Bonferroni multiple comparison tests or by *t*-test (Zar 1984). Mortality data were arcsine-square root transformed. All data are presented as treatment means.

Results

Azadirachtin Translocation in Conifers. Azadirachtin was detected by HPLC in terminal twig samples at both 2 and 7 d after application (Table 1). The concentrations averaged 313 ppm at 2 d and increased to 5,742 ppm after 1 wk. Results for the bark samples were less consistent, with one sample yielding 450 ppm after 2 d and two with no detectable azadirachtin. Only 120 ppm was detected in the 7-d bark extract. Azadirachtin levels in the needle samples could not be determined reliably because of confounding peaks from other compounds. Azadirachtin was not detected in any of the control twig or bark samples.

Table 1. Upward translocation of azadirachtin in young Douglas fir trees

Treatment	Area of tree		
	Bark	Terminal twig	Needles
Neem-less control			
Tree 1	ND ^a	ND	ND
Tree 2	ND	ND	ND
Tree 3	ND	ND	ND
2 d after neem introduction			
Tree 4	450	351	ND
Tree 5	ND	323	322
Tree 6	ND	266	2,183
7 d after neem introduction			
Trees 7, 8, 9 pooled	120	5,742	ND

Values are ppm azadirachtin detected after introduction of 1 ml of neem EC at 50,000 ppm azadirachtin per tree, or solvent and emulsifier only.

^a ND, not detected. Limit of detection was ≈ 10 ppm.

Field Test of Neem for Mountain Pine Beetle Control. Direct applications of neem EC to lodgepole pine attacked by mountain pine beetle caused decreases in the numbers of surviving larvae per unit area of bark and numbers of surviving larvae per unit area of adult gallery length, and an increased proportion of dead larvae at breast height relative to sham (frill treatment), and solvent-only controls (Figs. 1, 2). Effects increased in a dose-dependent manner. The effects of the highest neem dose tested, 1.9 g (AI) azadirachtin per tree, were less than those caused by applications of monosodium methane arsenate at the recommended dose. Neem did not affect total adult gallery length or weight of surviving larvae, but both measurements were significantly lower in the monosodium methane arsenate treatment ($F = 2.73$; $df = 6, 47$; $P = 0.02$). Neem treatment did not kill the adult mountain pine beetles within the galleries but monosodium methane arsenate did.

The negative effects of the highest neem dose and monosodium methane arsenate on mountain pine beetle larvae were not significant at 10 m above the application frill; however, values of larval numbers and density showed the same trend as those that were significant at breast height (Fig. 3). There was also no difference in total adult gallery length (methanol control: 27 cm; 1.90 g (AI) azadirachtin: 32 cm; monosodium methane arsenate: 18 cm [ANOVA, $F = 0.85$; $df = 2, 12$; $P = 0.45$]). There were not enough dead larvae found to allow comparisons of percentage mortality and not enough surviving larvae to allow comparisons of larval weight.

Fewer adult beetles emerged from the caged log sections from the neem-treated trees than from those treated with methanol only (0.8 ± 0.5 per 625-cm² bark sample versus 16 ± 7.2 in controls [t-test, $t = 4.73$, $P = 0.009$]). The total numbers of emerged and nonemerged but surviving

