

**AN ENVIRONMENTALLY FRIENDLY INSECTICIDE
FOR CONIFER SEED ORCHARDS**

by

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AN ENVIRONMENTALLY FRIENDLY INSECTICIDE FOR CONIFER SEED ORCHARDS

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ABSTRACT

An extract from seeds of the neem tree, *Azadirachta indica* (Juss.), enriched with azadirachtin, a proprietary product (NSE-5), was evaluated in three experiments (Exp.) for control of the green spruce aphid, *Elatobium abietinum* (Walker), on potted spruce trees in a greenhouse. The extract in Exp. 1 and 2 was first applied on 15 February 1993 when green spruce aphid populations were initially low; aphid populations were observed weekly for 15 weeks thereafter. In Exp. 1, foliar applications to the run-off point of 150 ppm azadirachtin applied three times (biweekly) held populations below 5.3 aphids per 6 cm twig samples; populations on trees treated at 75 ppm, or with the control (emulsifier) treatment only, reached 32.2 and 26.9 aphids per sample, respectively. When five applications were made weekly in Exp. 2, suppression to below 14 aphids per sample was achieved with both 150 and 75 ppm treatments, while populations on control trees peaked at 36.8 aphids per sample. NSE-5 at 150 ppm in Exp. 3 significantly reduced aphid numbers on trees that initially had high populations. The results indicate that neem has potential for operational use in conifer greenhouses and seed orchards.

Dedicated to the memory of my sister, Ellen Margaret Partridge.

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TABLE OF CONTENTS

<i>Approval</i>	<i>ii</i>
<i>Abstract</i>	<i>iii</i>
<i>Dedication</i>	<i>iv</i>
<i>Acknowledgements</i>	<i>v</i>
<i>List of Tables</i>	<i>viii</i>
<i>List of Figures</i>	<i>ix</i>
1.0 INTRODUCTION	1
1.1 Development of Neem as an Insecticide	2
1.2 Homoptera and Neem	8
1.3 Tests of Neem on Forest Insect Pests	10
1.4 Environmental and Health Effects of Neem	11
1.5 The Green Spruce Aphid	13
1.5.1 Hosts and Distribution	13
1.5.2 Damage	15
1.5.3 Life Cycle and Description	16
1.6 Objectives	18
2.0 MATERIALS AND METHODS	18
2.1 Experiments 1 and 2 - Low Initial Populations	18
2.2 Experiment 3 - High Initial Populations	23

3.0 RESULTS	26
3.1 Experiments 1 and 2 - Low Initial Populations	26
3.2 Experiment 3 - High Initial Populations	34
4.0 DISCUSSION	37
<i>References Cited</i>	43

LIST OF TABLES

- Table 1. Comparison of tree condition at completion of Exp. 1 and 2 as assessed by severity of chlorosis, rated on a scale of 0 to 10 (0 = no chlorosis; 10 = severe chlorosis with no new bud flush, tree probably dead). 33
- Table 2. Correlations between severity of chlorosis and green spruce aphid densities on "interior" spruce trees at the completion of Exp. 1 and 2. Chlorosis was rated on a scale of 0 to 10 (0 = no chlorosis; 10 = severe chlorosis with no new bud flush, tree is probably dead). 35
- Table 3. Effect of clonal variation on severity of chlorosis on pooled control trees at the completion of Exp. 1 and 2. 36
- Table 4. Effect on initially high green spruce aphid populations of a single spray to the run-off point with an aqueous formulation of a proprietary neem seed extract concentrate (NSE-5) containing 150 ppm of azadirachtin in Exp. 3. All trees had > 10 aphids per 6 cm segment of 1991 shoot growth at the time the spray was applied on 6 March 1993 (week 1). 38

LIST OF FIGURES

- Fig. 1. Portable protective barrier constructed from polyethylene and PVC tubing, showing placement around potted spruce tree to prevent overspray of adjacent trees with neem seed extract. 20
- Fig. 2. Examples of chlorosis ranked trees. Class 0 = no observable chlorosis; Class 10 = severe chlorosis, no new bud flush. Pictured are a 150 ppm treated tree in Exp. 2 ranked as chlorosis Class 1 and a control tree ranked as chlorosis class 9. 24
- Fig. 3. Population trends of green spruce aphids on potted spruce trees sprayed three times in Exp. 1 to the run-off point with an aqueous formulation of a proprietary neem seed extract concentrate (NSE-5) containing 75 or 150 ppm of azadirachtin. Arrows indicate weeks when sprays were applied. 27
- Fig. 4. Population trends of green spruce aphids on potted spruce trees sprayed five times in Exp. 2 to the run-off point with an aqueous formulation of a proprietary neem seed extract concentrate (NSE-5) containing 75 or 150 ppm of azadirachtin. Arrows indicate weeks when sprays were applied. 29

1.0 INTRODUCTION

Conifer seed orchards are an integral part of British Columbia's reforestation program. Seed orchards produce seed from stock selected for genetically inherited qualities such as tree height, growth rate, and wood density. The selection of parent trees, scion collection, propagation, planting, and maintenance of seed orchards, demands a considerable investment, making orchard-grown seed a very high value crop. Each tree is also important because of its contribution to the genetic balance of the orchard (Lavender *et al.* 1990).

Insect pests can adversely affect seed orchard yields through direct damage (by seed consumption) or indirect damage (by reducing tree vigour) (Ruth *et al.* 1982), but strategies and tactics for insect pest management in seed orchards are currently limited. To reduce the susceptibility of Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, to the directly damaging Douglas-fir cone gall midge, *Contarinia oregonensis* Foote, cold-water overhead irrigation in the early spring is used to delay flowering, thus disrupting oviposition-flowering synchrony. To control the indirectly damaging spruce gall adelgid, *Adelges cooleyi* (Gillette), current cultural practices include planting spruce, *Picea spp.*, away from Douglas-fir (the adelgid's alternate host) and hand-picking galls. When these methods are either not successful or not feasible, chemical insecticides are employed.

The insecticides currently registered for use against directly damaging insects on Douglas-fir and spruce are: dimethoate (Cygon), and oxydemeton-methyl (Metasystox-R), both organophosphorous insecticides. Several organophosphorous insecticides are registered for indirect pests including: oxydemeton-methyl, dimethoate, and diazinon. For aphid control, Safer's insecticidal soap can be used if aphid populations are low (Barnett 1993).

The reliance that seed orchard managers must place on organophosphorous insecticides for pest control is not desirable because organophosphorous insecticides are neurotoxicants, many of which are damaging to non-target organisms or pose a threat to public health (via ground water contamination or accidental exposure) (Isman *et al.* 1990a). Alternative, efficacious, environmentally safe compounds for pest management in seed orchards, especially those situated close to urban areas, need to be developed and, indeed, the focus of contemporary insecticidal research is to find pesticides that are environmentally "friendly". They must be pest-specific, nontoxic to humans, biodegradable, and not prone to pest resistance (Saxena *et al.* 1989).

