Control of conifer defoliators with neem-based systemic bioinsecticides using a novel injection device

Blair V Helson,[1] D Barry Lyons, Kevin W Wanner

Canadian Forest Service, Natural Resources Canada, PO Box 490, Sault Ste. Marie, Ontario, Canada P6A 5M7

Taylor A Scarr

Forest Management Branch, Ontario Ministry of Natural Resources, Suite 400, 70 Foster Drive, Sault Ste. Marie, Ontario, Canada P6A 6V5

The Canadian Entomologist **133**: 729 – 744 (2001)

Abstract—A systemic tree injection tube was designed to introduce the required volumes of neem-based bioinsecticides into conifer trees. The device consists of plastic tubing attached with hose clamps to a maple sap spile at one end and a tubeless automobile tire valve at the other end. A hole is drilled in the tree, the spile is hammered into the hole, the device is filled with the systemic insecticide, and the system is pressurized by attaching a bicycle pump to the tire valve. The parts are readily available, the device is simple to construct and easy and quick to install on a tree, application volumes are adjustable, and the device is reusable. This device has been used successfully to inject 188 trees representing four conifer species in either spring or fall, primarily with neem formulations but also with dimethoate, imidacloprid, and acephate. In most cases, all of the material was injected into the trees without leakage, although neem formulations were characteristically slow to enter the trees and certain neem formulations were not injected completely at volumes above 15 mL per injection tube. Dosages of 0.2 g azadirachtin / cm of diameter at breast height (dbh) or less provided control of pine false webworm, spruce budworm, cedar leafminers, gypsy moth, and introduced pine sawfly on red pine, white spruce, eastern white cedar, white pine, and white pine, respectively. Dosages as low as 0.005 g active ingredient / cm of dbh applied with injection tubes in either one or two holes per tree resulted in a 95% reduction in defoliation of mature (mean dbh ± SD = 23.4 ± 3.3 cm) red pine caused by pine false webworm.

Helson BV, Lyons DB, Wanner KW, Scarr TA. 2001. Lutte contre les défoliateurs des conifères au moyen de bioinsecticides systémiques à base de neem injectés à l'aide d'un nouveau dispositif. *The Canadian Entomologist* **133** : 729–744.

Résumé—Un tube d'injection systémique pour les arbres a été conçu pour introduire le volume voulu de bioinsecticides à base de neem dans des conifères. L'appareil est constitué d'un tube de plastique attaché à une goudrelle au moyen d'un collier de serrage à un bout et à une valve pour pneu sans chambre à air à l'autre bout. Il faut percer l'arbre avec une mèche, forcer la goudrelle dans le trou à coups de marteau et remplir l'appareil d'insecticide qui sera ensuite pressurisé à l'aide d'une pompe de bicyclette branché sur la valve. Les pièces sont faciles à obtenir, la construction et la fixation de l'appareil à l'arbre aisées et rapides, le volume d'insecticide à injecter est réglable et l'appareil est réutilisable. L'appareil a servi avec succès dans la lutte contre les défoliateurs sur 188 arbres représentant quatre espèces de conifères, au printemps et à l'été, surtout avec des préparations de neem, mais aussi avec du méthoate, de l'imidacloprid et de l'acéphate. Dans la plupart des

[1] Author to whom all correspondence should be addressed (E-mail: bhelson@nrcan.gc.ca).

cas, toutes les substances étaient injectées dans l'arbre sans fuites, bien que certaines préparations de neem se soient révélées particulièrement lentes à pénétrer et certaines n'ont pu être injectées entièrement à des volumes supérieurs à 15 mL par tube à injection. Des doses de 0,2 g d'azadirachtin / cm de diamètre d'arbre à hauteur de poitrine (dbh) ou plus basses ont permis une lutte efficace contre le Pamphile du pin sur des pins rouges, contre la Tordeuse des bourgeons de l'épinette sur des épinettes blanches, contre les mineuses sur des thuyas de l'est, contre la Spongieuse sur des pins blancs et contre la Tenthrède du pin introduite sur des pins blancs. Des doses aussi faibles que 0,005 g ingrédient actif / cm de dbh de poitrine injectées par les tubes dans un ou deux trous par arbre ont diminué de 95 % la défoliation de pins rouges matures (dbh moyenne ± écart type = 23,4 ± 3,3 cm) attaqués par des pamphiles.

[Traduit par la Rédaction]

Introduction

Seed kernel extracts of the neem tree, *Azadirachta indica* A. Juss (Meliaceae), containing azadirachtin have insecticidal and behavioural modifying effects against a broad range of pest insects (Schmutterer 1995). Foliar applications of neem-based insecticides have proven to be efficacious for the control of several forest insect pests in Canada (Thomas *et al.* 1992; Wanner and Kostyk 1995; Kostyk and Wanner 1997; Wanner *et al.* 1997; Helson *et al.* 1998, 1999; Lyons *et al.* 1998).

It has been demonstrated that neem seed kernel extracts containing azadirachtin possess systemic activity in plant tissues (Gill and Lewis 1971). There is a need to develop safer alternatives to the conventional, generally highly toxic, systemic insecticides for applications to trees. A neem-based bioinsecticide could be ideally suited for this purpose because of its very low mammalian toxicity and wide spectrum of insecticidal activity. A neem product would be safe to handle and would greatly reduce the risk due to bystander exposure. Several studies have demonstrated that systemic treatments of trees with neem seed kernel extracts were effective against birch leafminer, *Fenusa pusilla* (Lepeletier) (Hymenoptera: Tenthredinidae), in paper birch, *Betula papyrifera* Marsh. (Betulaceae) (Marion *et al.* 1990); mountain pine beetle, *Dendroctonus ponderosae* Hopkins, and pine engraver beetle, *Ips pini* Say (Coleoptera: Scolytidae), in lodgepole pine, *Pinus contorta* Douglas var. *latifolia* Engelmann (Pinaceae) (Naumann *et al.* 1994; Duthie-Holt *et al.* 1999); spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae), in white spruce, *Picea glauca* (Moench) Voss (Pinaceae) (Sundaram *et al.* 1997; Wanner *et al.* 1997); pine false webworm, *Acantholyda erythrocephala* (L.) (Hymenoptera: Pamphiliidae), in red pine, *Pinus resinosa* Aiton (Lyons *et al.* 1996); and twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), in potted trembling aspen, *Populus tremuloides* Michx. (Salicaceae) (Sundaram *et al.* 1995). Although these studies have clearly demonstrated the potential of systemic applications using relatively low doses of azadirachtin, application to large trees is a problem because relatively high volumes of neem-based insecticides are required to obtain comparable doses. All commercial and experimental liquid formulations of neem seed extract to date contain less than 4.5% azadirachtin. In the aforementioned studies, the treatments were performed either on small trees requiring low enough volumes that the neem extracts could be applied directly to holes drilled in the trees or on larger trees in which axe frills were cut at the base of the trees and the formulation was introduced directly into the frill (Naumann *et al.* 1994; Duthie-Holt *et al.* 1999). This technique would not be suitable for treating trees to improve their health and chances of survival. Wanner *et al.* (1997) used a technique that initially showed promise for applying the higher volumes that typically would be required for larger trees. They inserted plastic funnels into holes drilled at a downward angle in the tree

and sealed them with silicon cement. Twenty-five millilitres of an undiluted formulation of neem emulsifiable concentrate (EC) was poured into each funnel. In this trial, the delivery technique was successful, with all funnels being empty after 8 d. In subsequent experiments, this infusion technique was not consistent because not all of the neem entered the trees and the amounts remaining varied among trees (BV Helson, unpublished data).

