EVALUATION OF A NEEM-BASED INSECTICIDE FOR CONTROL OF THE MOUNTAIN PINE BEETLE.

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Marnie A. Duthie
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Name: Marnie Duthie

Degree: Master of Pest Management

Title of Thesis:

EVALUATION OF A NEEM-BASED INSECTICIDE FOR CONTROL OF THE MOUNTAIN PINE BEETLE.

Examining Committee:

Chair: Dr. G. Gries, Associate Professor

Dr. J. H. Borden, Professor, Senior Supervisor
Department of Biological Sciences, SFU

Dr. R. Nicholson, Associate Professor
Department of Biological Sciences, SFU

Dr. M. Isman, Professor
Department of Plant Science, UBC
Public Examiner

Date Approved: 21 April 1997

ABSTRACT

Mountain pine beetles (MPBs), *Dendroctonus ponderosae* Hopkins (Coleroptera: Scolytidae), are the most damaging forest insect in British Columbia, causing millions of dollars of damage yearly, particularly in lodgepole pines, *Pinus contora* var. *latifolia* Engelmann. Recent research suggests that MPBs may be controlled systemically by extracts from seeds of the neem tree, *Azadirachtin indica* A. Juss. (Meliacaeae). However, the extent of translocation in the bole of lodgepole pines has not been determined. Because attacks by the mountain pine beetle often do not occur high on the bole of standing trees, the extent of translocation was investigated by using pine engravers, *Ips pini* Say, as an indicator species for the presence of bioactive levels of neem constituents in trees that were treated and then felled. A proprietary emulsifiable concentrate formulation of neem seed extract containing 20,000 ppm azadirachtin was applied into a basal axe frill around the root collar of lodgepole pines. After one week the trees were felled and the logs were baited at 3, 9, and 15 m from the butt with the pheromone ipsdienol to induce attack by *I. pini*. Six weeks later bolts were removed from the trees at the bait positions, and the populations of beetles were evaluated after a further 16 weeks in rearing. At 3 and 9 m from the butt, populations of *I. pini* (emerged and remaining alive under the bark) were reduced by 87 and 77%, respectively, indicating that the active ingredients translocated at least 9 m up the bole and persisted for at least six weeks. Numbers of beetle holes in the bark were also significantly reduced by 89, 88, and 63% at 3, 9, and 15 m, suggesting that translocation extended beyond 9 m. Neem treatment, systemically or topically applied, had no impact on gallery construction and attack density, but significantly reduced the number of progeny per egg gallery. For two different emulsifiable concentrates, one containing DMSO and another with a proprietary formulation, acute toxicity tests in the laboratory resulted in respective LC_{50} values of 65 and 33 ppm azadirachtin, but these were confounded by excessive mortality caused by the constituents in the emulsifiers. In lodgepole pines, these constituents may be rapidly translocated into the crown. Crude neem oil and corresponding antennally-active volatiles did not cause repellency in baited multiple-funnel traps against both the MPB and *I. pini*. Since systemic application of a neem-based insecticide was effective at reducing the numbers of adult *I. pini* and the number of beetle holes up to 9 and 15 m from the butt,

respectively, it can be extrapolated that neem could be used operationally to as control tactic against mountain pine beetles. Neem could be effective in lethal trap tree treatments since it possesses no repellent or anti-feedant effects but effectively reduces the number of emergent broosd beetles from treated trees. Neem extracts, if used against the MPB, would have the following advantages over MSMA, the currently used systemic arsenical: virtually no toxicity to vertebrates and other on non-target organisms; short residual activity in the environment due to degradation by ultraviolet rays, heat, moisture, and, pH; and very low chance of resistance developing, possibly attributed to neem's multiple modes of action and the fact that only a small percentage of the total number of infested trees in a given year would ever be treated.

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1.0 INTRODUCTION

1.1 MOUNTAIN PINE BEETLE

1.1.1 PEST SIGNIFICANCE

The mountain pine beetle (MPB), *Dendroctonus ponderosae* Hopkins, is native to North America (Furniss & Carolin 1980). Since the early 1970's, MPBs have killed millions of trees per year throughout their range, in British Columbia and Alberta, through the Western United States, to northern Mexico (Furniss & Carolin 1980; Borden 1990). In British Columbia (BC), the MPB is the most important insect pest of lodgepole pine, *Pinus contorta* Douglas var. *latifolia* Engelmann (Cole & Amman 1980; Furniss & Carolin 1980; Maclauchlan & Brooks 1994).

Lodgepole pine is the most abundant conifer species in BC, accounting for 25% of available timber volume and 38% of yearly harvest (Miller *et al.* 1993). Therefore, large-scale mortality of lodgepole pine threatens local, regional, and national economies (Cole *et al.* 1985; Hall 1985). As of 1985, the total cumulative mortality of pines in BC caused by MPB damage was estimated at 50,000,000 m^3 (Hall 1985). Between 1972 and 1985, MPB killed approximately 195.7 million pines in BC, representing a postulated $14.4 - 19.6 billion (Canadian) loss to the economy (Klein *et al.* 1978). MPB continues to damage pines in B.C.; for example, in 1992 beetle-killed pines totaled 2.3 million m^3, equaling 18% of the annual lodgepole pine harvest, and nearly 11 times the area lost to forest fires (Wood & Van Sickle 1993). In addition to timber and economic losses caused by MPB, fire hazard is increased, wildlife habitat is affected, and recreation areas are significantly degraded (Shea *et al.* 1992).

1.1.2 BIOLOGY

MPB females, the pioneer sex, begin to fly in search of suitable hosts, in mid-July to early August, during high pressure and good weather conditions (Raffa 1987; Young 1988). Females tend to select physiologically-weakened trees when beetle populations are low, but as population densities reach outbreak levels, almost any host tree can be successfully colonized, because the beetles are numerous enough to overcome the host trees' defenses (Furniss & Carolin 1980; Koehler *et al.* 1978). MPBs preferentially

attack mature, large-diameter trees, >80 years old (Furniss & Carolin 1980; Borden *et al.* 1983b; Hall 1985). Such trees have thick phloem providing an abundant food resource (Cole & Amman 1969, 1980; Safranyik *et al.* 1974; Borden 1990; Shore & Safranyik 1992), and have less defensive resin production than younger trees (Koehler *et al.* 1978; Young 1988). As an infestation expands and the dominant trees are killed, MPBs will also attack intermediate and suppressed trees within a stand (Young 1988). MPB attacks occur along the bole of host trees, from ground-level up to the middle branches (Furniss & Carolin 1980).

Attacks are not always successful. Host tree resin flow may impede or halt attack altogether if there is a delay in colonization after initial attack by the pioneer female (Raffa 1988; Birch 1978; Borden *et al.* 1987). However, if massive numbers of beetles bore into the bark and inoculate the tree with their symbiotic fungi, an attack is usually successful (Safranyik *et al.* 1974; Birch 1978; Young 1988). Fungal establishment overcomes the tree's resistance by blocking xylem translocation, reducing the flow of constitutive resin, weakening the tree's ability to produce traumatic resin, and thus to restrict the advance of the fungal mycelium (Safranyik *et al.* 1974; Raffa 1988). Fungal spores are carried on the surface of beetles and in specialized maxillary mycangia (Whitney & Farris 1970). In the pupal chamber, fungi and yeasts are important as a food source for newly emergent adults (Safranyik *et al.* 1974).

Once established in the tree female MPBs excavate egg galleries, 30-90 cm long, vertically within the phloem, depositing 60-80 eggs singly in niches on alternate sides of a gallery (Furniss & Carolin 1980; Raffa 1988). In 2-3 days the eggs hatch and larvae begin feeding in tunnels excavated at right angles to the egg galleries (Furniss & Carolin 1980). Larvae pass through three molts, usually overwintering as third or fourth instars (Reid 1962). In the spring, fully grown larvae construct small oval cells at the ends of their mines, in which they pupate, and eclose later as callow adults (Furniss & Carolin 1980; Young 1988). After approximately a month of maturation feeding, brood adults emerge and seek new hosts (Reid 1962; Furniss & Carolin 1980; Young 1988). MPBs are usually univoltine, but may require two years to develop in cold climates or may undergo two generations and sometimes even a partial third generation in warm areas (Furniss & Carolin 1980).

Natural mortality factors which affect MPBs include: sub-zero winter and extremely high or low temperatures; nematodes; woodpeckers; and entomophagous insects (Reid 1958 & 1963; Safranyik *et al.* 1974; Furniss & Carolin 1980). The effectiveness of biotic mortality factors decreases as MPB populations increase to outbreak levels (Furniss & Carolin 1980).

As attacking beetles penetrate the bark, they server resin ducts, causing the release of large quantities of constitutive resin (Koehler *et al.* 1978; Young 1988). As it exudes from the entrance hole, which is kept open by the beetles, and begins to crystallize, this resin forms a characteristic pitch tube (Koehler *et al.* 1978; Furniss & Carolin 1980; Young 1988). These cream-colored pitch tubes aid in the early identification of MPB infestations, when ground surveys (beetle probes) are conducted (Maclauchlan & Brooks 1994). By late June in the year following attack the foliage on most of the attacked trees turns red. At this time aerial surveys are beneficial in detecting the previous years attack (Maclauchlan & Brooks 1994). In the second year after attack the discolored foliage eventually drops from the tree resulting in a dead tree that is grayish in color (Koehler *et al.* 1978; Furniss & Carolin 1980; Young 1988).

In natural ecosystems, MPB and wild fires serve to remove mature pines. However, effective fire suppression has created extensive stands of large mature pine, ideal for MPB outbreaks (Hall 1985; Borden 1990; Maclauchlan & Brooks 1994). In addition, harvesting in the first half of this century ignored lodgepole pine, which was considered a weed species. Because lodgepole pine was not favored, there were few roads that accessed these stands. Consequently when MPB populations began to rise in the 1970's to levels greater than any recorded in history they encountered vast areas of susceptible pine in inaccessible stands, where management of the beetle was virtually impossible (Borden 1990).

1.2 *IPS PINI*

Pine engravers, *Ips pini* (Say), are secondary bark beetles, widely distributed in North America, that characteristically attack downed trees along the entire length of the bole, unlike MPBs which only attacks live standing trees (Thomas 1961; Bright 1976; Livingston 1979). They commonly infest the thin-bark portions of logging slash, cull logs, windthrown, and dying trees. However, when populations are

high, e.g. when they are allowed to build up in downed stems after a pre-commercial thinning, *I. pini* can kill groups of trees, especially in unthinned young stands (Livingston 1979).