1.1 Development of Neem as an Insecticide

The search for biorational insecticides has led to increasing attention to insecticides derived from plants (Schmutterer 1990). Plants produce many defensive chemicals that act as repellents, feeding deterrents, ovipositional

deterrents, growth inhibitors, sterilants and toxicants (Saxena *et al.* 1989). Of special interest to researchers are chemicals produced by the neem tree [syn. Indian lilac, margosa tree, nim (Lowery 1992)], *Azadirachta indica* A. Juss. (syn. *Melia indica* Brandis, *Melia azadirachta* L. and *Melia parviflora* Moon.) (Meliaceae) (Mohan Ram and Nair 1993).

The neem tree is native to arid areas of the Indian subcontinent but is now widely distributed throughout Africa, southeast Asia, the Caribbean, Central and South America and Australia (Koul *et al.* 1990). Its uses date back 4000 years to the Vedic period of India when different neem tree parts were processed into Ayurvedic medicine. Ayurvedic medicine is treatment using natural herbs and specialized plant parts, such as seeds, leaves and bark. It was developed by a caste of nomadic mendicants (Larson 1989). Uses claimed include curatives for diabetes, stomach aches, malarial fever, stomach worms, and skin disorders. Research is in progress to test products from neem trees for their contraceptive and antitumour effects. Twigs from the tree are traditionally used as a very effective dentifrice (Larson 1989). Neem cake (the remaining residue after oil is extracted from the seed) is an effective fertilizer and animal feed. The oil from the seed kernels is made into lamp fuel and soap. The tree's timber is made into furniture and poles.

The remarkable pesticidal attributes of neem are well known in India. Traditionally neem seeds are combined with stored grains to repel seed-eating insects; leaves are placed in books for protection against silverfish, inserted in

wool clothing to protect against moths, and placed under mattresses to repel crawling insects (Larson 1989). In 1942, pioneering work on the isolation and identification of neem constituents was started in India (Koul *et al.* 1990). Pradhan *et al.* (1962) working at the Indian Agricultural Research Institute, New Delhi, clearly demonstrated the antifeedant properties (100% deterrence) of an aqueous neem extract (0.01%) against the desert locust, *Schistocerca gregaria* (Forskål). This research triggered a worldwide interest in neem. Today, numerous studies describe the insecticidal, antifeedant, growth inhibitory, oviposition deterring, antihormonal, and antifertility properties of neem against a broad spectrum of insects (Jacobson 1986, 1989b; Schmutterer 1988, 1990; Warthen 1989; Arnasan *et al.* 1989; Subrahmanyam 1990; National Research Council 1992; Ascher 1993). There have been three international neem conferences (Schmutterer *et al.* 1981; Schmutterer and Ascher 1984, 1987), and two American conferences (Locke and Lawson 1990, Ahmed 1993).

Over 100 constituents have been isolated from different parts of the neem tree and their chemical structures described. Included are protolimonoids, limonoids or tetranortriterpenoids, pentanortriterpenoids, hexanortriterpenoids, and nonriterpenoidal constituents (Koul *et al.* 1990). Because of its insect feeding and growth-disruptant properties, the crude seed oil has become the primary material for study, followed by the leaves (Jones *et al.* 1989). The major active principal of neem oil is azadirachtin, a tetranortriterpenoid, which is possibly the most potent natural insect antifeedant

discovered to date (Isman *et al.* 1990a). It also interferes strongly with molting and reproduction in several species of insects.

Azadirachtin was isolated from neem seeds by Butterworth and Morgan (1968). Its structure was reported by Zanno *et al.* (1975), and later corrected by Kraus *et al.* (1985) using X-ray crystallography. Not until 1983, was "azadirachtin" determined to be a mixture of seven isomers named azadirachtin A to G (Rembold *et al.* 1984). The isomers share similar chemical structures and biological activity. Azadirachtin-A is the most prevalent, while azadirachtin-E is the most effective insect growth regulator. The complex chemical nature of azadirachtin presents extreme difficulties in the synthesis of this molecule or bioactive analogues, although such efforts are currently underway (Ley *et al.* 1987, 1993).

It is unlikely that pure azadirachtin will be applied in the field because it is highly unstable and must be kept under nitrogen at -40°C and in a desiccator to maintain its purity (Larson 1989). Other potentially active constituents and/or stabilizers may be present in crude extracts that enhance the bioactivity of azadirachtin (Kraus *et al.* 1987, Balandrin *et al.* 1988). Also, azadirachtin and other biologically active compounds may be stabilized by the oil in oil-based extracts or emulsions. Therefore the commercial production of an azadirachtin-rich neem preparation may be advantageous over pure azadirachtin for field applications (Isman *et al.* 1990a).

Isman *et al.* (1990b) found that azadirachtin may vary from 0 to 4300 ppm in oil samples obtained from various suppliers. Because of this variability (not unexpected in a botanical pesticide), Koul *et al.* (1990) recommend that protocols be implemented for processing neem seeds and other parts of the tree, including standardization of specifications for derivatives such as neem oil. Isman *et al.* (1991a) recommend that the azadirachtin content be given in all published work so that effects of neem products on various insects can be compared. Even with known azadirachtin concentration, Mordue (Luntz) and Blackwell (1993) found that detailed comparisons of efficacy against different pest species remain extremely difficult, due to differing neem formulations and application methods.

Mordue (Luntz) and Blackwell (1993) assessed published data with comparable protocols and found that antifeedant sensitivity ranged widely between species, whereas insect growth regulatory effects were relatively consistent. They found that for antifeedant effects, lepidoptera are extremely sensitive (< 1-50 ppm azadirachtin sensitivity depending on species); coleoptera, hemiptera and homoptera are less sensitive (100-600 ppm azadirachtin); and orthoptera have a wide range of sensitivity (0.05-1000 ppm azadirachtin). In contrast, the effective dose for 50% mortality (ED_{50}) after injection into the haemolymph varies between only 1 and 4 μg per g body weight in all species tested (lepidoptera, hemiptera, orthoptera) except the milkweed bug, *Oncopeltus fasciatus* (Dallas) ($ED_{50} \leq 0.6 \mu\text{g}$ per g), which is

known to be highly susceptible to endocrine disruption. The variability of neem's effects on a given species is attributed to formulation, dose, route of application, life stage and sex (Dorn *et al.* 1987, Schmutterer 1988).

Differences in systemic activity within a plant is a particularly important factor for the control of phloem-feeding insects such as aphids (Lowery 1992). Different formulations, application rates, and spray coverage, will also influence the degree of systemic activity. Systemically-treated plants require concentrations of > 100 ppm of azadirachtin to produce primary antifeedant effects on aphids (Griffiths *et al.* 1978; Nisbet *et al.* 1993). Azadirachtin concentrations of > 250 ppm prevented cereal aphids, *Rhopalosiphum padi* (L.) and *Sitobion avenae* (F.), from settling on systemically-treated barley seedlings, although lower doses (50 ppm) produced the same effects when applied topically to the leaves [West and Mordue (Luntz) 1992]. Schmutterer (1985) states that low sensitivity of aphids to the primary antifeedant effect of azadirachtin may result from poor phloem-mobility of the compound, although concentrations up to 75 ppm in artificial diets do not initially deter aphids from feeding (Nisbet *et al.* 1994).