Both macroinjection and microinjection techniques using pressure to introduce liquids into trees have been developed (Kielbaso 1979; Tattar 1999). Macroinjection techniques are designed to treat large trees with high volumes of fluids, primarily to control vascular diseases such as Dutch elm disease (Kondo 1972). Although macroinjection techniques could introduce the volumes of neem formulations required, these techniques are labour-intensive and are not practical for rapid treatment of numerous trees. The devices are also not readily portable in rough terrain. Microinjection devices such as those developed by the JJ Mauget Company, Arcadia, California, are portable, and numerous trees can be treated quickly, but the injector is designed to hold a small volume of concentrated chemical. The number of units required to treat a large tree with a neem formulation would be impractical and so would the number of holes and time required. Tree Technology Inc, Mokena, Illinois, has developed another microinjection device which holds larger volumes, but both these microinjection devices must be preloaded by the commercial manufacturer, are expensive, and cannot be reused, which would be impractical, particularly for research purposes. The ideal technique would be quick to set up, inexpensive, and reusable, could deliver variable volumes of 5–50 mL depending on tree size, and would not leak. Many devices were required to treat large numbers of trees simultaneously in replicated experiments. McCoy (1979) described a pressure-tank injector constructed of metal parts for treating coconut palms with oxytetracycline. Although this device was effective for injecting 1-L volumes of solution into only one hole per tree, it was apparently not pursued further because it was not commercially available. We have developed a systemic tree injection tube based on this pressure-tank principle which was constructed from inexpensive and easily obtained components.

Several independent field trials were conducted to test the suitability of the injection tubes for introducing neem seed formulations and other systemic insecticides into conifer trees and to assess the effectiveness of these products for the control of the introduced pine sawfly, *Diprion similis* (Hartig) (Hymenoptera: Diprionidae), on white pine, *Pinus strobus* L.; cedar leafminers, *Argyresthia thuiella* (Packard) and *Argyresthia aureoargentella* Brower (Lepidoptera: Argyresthiidae), on eastern white cedar, *Thuja occidentalis* L. (Cupressaceae); spruce budworm on white spruce; and pine false webworm on red pine. The effects of timing of application (*i.e.*, spring *versus* fall; time in spring), formulation and dose of azadirachtin, and comparison of neem with other systemic products were also examined using the pine false webworm system.

The introduced pine sawfly is an open-feeding pest of pines. Use of a systemic insecticide ensures that trees have prolonged protection against this migratory multivoltine sawfly. Eggs of cedar leafminers are laid on new foliage in the summer, and larvae mine the leaves until late September and usually overwinter in the mines as fifth instars. Feeding resumes in the spring; the larvae complete development and pupate in late May. Unlike the previous species, cedar leafminer larvae are internal feeders, thus our strategy was to treat trees systemically with neem in late summer after the eggs were laid and when small larvae were present in the cedar leaflets. Spruce budworm is also concealed when mining needles, unopen and expanding buds, and flowers of spruce or balsam fir, *Abies balsamea* (L.) Miller (Pinaceae), in spring. Adults of the pine false webworm, a univoltine species, emerge from overwintering sites in the soil in early spring (Lyons and Jones 2000). Females oviposit on the needles of trees of

the genus *Pinus*. The larvae feed during June and early July on needles from within webs, which are formed by the larvae from silk and debris along the branches of the host plant. The cryptic nature of these larvae also makes them suitable candidates for control by systemic insecticides. Furthermore, other than aerial application, systemic treatment is the only practical method for controlling larvae in trees 20 m or more tall.

Materials and methods

Systemic tree injection tube

The components for a systemic tree injection tube (Fig. 1) consisted of a maple sap spile for tubing systems (Interprovincial Plastics Limited, Québec City, Quebec), a 20 mm long piece of plastic tubing (11 mm i.d. × 14 mm o.d.), the main piece of plastic tubing (13 mm i.d. × 17.5 mm o.d.) cut to length for the required volume, a tubeless automobile tire stem (51 or 63.5 mm length) with valve, and two hose clamps (8–22 mm). The main piece of plastic tubing served as the fluid reservoir and pressure tank. The rubber bulb at the base of the tire stem was removed to fit inside the tubing. Initial assembly before field use involved attaching the main piece of tubing to the spile and clamping tightly with a hose clamp (Fig. 1).

The equipment required to set up the device on a tree (Fig. 2) included a drill with a 9.5-mm bit, a hammer, and a screwdriver or nutdriver for tightening the hose clamps. A 6 cm deep hole was drilled at the base of the tree at a slight downward angle. Most of the wood filings were removed from the hole while withdrawing the drill. The maple sap spile was hammered into the hole to within about 1 cm of the base of the spile until there was solid resistance to further hammering. The plastic tube was filled with the desired volume of fluid and the tire valve stem was inserted into the top of the tubing and sealed with a hose clamp. A bicycle air pump was connected to the tire valve and the injection tube was pressurized to 275 kPa. The pump was disconnected in a rapid motion to prevent depressurization. The plastic tubing was checked for rigidity to ensure that the system was pressurized. For most injections (except where noted), two holes at the base of each tree were drilled on opposite sides and an injection tube was placed in each hole. The required amount of insecticide was measured into each tube and the system was pressurized.

Control of introduced pine sawfly on white pine

A trial was conducted near Sault Ste Marie, Ontario (46°48′N, 82°52′W), to assess the effectiveness and residual activity of an experimental neem formulation (Neem EC, 3% azadirachtin, Neem International Enterprises, Surrey, British Columbia), injected *via* injection tubes into white pines. Efficacy was evaluated using larvae of *D. similis* in foliage-feeding bioassays in the laboratory. On 27 August 1997, this undiluted formulation was injected into three white pines [20, 20, and 23 cm diameter at breast height (dbh)] at 0.1 g active ingredient (a.i.) / cm of dbh (*i.e.*, 33–38 mL). The devices were examined 18 h after set up, and three of the six tubes still contained neem. These were repressurized, although they still had some pressure at this time. After 24 h, all of the contents had entered the trees.