Male beetles are the pioneers. They penetrate the bark, construct nuptual chambers in the phloem tissue (Anderson 1948; Thomas 1961) and release an aggregation pheromone comprised of ispdienol (Plummer *et al.* 1976) and lanierone (Teale *et al.* 1991). Adult males are polygamous, mating with 1-8 females (Anderson 1948; Thomas 1961; Schmitz 1972). After mating, females begin construction of egg galleries, which radiate from the nuptual chamber and become more or less parallel to the wood grain (Thomas 1961; Schmitz 1972), typically forming an overall X or Y gallery pattern (Bright 1976). Individual egg niches are cut alternately on each side of the egg gallery, with hatching taking place about 6-10 days following oviposition (Anderson 1948; Thomas 1961; Bright 1976; Schmitz 1972).

Larvae then begin to mine at right angles to the egg gallery, mostly in the phloem tissue (Thomas 1961; Schmitz 1972; Bright 1976). Larvae proceed through two molts, before constructing pupal cells. The pupal stage lasts from 7-10 days before the callow adults eclose (Thomas 1961; Bright 1976). Callow adults usually feed in the inner bark for 2-4 weeks before emerging and invading new feeding or breeding material (Anderson 1948; Thomas 1961). *Ips pini* usually has two broods a year in BC. The first generation attacks in mid- to late-May and produces the first brood. Parents re-emerge in mid-June and produce a second brood (Thomas 1961; Bright 1976; Miller 1990).

1.1.3 CONTROL TACTICS FOR THE MPB

Strategies for management of the MPB may involve long-term, ecologically-based tactics which reduce stand susceptibility, or short-term direct control tactics which reduce infestation expansion, providing time for long-term tactics to be implemented (Hall 1985). Short-term tactics primarily include removing as many beetles as possible by either sanitation-salvage clearcutting (Safranyik *et al.* 1974; McMullen *et al.* 1986; Borden 1990) or partial cutting (Cahill 1978), and disposing of single infested trees. These trees are either cut and burned (Klein 1978; Whitney *et al.* 1978; Borden 1990), cut and sprayed with insecticides (including over the years DDT, ethylene dibromide, orthodichlorobenzene, lindane, chlorpyrifos, and carbaryl) (Bedard 1938; Craighead & St. George 1938; Kinghorn 1955; Smith *et al.* 1977;

Cole & Amman 1980; Fuchs & Borden 1985), or injected while still standing with systemic insecticides such as monosodium methanearsonate (MSMA) and cacodylic acid (Kinghorn 1955; Hall 1985; Maclauchlan *et al.* 1988; Manville *et al.* 1988; Borden 1990; Maclauchlan & Brooks 1994).

1.1.4 ALTERNATIVE CONTROL OF MPB

The only direct chemical agent used operationally to control the MPB in BC is MSMA (Holsten 1985). MSMA is applied in liquid formulation into an axe frill around the base of a recently-attacked tree. It then translocates up the xylem and kills developing eggs and larvae (Maclauchlan *et al.* 1988). To be completely effective, arsenicals must be applied within three weeks of MPB attack (Stevens *et al.* 1974). Thereafter, fungal growth in the sapwood will inhibit translocation, rendering MSMA increasingly ineffective (Newton & Holt 1971; Maclauchlan *et al.* 1988). Pre-attack treatment with MSMA simply killed the trees, which were then ignored by the MPB and attacked by *Ips* spp. (J.H. Borden pers. comm.).

Despite MSMA's efficacy if used properly, it is a simple derivative of arsenic acid (Wauchope & Yamamoto 1980), and is therefore a potentially environmentally insensitive chemical, possessing lengthy residual activity and moderate toxicity to vertebrates (B.C. Ministry of Forests 1989). Thus, use of MSMA is restricted to uninhabited areas, at least 10 m away from water bodies.

An alternative to MSMA may be needed, even if there are no demonstrable adverse environmental effects. One possible alternative, that may displace MSMA and provide an additional pre-attack treatment option, are the extracts from the seeds of neem trees, *Azadirachta indica* A. Juss. (Meliacaeae).

1.3 NEEM

The insecticidal and growth regulation properties of *A. indica* (syn. *Melia indica* Brandis, *Melia azadirachta* L., and *Melia parviflora* Moon.), have elicited great interest world-wide (Saxena 1989; Schmutterer 1990; Govindachari 1992; Stone 1992; Mohan Ram & Nair 1993; Quaries 1994). The neem tree is a hardy, broadleaf, evergreen, related to mahogany (NRC 1992; Ley *et al.* 1993). It can grow in dry nutrient-poor soils (NRC 1992; Ley *et al.* 1993). It is native to arid areas of the Indian subcontinent at elevations <1000 m (Koul *et al.* 1990; Schmutterer 1995), but has been introduced into the tropical and

subtropical zones of Asia, Africa, Australia, the Americas, and the South Pacific Islands (NRC 1992; Quaries 1994; Schmutterer 1995). The Board on Science and Technology of the International Development Research Council of Canada (BOSTID) (1992) believes that neem extracts may have wide acceptance in future pest control, based on their biorational qualities including: 1) efficacy against various pests; 2) multiple modes of action; 3) systemic action in numerous plant species; 4) apparent non-toxicity to vertebrates; and 5) traditional use over many centuries by indigenous cultures (Larson 1987).

1.3.1 EFFICACY OF NEEM

Neem extracts have proven to be effective against > 200 insect species, representing the orders Orthoptera, Heteroptera, Homoptera, Thysanura, Hymenoptera, Coleoptera, Lepidoptera, and Diptera (Schmutterer 1990; Shiparo *et al.* 1994), as well as some mites, nematodes, fungi, bacteria, protozoa, and even a few viruses (BOSTID 1992; NRC 1992; Ishida *et al.* 1992; Stone 1992; Ascher 1993; Mordue & Blackwell 1993). Efficacy has been demonstrated for > 90% of the species tested (Isman *et al.* 1990; NRC 1992). Among the coleoptera affected by neem are representatives of the families Scarabacidae, Chrysomelidae, Tenebrionidae, Curculionidae, and Scolytidae (Jilani & Malik 1973; Steets & Schmutterer 1975; Steets 1976/77; Ladd *et al.* 1978; Reed *et al.* 1982; Jilani & Saxena 1990; Kaethner 1991; Beitzen-Heineke & Hofmann 1992; Kaethner 1992; Naumann *et al.* 1994; Palaniswamy & Wise 1994; Schmutterer 1995; Xie *et al.* 1995).

1.3.2 MULITPLE MODES OF ACTION

Pesticidal activity has been found in all parts of the neem tree (Quaries 1994). However, seeds show the highest concentrations of activity. Many of the biologically active compounds are triterpenoids, specifically limonoids (Jones *et al.* 1989; Koul *et al.* 1990; NRC 1992; Ley *et al.* 1993). New limonoids are still being isolated from the neem tree, but azadirachtin, salannin, meliantriol, and nimbin are the best known and most biologically significant (NRC 1992). Of these, azadirachtin is the single most important compound in terms of insecticidal activity (Govindachari 1989; Rembold 1989; Isman *et al.* 1990; Mordue & Blackwell 1993; Quaries 1994). However, extracts containing limonoid mixtures may be more effective

than azadiracthin alone (Mordue & Blackwell 1993; Quaries 1994), and neem oil possesses more insecticidal properties than pure azadirachtin (Mordue & Blackwell 1993). Azadirachtin itself is not a single substance, but a mixture of at least nine closely related chemical and structural isomers, of which azadirachtin A has been shown to comprise 83% in some cases (Jones *et al.* 1989; Rembold 1989; Isman *et al.* 1990; Govindachari 1992; Ley *et al.* 1993; Mordue & Blackwell 1993; Quaries 1994). The chemical and physical properties of azadirachtin isomers are so closely related that it was not until 1983 that the mixture was detected (Rembold 1989). Neem, including azadirachtin and the other limonoids, has two main modes of insecticidal action, morphological disturbance (at low concentrations, specifically 1-5 ppm for the Mexican bean beetle), and feeding deterrency (at high concentrations, specifically 10-100 ppm for the Mexican bean beetle) (Rembold 1989).

Morphological disturbances occur mainly in immature insects, on which neem acts as an insect growth regulator (IGR) (Stone 1992; Quaries 1994). It decreases growth in general (Saxena 1989), inhibits chitin synthesis (Stone 1992; Quaries 1994), interrupts ecdysis (Schmutterer 1990), and increases mortality at molting (Schmutterer 1990). In adult insects, neem can decrease fecundity (Schmutterer 1987; Rembold 1989; Mordue & Blackwell 1993; Nisbet *et al.* 1994; Stark & Rangus 1994) by inhibiting egg maturation (Schmutterer 1987) and inducing sterility (Govindachari 1989; Schmutterer 1990; Pathak & Krishma 1991).

Because azadirachtin and the insect molting hormone, ecdysone are structurally similar (Rembold 1989; NRC 1992; Stone 1992; Ascher 1993), azadirachtin is often called an anti-hormone compound (Rembold 1989). Azadirachtin is able to cause inhibition of metamorphosis mimicking the ecdysteroid metabolite of ecdysone involved in the hormone titre feedback control (Rembold 1989; Stone 1992). The main site of action, however, is not the prothoracotropic gland, which is the site of ecdysone synthesis, but rather the neurosecretory cells of the brain, which synthesize the prothoracicotropic hormone (Rembold 1989; Isman 1994). In larval insects, azadirachtin causes reduced and delayed synthesis of both juvenile hormone and ecdysone (Rembold 1989); in adults, it affects only the level of ecdysone (Sieber & Rembold 1983). In the immature stages metamorphosis is affected. In adults ovarian development is reduced and sterility occurs due to prevention of vitellogen production (Rembold 1989; Ascher 1993).

Azadirachtin can also inhibit digestion and utilization of ingested proteins (Timmins & Reynolds 1992). Growth is decreased because trypsin activity in the midgut is diminished, thereby affecting an insect's ability to digest protein (Timmons & Reynolds 1992).

Neem functions as both a contact and a systemic pesticide (Olkowski *et al.* 1991). The systemic activity of neem enables it to be used in controlling sucking insects, and cryptic stem and root-feeding pests (Isman *et al.* 1991), such as the MPB when neem formulation is applied into an axe frill at the base of an attacked lodgepole pine (Naumann *et al.* 1994).

Neem also acts as a repellent and a feeding deterrent (Whitehead & Bowers 1983; Taylor 1984; Schmutterer 1990; Serit *et al.* 1992; Ascher 1993; Mordue & Blackwell 1993; Quaries 1994), rendering plants unattractive or unacceptable to insects (Lowery & Isman 1993). Certain insects will starve to death rather than eat neem-treated plants (Taylor 1984).