The potential for a target insect species to develop resistance is an important factor when developing and prescribing an insecticide. To date there has been no reported resistance to neem (Ascher 1993). A laboratory attempt to develop resistance in the diamondback moth, *Plutella xylostella* (L.), which develops resistance rather quickly to all major groups of pesticides, including

Bacillus thuringiensis (Berliner) (Tabashnik *et al.* 1990), was unsuccessful after 42 generations of selection pressure (Völlinger 1987). The apparent inability of insects to develop resistance to neem is attributed to its many constituents and also to its complex mode of action (Schmutterer 1988).

1.2 Homoptera and Neem

Over 200 insect species in seven orders including Coleoptera, Diptera, Heteroptera, Homoptera, Hymenoptera, Isoptera, Lepidoptera and Orthoptera are known to be susceptible to neem (Warthen 1989, Saxena *et al.* 1989, Mordue [Luntz] and Blackwell 1993). Affected homoptera include aphids (see above), leafhoppers, planthoppers, psyllids, whiteflies, and scale insects. Neem may have negligible impact, or repellent, growth disruptant or toxic effects, depending on insect species, plant host, route of administration, concentration, and formulation. Saxena and Khan (1986) using neem oil diluted with 1.7% aqueous detergent ('Teepol') solution found that neem odour alone reduced feeding by the green leafhopper, *Nephotettix virescens* (Distant), on rice plants in no-choice experiments in the laboratory. The leafhoppers increased their probing frequency, decreased their intake from phloem tissue and increased intake from xylem tissue. In laboratory tests Coudriet *et al.* (1985) found that 0.2 and 2.0% of an ethanolic extract deterred oviposition and was toxic to eggs and larvae of the sweetpotato whitefly, *Bemisia tabaci* (Gennadius). In contrast, Price and Schuster (1990) found only

4% mortality of sweetpotato whitefly eggs on ornamental poinsettia plants, but 96% mortality of 2nd - 3rd instars using a single foliar spray of 20 ppm azadirachtin (Margosan-O, Grace-Sierra, Fogelsville, PA.) Flint and Parks (1989) found that 160 ppm azadirachtin (Margosan-O formulation) was needed for efficacy against the sweetpotato whitefly on cotton when tested in the field.

Aphids must ingest azadirachtin before expressing negative effects, because they are generally not repelled or otherwise adversely affected by topical applications. Schmutterer (1985) states that aphids are difficult to control with aqueous or methanolic products because there is no contact effect; therefore, efficacy demands systemic uptake and translocation in the plant followed by ingestion.

However, crude neem extracts containing 100 ppm azadirachtin applied topically repelled the green peach aphid, *Myzus persicae* (Sulzer) (Griffiths *et al.* 1978). West and Mordue (Luntz) (1992) found that topical treatments with 50 to 500 ppm azadirachtin solutions caused a significant rejection of treated wheat seedlings by flying cereal aphids, *Rhopalosiphum padi* (L.) and *Sitobion avenae* (F.). Probing behaviour of aphids was also significantly reduced for up to four days, whether azadirachtin was applied topically or systemically at either 250 or 500 ppm. Azadirachtin at 100 ppm in artificial diets deterred feeding by the green peach aphid, whereas a dosage of 300 ppm was required when it was applied systemically (Nisbet *et al.* 1992, 1993). In general, higher

concentrations of azadirachtin are needed to repel or deter feeding by aphids than are required for similar effects on orthoptera or lepidoptera.

Lowery *et al.* (1993) found that in laboratory studies, applications of neem seed oil (10, 20 and 40 ppm azadirachtin) whether applied before or after aphid infestation, decreased aphid numbers to a similar extent, thus implying no contact effect. Also, mortality of *M. persicae* was greater on pepper than on rutabaga, demonstrating an effect of host plant species on efficacy.

1.3 Tests of Neem on Forest Insect Pests

Research on neem's effect on forest insect pests is only now beginning. Foliar applications were tested by Shapiro *et al.* (1994) and Thomas *et al.* (1992) against larval gypsy moths, *Lymantria dispar* (L.), and Eastern spruce budworms, *Choristoneura fumiferana* (Clem.), respectively. Shapiro *et al.* (1994) found that 23 to 230 ppm azadirachtin solutions of a viscous 50% concentrate of neem seed extract inhibited both growth and development of larval gypsy moths, and enhanced the lethal effect of a nuclear polyhedrosis viral (NPV) disease. They recommend the use of neem as an additive to the gypsy moth NPV. Thomas *et al.* (1992) found feeding was reduced by 50% by larval spruce budworms when fed a 19 ppm azadirachtin-treated artificial diet but the LC_{50} was only 0.15 ppm. The high toxicity of azadirachtin to Eastern spruce budworms compares favourably with that of fenitrothion, an

organophosphorous insecticide still used in parts of eastern Canada.

Therefore neem could potentially be a biorational alternative to conventional chemical insecticides for controlling spruce budworms.

Marion *et al.* (1990) found that microinjections of 1.5 and 3.0% neem seed extract concentrate into the bole was as effective as Metasystox-R in reducing the number of adult birch leafminers, *Fenusa pusilla* (Lepeletier), reared from foliage of treated seven-year-old paper birch trees. Naumann *et al.* (1994) found that injections of neem into the sapwood at the root collar of lodgepole pines, *Pinus contorta* var. *Latifolia* (Engelm.), caused a significant reduction in densities in the phloem of larval mountain pine beetles, *Dendroctonus ponderosae* Hopkins; however, the effect was not as great as that of the arsenical herbicide, monosodium methane arsonite (MSMA).

No studies of the effects of neem on forest homopteran pests appear in the literature.

1.4 Environmental and Health Effects of Neem

One of neem's most important characteristics for use in IPM programs is its relative non-toxicity to beneficial arthropods (Schmutterer 1990). Some important predators that are not adversely affected by neem include wolf spiders, predaceous mites, and coccinellids (Schmutterer 1990). The parasitoid *Telenomus remus* Nixon emerged normally from neem-treated eggs of *Spodoptera litura* (F.) and, if treated after oviposition, their longevity was

increased (Joshi *et al.* 1982). Parasitization of larval rice leaffolders, *Cnaphalocrocis medinalis* (Guenee), was higher in rice fields sprayed weekly with neem oil than in unsprayed fields, and adult parasitoids emerged normally (Saxena *et al.* 1981). Isman *et al.* (1991a) found parasitization rates and predator numbers to be higher in neem-treated field plots than in control plots. Mordue (Luntz) and Blackwell (1993) document adverse effects of neem on: *Cotesia congregata* (Say), a hymenopteran parasitoid of the tobacco hornworm, *Manduca sexta* (L.); the two-spotted stink bug, *Perillus bioculatus* (F.), a predator of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say); and two parasitoids, *Encarsia spp.* and *Aleurodiphiulus spp.*, of the sweet-potato whitefly, *Bemisia tabaci* (Gennadius). However, the effects are either dependent on larval stage of the beneficial insect at time of application or they are over-ridden by a lack of adverse effect on other important beneficial species.