Fourteen, 28, 41, 72, and 77 d after treatment, two branch tips were collected from random aspects of each tree at a height of 4–5 m. Branch tips were also collected from comparable untreated trees. In the laboratory, a twig with both 1-year-old and current-year foliage was removed from each branch and the cut end placed in a vial containing water. Five third-instar, introduced pine sawfly larvae from a laboratory colony were placed on each twig for 3 d. The larvae were placed on untreated foliage and

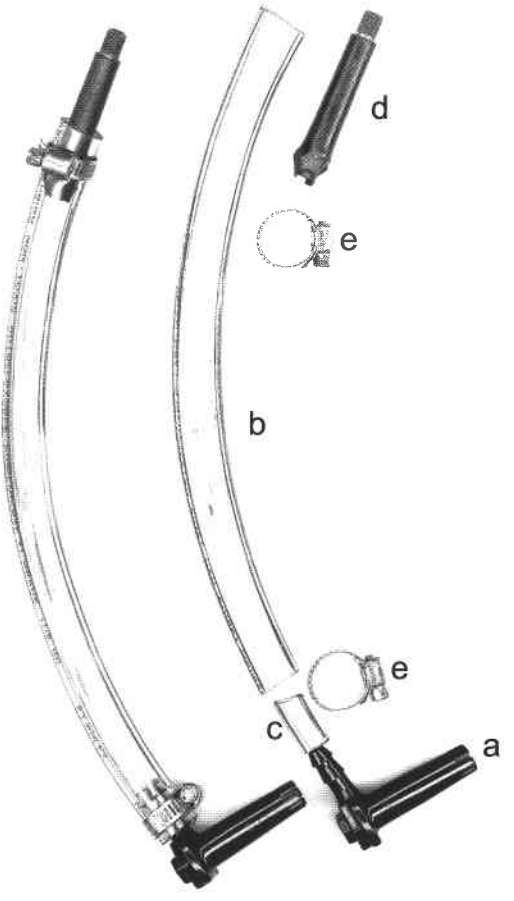

FIGURE 1. The assembled and unassembled components of the systemic tree injection tube: (*a*) maple sap spile; (*b*) chemical reservoir tube; (*c*) coupling tube; (*d*) tubeless automobile tire stem with valve; and (*e*) hose clamps.

checked at 3-d intervals thereafter until they pupated or died. Larvae were kept under a photoperiod of 16L:8D at about 21°C and 70% RH. Larvae from each tree were pooled for analysis. An arcsine transformation of the square root of the proportion dead after 15 d in each bioassay was performed and differences between treatments and times after treatment were compared by a two-way analysis of variance using SigmaStat 2.0 (Jandel Corporation 1995). A similar bioassay with fourth-instar gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), larvae was performed 56 d after treatment when sawfly larvae were unavailable.

Control of cedar leafminers on eastern white cedar

On 10 September 1997, the Neem EC formulation was applied to five 8-cm dbh eastern white cedars in Fort St. Joseph National Historical Site, St. Joseph Island, Ontario (46°4′N, 83°56′W), in a stand of cedars which was heavily infested with cedar leafminers. The formulation was applied at a rate of 0.2 g a.i./cm of dbh. For comparison, dimethoate (Cygon 2E, 240 g a.i./L, NU-GRO Corporation, Woodstock, Ontario) was injected into five comparable trees at 0.2 g a.i./cm of dbh.

https://doi.org/10.4039/Ent133729-5 Published online by Cambridge University Press

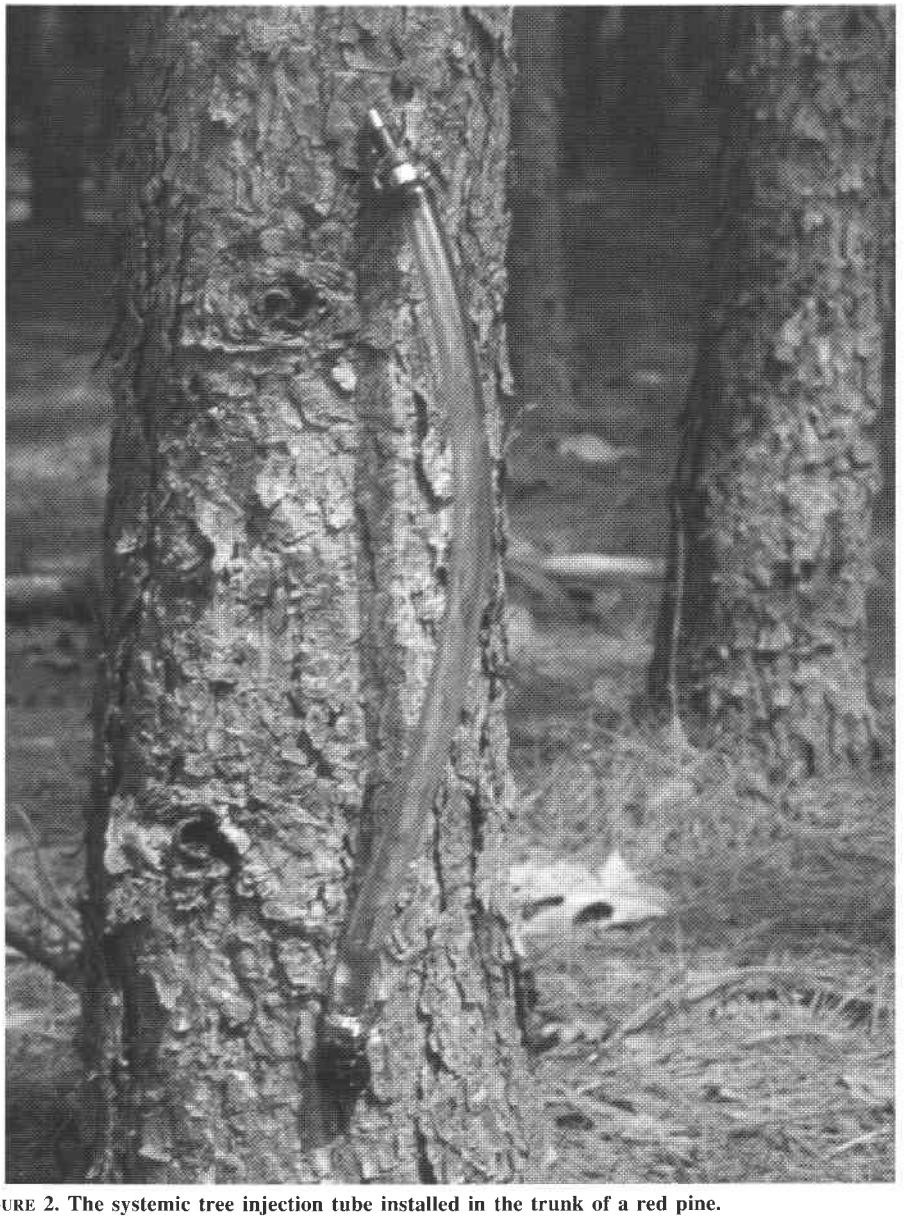

FIGURE 2. The systemic tree injection tube installed in the trunk of a red pine.