The long-range repellent effect of neem may reside in the organosulfur constituents principally derivatives of di-*n*-propyl- and *n*-propyl-1-propenyl di-, tri-, and tetrasulfide that make up the majority of neem volatiles (Balandrin *et al.* 1988; Saxena 1989) and give neem seed extracts their characteristic garlic odor (Balandrin *et al.* 1988). The similarity between neem volatiles and those of onions, *Allium sativum* L. (Amonkar & Banerji 1971), suggests that similar biosynthetic pathways occur in both species (Balandrin *et al.* 1988).

The feeding deterrent effect of neem is caused by azadirachtin (Blaney & Simmonds 1995), the most potent natural insect antifeedant discovered to date (Isman *et al.* 1990). In addition to suppressing feeding behavior (Taylor 1984), azadirachtin may act on the gut musculature, inhibiting swallowing and peristalsis (Mordue *et al.* 1985; Dorn & Trumm 1993; Blaney & Simmonds 1995). Neem also possesses a minor ingredient, deacetyladiraachtinol, that paralyzes the swallowing mechanism (NRC 1992). Despite the effectiveness of neem as a repellent, feeding deterrent, and digestive system inhibitor, herbaceous insects will move to untreated parts of neem treated plants and recover if plants are not re-treated (Rembold 1989). Mordue and Blackwell (1993) report wide differences in feeding deterrent sensitivity between insect species, whereas doses that cause growth regulatory effects remained relatively constant. Lepidoptera are

extremely sensitive (<1-50 ppm azadirachtin); coleoptera, hemiptera, and homoptera are less sensitive (100-600 ppm azadirachtin); orthoptera have a wide range of sensitivity (0.05-1000 ppm azadirachtin).

1.3.3 TRADITIONAL USE AND SAFETY

One of the most significant attributes of neem is that it is non-toxic to vertebrates (Radwanski & Wickins 1981), suggesting that its US registration for use on food crops, should be emulated elsewhere. For at least 4,000 years, various parts of the neem tree including the leaves, fruit, and bark, have been part of traditional Hindu folklore in India (Jacobson 1988; Stone 1992; Quaries 1994). The neem tree has been called the village pharmacy due to its numerous medicinal properties (Larson 1989; Koul *et al.* 1990; BOSTID 1992; Govindachari 1992; Stone 1992; Quaries 1994). Contained within neem seeds and leaves, are compounds that act as antiseptics, antiviral, anti-inflammatory, hypotensive, antiulcer, antiitch, antiindigestion, and antifungal agents (Patrao 1984; Mordue & Blackwell 1993). The antibiotic effects against the causative agents of tuberculosis, typhoid fever, cholera, and plague (Chopra *et al.* 1952) may be partially attributed to the organosulfur constituents (Balandrin *et al.* 1988; Olkowski *et al.* 1991). Neem leaves are specifically used to treat constipation, diabetes, sleeplessness, stomach aches (Patrao 1984), smallpox (Nadkarni & Nadkarni 1954), head lice, maggots in open wounds (BOSTID 1992), tetanus, eczema, and the early stages of leprosy (Govindachari 1992). Neem seed oil has been ingested as an antihelminthic, antiseptic, anti-inflammatory, and antimicrobial agent (Balandrin *et al.* 1988; Koul *et al.* 1990). Externally, neem seed oil was applied for treatment of acute eczema, scabies (Koul *et al.* 1990) and boils (Balandrin *et al.* 1988). Reduced tooth decay and inflammation of gums can result from chewing neem bark (BOSTID 1992), and ingestion of neem bark reduces fever and rheumatism (Govindachari 1992).

1.4 OBJECTIVES

Naumann *et al.* (1994) demonstrated that emergence of MPB brood was greatly reduced in logs taken at 1.3 m above the ground from lodgepole pines to which various neem concentrations corresponding to 0.25, 0.63 and 1.90 g azadirachtin per tree had been applied in a basal axe frill. They also found by chemical analysis that azadirachtin was translocated into terminal twigs and bark of similarly-treated Douglas-fir saplings within two days. However, no attempt has been made to measure the ability of mature lodgepole pine trees to translocate the constituents of neem, to determine the effect on brood survival, or to assess the toxicity or repellent-feeding deterrent properties for the MPB. Therefore, my objectives were to test three characteristics of neem seed extract related to their efficacy the MPB, specifically to:

1. determine the upward extent of translocation of the active ingredients in neem seed extract in lodgepole pine trees;
2. determine the toxicity of two neem seed extract emulsifiable formulations and the corresponding formulation controls without the active ingredient against MPB larvae; and
3. test the efficacy of crude neem seed extract and an emulsifiable-concentrate formulation as a repellent against MPBs.

2.0 METHODS AND MATERIALS

2.1 TRANSLOCATION

Because MPBs often do not attack trees above a height of 5-6 m, it is difficult to asses the height of translocated neem constituents by determining the success of MPB broods at different heights in neem-treated trees. Assessment by chemical methods (Naumann *et al.* 1994) would be possible, but would be expensive and laborious, and would not indicate if effective doses were present. Therefore, I elected to measure effective translocation by treating unattacked trees, allowing time for translocation to occur, felling the trees (terminating translocation) and then inducing attack by a secondary bark beetle, *I. pini*. The attacking *I. pini* would then be exposed to whatever dose of neem constituents remained at the given height

on the bole at the time of felling. It was necessary to use *I. pini* as an indicator species because MPBs attack only standing trees.

On 4 June 1996, 30 lodgepole pine trees [mean diameter (± SE) at breast height (dbh = 1.3m) and height of 22 measured trees were 22.3 ± 0.4 cm and 19.8 ± 0.3 m, respectively] were selected at a site 12.6 km southeast of Moffatt Lake on Redeau Road, near 150 Mile House, BC. The selection criteria were as follows: >15 cm dbh; not infested by bark beetles; free of cankers and other bole flaws that could inhibit translocation; and >10 m apart from one another. Ten trees were randomly assigned to each of three treatment groups: untreated control, formulation control and neem. There was no significant difference in dbh (GLM, F= 0.48, df= 21, *P*= 0.6264) or tree height (GLM, F= 1.33, df=21, *P*= 0.2871) between treatment groups. A frill was cut at 45°, just into the xylem tissue using a hatchet, around the entire circumference at the base of the bole of each tree (Frye & Wygant 1971; Lister *et al.* 1976). The formulation control consisted solely of an emulsifiable concentrate formulation and the neem treatment had 2% (20,000 ppm) azadirachtin added to the emulsifiable concentrate formulation (Neem International Enterprise, Surrey, BC, Canada). Oils had been removed using cold pressure and then proprietary processes were used to solvent extract azadirachtin from the seed meal. The ultimate result was a dry powder with concentrates of azadirachtin between 20-30% (Putland pers. comm.). The concentrated azadirachtin was then transported to the manufacturer and formulated into emulsifiable concentrates containing 3-5% azadirachtin as the active ingredient (Putland pers. comm.). The formulation control and neem treatments were applied into the frill with a hand-held plastic squirt bottle at a rate of 2 mL per 1 cm tree circumference. The mean dose in the neem treatment was 783.2 ± 2.0 mg of azadirachtin per tree.

One week later all 30 trees were felled and immediately baited at 3, 9, and 15 m, on the north or shaded side with bubble cap baits containing ipsdienol, an aggregation pheromone of *I. pini* (Phero Tech Inc., Delta, BC).. The felled trees were monitored weekly for *I. pini* attack, which occurred sporadically over the next six weeks. At this time (29 July 1996), 50 cm long bolts were taken at 3, 9, and 15 m, from the butt, where *I. pini* attack was present. The numbers of bolts removed for each treatment and height (3, 9, and 15 m), respectively were: untreated control 6, 8, and 8; formulation control 7, 7, and 7; and neem 6, 7, and 5.

All 61 bolts were transported to Burnaby, BC and placed into individual screen mesh cages held in a roofed outdoor enclosure. Emergence of *I. pini* was monitored intensively in August approximately every two days, then weekly in September and October, and monthly in November, over a four month period from the beginning of August to the end of November 1996. Bark surface area and number of holes including entrance, ventalation, and emergence holes, were measured and number and sex of *I. pini* at each collection was recorded. At the end of November, bark was striped from all bolts and any remaining *I. pini* adults were removed, counted, and sexed. Numbers of nuptual chambers and egg galleries for each bolt were counted and egg gallery length was measured.

Data were transformed by $\log_{10}(x+1)$ to satisfy criteria for normality and homoscedasticity (Zar 1994), and analyzed by GLM and the Ryan-Einot-Gabriel-Welsch (REGW) Multiple Q-test ($\alpha = 0.05$) (Day & Quinn 1984) to determine differences between means (SAS Institute Inc. 1988).

2.2 ACUTE TOXICITY

MPBs were collected from infested lodgepole pines felled in November 1995 and October 1996 from the valleys of Sunday and Willis Creek respectively, near Princeton, BC. Bark was striped from infested bolts and second and third instar larvae were removed and placed immediately on moistened filter paper until used later the same day.

Ground phloem medium was prepared, using methodology slightly modified from Bedard (1966) and Hunt (1987), from phloem tissue striped from trees felled in November 1995 and September 1996. Immediately after removal, phloem was oven-dried in four batches at 225 ^{o}C for approximately one day. Dried phloem was ground to a fine powder using a Willey Mill with a #12 screen. For an initial series of tests involving a neem formulation containing dimethylsulfoxide (DMSO) the ground phloem was also autoclaved at a temperature of 121 ^{o}C for 30 min and dried for an additional 15 min prior to adding dehydrated brewers yeast and either the formulation control or the neem containing formulation. Dehydrated brewers yeast (ICN Biomedical, Aurora, Ohio) and ground phloem were respectively combined in a 1:6, vol.:vol. ratio. The medium was transferred aseptically to 60 x 15 mm plastic petri dishes, 5 g per dish, and pressed gently into the bottom of the dishes.

Two proprietary emulsifiable-concentrate formulations, with no active ingredient or with neem seed extract added (20,000 ppm azadirachtin) were tested for toxicity to MPB larvae. The first formulation (Phero Tech Inc., Delta, BC) contained DMSO along with other proprietary compounds. No ingredients of the other proprietary formulation were disclosed by the manufacturer, Neem International Enterprises, Surrey, BC. Dilutions were made with distilled water, resulting in treatments of 0.01, 0.1, 1, and 10% DMSO formulation, 0.01, 0.05, 0.1, 0.5, 1, 5, and 10% for the other EC formulation, 2, 20, 200, and 2000 ppm azadirachtin in DMSO formulation, and 2, 10, 20, 100, 200, 1000, and 2000 ppm of azadirachtin in the other EC formulation. The untreated control consisted of only distilled water added to the phloem media. Aliquots of each dilution (2.5 mL) were dripped from a Pasteur pipette onto the pressed phloem medium in the petri dishes.