Other important considerations when developing an insecticide are environmental safety (including persistence) and toxicity to non-target organisms other than beneficial insects. Azadirachtin degrades in the presence of ultraviolet light (including sunlight) with a half-life (pure compound on glass surface or in solution) of about 24 h (Barnby *et al.* 1989). But azadirachtin in neem oil may degrade much more slowly (Isman *et al.* 1991b). Also, although pure azadirachtin degrades rapidly, the degradation products remain biologically active. Azadirachtin exposed to ultraviolet light retained full

bioactivity for 90 h and partial activity at 400 h (Barnby *et al.* 1989). Osman and Port (1990) hypothesize that the half-life of azadirachtin is increased when it is applied as a soil drench (either as neem seed powder or solution) because of protection from sunlight. Temperature and pH also affect azadirachtin, and neem extracts rapidly lose their potency if not stored and handled properly (Larson 1989; Barnby *et al.* 1989; Walter and Knauss 1990). In general, neem can be considered to be non-persistent in the environment (Schmutterer 1990).

When tested under rigid conditions neem formulations were relatively non-toxic to mammals (including humans) but consumption of neem oil by children or neem leaves by goats, sheep, or guinea pigs was detrimental and even fatal (Jacobson 1989a). Grant and Schmutterer (1987) report that ostracod crustaceans had abnormal growth and moulting after exposure to neem seed kernel extracts. Thus there are cases of detrimental effects on non-target organisms and care must be taken when developing and using a neem product. But, overall, when weighed against the detrimental effects of other types of insecticides, neem remains an insecticide with great potential, especially for IPM programs.

1.5 The Green Spruce Aphid

1.5.1 Hosts and Distribution

The green spruce aphid, *Elatobium abietinum* (Walker), is found in Europe, the United Kingdom, Eurasia, East Asia, New Zealand and North

America (von Kloft *et al.* 1964). In North America it occurs only throughout the range of Sitka spruce, *Picea sitchensis* (Bong.) Carr., on the Pacific Coast from Alaska to California. It has been a chronic pest in the Queen Charlotte Islands and adjacent mainland of British Columbia since 1960. In 1981, > 5,000 ha of spruce were severely defoliated in coastal areas of the Queen Charlotte Islands and 67% of trees died (Koot 1992). It is generally agreed that the aphid was introduced to British Columbia from Europe, probably on contaminated Norway spruce, *P. abies* (L.) Karst, nursery stock (von Kloft *et al.* 1964). Evidence supporting this hypothesis include: 1) a historical deduction that *E. abietinum* probably originated in natural stands of Norway spruce growing in northern Europe (Nichols 1987); 2) morphological comparisons indicating close homology between Eurasian and North American aphids (von Kloft *et al.* 1964); 3) restricted distribution of the aphid in North America but not in Europe; 4) the high susceptibility of North American spruces to aphid attack compared with the low susceptibility of Eurasian, Chinese and Japanese species (Nichols 1987); and 5) the high degree of tolerance that Norway spruce has to high aphid attack compared with the low tolerance of Sitka spruce.

The green spruce aphid is a pest almost exclusively of spruce trees but has been found on pine and Douglas-fir (Furniss and Carolin 1980). No species of spruce is immune to aphid attack but there is variability in degree of attack as well as the degree of defoliation for a given aphid density (Hanson

1951). For example, Norway spruce is highly susceptible to attack, but suffers minimal defoliation, whereas Sitka spruce, which is also highly susceptible to attack, suffers severe defoliation. On the other hand, white spruce, *P. glauca* (Moench) Voss, is not very susceptible to attack. Susceptible North American native spruce trees include: Sitka spruce; white spruce; black spruce, *P. mariana* (Mill.) B.S.P.; blue spruce, *P. pungens* Engelm.; and Engelmann spruce, *P. engelmannii* Parry ex Engelm. (Hanson 1951).

1.5.2 Damage

The green spruce aphid is a gregarious phloem feeder preferring needles that are ≥ 1 year old. It usually aggregates on the lower side of needles in the shaded portions of the lower crown, but will colonize the upper crown and new growth when epidemic (Hussey 1952). Feeding by aphids results in chlorosis, defoliation, and decreased growth (Carter 1977, Ruth *et al.* 1982). The first signs of feeding are yellow spots on the needles. The spots become patches, which spread over the whole needle which eventually turns brown and then drops (Hussey 1952, Fisher 1987). Needle discoloration and drop varies according to attack density and weather. Severe attack may completely defoliate and kill trees (Koot 1992). Usually death of trees is attributed to successive years of aphid attack or the combined damage of the aphid and other pests such as the Cooley spruce gall adelgid, *Adelges cooleyi* (Gillette), (Hussey 1952). Outbreaks usually occur after a mild winter. Susceptible stages range from saplings to mature trees (Koot 1992).

Effects of *E. abietinum* on cone and seed production are not known (Ruth *et al.* 1982) but reduced seed production, and particularly death of a tree are undesirable because of the value of seed from each genetically selected tree (Lavender *et al.* 1990). It is generally believed the combined damage of the green spruce aphid and other "twig" pests results in fewer cones on infested than uninfested trees. For example, branches weakened by the green spruce aphid may break if infested with *A. cooleyi*, resulting in reduced future cone production for that tree.

1.5.3 Life Cycle and Description

In North America and Great Britain there are two aphid morphs - apterous and alate viviparous females (anholocyclic populations) (Hanson 1951). The apterous morph is from 1 to 1.5 mm in length, green, oval and convex, with a dark line on each side of the body. The head is yellowish-green to fawn colour; the antennae are about half the length of the body, pale yellowish-green, and dark at the tips. The alate female is slightly larger than the apterous morph, with antennae nearly as long as her light brown body. Her abdomen is bright green with two rows of four dark green spots on the upper surface, and four smaller and less distinct spots along each side. The wings are large and much longer than the body (Hanson 1951).

The apterous female and her progeny are confined to a single tree where they feed and reproduce by parthenogenesis during periods of favourable ambient temperature (Day 1986). The greatest population increase

occurs from late winter to early spring. In spring, alate females are produced. Induction of alates is determined by tree phenology (phloem amino acid concentration), aphid density and increasing photoperiod (Fisher 1982). At bud burst, aphid populations peak and then decline sharply. Population decline is attributed to: emigration of alates; reduced fecundity due to changes in phloem nutritional quality (Day and Crute 1990); and mortality due to starvation, predation and parasitism (Hussey 1952). In coastal B.C. only apterous females have been observed reproducing, and these only in late winter and spring. In contrast, alate and apterous aphids produce nymphs all year in milder areas of Europe (Koot 1992). Also, in areas with severe winters, holocyclic populations produce dormant, overwintering eggs as a survival strategy (von Scheller 1963). It is thought that anholocyclic populations, found in mild climates, have been selected for because of their greater reproductive capacity than holocyclic populations. By-passing the dormant egg stage enables anholocyclic parthenogenetic viviparae to reproduce year round. This can cause outbreaks after just one favourable winter (Hanson 1951).