In late May 1998, two, 30-cm branch tips were collected from each treated tree and five comparable untreated trees. Branches were sampled with pole pruners from a height of approximately 3 m above the ground from random aspects of the trees. The total numbers of larvae, pupae, and active mines on all pairs of branch tips was determined. Following a $(x + 0.5)^{1/2}$ transformation of the data, mean numbers of larvae, pupae, and mines per pairs of branch tips were compared using analysis of variance followed by Tukey's test using SigmaStat 2.0 (Jandel Corporation 1995).

https://doi.org/10.4039/Ent133729-5 Published online by Cambridge University Press

Control of spruce budworm on white spruce

Neem EC was introduced into white spruces, which had been defoliated in the spring of 1997 by spruce budworm larvae, using injection tubes in the fall of 1997 to determine if neem would carry over the winter in these trees and provide protection against larval feeding in the spring of 1998.

On 30 October 1997 at Balsam Lake Provincial Park near Orillia, Ontario (44°36′N, 78°53′W), the undiluted formulation (19–33 mL) was injected into five 15–20 cm dbh trees at 0.006 g a.i. per trunk cross-sectional area (cm^2) at breast height (= 0.04–0.05 g a.i./cm of dbh).

On 8 May 1998, two 45-cm branch samples were randomly collected from each treated tree and five untreated trees. The cut ends were placed in jars with water in the laboratory. The bud caps of the new shoots were breaking at this time and wild spruce budworm larvae on these branches were predominantly third instars. The number of spruce budworm larvae from natural attack in the field was determined for these branches. After 3 d, two 8-cm tips with flushed buds were cut from each branch and placed in plastic cups with 15 fourth-instar, laboratory-reared spruce budworm. After 5 d, the larger laboratory-reared larvae were removed from this foliage and placed on fresh, untreated white spruce foliage. The larvae were observed every 3 d until they died or pupated. Data for branch pairs from each tree were combined and mean numbers of wild larvae on naturally attacked treated and untreated branches and mortality of laboratory-reared larvae on treated and untreated branch tips were compared by *t* tests using SigmaStat 2.0 (Jandel Corporation 1995).

Control of pine false webworm on red pine

Fall versus spring treatments

Systemic injections of neem were performed in the fall of 1997 and spring of 1998 on about 20 m tall, 25–30 cm dbh red pines, which had been severely defoliated by pine false webworm larvae, to determine if neem would remain in trees through the winter and provide comparable protection to those treated in the spring against larvae which emerged in the spring of 1998.

Twenty trees were selected in the fall of 1997 in a red pine plantation (Simcoe County Forest, Craighurst Tract) 15 km southwest of Orillia, Ontario (44°32′N, 79°34′W), and trees were randomly assigned to the four treatments. On 30 October 1997, the Neem EC formulation (21–25 mL) was injected into five of the trees at 0.05 g a.i./cm of dbh. On 20 May 1998, about 2 weeks before egg hatch, five trees were treated with this formulation by the same method and another five trees were treated with another formulation of neem, Fortune Aza EC (3% azadirachtin, Fortune Bio-tech Ltd, Secunderabad, India).

In September 1998, the defoliation of these treated trees was compared with the defoliation of five nearby, untreated trees by randomly collecting two branches from the midcrown of each tree with pole pruners and estimating the percentage of 1-year-old needles consumed to the nearest 10% and determining the average for the two branches. Differences in defoliation between treatments were compared by one-way analysis of variance following arcsine transformation of the square root of the data and Tukey's test for all pairwise multiple comparisons using SigmaStat 2.0 (Jandel Corporation 1995).

Influence of treatment time in spring

Tree injections were performed on 25 March, 1 April, 14 April, and 12 May 1998 to determine the length of time before egg hatch systemic injections would be effective

against pine false webworm larvae. The first treatment, on 25 March, was performed soon after the snow had melted in the plantation. On each date, five 15–20 cm dbh red pines in a plantation 15 km north of Barrie, Ontario (44°30′N, 79°59′W), were injected with the Fortune Aza EC at 0.05 g a.i./cm of dbh. Absolute ethanol was added to the Fortune Aza EC formulation in each injection tube for the treatments on 25 March because a preliminary trial had indicated that the uptake of this formulation was slow. On subsequent treatment dates, no ethanol was added. Defoliation estimation and statistical analysis were as described earlier.

Influence of insecticide

The effectiveness of systemic injections of Fortune Aza EC and three other systemic insecticides, acephate (Orthene 70% SP, 700 g a.i./kg, United Agri Products, Dorchester, Ontario), dimethoate (Cygon 2E, 240 g a.i./L, Chipman, Stoney Creek, Ontario), and imidacloprid (imidacloprid technical, 977 g a.i./kg, Bayer Agricultural Products, Kansas City, Missouri), for controlling pine false webworm larvae was compared. Each product was injected into five 15–20 cm dbh red pines on 12 May 1998 in the plantation 15 km north of Barrie, Ontario. Five comparable, untreated trees were used as controls. All products were applied at a rate of 0.05 g a.i./cm of dbh. Fortune Aza EC was applied undiluted in volumes of 0.83 mL/cm of dbh per injection tube. The other products were diluted or dissolved in an equivalent volume of water to standardize the volume injected and achieve the appropriate dose. Defoliation estimation and statistical analysis were as described earlier.

Influence of reduced dosage

In the previous trials, a dosage of 0.05 g a.i./cm of dbh was used against pine false webworm. In this trial, 20 red pine trees were injected on 13 May 1998 with the Fortune Aza EC formulation at 0.02 g a.i./cm of dbh to determine the effectiveness of this reduced dosage over the typical range of larger tree sizes infested by *A. erythrocephala* in Ontario. In the plantation 15 km north of Barrie, Ontario, ten 15–20 cm dbh trees were treated using injection tubes. Ten untreated trees of similar size were used as controls in this site. In another red pine plantation (Simcoe County Forest, South Barr Tract) about 5.5 km away (44°31.6′N, 79°40.3′W), ten 25–30 cm dbh trees were injected and 10 untreated trees of similar size were used as controls. Methods for estimating defoliation were the same as reported earlier. Differences between sites and treatments were compared by a two-way analysis of variance, following arcsine transformation of the square root of the data, using SigmaStat 2.0 (Jandel Corporation 1995).