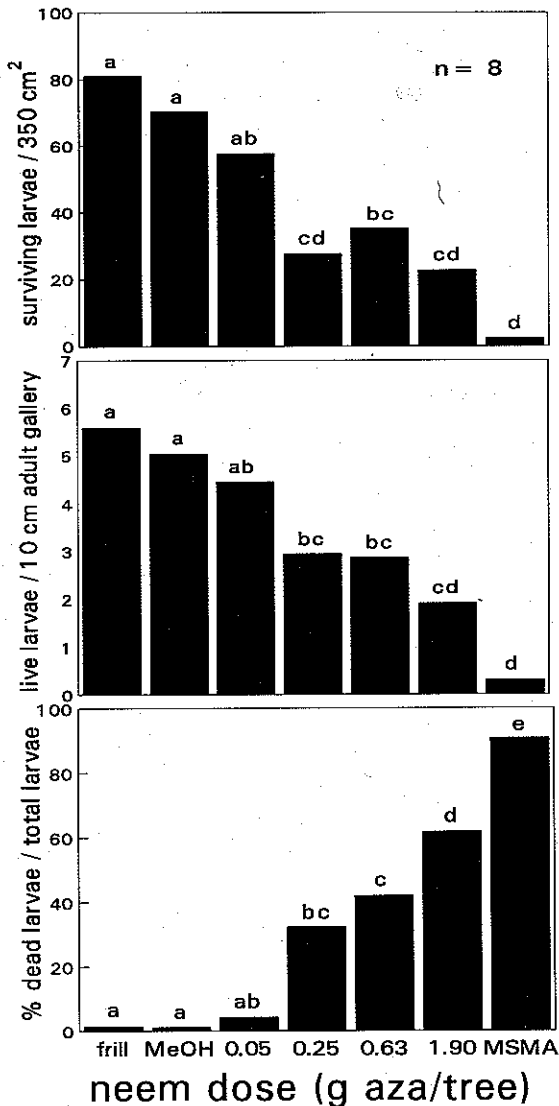


Fig. 1. Mean numbers and relative densities of surviving larvae and percentage mortality of beetle larvae 10 wk after host trees were treated with different doses of neem, monosodium methane arsenate (MSMA), solvent only (MeOH), or a sham operation (frill). Samples were collected at breast height. Larval densities are mean numbers of larvae per 10 cm of adult gallery length and are based upon a 350-cm² sample of bark per tree. Mortality data were for visible live or dead larvae and did not include unoccupied larval galleries. Columns marked by different letters are significantly different (ANOVA, Bonferroni multiple comparison test, mortality data were arcsine-square root transformed).

individuals were also less in the neem-treated logs (2.2 ± 2.8 per 625-cm² bark sample versus 21.6 ± 7.7 in controls [$t = 5.32$, $P = 0.003$]). No malformations typical of neem-induced growth regulation effects were seen in any of the emerged adults.

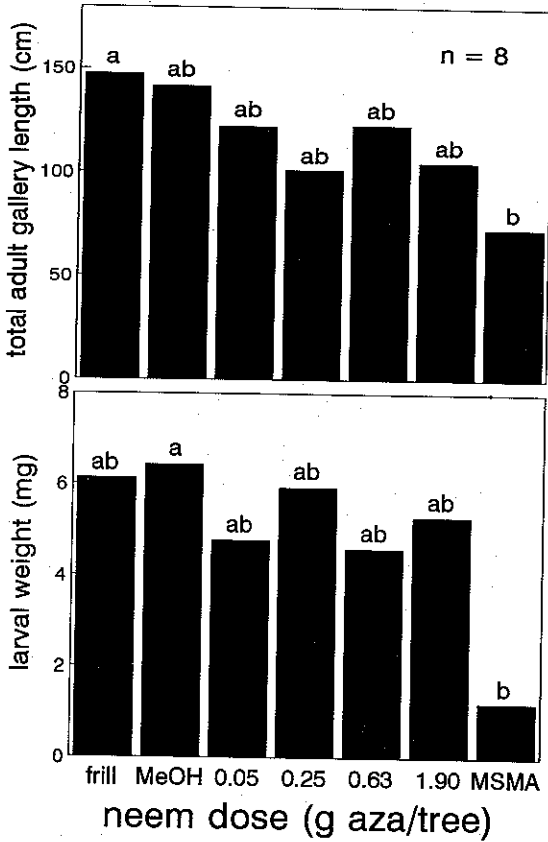


Fig. 2. Total adult gallery length per 350-cm² bark sample and larval weight of surviving larvae 10 wk after host trees were treated with different doses of neem, monosodium methane arsenate (MSMA), solvent only (MeOH), or a sham operation (frill). Samples were collected at breast height. Columns marked by different letters are significantly different (ANOVA, Bonferroni multiple comparison test). For larval weights, sample sizes were frill, 8; MeOH, 8; 0.05 g, 8; 0.25 g, 7; 0.63 g, 8; 1.9 g, 4; MSMA, 2.

Discussion

The results of this study suggest that neem seed extract has potential for the control of localized outbreaks of mountain pine beetle in lodgepole pine, although it is not as efficacious nor as reliable as monosodium methane arsenate using the formulation and concentrations tested here. Reductions in numbers of beetle larvae after neem treatment are the result of upward translocation of azadirachtin from the point of application. Translocation of azadirachtin (or the toxic effects of neem extracts) has previously been reported to occur in vegetable crops (Osman & Port 1990) and in birch, *Betula* (Marion et al. 1990).

Neem-induced mortality may have continued to occur after the initial field samples were taken; compared with methanol controls, a 90% reduction in the numbers of beetles emerged or still living was observed in the caged log sec-