A MPB larva was transferred as aseptically as possible to each dish. The dishes were held in the dark at 22 ^{o}C. and the survival of 5, 10, or 20 larvae per treatment was evaluated for DMSO formulation blank, DMSO formulated neem, and proprietary EC control and formulated neem, respectively, after one week.

Percent moralities of MPB larvae were converted to probit values and concentrations were transformed to log concentrations (Chan & Hayes 1989; Nicholson pers. comm. 1996). Regression lines were fitted on probit curves (α =0.05) to determine the best-fitting line through the percent mortality at each of the log concentration (SAS Institute Inc. 1988). From the line of best fit the lethal concentration that killed 50% of the population (LC_{50}) was determined using the formula regenerated by the line. Because the emulsifiable concentrate formulations were toxic alone, data for the formulations with neem seed extract added were analyzed, as were corrected data with the mortality caused by the formulation controls deducted. Abbott's formula was used to subtract the effects of the formulation and the % mortality was again converted to probit values (Chan & Hayes 1989; Nicholson pers. comm. 1996).

2.3 REPELLENCY

2.3.1 VOLATILES FROM CRUDE NEEM OIL

Crude neem oil (Neem International Inc.) (approximately 500 mL) was placed in a modified 6 L Erlenmeyer flask. Charcoal-filtered air was drawn through the flask at 2 L per min for 8 days and the headspace volatiles were collected a Porapak Q (Pierce *et al.* 1981). Volatiles were eluted from the Porapak Q with 150 mL of pentane, and the eluent was concentrated to 2 mL by distilling off the solvent through a Dufton column. (Pierce *et al.* 1981). Captured neem oil volatiles were analyzed by coupled gas chromatographic-electroantennographic (GC-EAD) detection (Arn *et al.* 1975; Gries *et al.* 1993), using MPBs that emerged from infested lodgepole pine bolts collected in November 1995 at Sunday Creek, near Princeton, BC. All GC-EAD analyzes were performed by R. Gries, Chemical Ecology Research Group, S.F.U. Eight antennally active components were identified using coupled GC-mass spectroscopy and confirmed by GC-EAD analysis. Two of the 10 antennally active volatiles were not identified.

Three randomized, with 17, 10 and 7 replicates respectively, complete block trapping experiments were conducted from 12 August to 1 October 1996 in clearcuts adjacent to MPB-infested lodgepole pine, at Willis Creek approximately 20 km south of Princeton, BC. All experiments utilized 12-unit multiple funnel traps (Lindgren 1983) hung from metal poles. Traps were set-up in lines at least 10 m into the cutblock, and with at least 15 m between traps. One experiment tested the ability of individual antennally active crude neem oil components to repel MPB. The second experiment tested the ability of crude neem oil to repel MPB and the third experiment tested the ability of crude neem oil to repel *I. pini*.

Eight antennally active compounds identified were tested (Table 1) for their ability to deter response to traps baited with MPB baits (Phero Tech Inc.) comprising myrcene, exo-brevicomin, and trans-verbenol released at 95 mg @ 23°C, 1.5 mg @ 20°C, and 280 *u*g @ 20°C per 24 h, respectively. They were released individually from 400 *u*L polypropylene eppendorf tubes, with a 1.5 mm diam. hole in the side to facilitate release. Release rates (Table 1) were calculated by holding 10 tubes of each chemical at 22.4 $\pm$ 0.6 °C for at least 14 days, and weighing each tube every day. The three aldehydes (heptanal, octanal, and nonanal) and two alcohols (heptanol and octanol) were tested in groups and all other

compounds were tested singly. Captured MPBs and *I. pini* were collected and stored in a freezer until they could be sexed and counted.

Table 1. Chemicals identified by GC-EAD and tested in field trapping experiments with MPB, *Dendroctonus ponderosae* and *Ips pini* .

CHEMICAL	SOURCE [a]	PURITY (%) [b]	RELEASE RATE mg per 24h[c]
xylenes (*o*-xylene, *m*-xylene, & *p*-xylene) $C_6H_4(CH_3)_2$	Anachemia	99	0.362 ± 0.000
methyl tiglate	Bedoukian Research Inc.	98.8	0.032 ± 0.001
heptanal (heptaldehyde) $CH_3(CH_2)_5CHO$	Aldrich Chemical Company Inc.	95	0.014 ± 0.003
octanal $CH_3(CH_2)_6CHO$	S.F.U. Chemistry Department	81	0.006 ± 0.000
nonanal (nonyl aldehyde) $CH_3(CH_2)_7CHO$	Aldrich Chemical Company Inc.	95	0.003 ± 0.001
heptanol (1-heptanol) $CH_3(CH_2)_6OH$	Aldrich Chemical Company Inc.	99	0.003 ± 0.001
octanol (octanol-1, n-octanol) $CH_3(CH_2)_6CH_2OH$	BDH Chemicals	97	0.002 ± 0.001
n-hexoic acid (*n*-caproic acid) $CH_3(CH_2)_4COOH$	BDH Chemicals	98-101	0.002 ± 0.000
crude neem oil	Neem International Inc.	100	-

[a] Chemical sources: Aldrich Chemical Co., Milwaukee, WI; Anachemia Chemical Co., Vancouver, BC; BDH Chemicals, The British Drug Houses Ltd., Poole, England; Bedoukian Research Inc., Danbury, Connecticut; Neem International Inc., Surrey, BC; synthesized by G.G.S. King, Department of Chemistry, Simon Fraser University, Burnaby, BC V5A 1S6.

[b] Purity as listed by manufacturer.

[c] Determined in laboratory over a period of 10 days at 22.4 ± 0.6 °C (n =10) .

2.3.2 TOPICAL BARK SPRAY

Forty five uninfested lodgepole pines (mean dbh = 27.3 $\pm$ 3.6 cm) were selected at 25 m intervals in a mature stand on Commander Road, in the Willis Creek drainage. The trees were randomly assigned to one of three treatment groups; untreated control, formulation control (10% emulsifiable concentrate formulation in water, with no neem), and neem (2,000 ppm azadirachtin in 10% emulsifiable concentrate formulation in water). The formulation control and neem treatments were supplied by Neem International Enterprises Inc.

Two separate back pack sprayers each with 1.5 m wand extensions and flat fan nozzles were added to apply the formulation control and neem treatments. On 25 July 1996 trees were sprayed with 1.0 to 1.3 L treatments approximately 5 m up the bole to the run off point around the entire bole circumference. Mountain pine beetle tree baits (Phero Tech Inc.) were then stapled approximately 1.5 m high on the north face of each of the 45 trees to challenge MPBs to attack the trees. The baits contained *trans*-verbenol and *exo*-brevicomin released at 1.5 mg and 280 *ug* per 24 h @ 20^{o}C, respectively.

Attack densities were evaluated on; 26 July, 1 August, and 7 August 1996 by counting the numbers of MPB entrance holes in 20 x 40 cm areas at eye level on the east and west faces of each tree. In October, 20 x 20 cm bark samples were removed at eye level from the east and west faces of each tree. The numbers of entrance holes, mature adults, larvae, eggs, egg galleries, and exit holes, were counted, and the total egg galleries in each sample was measured.

Statistical Analysis

To satisfy criteria for normality and homoscedasticity (Zar 1984) data on trap catches were transformed by $\log_{10}(x+1)$. GLM and Ryan-Einot-Gabriel-Welsch Multiple Q-test ($\alpha = 0.05$) (SAS Institute Inc. 1988) both the trapping and spraying experiments.

3.0 RESULTS

3.1 TRANSLOCATION

Basal axe frill treatment with an emulsifiable concentrate neem formulation significantly decreased *I. pini* brood production at 3 and 9 m heights, as evidenced by reductions in numbers of total male and female beetles (Fig. 1), numbers of male and female beetles produced (Table 2), and numbers of holes in the bark (Fig. 2). The highest percent reductions in numbers of emergence beetles, 90 and 86% for males and females, respectively occurred at 3 m. The numbers of holes were significantly reduced by 89 and 88% at 3 and 9 m, but were also significantly reduced by 63% at 15 m. Because the neem treatment had no effect on *I. pini* gallery construction, including numbers of nuptual chambers, or number and length of egg galleries (Table 3), the entire impact of the treatment can be attributed to a reduction in brood production per gallery. In no case did the formulation control have any effect.

Totals of respective numbers of beetles emerged or remaining alive under the bark for the untreated control, formulation control, and neem treatments were 242, 295, and 61 for males and 353, 393 and 91 for females. The respective percents of the total population remaining alive under the bark for the untreated control, and neem treatments were 0.36, 0.32, and 0.11 for males and 0.27, 0.29, and 0.09 for females. In no case at any height was there a significant difference in percent of the population remaining unemerged after 104 days in rearing, GLM test, $P>0.05$. The inflection point in emergence at 60 days in rearing probably represents the onset of brood emergence (Fig. 3). By this criterion, a higher percentage of parent beetles emerged from the neem-treated bolts during the first 60 days in rearing than from bolts in either control treatment. However, reduced brood survival would artificially elevate parent adult emergence as a percentage of total emergence.

Figure 1. Numbers of brood adult *Ips pini* males and females per m^2 (emerged plus remaining under bark) in bolts taken at three heights from lodgepole pines treated in basal axe frills with emulsifiable neem formulation (20,000 ppm azadirachtin) or the formulation alone, Moffatt Lake, near 150 Mile House, B.C., Trees treated on 4 June 1996, felled on 11 June, and attacked by *I. pini* over the following six weeks. Bars with same letter are not significantly different, REGW test, $P<0.05$.