Influence of neem formulation at low dosages

On 12–13 May 1999, an extensive trial was performed to evaluate the effectiveness of systemic injections of three formulations of neem seed extract (Amvac Aza EC, Neemol, and CFS EC) at three low dosages (0.005, 0.01, and 0.02 g a.i./cm of dbh) against pine false webworm defoliating mature red pines (mean dbh ± SD = 23.4 ± 3.3 cm) in a plantation (Simcoe County Forest, South Barr Tract) near Craighurst, Ontario (44°31.7′N, 79°40.3′W). Tree diameters did not differ among the treatment groups (ANOVA, $F_{12,110} = 0.716$, $P = 0.733$). Amvac Aza EC (3% azadirachtin) was obtained from Amvac Chemical Corp, Los Angeles, California. Neemol (experimental formulation) was prepared by adding 25 g of technical azadirachtin (30% azadirachtin, Vikwood Ltd, Sheboygan, Wisconsin) to 86 mL of absolute ethanol and stirring well for 1 h followed by centrifugation at 10 000 rpm for 30 min and collection of the supernatant. This supernatant (Neemol) contained 7.5% azadirachtin as determined by high-pressure liquid chromatography analysis (DG Thompson, Canadian Forest Service,

Sault Ste. Marie, Ontario). CFS EC (experimental proprietary formulation, 5% azadirachtin, Canadian Forest Service, Sault Ste. Marie, Ontario) contained Neemol plus an emulsifier and oil. Ten trees were treated with each dose of each formulation in a randomized block design and 10 untreated trees served as controls. The CFS EC formulation was also injected into five trees at each of these dosages using one hole per tree. In September 1999, the defoliation of these trees was assessed and statistically analyzed as described earlier.

Results

Control of introduced pine sawfly on white pine

The injection tubes performed well in delivering the Neem EC formulation to the white pine trees. All of the formulation entered the trees within 24 h. No leakage occurred around the injection holes in this or subsequent experiments except where noted. This formulation went in slowly under pressure, requiring several hours for volumes of 33–38 mL to enter the trees. These injections were effective, providing 83–100% mortality of introduced pine sawfly larvae up to 77 d after treatment (Table 1). Mean mortality of larvae feeding on foliage from treated trees was higher than on untreated trees ($F_{1,29}$ = 194.3, P = 0.001). There was no difference in mean mortalities at different times after treatment ($F_{4,29}$ = 0.133, P = 0.968), and there was no interaction between time and treatment ($F_{4,29}$ = 0.882, P = 0.493). These systemic treatments were also effective against gypsy moth larvae 56 d after treatment. Mortality (mean ± SD) after 30 d following exposure to foliage from treated trees was 91.7 ± 11.7% compared with 10 ± 0.0% in controls (t_6 = 5.8, P = 0.001).

Control of cedar leafminer on eastern white cedar

Neem EC was introduced into these cedar trees slowly. After repressurizing the injection tubes over a 6-d period, 80–100% of the neem was injected into each tree. Six of the 10 tubes were empty and the other four tubes, all on different trees, had 10, 15, 30, and 40% remaining after 6 d. Neem and dimethoate greatly reduced the numbers of leafminer larvae ($F_{2,14}$ = 37.4, P <0.001), pupae ($F_{2,13}$ = 54.8, P < 0.001), and mines ($F_{2,12}$ = 61.7, P < 0.001) in the treated branch samples by 90% or more (Table 2). The 3–4 mL of the undiluted dimethoate formulation per injection tube entered the trees in less than 1 min.

Control of spruce budworm on white spruce

The Neem EC formulation was completely injected into five white spruce trees within 1 d in the fall of 1997. The following spring, the mean (± SD) number of spruce budworm larvae was lower on the 45-cm branch samples from the neem-injected (5.9 ± 3.5) trees than from the control (25.3 ± 14.6) trees (t_8 = –2.89, P = 0.02). This indicated that neem carried over in the trees during the winter and provided control of larvae before they reached third instars. The mortality of fourth-instar larvae placed on buds of these branches 3 d after collection was not different between control (10.2 ± 6.5%) and treated (25.7 ± 27.3%) trees (t_8 = 1.24, P = 0.25).

https://doi.org/10.4039/Ent133729-5 Published online by Cambridge University Press

TABLE 1. Mortality of *Diprion similis* larvae in bioassays of branches from *Pinus strobus* treated with Neem EC at 0.1 g a.i./cm of dbh using systemic tree injection tubes, 27 August 1997.

	Mean mortality ± SD (%)	
Days after treatment	Treated trees	Untreated trees
14	100±0	6.7±5.9
28	91.7±14.4	14.8±12.7
41	100±0	10.0±10.0
72	83.3±28.9	14.1±6.9
77	96.7±5.8	6.1±5.3

NOTE: Means (n = 5) within a column are not significantly different (ANOVA with Tukey's test after arcsine square-root transformation of the data, $P > 0.05$).

TABLE 2. Comparison of the mean numbers of larvae, pupae, and mines of *Argyresthia thuiella* and *Argyresthia aureoargentella* per two 30-cm branch tips of *Thuja occidentalis* treated with insecticides at 0.2 g a.i./cm of dbh using systemic tree injection tubes.

Treatment	Mean no. of larvae ± SD	Mean no. of pupae ± SD	Mean no. of mines ± SD
Neem EC	1.8±1.6*b*	0.4±0.5*b*	5.0±4.1*b*
Cygon 2E	0.4±0.5*b*	2.8±1.9*b*	1.6±1.1*b*
Untreated control	14.4±5.5*a*	26.5±10.9*a*	45.0±1.7*a*

NOTE: Means (n = 5) within a column followed by the same letter are not significantly different [ANOVA with Tukey's test after $(x + 0.5)^{1/2}$ transformation of the data, $P > 0.05$].

Control of pine false webworm on red pine

Fall versus spring treatments

All of the Neem EC formulation entered the red pines within 24–48 h in both spring and fall treatments. The Fortune Aza EC formulation was taken up much slower, and approximately 25% had not entered the trees after a week or more. For this reason, this treatment has not been included in our analysis. Both the fall and spring treatments with the Neem EC formulation reduced defoliation compared with untreated trees (Table 3; $F_{2,14} = 13.2$, $P < 0.001$). There was no difference in mean defoliation between the fall and spring treatments (Table 3).

Influence of treatment time in spring

The treatments were carried out 72, 65, 52, and 24 d before 97% egg hatch on 5 June. The Fortune Aza EC formulation was slowly introduced into the red pines. In most trees, it was completely injected by 48 h but took longer in five trees. All treated trees were less defoliated than the control trees (Table 4; $F_{4,22} = 12.23$, $P < 0.001$). There were no differences in mean defoliation on the different treatment dates. The treatments provided 75–80% foliage protection at this dosage when treated as much as 10–11 weeks before egg hatch.