The rates of development and reproduction in *E. abietinum* may vary considerably (Hanson 1951) but, on average at 15.5°C, anholocyclic apterous nymphs mature to adults in 18.5 days after four moults (Hussey 1952). Nymphs are produced three days later. Adults live an additional 17 days (total longevity 35.5 days) during which an average of 12 young are produced (Hanson 1951). However, Hussey (1952) reports the total longevity of

apterous females to be 50 days, also with 12 young produced. Alate nymphs take longer to reach maturity (23 days) and undergo five moults. Cunliffe (1924) estimated the average rate of reproduction to be 1,200% in 25 days at 15.5°C in the laboratory.

1.6 Objectives

My objectives were: 1) to determine if applications of neem will reduce populations of the green spruce aphid; 2) to establish the doses and frequency of applications required to achieve efficacy; and 3) to observe any effects on treated trees. Experiments conducted in 1992 on the green spruce aphid were not definitive. Three successful experiments were completed in 1993 and are reported herein.

2.0 MATERIALS AND METHODS

2.1 Experiments 1 and 2 - Low Initial Populations

A proprietary neem seed extract concentration (NSE-5) containing 5% azadirachtin as the active ingredient was supplied by Phero Tech Inc., Delta, B.C. Doses are expressed in terms of ppm (volume to volume) of azadirachtin.

A concentrate solution was prepared by first mixing NSE-5 in a ratio of 1.0 mL NSE-5 to 9.0 mL I-SOL_{RB} emulsifier (Leo-Chem Enterprises, Oakville, Ontario), then slowly adding water while stirring constantly using a separator

funnel and magnetic stirrer to make a solution of 18 mL NSE-5 in 162 mL emulsifier and 1,620 mL water. Further dilutions in water were made to prepare 75 ppm and 150 ppm azadirachtin solutions.

Potted interior spruce trees¹ and greenhouse facilities at the Saanich Test Nursery, Saanichton, B.C., were provided by British Columbia Forest Service, Silviculture Branch. Seventy-two trees, in apparent good health and with no to low observable spruce aphid populations, were selected in January 1993 from a pool of 105 trees originating from two Forest Service research facilities on southern Vancouver Island (North Road, and Cobble Hill). Conditions in the greenhouse were: natural lighting, drip irrigation three times per week (to saturation), air circulation system, and temperature maintained above 4° C.

Trees were sprayed to the run-off point using three hand-held sprayers (Home Gardener Sprayer, Home Hardware Stores Ltd., St. Jacobs, Ontario). Trees were spaced to ensure that branches were neither touching nor overlapping. To prevent overspray a portable protective barrier constructed of polyethylene and PVC tubing (Fig. 1) was placed around each tree during spraying. Each concentration of neem was assigned to a sprayer, colour

¹Interior spruce is the operational forestry term applied to all spruces taken from the natural zone of hybridization of white and Engelmann spruce, *Picea glauca* (Moench) Voss and *P. engelmannii* Parry, respectively, in southern British Columbia.

Fig. 1. Portable protective barrier constructed from polyethylene and PVC tubing, showing placement around potted spruce tree to prevent overspray of adjacent trees with neem seed extract.



coded by treatment, to reduce accidental error when filling or when spraying trees.

Treatments were: 0 ppm (emulsifier control), 75 ppm, and 150 ppm of azadirachtin applied either biweekly (Exp. 1) or weekly (Exp. 2), comprising three or five applications, respectively, with 12 replicates (trees) per treatment. Trees were to be sprayed until a difference in aphid numbers between control and azadirachtin-treated trees was noticeable and significant, i.e. the number of sprays was not pre-set.

Aphid populations were assessed on five branches randomly selected from the lower to mid crown of each tree. Aphids were counted on a 6 cm segment of the 1991 shoot growth measured distally from the proximal internode. Counts for all replicates took two days to complete. The first (pre-spray) count was done on 15 and 16 February 1993. Aphids were counted weekly on all trees for 15 weeks, except for week 14. Aphids were counted just before the application of NSE-5. The mean number of aphids per segment was calculated for each tree. Assessment continued until aphid populations on the control trees declined below 50% of the peak population density.

To control for location in the greenhouse and clonal type, trees for both experiments were combined and arranged in a six-by-six double Latin square with block and clone as row and column effects. There were insufficient numbers of ramets of each clone to include ramets in a statistical model. Hence data were analyzed by an analysis of variance, General Linear Model

with two factors (treatment and block) (Wilkinson 1990) to determine when to stop spraying. Subsequently differences in magnitude of population peaks for each treatment were cube-root transformed and analyzed by One-way analysis of variance followed by Tukey's HSD test (Wilkinson 1990). In all cases $\alpha = 0.05$.

Tree condition was assessed on 4 June 1993 by rating each tree according to severity of chlorosis. Severity of chlorosis ranged from Class 0 = no observable chlorosis to Class 10 = severe chlorosis with no new bud flush. Examples of trees in Classes 1 and 9 appear in Fig. 2. Differences between mean indices of chlorosis were compared with Kruskal-Wallis one-way analysis of variance, $\alpha = 0.05$ (Wilkinson, 1990). The relationship between aphid densities and severity of chlorosis was determined for each treatment in Exp. 1 and 2 by Spearman's rank correlation procedure (Zar 1984, Wilkinson 1990). The influence of host genotype on severity of aphid impact was assessed by pooling the control trees in Exp. 1 and 2, and ranking them by clone according to severity of chlorosis. There were eight clones with two ramets and seven clones with only one ramet.

2.2 Experiment 3 - High Initial Populations

In March 1993, 12 trees were selected which had high populations of > 10 aphids per branch segment. Treatments were 0 ppm (emulsifier control) and 150 ppm azadirachtin. NSE-5 solutions were prepared as above.

Fig. 2. Examples of chlorosis ranked trees. Class 0 = no observable chlorosis; Class 10 = severe chlorosis, no new bud flush. Pictured are a 150 ppm treated tree (on the left) in Exp. 2 ranked as chlorosis class 1 and a control tree (on the right) ranked as chlorosis class 9.



us (big) and 4-5' or more

Not a good idea to

de-branch or to

cut

to keep it

up

Treatments were randomly assigned to each tree (six trees per treatment). Trees in each treatment were sprayed in groups of three to reduce the time required to spray them. Trees were sprayed once on 6 March using the methods described in Exp. 1 and 2.

Aphids were assessed prior to spraying and weekly for six weeks following the spray. Because it is extremely difficult and time consuming to count large populations of green spruce aphids, aphid numbers per segment were assessed according to four classes: Class 1 = 0 - 10 aphids; Class 2 = 11 - 30; Class 3 = 31 - 50; Class 4 = > 51 aphids. Classes were summed for each tree (i.e. if a tree had three segments each rated as Class 3 then the tree received a rating of 9) and differences between treatments for each week were tested for significance using a Mann-Whitney U-test, $\alpha = 0.05$ (Sokal and Rohlf 1981).