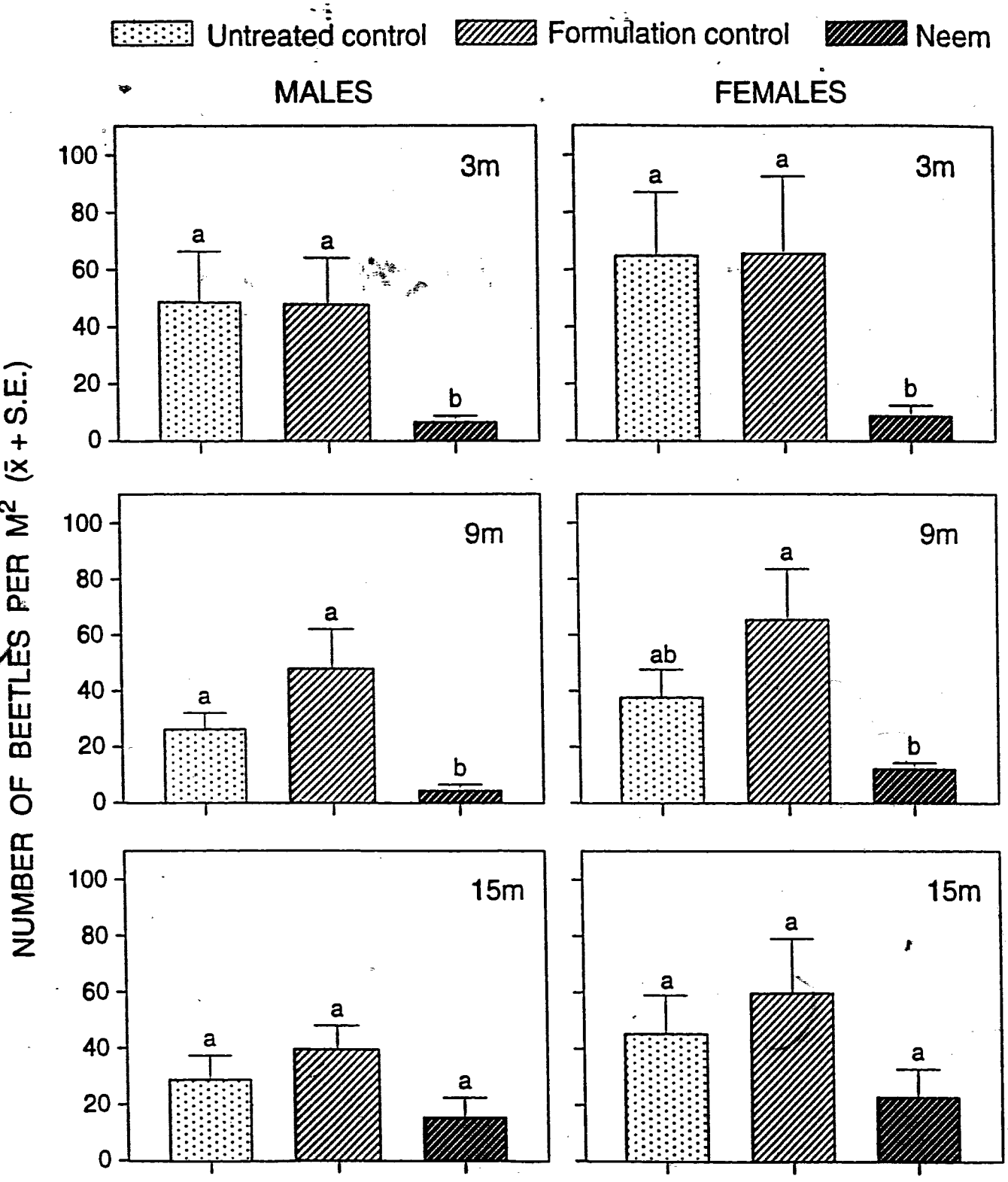

Untreated control
Formulation control
Neem
MALES
FEMALES
NUMBER OF BEETLES PER M² (x̄ + S.E.)
3m
9m
15m
100
80
60
40
20
0
a
a
b
a
a
b
a
a
b
ab
a
b
a
a
a
a
a
a

Table 2. Numbers of adult male and female *I. pini* per egg gallery emerged from or remaining in bolts taken at three heights from neem- or formulation control-treated lodgepole pine trees. Moffatt Lake, near 150 Mile House, BC. Trees treated on 4 June 1996, felled on 11 June and attacked by *I. pini* over the following six weeks.

Bolt height (m)	Treatment	No. of bolts	Number of live beetles per egg gallery ($\bar{x}$±S.E.)[a]	
			Males	Females
3	Untreated control	6	0.98 ± 0.29a	1.31 ± 0.37a
	Formulation control	7	1.19 ± 0.31a	1.68 ± 0.50a
	Neem	6	0.17 ± 0.05b	0.19 ± 0.07b
9	Untreated control	8	0.95 ± 0.23a	1.16 ± 0.25a
	Formulation control	7	1.38 ± 0.35a	1.97 ± 0.60a
	Neem	7	0.17 ± 0.06b	0.38 ± 0.09b
15	Untreated control	8	1.33 ± 0.35a	1.94 ± 0.56a
	Formulation control	6	1.34 ± 0.19a	2.04 ± 0.53a
	Neem	6	0.60 ± 0.16a	0.92 ± 0.23a

[a] Means within a height followed by the same letter are not significantly different, REGW test, $P<0.05$.

Figure 2. Numbers of *I. pini* holes per m^2 for neem and two control treatments at the three heights on lodgepole pines. Moffatt Lake, near 150 Mile House, BC, treated on 4 June 1996.

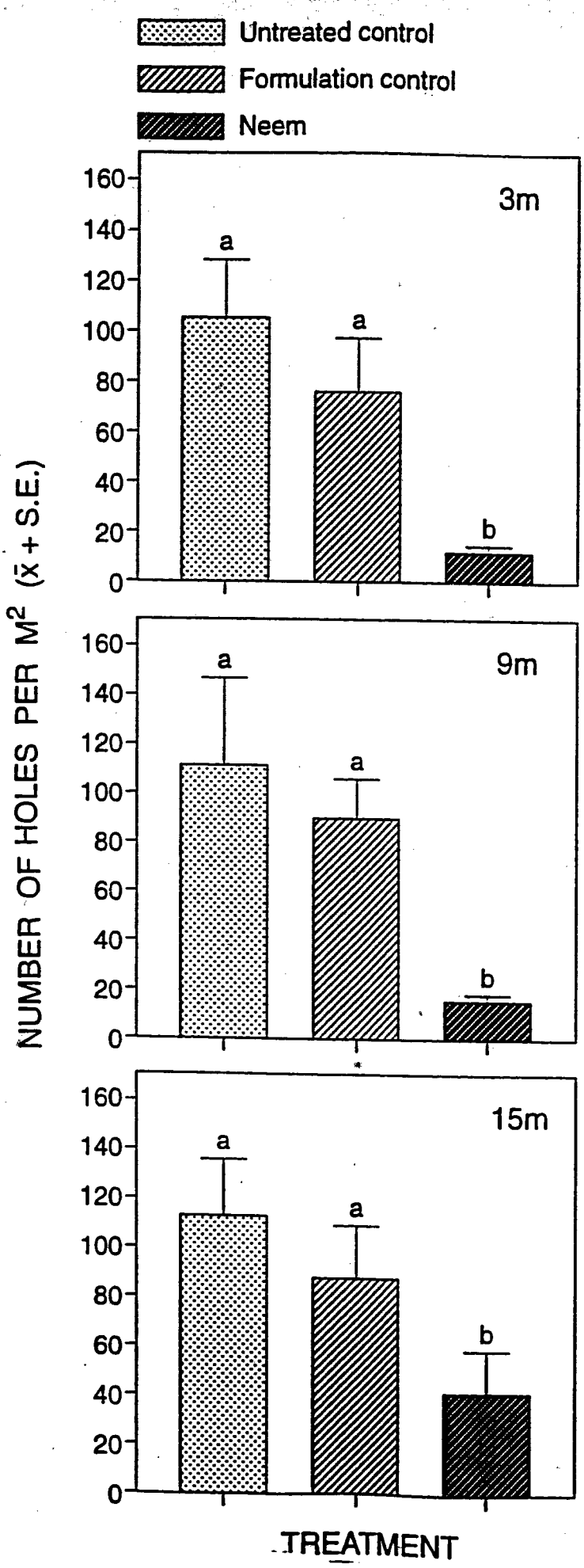
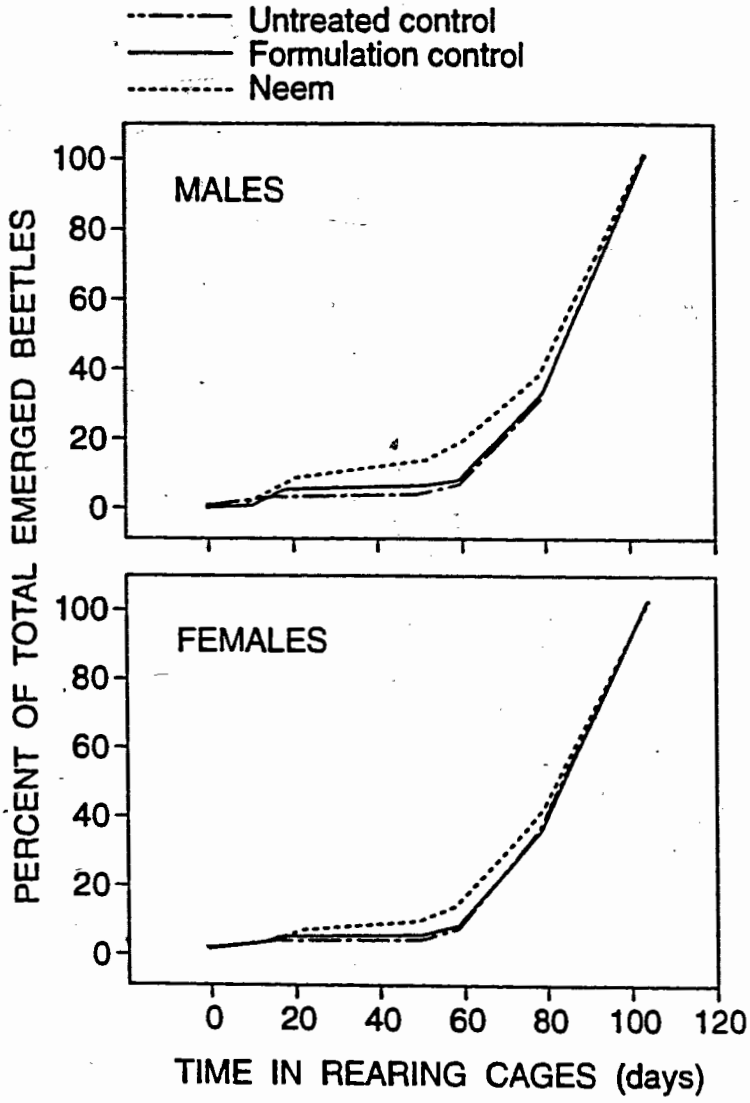

Untreated control
Formulation control
Neem
3m
9m
15m
a
a
b
160
140
120
100
80
60
40
20
0
NUMBER OF HOLES PER M2 (x̄ + S.E.)
TREATMENT

Table 3. Summary of data on gallery construction by *I. pini* at three heights in neem-treated or control lodgepole pines. Moffatt Lake, near 150 Mile House, BC. Trees treated on 4 June 1996, felled on 11 June and attacked by *I. pini* over the following six weeks.