Influence of insecticide

All three of the formulations dissolved in or diluted with water (*i.e.*, acephate, dimethoate, and imidacloprid) entered the trees quickly and were completely taken up

TABLE 3. Mean defoliation in September 1998 by *Acantholyda erythrocephala* larvae of *Pinus resinosa* treated with Neem EC at 0.05 g a.i./cm of dbh using systemic tree injection tubes in fall 1997 and spring 1998.

Treatment	Treatment date	Mean defoliation ± SD (%)
Neem EC	30 October 1997	0±0*b*
	20 May 1998	3±4.5*b*
Untreated control	—	56±35.3*a*

NOTE: Means (n = 5) followed by the same letter are not significantly different (ANOVA with Tukey's test after arcsine square-root transformation of the data, $P > 0.05$).

TABLE 4. Mean defoliation in September 1998 by *Acantholyda erythrocephala* larvae of 15–20 cm dbh *Pinus resinosa* treated on different dates with 0.05 g a.i./cm of dbh using systemic tree injection tubes.

Treatment	Treatment date	Mean defoliation ± SD (%)
Fortune Aza EC	25 March 1998	25.0±30.8*b*
	1 April 1998	21.3±23.2*b*
	14 April 1998	21.0±16.4*b*
	12 May 1998	20.0±17.0*b*
Untreated control	—	95.0±11.2*a*

NOTE: Means (n = 5) followed by the same letter are not significantly different (ANOVA with Tukey's test after arcsine square-root transformation of data, $P > 0.05$).

in 24 h. Injection of the Fortune Aza EC formulation was slower. Only one of the five trees was finished after 24 h. All of the neem had entered the remaining four trees after 48 h. Mean defoliation differed among the treatment groups ($F_{4,22} = 4.19$, $P = 0.014$). All systemic treatments had less defoliation than the control trees, but imidacloprid was not significantly different from the control (Table 5). Mean defoliation for the four products was not different. Neem provided the most protection and the least variation among trees.

Influence of reduced dosage

In this trial, the contents of all injection tubes entered the trees within 24–48 h. Although the same Fortune Aza EC formulation was used in this trial as in previous experiments, the reduced volumes needed for this lower dosage probably accounted for the quicker uptake. A maximum of 10 mL per injection tube was required to treat 30-cm trees with 0.02 g a.i./cm of dbh. Mean percent defoliation differed between sites ($F_{1,39} = 69.5$, $P < 0.001$) and between treated and untreated trees ($F_{1,39} = 227.7$, $P < 0.001$). In both sites, this lower dosage of azadirachtin greatly reduced defoliation and provided foliage protection of 91–97% (Table 6). The interaction between site and treatment was also significant ($F_{1,39} = 27.5$, $P < 0.001$). The defoliation of both untreated trees and treated trees was lower in the South Barr Tract site than in the Craighurst Tract site (Tukey test: untreated, $p = 2$, $q = 13.6$, $P < 0.05$; treated, $p = 2$, $q = 3.1$, $P < 0.05$).

Influence of neem formulation at low dosages

The volumes used in this trial were low compared with those of previous experiments; the maximum volume was 11 mL per injection tube with the CFS EC formulation at 0.02 g a.i./cm of dbh in one hole, and the minimum volume was 0.6 mL per injection tube with the 7.5% ethanolic extract at 0.005 g a.i./cm of dbh. All the tubes were completely empty within 24 h. Three of 195 tubes leaked initially because the

https://doi.org/10.4039/Ent133729-5 Published online by Cambridge University Press

TABLE 5. Mean defoliation in September 1998 by *Acantholyda erythrocephala* larvae of *Pinus resinosa* treated with four systemic insecticides at 0.05 g a.i./cm of dbh using systemic tree injection tubes, 12 May 1998.

Treatment	Mean defoliation ± SD (%)
Fortune Aza EC	20.0±17.0*b*
Cygon 2E	30.0±36.7*b*
Orthene	27.5±48.6*b*
Imidacloprid technical	47.5±42.9*ab*
Untreated control	95.0±11.2*a*

NOTE: Means (n = 5) followed by the same letter are not significantly different (ANOVA with Tukey's test after arcsine square-root transformation of the data, $P > 0.05$).

TABLE 6. Mean defoliation in September 1998 by *Acantholyda erythrocephala* larvae of 15–20 and 25–30 cm dbh size class *Pinus resinosa* in two sites treated on 13 May 1998 at 0.02 g a.i./cm of dbh using systemic tree injection tubes.

Site	Tree size class (cm dbh)	Treatment	Mean defoliation ± SD (%)
Craighurst Tract	15–20	Fortune Aza EC	9.0±11.3*a*
		Untreated control	97.5±7.9*b*
South Barr Tract	25–30	Fortune Aza EC	1.0±2.1*c*
		Untreated control	41.0±20.4*d*

NOTE: Means (n = 10) followed by the same letter are not significantly different (ANOVA with Tukey's test, after arcsine square-root transformation of the data, $P > 0.05$).

spile was not seated in the hole properly. The leaking was stopped in all three cases by releasing the pressure and hammering the spile all the way into the hole. Mean defoliation differed among treatments ($F_{9,96}$ = 27.0, P <0.001). All treated groups were less defoliated than the control group (Table 7). All treatments reduced defoliation 95–100% compared with controls, and there were no differences between formulations or dosages. Defoliation of trees treated with the CFS EC formulation in one hole was 0% at all three dosages and not different ($F_{2,28}$ = 0.885, P = 0.426) from the defoliation for comparable trees using two holes (0.7%).

Discussion

The systemic tree injection tube has proven to be a reliable and effective device for injecting neem formulations and other systemic insecticides into conifers for research purposes. In total, 188 trees from four conifer species were successfully injected in either spring or fall. Usually, all of the material was injected into the trees without leakage, including the neem formulations in most red pine, white pine, and white spruce and other systemic insecticides in red pine and eastern white cedar. The Neem EC formulation in eastern white cedar and Fortune Aza formulation in 25–30 cm dbh red pine at 0.05 g a.i./cm of dbh were not completely taken up. Available commercial or experimental neem formulations were not entirely suitable for systemic injections. They entered trees slowly and not always completely depending on the type of formulation, the quantity applied, and the tree species. Volumes of 15 mL or less consistently went into trees typically within 48 h. Initial trials with a solution of neem seed powder in ethanol (Neemol) indicated that this product can be injected quickly and completely using injection tubes. Research is currently in progress to develop and evaluate neem

TABLE 7. Mean defoliation in September 1999 by *Acantholyda erythrocephala* larvae of *Pinus resinosa* treated with three formulations of neem at different dosages on 12–13 May 1999 using systemic tree injection tubes.