3.0 RESULTS

3.1 Low Initial Populations

Azadirachtin at 150 ppm was efficacious at suppressing green spruce aphid populations in a greenhouse with low initial populations on potted spruces (Figs. 3 and 4). Suppression occurred whether applications were made three times (biweekly sprays, Exp. 1) or five times (weekly sprays, Exp. 2) (Exp. 1: $F = 3.88$; $df = 2,33$; $P = 0.03$; and Exp. 2: $F = 3.77$; $df = 2,32$; $P = 0.03$).

Fig. 3. Population trends of green spruce aphids on potted spruce trees sprayed three times in Exp. 1 to the run-off point with an aqueous formulation of a proprietary neem seed extract concentrate (NSE-5) containing 75 or 150 ppm of azadirachtin. Arrows indicate weeks when sprays were applied.

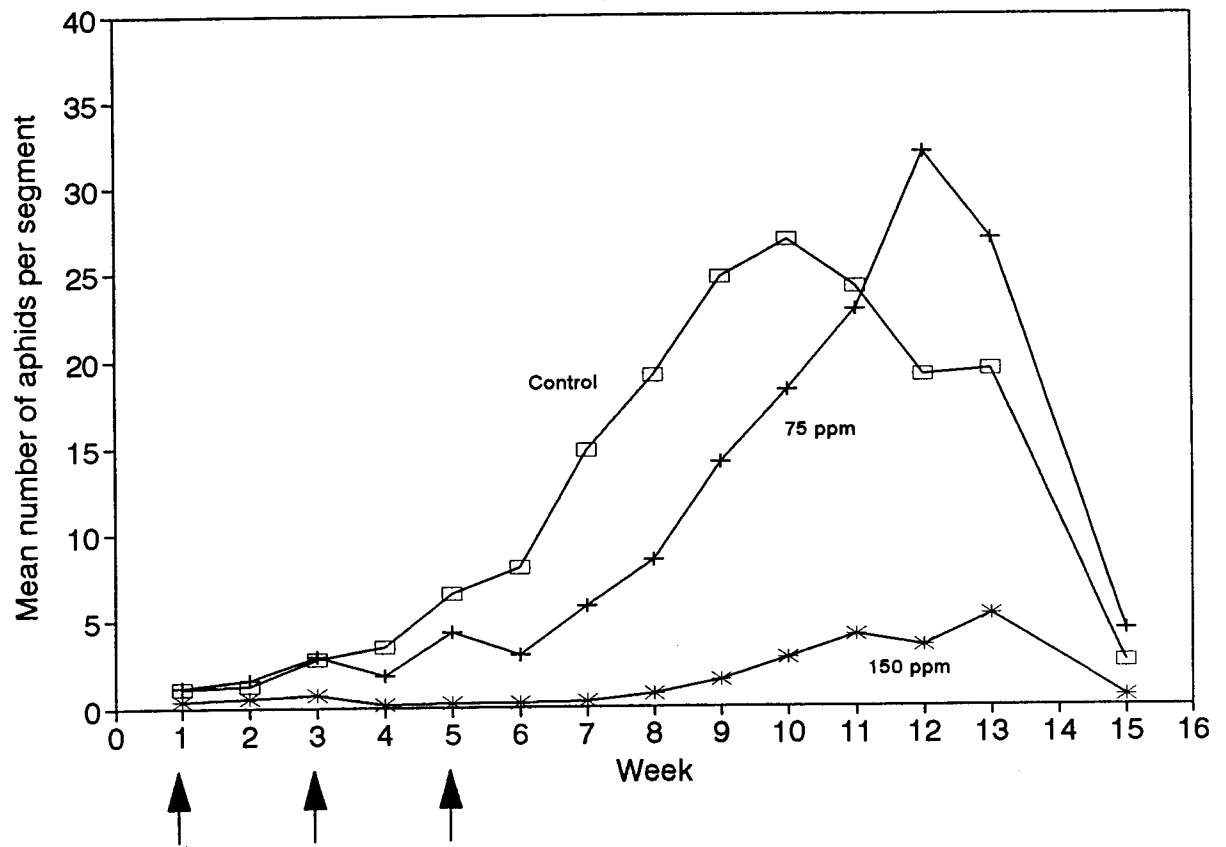
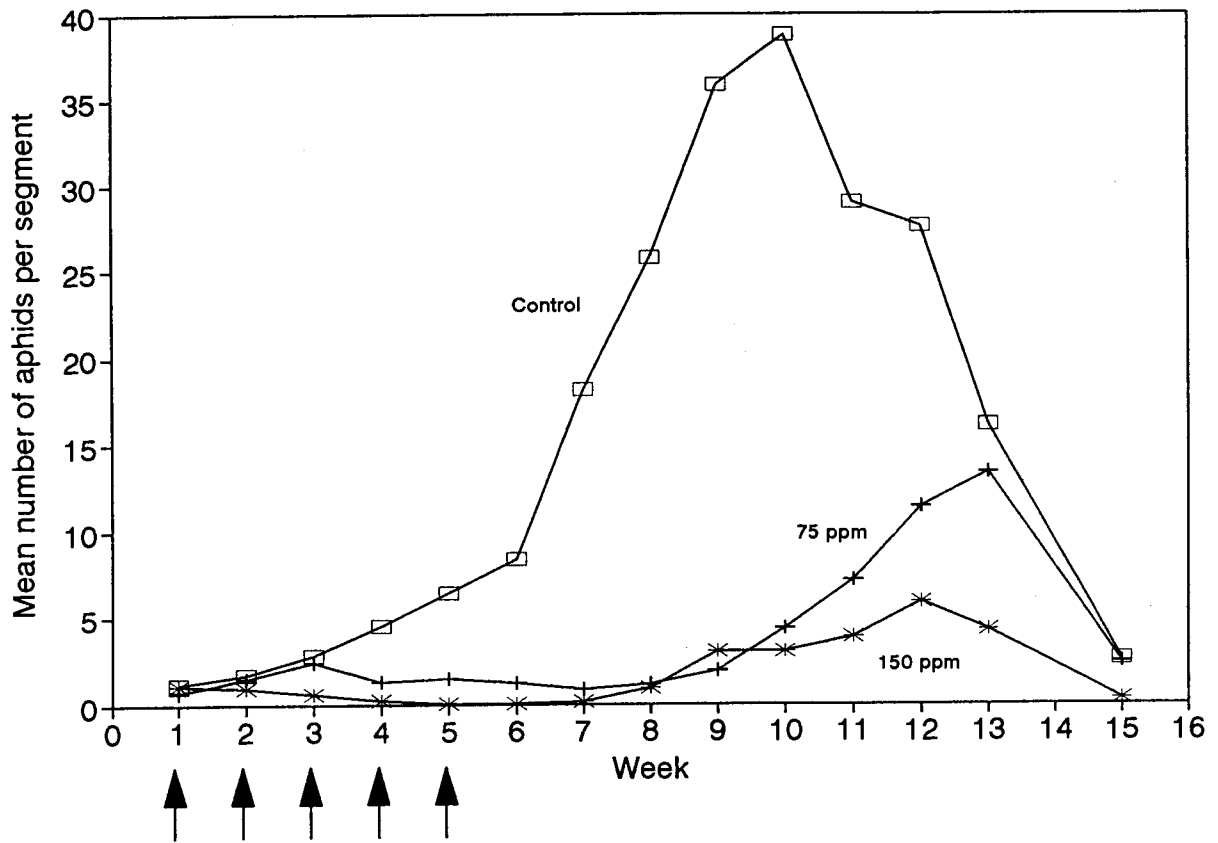


Fig. 4. Population trends of green spruce aphids on potted spruce trees sprayed five times in Exp. 2 to the run-off point with an aqueous formulation of a proprietary neem seed extract concentrate (NSE-5) containing 75 or 150 ppm of azadirachtin. Arrows indicate weeks when sprays were applied.