			Criteria Measured[a]		
Bolt height (m)	Treatment	No. of bolts	Number of nuptual chambers per m^2 ($\bar{x}\pm$S.E.)	Number of egg galleries per m^2 ($\bar{x}\pm$S.E.)	Length of egg galleries per m^2 ($\bar{x}\pm$S.E.)
3	Untreated control	8	61.4 ± 9.0	149.6 ± 17.4	939.4 ± 117.6
	Formulation control	7	53.1 ± 15.3	114.0 ± 33.3	965.6 ± 272.2
	Neem	6	46.0 ± 15.0	107.1 ± 33.4	738.1± 219.2
9	Untreated control	8	68.5 + 17.0	150.6 + 32.9	1019.7 + 238.2
	Formulation control	7	61.5 + 11.0	144.8 + 25.2	1041.7 + 178.8
	Neem	7	74.8 + 14.5	157.4 + 25.0	1080.7 ± 178.1
15	Untreated control	8	59.0 + 12.3	147.4 + 22.8	1008.0 + 143.5
	Formulation control	6	72.9 + 12.7	152.1 + 21.7	1117.4 + 217.6
	Neem	6	75.3 + 16.4	167.1 + 29.2	1244.4 + 223.5

[a]No significant difference between any means within a height, GLM, test, $P<0.05$.

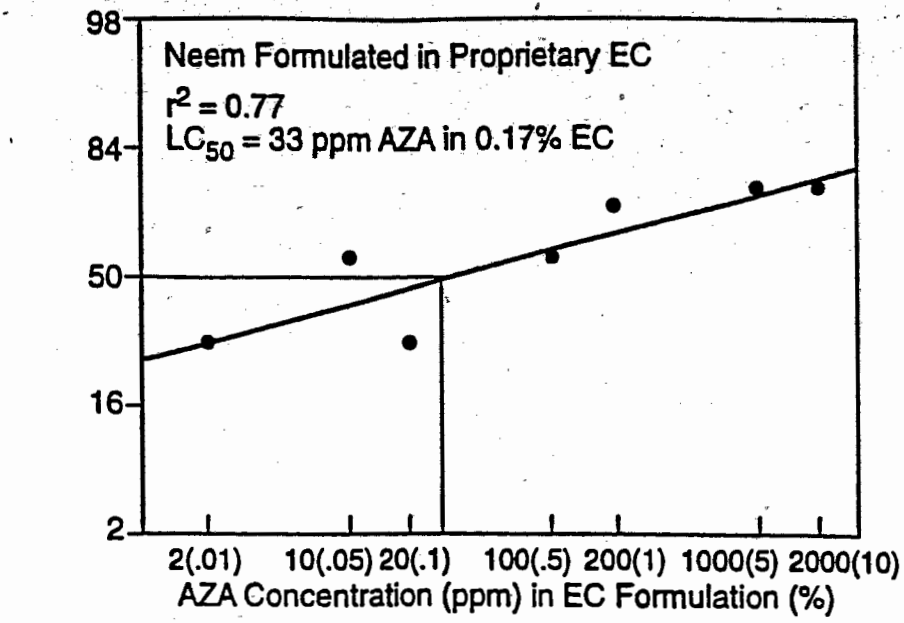
Figure 3. Percents of total *I. pini* emerged from bolts taken from neem-treated or control lodgepole pines from, 12 August to 28 November 1996. Moffatt Lake, near 150 Mile House, BC. Trees treated on 4 June 1996, felled on 11 June, and attacked by *I. pini* over the following six weeks.

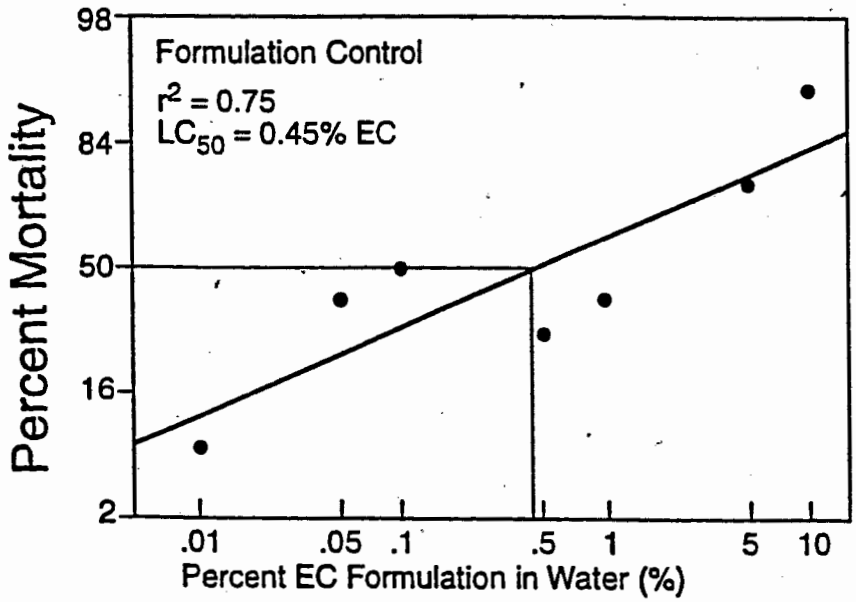

Untreated control
Formulation control
Neem
MALES
FEMALES
PERCENT OF TOTAL EMERGED BEETLES
0
20
40
60
80
100
120
TIME IN REARING CAGES (days)

3.2 ACUTE TOXICITY

Both formulations of neem seed extract were acutely toxic to MPB larvae within one week of exposure to treated ground phloem medium (Figs. 4, 5). DMSO-formulated neem was less toxic than the other proprietary formulation with undisclosed ingredients. As well, both formulation controls, were also toxic to MPB larvae, but to a lesser extent than the formulated neem. When the formulation control mortality was subtracted from the corresponding formulated neem mortality, the neem alone had an LC_{50} of 570 ppm azadirachtin for the DMSO formulation, and apparently no impact on mortality in the proprietary formulation. A regression line fitted to the points for neem alone was weekly negative in slope. These observations indicate that in both cases the emulsifiable concentrate formulations alone were mainly responsible for the mortality of MPB larvae.

Figure 4. Probit mortality curves and calculated LC_{50} values for MPB larvae feeding in treated ground phloem medium for one week with various concentrations of emulsifiable concentrate formulations with and without (control) neem seed extract. AZA = azadirachtin, EC = emulsifiable concentrate.


Neem Formulated in Proprietary EC
$r^2 = 0.77$
LC_{50} = 33 ppm AZA in 0.17% EC
98
84
50
16
2
2(.01) 10(.05) 20(.1) 100(.5) 200(1) 1000(5) 2000(10)
AZA Concentration (ppm) in EC Formulation (%)


Percent Mortality
Formulation Control
$r^2 = 0.75$
LC_{50} = 0.45% EC
98
84
50
16
2
.01 .05 .1 .5 1 5 10
Percent EC Formulation in Water (%)

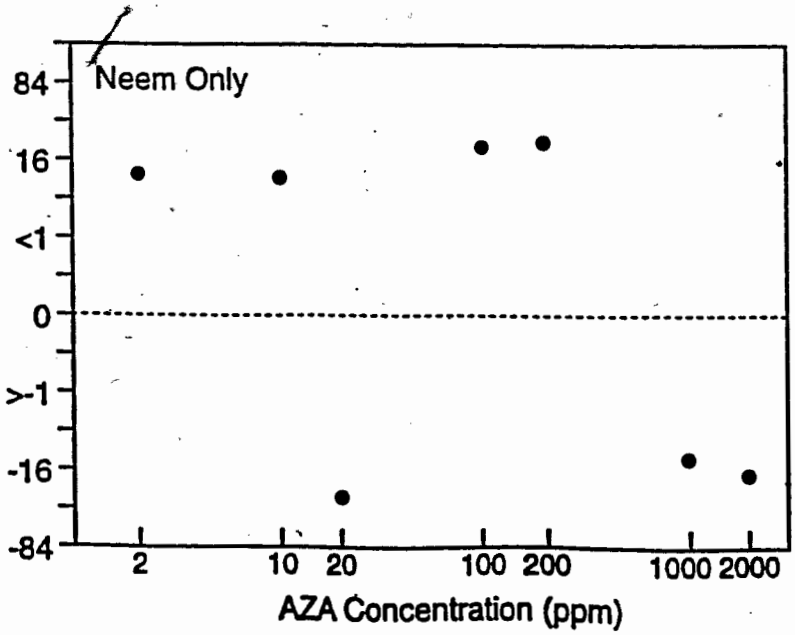
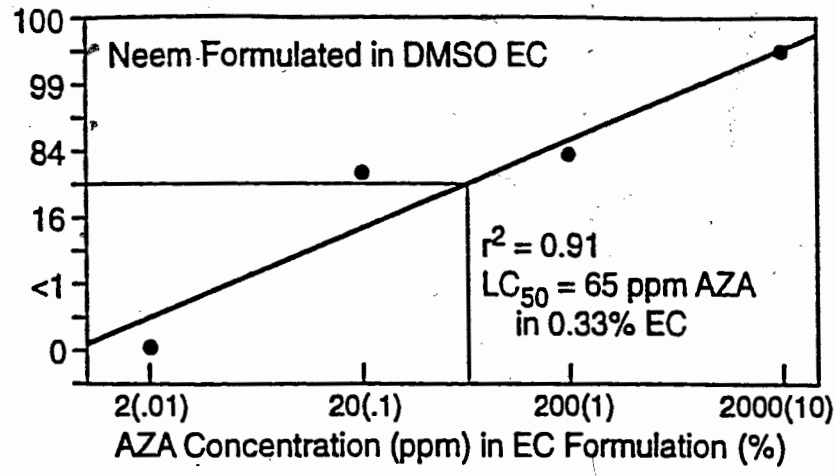
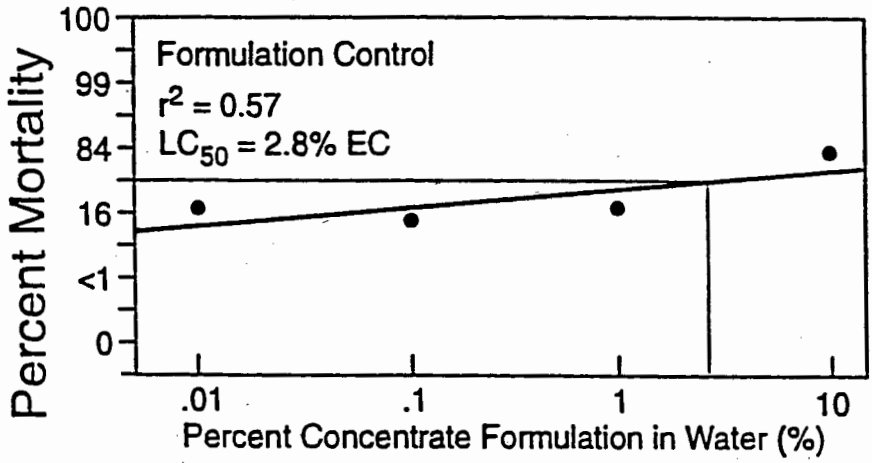
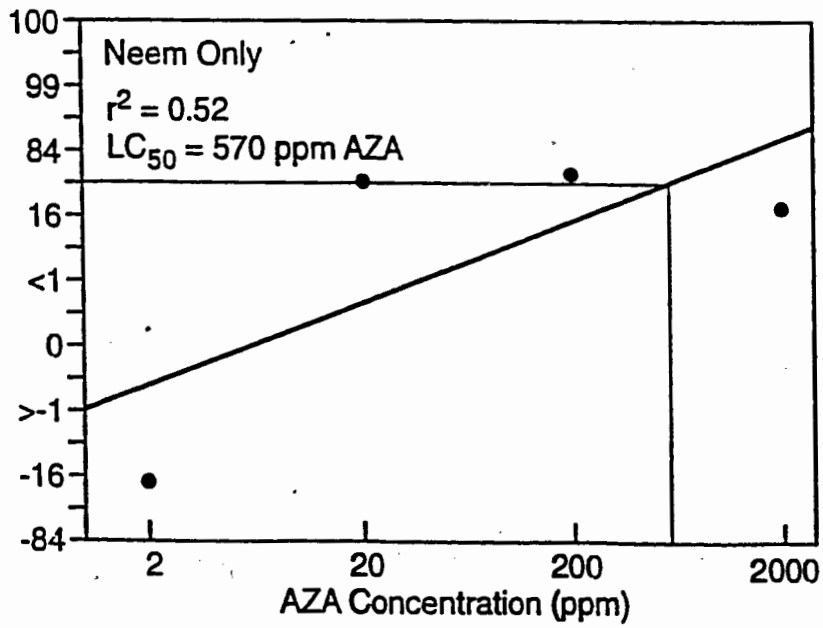