Treatment	Dosage (g a.i./cm of dbh)	Mean defoliation ± SD (%)
Amvac Aza EC	0.005	0.0±0.0*b*
	0.01	0.0±0.0*b*
	0.02	1.0±3.2*b*
Neemol	0.005	2.5±7.9*b*
	0.01	0.6±1.7*b*
	0.02	0.0±0.0*b*
CFS EC	0.005	0.0±0.0*b*
	0.01	0.0±0.0*b*
	0.02	2.2±5.1*b*
Untreated control	—	57.8±35.8*a*

NOTE: Means (n = 10) followed by the same letter are not significantly different (ANOVA with Tukey's test after arcsine square-root transformation of the data, $P > 0.05$).

formulations specifically for systemic injections into trees which can be introduced rapidly and completely, and which translocate within the trees efficiently.

The injection tubes proved to be easy and quick to install. With practice, two devices could be installed on a tree in 5 min. A concern about using this type of application technique, where drilling holes is required, is the damage caused to the trees. To date, we have not seen any phytotoxic effects (*e.g.*, tissue necrosis or foliage chlorosis) due to these treatments after 2 years.

The systemic tree injection tube could be a versatile tool for tree injections. In addition to being inexpensive and reusable, it can be used for the application of not only a variety of insecticides but also fertilizers, fungicides, or antibiotics to trees. It can be used to apply mixtures of products or a series of products sequentially in any proportion or quantity desired. These devices are capable of delivering at least several hundred millilitres of solutions which readily enter trees. For operational use, the device could be easily modified by placing volume graduations on the plastic tubing and adding a branch tube with screw cap for quick loading.

Systemic injections of neem EC formulations were effective against several forest insect pests. Dosages of 0.2 g a.i./cm of dbh or less provided control of pine false webworm, spruce budworm, cedar leafminers, gypsy moth, and introduced pine sawfly on red pine, white spruce, eastern white cedar, white pine, and white pine, respectively. Based on these trials and other studies (Marion *et al.* 1990; Lyons *et al.* 1996; Sundaram *et al.* 1997; Wanner *et al.* 1997), systemic applications of azadirachtin have the potential of managing many lepidopteran and hymenopteran defoliators and miners on conifer tree species and may also have potential for controlling scolytid beetles (Naumann *et al.* 1994; Duthie-Holt *et al.* 1999). Although no trials with injection tubes have been conducted yet on deciduous species, a neem seed extract was effective against birch leafminers in small birch trees by inoculation directly into holes (Marion *et al.* 1990; BV Helson, unpublished data), which suggests that azadirachtin has potential for control of some important pests of deciduous trees. The suitability of the devices will need to be evaluated for injections of various deciduous tree species. In ring porous species such as oak, elm, and ash, water conduction occurs primarily in the xylem vessels of the outermost growth ring. The maple sap spiles would potentially block this ring and deliver fluid to older growth rings that do not conduct water. Consequently, this injection device may be less efficient in ring porous than diffuse porous species

such as birch and maple and nonporous conifers where several growth rings are active in water conduction (Chaney 1991; Ellmore *et al.* 1991). Trials in deciduous tree species are planned as soon as a suitable neem formulation for tree injections is developed.

The effectiveness of neem seed extracts against two pests, cedar leafminers and pine false webworm, was comparable to that of systemic insecticides (*i.e.*, Cygon 2E, Orthene, imidacloprid technical) which are currently in common use. In both comparisons, the level of control was high, which could indicate that the dosages used for some or all of these products were above the minimum required. It is not known what the relative activity of neem seed extracts and these systemic insecticides would be at lower dosages. The dosage of azadirachtin required against pine false webworm is probably much lower than 0.05 g a.i./cm of dbh because comparable foliage protection was achieved at a dosage of 0.02 g a.i./cm of dbh in another experiment at the same site. The minimum effective dosage may even be as low or lower than 0.005 g a.i./cm of dbh because almost complete protection was obtained at this dosage in one experiment with large red pine experiencing moderate defoliation. Injections into one hole per tree also provided protection comparable to that of two holes in the final experiment of this study. Rediske *et al.* (1970) found that radiolabelled dimethoate was uniformily distributed through most of the upper third of the crown of young Douglas-fir, *Pseudotsuga menziesii* (Mirbel) Franco (Pinaceae), 4–7 m above a single inoculation hole at breast height, but lateral displacement was not complete in lower parts of the crown. In our trial, the red pines were injected at least 10 m below the lowest branches, which may have provided a sufficient height for relatively uniform distribution through the entire crown. Once a suitable systemic neem formulation is developed, trials to determine minimum effective dosages of azadirachtin for different pest and tree species injected into one or more holes are planned.

These systemic injections with formulations containing azadirachtin provided long-lasting insecticidal activity. A dosage of 0.1 g a.i./cm of dbh in white pine was effective against introduced pine sawfly larvae for at least 11 weeks. Similarly, treatments of red pine 10–11 weeks before egg hatch provided excellent protection against feeding damage by pine false webworm larvae. Such long residual activity offers some valuable features. One application at the beginning of the season could provide protection against a multivoltine species such as the introduced pine sawfly or against more than one species with different feeding activity periods on the same tree. Injections can be performed over an extended period of time before the onset of larval feeding. In fact, our results have shown that in north temperate climates such injections can be performed before winter to provide protection the following spring. With such a wide window for treatment, large numbers of trees could be treated with neem over a period of several months, even though systemic injections are somewhat labour-intensive. Furthermore, preliminary results indicate that systemic applications of neem by infusion in red pine can provide at least two seasons of protection against pine false webworm (BV Helson, unpublished data). We intend to assess the duration of protection beyond the first season obtained against pine false webworm by continuing to monitor defoliation of trees treated with the injection tubes in this study.

With the injections of white spruce in the fall for control of spruce budworm, natural populations were significantly reduced the following spring. At the time of treatment on 30 October, second-instar larvae in hibernaculae would have been present on external surfaces of the trees. These larvae would not have been exposed to neem either by contact or ingestion because they are stationary and do not feed during this stage. The following spring in late April to early May, these larvae emerge from hibernaculae and typically mine 1-year-old needles. Larvae begin to feed at this time and would potentially ingest azadirachtin that had translocated to the needles. Thereafter, the larvae move to the growing shoots where they continue feeding and would ingest azadirachtin,

which had moved to the shoots. Sundaram *et al.* (1997) showed that azadirachtin translocated to the 1-year-old needles and new shoots of white spruce when treated on 26 June by trunk infusion. Interestingly, the fourth-instar larvae exposed to the shoots from the same branches in the bioassays were not significantly affected. Factors which could explain this result include degradation of azadirachtin in the shoots by the time the larvae were exposed or lower susceptibility of fourth-instar larvae to neem compared to second and third instars or a shorter exposure period. It is also possible that the dynamics of translocation and distribution of azadirachtin is different in the fall than in the spring. In a spring treatment, the concentrations in the shoots were higher than in the needles (Sundaram *et al.* 1997). In a fall treatment, however, the injected azadirachtin would remain in woody tissues, move to mature needles, or both. How much moves to the new shoots the following spring will depend on its location in the tree and its distribution from these locations. The dynamics of translocation and distribution of azadirachtin in injected trees needs to be studied in relation to its bioactivity on different insect pest species.