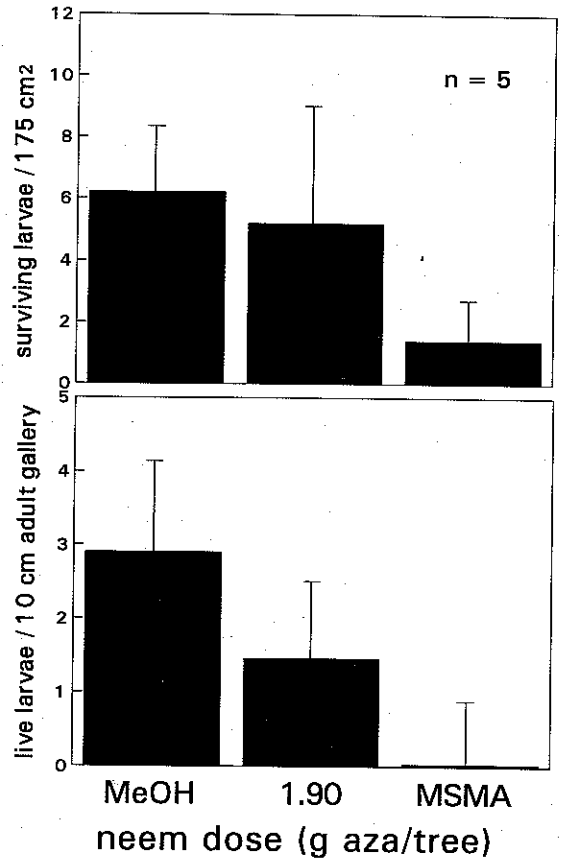


Fig. 3. Mean \pm SEM numbers and relative densities of surviving beetle larvae and percentage mortality of beetle larvae 14 wk after host trees were treated with neem, monosodium methane arsenate (MSMA), or solvent only (MeOH). Samples were collected from 10 m above ground. Larval densities are mean numbers of larvae per 10 cm of adult gallery length and are based upon a 175-cm² sample of bark per tree.

tions from the trees treated with the highest neem dose versus a 68% reduction at the first field sample. Any growth regulation effects of the neem treatments were not carried through to eclosion, because no deformed adults were seen.

The level of mountain pine beetle control achieved by the highest neem dose used in this study was inconsistent and therefore not satisfactory for commercial use. The highly fluid, methanol-based formulation may have run out of the frills on some trees, with the result that little neem was actually acquired and translocated in those trees. Efficacy might increase by using a higher dose or by producing a thicker formulation. A thicker product would be less prone to run out of the frill and would be available for entry into the vascular tissues for a longer period of time. Alternatively, azadirachtin may not remain in the trunk tissues long enough to affect all larvae before being carried farther up the tree.

Azadirachtin may also be more rapidly broken down than monosodium methane arsenate.

Near-total extermination of mountain pine beetle within treated trees may not be required for the control of spot infestations; the spread of an outbreak can be slowed or halted if beetle numbers are reduced to levels where adult attack densities are insufficient to overcome tree defenses. Control may be achieved even if some larvae survive insecticide applications. Thus, a more consistent neem effect, possibly from a thicker formulation, may provide an effective alternative to monosodium methane arsenate for the control of spot infestations of mountain pine beetle.

Effects of the higher neem doses were apparent only as larval mortality, unlike the monosodium methane arsenate treatment, which also killed the adults. Effects of neem and monosodium methane arsenate on unhatched eggs were not evaluated.

The cost of neem products (currently estimated at U.S.\$2–3 per g of azadirachtin) prohibits their use over large areas. However, spot applications of neem, if improved in efficacy, may be suited to localized control of mountain pine beetle outbreaks. Currently, control of such outbreaks utilizes ground crews who treat previously baited trees and overflow attacked trees with monosodium methane arsenate, or fall and burn infested trees. Monosodium methane arsenate-treated trees are normally cut down several weeks after application. In British Columbia, the cost per tree under such a system ranges from U.S.\$3–4 for sites with road access to U.S.\$9–11 for sites requiring helicopter access. Costs for inaccessible sites can be as high as U.S.\$17 per tree. This includes costs of checking lines, injection, and chemicals but does not include initial baiting and probing costs. In 1991, monosodium methane arsenate cost \approx U.S.\$10 per liter. With an average tree breast height diameter of 45 cm, and a monosodium methane arsenate application at the rate of 1 ml/2.5 cm of circumference, the actual monosodium methane arsenate cost per tree was less than U.S.\$1. Probable neem costs of U.S.\$3–6 per tree would result in a doubling of total treatment cost per tree in easily accessible sites and approximately a one-third increase in more remote sites. However, these increased costs may be offset by the increased safety to the applicators and the likely decrease in detrimental effects on nontarget organisms (Hoelmer et al. 1990, Stark 1992, McCloskey et al. 1993). Improved formulations may also decrease the amount of neem required for more effective control.

Further work is required to determine if different rates and formulations can improve the efficacy of neem for controlling mountain pine beetle and if neem controls other bark beetle species.

Acknowledgments

We thank M. Bomford, N. Brard, C. Haeussler, D. Heppner, and S. F. Seward for help with this project. Funding was provided by the British Columbia Ministry of Forests, a postdoctoral fellowship from the Natural Sciences and Engineering Research Council of Canada (NSERC) to K.N., and NSERC grants to M.B.I.

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Received for publication 10 February 1994; accepted 20 July 1994.