Neem Only
84
16
<1
0
>-1
-16
-84
2 10 20 100 200 1000 2000
AZA Concentration (ppm)

Figure 5. Probit mortality curves and calculated LC_{50} values for MPB larvae feeding in treated ground phloem medium for one week with various concentrations of emulsifiable concentrate formulations with and without (control) neem seed extract. AZA = azadirachtin, EC = emulsifiable concentrate.


Neem Formulated in DMSO EC
$r^2 = 0.91$
LC_{50} = 65 ppm AZA
in 0.33% EC
100
99
84
16
<1
0
2(.01)
20(.1)
200(1)
2000(10)
AZA Concentration (ppm) in EC Formulation (%)


Percent Mortality
Formulation Control
$r^2 = 0.57$
LC_{50} = 2.8% EC
100
99
84
16
<1
0
.01
.1
1
10
Percent Concentrate Formulation in Water (%)


Neem Only
$r^2 = 0.52$
LC_{50} = 570 ppm AZA
100
99
84
16
<1
0
>-1
-16
-84
2
20
200
2000
AZA Concentration (ppm)

3.3 REPELLENCY

3.3.1 VOLATILES FROM CRUDE NEEM OIL

GC-EAD analysis of crude neem oil revealed 10 antennally-active compounds. Eight of these were identified as: xylenes (*o*-xylene, *m*-xylene, and *p*-xylene), methyl tiglate, three aldehydes (heptanal, octanal, and nonanal), two alcohols (heptanol and octanol), and *n*-hexoic acid.

Neither crude neem oil (Fig. 6) nor any of the antennally-active components of crude neem oil (xylenes, methyl tiglate, aldehydes, alcohols, and *n*-hexoic acid) (Fig. 7) were repellent to MPBs when placed in baited multiple funnel traps. Crude neem oil also showed no repellency against *I. pini* when placed in ipsdienol-baited multiple funnel traps (Fig. 6).

3.3.2 TOPICAL BARK SPRAY

The proprietary neem formulation had no effect on MPB attack when applied as a spray to the bole of lodgepole pines. All treated and control trees were attacked within one day of treatment, and mass attacked ($\geq$ 31.25 attacks per m^2) within one week of treatment. There were no differences between treatments in MPB attack densities, densities or length of egg gallery or numbers larvae per m^2 (Table 4).

Figure 6. Response of *D. ponderosae* and *I. pini* to multiple funnel traps, respectively baited with MPB lures, ipsdienol alone, or either in combination with 400 *u*L of crude neem oil. Willis Creek, near Princeton, BC, 29 Aug to 1 Oct, 1996. For each sex, bars associated with the same letter are not significantly different, REGW test, $P<0.05$.

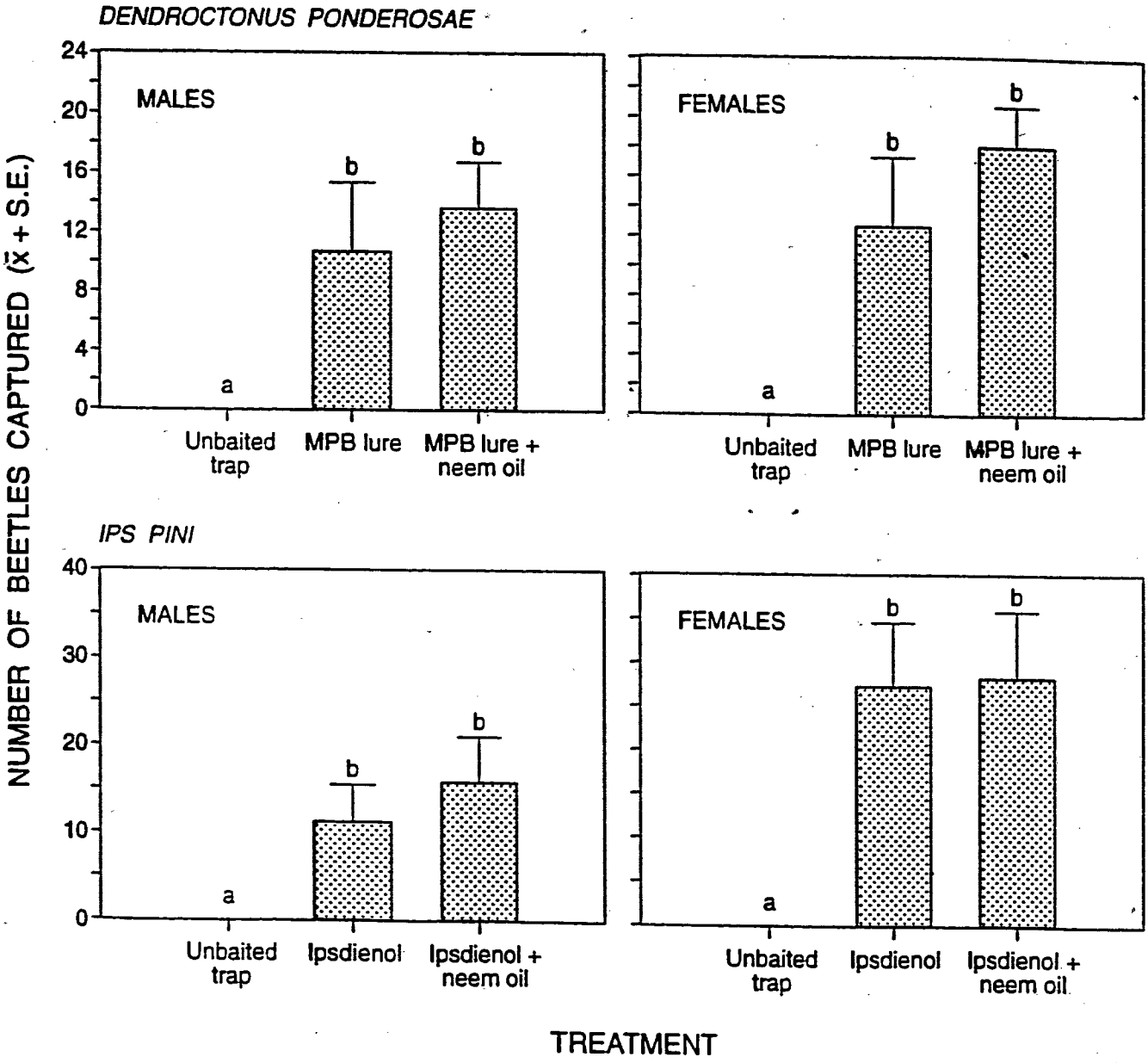

DENDROCTONUS PONDEROSAE
MALES
FEMALES
IPS PINI
MALES
FEMALES
NUMBER OF BEETLES CAPTURED (x̄ + S.E.)
0
4
8
12
16
20
24
0
10
20
30
40
a
b
Unbaited trap
MPB lure
MPB lure + neem oil
Ipsdienol
Ipsdienol + neem oil
TREATMENT

Figure 7. Response of MPB to multiple funnel traps, baited with MPB lures alone, and in combination with xylenes (*o*-xylene, *m*-xylene, and *p*-xylene), methyl tiglate, aldehydes (heptanal, octanal, and nonanal), alcohols (heptanol and octanol), and hexoic acid. Willis Creek, near Princeton, BC, 12-29 Aug. 1996, n = 10 replicates. For each sex, bars associated with the same letter are not significantly different, REGW test, $P<0.05$.