Systemic injection of neem seed extracts with the systemic tree injection tube is a very promising new technology for the management of many defoliators, miners, and borers of trees.

Acknowledgements

We thank D Comba, G Jones, and J McFarlane for technical assistance and DG Thompson for determining the concentration of azadirachtin in Neemol. Funding for this project was provided in part by the Forest Management Branch, Ontario Ministry of Natural Resources.

References

Chaney WR. 1991. Anatomy and physiology of chemical movement in trees. pp 1–10 *in* KB Miller (Ed), Proceedings of the 2nd Symposium on Systemic Chemical Treatments in Tree Culture, 5–7 October 1987, Michigan State University, East Lansing

Duthie-Holt MA, Borden JH, Rankin LJ. 1999. Translocation and efficacy of a neem-based insecticide in lodgepole pine using *Ips pini* (Coleoptera: Scolytidae) as an indicator species. *Journal of Economic Entomology* **92**: 180–6

Ellmore GS, Phair WE, Gill C, Skinner D. 1991. Fluid delivery in injected ring-porous trees. pp 32–42 *in* KB Miller (Ed), Proceedings of the 2nd Symposium on Systemic Chemical Treatments in Tree Culture, 5–7 October 1987, Michigan State University, East Lansing

Gill JS, Lewis CT. 1971. Systemic action of an insect feeding deterrent. *Nature (London)* **232**: 402–3

Helson B, de Groot P, McFarlane J, Zylstra B, Scarr T. 1998. Leader and systemic applications of neem EC formulations for control of white pine weevil (Coleoptera: Curculionidae) on jack pine and white pine. *Proceedings of the Entomological Society of Ontario* **129**: 107–26

Helson B, Lyons B, de Groot P. 1999. Evaluation of neem EC formulations containing azadirachtin for forest insect pest management in Canada. pp 79–89 *in* RP Singh, RC Saxena (Eds), *Azadirachta indica* A. Juss, International Neem Conference, Gatton, Australia, February 1996. New Delhi: Oxford & IBH Publishing Co PVT Ltd

Jandel Corporation. 1995. *SigmaStat statistical software user's manual (version 2.0)*. San Rafael, California: Jandel Corporation

Kielbaso JJ (*Editor*). 1979. Proceedings of the Symposium on Systemic Chemical Treatments in Tree Culture. East Lansing: Michigan State University

Kondo ES. 1972. A method for introducing water soluble chemicals into mature elms. *Canadian Forestry Service Information Report* **O-X-1971**

Kostyk BC, Wanner KW. 1997. Control of insect damage to black spruce seed cones with neem. *Northern Journal of Applied Forestry* **14**: 40–3

Lyons DB, Jones GC. 2000. What do we know about the biology of the pine false webworm in Ontario? pp 3–12 *in* DB Lyons, GC Jones, TA Scarr (Eds), Proceedings of a Workshop on the Pine False

Webworm, *Acantholyda erythrocephala* (Hymenoptera: Pamphiliidae). Sault Ste. Marie, Ontario: Canadian Forest Service, Natural Resources Canada

Lyons DB, Helson BV, Jones GC, McFarlane JW, Scarr T. 1996. Systemic activity of neem seed extracts containing azadirachtin in pine foliage for control of the pine false webworm, *Acantholyda erythrocephala* (Hymenoptera: Pamphiliidae). *Proceedings of the Entomological Society of Ontario* **127**: 45–55

Lyons DB, Helson BV, Jones GC, McFarlane JW. 1998. Effectiveness of neem- and diflubenzuron-based insecticides for control of the pine false webworm, *Acantholyda erythrocephala* (L.) (Hymenoptera: Pamphiliidae). *Proceedings of the Entomological Society of Ontario* **129**: 115–26

Marion DF, Larew HG, Knodel JJ, Natoli W. 1990. Systemic activity of neem extract against the birch leafminer. *Journal of Arboriculture* **16**: 12–6

McCoy RE. 1979. Systemic treatment of coconut palm with oxytetracycline-R. pp 215–22 *in* JJ Kielbaso (Ed), Proceedings of the Symposium on Systemic Chemical Treatments in Tree Culture. East Lansing: Michigan State University

Naumann K, Rankin LJ, Isman MB. 1994. Systemic action of neem seed extract on mountain pine beetle (Coleoptera: Scolytidae) in lodgepole pine. *Journal of Economic Entomology* **87**: 1580–5

Rediske JH, Gauditz I, Johnson NE. 1970. Distribution of dimethoate-P^{32} in Douglas-fir following stem injection. *Forest Science* **16**: 106–12

Schmutterer H (*Editor*). 1995. *The neem tree,* Azadirachta indica *A. Juss, and other meliaceous plants: sources of unique natural products for integrated pest management, medicine, industry and other purposes*. New York: VCH Publishers Inc

Sundaram KMS, Campbell R, Sloane L, Studens J. 1995. Uptake, translocation, persistence and fate of azadirachtin in aspen plants (*Populus tremuloides* Michx.) and its effect on pestiferous two-spotted spider mite (*Tetranychus urticae* Koch). *Crop Protection* **14**: 415–21

Sundaram KMS, Sundaram A, Curry J, Sloane L. 1997. Dissipation kinetics of azadirachtin in some forest matrices and its systemic translocation in conifers for spruce budworm control. *Journal of Environmental Science and Health Part B* **32**: 803–29

Tattar TA. 1999. Tree health care using microinjection technology. Available at http://www.bio.umass.edu/micro/systemic.html [accessed on November 1999]

Thomas AW, Strunz GN, Chiasson M, Chan TH. 1992. Potential of Margosan-O, an azadirachtin containing formulation from neem seed extract, as a control agent for spruce budworm, *Choristoneura fumiferana*. *Entomologia Experimentalis et Applicata* **62**: 37–46

Wanner KW, Kostyk BC. 1995. Evaluation of neem seed extract against spruce budworm, *Choristoneura fumiferana* (Clem.), in white spruce, *Picea glauca* (Moench) Voss., seed orchards. *Proceedings of the Entomological Society of Ontario* **126**: 91–3

Wanner KW, Helson BV, Kostyk BC. 1997. Foliar and systemic applications of neem seed extract for control of spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae), infesting black and white spruce seed orchards. *The Canadian Entomologist* **129**: 645–55

(Received: 9 February 2001; accepted: 18 June 2001)