Aphid populations (aphids per segment) on trees treated with azadirachtin at 150 ppm peaked at 5.3 (three sprays) and 5.9 (five sprays), significantly lower than the peak populations of 26.9 (three sprays) and 36.8 (five sprays) aphids per segment for the control groups (Tukey's HSD test, $P < 0.05$). Azadirachtin at 75 ppm was not as effective as at 150 ppm. Aphid populations on trees sprayed three or five times at 75 ppm peaked at 32.2 and 13.4 aphids per segment, respectively; these peak numbers were not significantly different from the population peaks in control trees (Tukey's HSD test, $P > 0.05$). Aphid populations on trees sprayed three times at 75 ppm differed significantly from trees sprayed three times at 150 ppm (Tukey's HSD test, $P = 0.03$), whereas populations on trees sprayed five times at 75 ppm did not differ significantly from trees sprayed five times at 150 ppm (Tukey's HSD test, $P = 0.82$).

The decision to "stop spraying" was made after qualitatively judging and then quantitatively confirming (by statistical analysis) that populations on control trees were significantly greater than those on azadirachtin-treated trees. This judgement was made after six weeks. At week six, populations on trees treated with 150 ppm azadirachtin differed significantly from those on control trees, but not from those on trees treated with azadirachtin at 75 ppm ($F = 4.191$; $df = 2,22$; $P = 0.03$ and $F = 5.908$; $df = 2,21$, $P = 0.01$ for Exp. 1 and 2, respectively; Tukey's HSD test, $P < 0.05$). At this time, there was no difference between populations on trees sprayed three times with azadirachtin

at 75 ppm and those on control trees (Tukey's HSD test; $P > 0.05$) whereas populations on trees sprayed five times with 75 ppm were significantly different from control trees (Tukey's HSD test; $P < 0.05$).

Populations on control trees treated only with the emulsifier increased gradually at first, rose exponentially after week six to a peak at week 10 and declined sharply thereafter (Figs. 3 and 4). In contrast, the 150 ppm treatments effectively suppressed aphid populations to below 1.0 aphid per segment until week eight (three weeks post-spray). After week eight, aphid populations gradually rose to greatly suppressed peaks on weeks 13 and 12 for three and five sprays, respectively, and declined to near zero by week 15.

Aphid populations on trees treated three times with azadirachtin at 75 ppm paralleled those on the control trees but had a two-week delay (Fig. 3). There appeared to be a minor "knockdown" effect after sprays on weeks three and five (Fig. 3). However, five sprays at 75 ppm suppressed populations to levels obtained with the 150 ppm treatments, but the population "escaped" to a higher peak (13.4 aphids per segment) than in the 150 ppm trees (5.9 aphids per segment).

In Exp. 1 and 2 mean indices of chlorosis ranged between 3.2 and 5.3 (Table 1), but there were no significant differences between treatments. All trees with aphids suffered some degree of chlorosis. Trees with high aphid densities turned yellow, then brown and then shed their needles, whereas some trees with low aphid densities turned from green to red; they did not turn

Table 1. Comparison of tree condition at completion of Exp. 1 and 2 as assessed by severity of chlorosis, rated on a scale of 0 to 10 (0 = no chlorosis; 10 = severe chlorosis with no new bud flush, tree probably dead).

Exp.	No. of Sprays	Assessment Week	Treatment	N	Mean
					Chlorosis Class ^a
1	3	18	Control	12	4.4
			75 ppm	12	4.6
			150 ppm	11	3.2
2	5	18	Control	12	5.3
			75 ppm	12	4.0
			150 ppm	12	4.8

^aNo significant difference between means within either experiment, Kruskal Wallis one-way analysis of variance, $P > 0.05$.

yellow. Red coloration is characteristic of drought stress rather than aphid damage. The relationship between degree of chlorosis and aphid densities was highly significant for each control treatment in Exp. 1 and 2, and was weakly significant for trees treated with 75 ppm azadirachtin three times biweekly in Exp. 1 or five times weekly in Exp. 2 (Table 2). However, the relationship between aphid densities and chlorosis was not significant for trees treated with 150 ppm azadirachtin three times biweekly in Exp. 1 or five times weekly in Exp. 2 (Table 2). Thus, when aphid populations were suppressed, chlorotic effects still occurred, and the lack of a relationship between aphid numbers and severity of chlorosis for the 150 ppm azadirachtin treatments indicates that factors other than aphids, e.g. drought stress, were adversely affecting tree condition. There were indications that some clones were more susceptible to chlorosis than others (Table 3). When two ramets were represented, each received similar if not identical rankings, except for clone 006, which had ranks of 2 and 8.

During counts of green spruce aphids, I found alate morphs after March 26, week 6. On several occasions, I observed parturition by an alate morph. This is the first record of reproduction by alate females in British Columbia.

3.2 High Initial Populations

Pre-treatment assessment on 6 March, 1993 confirmed that there was no significant difference in high populations between treatment and control

Table 2. Correlations between severity of chlorosis and green spruce aphid densities on "interior" spruce trees at the completion of Exp. 1 and 2.

Chlorosis was rated on a scale of 0 to 10 (0 = no chlorosis; 10 = severe chlorosis with no new bud flush, tree is probably dead).

Exp. no.	Treatment	n	r_s	P^a
1	Control	12	0.90	< 0.001
	75 ppm	12	0.75	< 0.01
	150 ppm	11	0.41	> 0.10
2	Control	12	0.86	< 0.002
	75 ppm	12	0.71	< 0.02
	150 ppm	12	-0.21	> 0.50

^aSpearman's rank correlation

Table 3. Effect of clonal variation on severity of chlorosis on pooled control trees at the completion of Exp. 1 and 2.

Clone	No. of ramets	Ranked severity of chlorosis
52	1	10
150	2	10,8
28	1	9
113	1	9
170	1	8
024	2	7,8
006	2	2,8
017	1	7
048	1	6
002	1	4
090	2	2,2
029	2	2,2
003	2	2,1
005	2	1,1
081	2	1,1

trees in Exp. 3 (Table 4). At one week post-spray after only one application of azadirachtin at 150 ppm, aphid populations on treated trees were reduced significantly and remained so through week five, although there was a slight increase four weeks post-spray. Many dead aphids, distinguished by their black coloured bodies, were visible. At week six, populations were declining on the control trees while those on the azadirachtin-treated trees increased slightly, before declining to a level similar to that on control trees at week seven. Although not assessed, populations on both treated and control trees "crashed" by week eight.