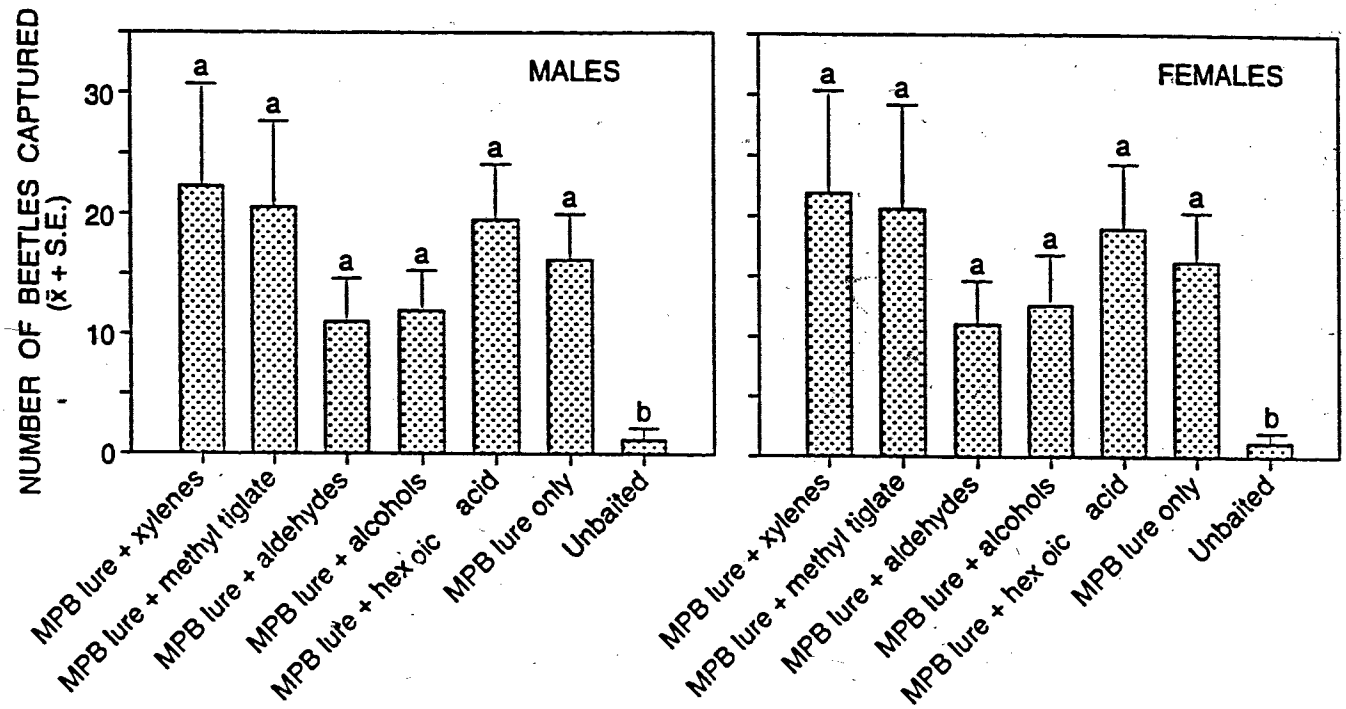

NUMBER OF BEETLES CAPTURED
(x̄ + S.E.)
30
20
10
0
MALES
FEMALES
a
b
MPB lure + xylenes
MPB lure + methyl tiglate
MPB lure + aldehydes
MPB lure + alcohols
MPB lure + hex oic acid
MPB lure only
Unbaited

Table 4. Summary of MPB attack characteristics on untreated lodgepole pines or trees sprayed to the run-off point with a formulation control (10% proprietary emulsifiable-concentrate formulation diluted with water), and neem (2,000 ppm azadirachtin in a 10% emulsiable-concentrate formulation). Commander Road, near Princeton, BC, treated 24 July 1996, 15 trees per treatment. Attack density data and bark samples taken on 8 and 20 October 1996.

		Criteria measured[a]			
Treatment	N[a]	Attack density per m^2 ($\bar{x}\pm$S.E.)	Number of egg galleries per m^2 ($\bar{x}\pm$S.E.)	Length of egg gallery (cm) per m^2 ($\bar{x}\pm$S.E.)	Number of larvae per m^2 ($\bar{x}\pm$S.E.)
Untreated control	15	127.5 ± 16.5	345.0 ± 30.0	4271.3 ± 463.5	1505.0 ± 347.1
Formulation control	15	126.7 ± 11.3	348.3 ± 25.3	4298.8 ± 448.3	1895.0 ± 325.3
Neem	15	155.8 ± 13.6	336.7 ± 24.9	4182.8 ± 401.3	1866.7 ± 278.3

[a]No significant difference between means within any column, GLM test, $P<0.05$.

4.0 DISCUSSION

My results, using *I. pini* as an indicator species, show that within one week after a basal axe frill treatment, the active ingredients in neem seed extract are translocated at least 9 m up the bole (Fig. 1), and possibly up to 15 m (Fig. 2). Moreover efficacy persists for at least six weeks after felling of the treated trees (Fig. 1). Because gallery elongation is associated with oviposition (Schmitz 1972), and neem treatment had no effect on gallery characteristics (Table 3), the low numbers of beetles per gallery in neem-treated trees (Table 2) apparently resulted from reduced survival of brood beetles. It is not known at what stage(s) mortality occurred. Azadirachtin has limited phloem mobility and only small amounts of azadirachtin are carried in the phloem (Schmutterer 1985). Therefore, as occurs for MSMA (Maclauchlan 1986), azadirachtin is probably xylem-translocated in lodgepole pines when applied into axe frills that penetrate the xylem tissue, but may reach the phloem tissue through the ray elements. Neem also was an effective systemic insecticide against the birch leafminer, *Fenusa pusilla* (Lepeletier) when injected into the bole (Marion *et al.* 1990). This was the first demonstration that neem translocated and had insecticidal action in woody plants (Helson 1992). Neem was effective in lodgepole pine against the MPB when 0.25, 0.63, or 1.9 g of azadirachtin per tree were applied into axe frills (Naumann *et al.* 1994), but populations were not sampled above 1.3 m. Because MPBs most commonly attack trees within the first 10 m of the bole (Cahill 1960), my data suggest that neem treatments could be operationally effective against the MPB, as long as they were applied before the symbiotic fungi associated with the MPB disrupted xylem translocation. For MSMA, this period extends to three weeks after attack (Stevens *et al.* 1974). The persistent insecticidal activity of neem for six weeks post felling in the treated trees before mass attack of *I. pini* occurred suggests that neem is protected from breakdown within fallen lodgepole pines. In general, neem is considered to be non-persistent in the environment (Schmutterer 1990). In open field conditions degradation of neem extracts is usually rapid with a half life of between 7-10 days (Barnby *et al.* 1989). Degradation is due to the presence of ultraviolet light, heat, moisture, and pH changes (Barnby *et al.* 1989; Larson 1989; BOSTID 1992; Mordue & Blackwell 1993; Isman 1994). Ultra violet light and high temperatures are not likely to be significant causes of degradation when neem is applied systemically to

trees in a temperate forest. In other systemic applications, neem was found to be persistent for three weeks (Reed & Reed 1985).

The observation that neem did not effect gallery construction by *I. pini* (Table 3) is consistent with the results of Naumann *et al.* (1994) for the MPB. Neem's specific effects on brood production in coleoptera include reduced fecundity in the Mexican bean beetle, *Epipachna varivestis* Mulsant (Steets & Schmutterer 1975) and colorado potato beetle, *Leptinotarsa decemlineata* Say (Steets 1976/77) and sterilization of female cockchafers, *Melolontha* spp. (Kaethner 1991; Schmutterer 1995). While some mortality of parent adult *I. pini* might have occurred, evidence of larval mining in debarked bolts indicates that neem also caused reduced brood survival. Neem seed extracts, specifically azadirachtin and the other limonoids, are known to act as growth regulators on immature insects (Stone 1992; Quaries 1994). Disruption of metamorphosis and high mortality between molts has been observed in other coleoptera such as the Colorado potato beetle (Steets 1976/77), the khapra beetle, *Trogoderma granarium* Everts (NRC 1992), the Mexican bean beetle, and the Japanese beetle, *Popillia japonica* (Ladd *et al.* 1978). It is likely that mortality of *I. pini* was also latent, possibly as late as the pupal-adult molt, as observed by Naumann *et al.* (1994) for the MPB. If mortality is characteristically latent, then acute toxicity tests in the laboratory (Figs. 5, 6) might tend to overestimate the amount of active ingredient needed to achieve efficacy as a systemic insecticide in the bole of a conifer.

Even though both formulation controls were acutely toxic to MPB larvae (Figs. 5, 6), the formulation control within the bole of lodgepole pines (Figs. 1, 2) had no effect on *I. pini*. I hypothesize that the toxic constituents in the formulation translocated very quickly, possibly ending in the terminal twigs, bark, and foliage, as also occurs for MSMA (Maclauchlan *et al.* 1988). If this hypothesis is valid, acute toxicity in the laboratory of the complete formulation (Figs. 5, 6) would expose larvae to lethal ingredients in the carrier that they would not encounter in a tree. When injected into Douglas-fir sapplings, *Pseudotsuga menenzeii* Mirb (Franco), azadirachtin translocated into the foliage within two days (Naumann *et al.* 1994). It might also have reached the foliage of mature lodgepole pines in my experiment, but translocation was halted after one week by felling the trees. Because azadirachtin has limited phloem

mobility (Schmutterer 1985), it may also move fairly slowly in the living sapwood of a conifer, and thus would not have traveled as far up the bole of the tree as the formulation constituents.

Concerns regarding the toxicity of the formulation arise during registration of a pesticide. In Canada, many formulations are toxic to some extent, but are registered nonetheless. However, it has been proposed that for biochemicals there may be reduced data required for registration, such as toxicological and environmental fate information, thereby reducing cost and time for registration (Helson 1992). It has not been decided if natural insecticides like neem fit the criteria for biochemicals. If the neem formulation is toxic then complications may arise and neem could be required to go through all of the testing necessary for other pesticides. Presently, no neem-based pesticides are registered in Canada. This creates a huge incentive to develop non-toxic formulations so that neem-based pesticides can be registered as soon as possible.

Neem-induced repellency and/ or feeding deterrency have been found in the following families of Coleoptera: Bostrichidae (Jilani & Saxena 1990), Chrysomelidae (Steets 1976/77, 1978; Reed *et al.* 1982; Kaethner 1992; Palaniswamy & Wise 1994); Curculionidae (Beitzen-Heineke & Hofmann 1992); Scarabeidae (Ladd *et al.* 1978; Schmutterer 1995); Scolytidae (Sponagel 1993); and Tenebrionidae (Schmutterer 1995). However, no such effects were seen in experiments conducted on the MPB and *I. pini*, either with crude neem oil (Fig. 7) and the antennally-active volatiles therein (Fig. 8), or with an emulsifiable-concentrate formulation sprayed on the bole (Table 4). Because MPBs and *I. pini* were not repelled by neem extracts, bark beetles would probably not be repelled by the exposed neem formulation applied into open axe frills in systemic treatment of coniferous trees.

If neem is adopted into pest management programs for bark beetles, pest managers should be aware of neem's different modes of action, and immediate mortality of the larvae should not be expected. Neem extracts, if used against the MPB, would have the following advantages over MSMA: no toxicity to vertebrates (Radwanski & Wickins 1981); no effect on non-target organisms (Olkowski *et al.* 1991; Ascher 1993; Quaries 1994); short residual activity in the environment due to degradation by ultraviolet rays, heat, moisture, and, pH (Larson 1989; BOSTID 1992; Mordue & Blackwell 1993); and very low chance of resistance developing, possibly attributed to neem's multiple modes of action (Vollinger 1987; Saxena

1989; BOSTID 1992) and the fact that only a small percentage of the total number of infested trees in a given year would ever be treated. As well, neem has already gained public acceptance in developed countries for use on food crops (Isman 1994). A possible drawback of using neem is the cost of $1500 per ton of neem oil (Stone 1992) and the further cost of formulation. The price of neem-based pesticides in the future will depend on the cost of production, development, dosages required for effective control, and size of the market. Unless neem-based pesticides find wide acceptance in agricultural and urban markets, their high cost will likely preclude their use in forestry.

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6.0 PERSONAL COMMUNICATIONS

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