4.0 DISCUSSION

My results demonstrate that neem (formulated as NSE-5) is efficacious at reducing green spruce aphid populations. However, my results also indicate that dosage, application frequency and timing, clonal variation among infested trees, and environmental conditions could affect neem's potential for use in seed orchards and other forestry systems. The mechanism by which neem reduced green spruce aphid abundance is not known. Increased mortality is involved because populations in Exp. 3 were reduced (Table 1). Reduced fecundity is suggested by inhibition of population growth in Exp. 1 and 2 (Figs. 3, 4). The mode of action is unclear but either a repellent or antifeedant effect, direct toxicity, disruption of molting or some combination thereof are probable mechanisms. Because neem is not considered to be a contact aphicide and

Table 4. Effect on initially high green spruce aphid populations of a single spray to the run-off point with an aqueous formulation of a proprietary neem seed extract concentrate (NSE-5) containing 150 ppm of azadirachtin in Exp. 3. All trees had > 10 aphids per 6 cm segment of 1991 shoot growth at the time the spray was applied on 6 March 1993 (week 1).

Week	Mean aphid population index (max = 4.00)		<i>P</i> ^a
	Emulsifier control	150 ppm azadirachtin	
1	2.00	2.11	0.60
2	1.11	0.61	0.05
3	2.11	0.00	0.00
4	3.00	0.28	0.00
5	3.00	0.44	0.00
6	1.56	1.11	0.46
7	0.83	0.83	0.63

^aProbability of significant difference between treatments (n = 6) determined by Mann-Whitney U-tests.

because aphids are phloem feeders, any effect other than repellency must have been caused by the presence of neem in the phloem as a result of systemic uptake, probably through sprayed foliage.

The failure of NSE-5 at 75 ppm azadirachtin applied three times biweekly in Exp. 1 to suppress aphid densities (Fig. 3) probably occurred because of an insufficient dose. Mortality of aphids is positively correlated with dose (Lowery *et al.* 1992), and dose is related to amount of active ingredient present over time. As Lindquist *et al.* (1990) emphasized, dose is more important than concentration. Neem's residual activity ranges from four to eight days; therefore, there was probably little, if any, active ingredient remaining at one week post spray. This hypothesis is supported by the apparent "knock down" and "escape" effect on trees treated with 75 ppm azadirachtin in Exp. 1 (Fig. 3). NSE-5 applied at the same concentration of 75 ppm azadirachtin but applied weekly in Exp. 2 was probably effective because it was constantly available for ingestion by the green spruce aphid for a six-week duration. Conversely, NSE-5 at 150 ppm azadirachtin applied biweekly in Exp. 1 must have been an effective dose because of its greater concentration; there was evidently an effective dose remaining during the week between sprays.

The differences in magnitude of population growth among treatments is likely related to population levels six and nine weeks after the first treatment, at the start of the population growth phase, as can be seen in Fig. 4 when 75

ppm azadirachtin and control treatments are compared. Therefore azadirachtin should be applied at a rate that will suppress or knock down initial aphid populations to a level that will recover slowly post spray. Careful monitoring should follow and additional sprays, if required, should effectively suppress aphid populations to below damaging levels.

The severity of chlorosis observed on potted control trees with low aphid populations (Table 1) is due to other factors as well as aphid abundance. These other factors are revealed by differences in the strength of the relationships between severity of chlorosis and aphid density for different treatments (Table 2). Drought stress, heat stress, temperature fluctuations, NSE-5, emulsifier, previous tree management, clonal variation, provenance (genotypic) differences, or some interaction of these factors are all possible causes. Trees that turned red likely suffered from drought stress, because red foliage is a drought stress symptom. It is possible that some trees with low aphid densities retained sufficient foliage to be able to transpire at a higher rate than trees with high aphid densities; hence, they may have received insufficient water to meet their transpiration losses. This may explain why there were more red trees in the "best" neem treatments than in the controls. As well, red trees were "clumped" in Blocks 1, 3, and 6 so some "position" effect may have caused drought stress. The small containers in which some trees were planted may have held insufficient water to meet the tree's needs, and thus enhanced drought stress, despite frequent watering. Trees with low

aphid densities but high severity of chlorosis, and which had typical chlorotic symptoms, were probably adversely affected by some other factor than drought.

Trees treated five times with 150 ppm azadirachtin in Exp. 2 had the weakest correlation between aphid numbers and severity of chlorosis (Table 2). Because the severity of chlorosis was the same as with other treatments, it is unlikely that the NSE-5 formulation used in these experiments may have interacted with the trees or some other factor to cause chlorosis. Neem seed oil is phytotoxic to some plants (Lowery *et al.* 1992; Schmutterer 1990), but there are no reports of phytotoxicity due to refined neem seed extracts.

A knowledge of provenance and clonal variability in aphid susceptibility would be useful in conifer seed orchards. Day (1984) demonstrated that aphid density appeared to be related to the latitudinal origin of trees with southerly provenances being more susceptible than northern ones. The seed orchard manager could avoid planting susceptible genotypes in seed orchards located in areas that have mild winters (such as southern Vancouver Island). Thus the risk of outbreaks due to warm temperatures could be reduced. Trees in seed orchards could be identified and mapped according to aphid susceptibility; the seed orchard manager could focus on monitoring and treating only susceptible genotypes and if treated early, aphid damage to trees could be reduced. This would be a great improvement over the current system where: 1) trees must first show signs of aphid attack (chlorosis) before monitoring is initiated; 2)

mean aphid densities of 10 chlorotic and 10 non-chlorotic trees combined is used before deciding to treat; and 3) only heavily attacked (chlorotic) trees are treated; thus, trees must first suffer aphid damage before the aphids are "managed".

For reforestation, the knowledge of clonal variation could be used by silviculturalists to avoid planting high-hazard sites with genotypes susceptible to green spruce aphids, thereby reducing tree losses. At the same time, indigenous levels of aphids in high-hazard areas would be reduced and the occurrence of outbreaks would be lessened. The reduction of indigenous populations and the employment of non-susceptible or tolerant genotypes will be especially important if the predicted greenhouse effect occurs because warm conditions favour green spruce aphid survival and developmental rates (Day and Crute 1990, Fisher 1992).

Neem's demonstrated efficacy at reducing green spruce aphid populations justifies its potential use in the management of seed orchard pests. Control of seed orchard pests will maintain tree health and cone crop yield, thus ensuring high yields of genetically-improved seed for reforestation. The use of neem, a demonstrated environmentally-friendly product, should be publicly acceptable as an alternative to broad-spectrum, highly-toxic organophosphorous insecticides. Also, neem should enhance natural biological control agents as part of an integrated pest management strategy.

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