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ENTOMOLOGY

② **Biocidal Effect of Fatty Acids and Soaps on the Balsam Woolly Aphid.** — During a bioassay of todomatuic acid, a juvenile hormone analogue found in certain aphid infested trees (Puritch and Nijholt, Can. J. Bot., in press), we used the unsaturated lipid, oleic acid (C_{18}:1) as a control. To our initial surprise, oleic acid affected the aphid's wool and caused mortality of all stages, including eggs. We then realized that fatty acids and their salts, i.e., soaps, were among the oldest insecticides and had been commonly used for centuries to control garden and orchard pests. Several scientific reports dealing with the use of these compounds as contact insecticides were presented during the 1920's and 30's (Shepherd, H. H. ed. The chemistry and toxicology of insecticides, Burgess Pub. Co. Minn. 1947) but few, if any, appeared since that time. We therefore investigated more fully the effect of these compounds on the balsam woolly aphid and their usefulness as insect control agents.

To further test the effects of oleic acid on various stages of non-dormant balsam woolly aphids [*Adelges piceae* (Ratz.)], 6-year-old *Abies grandis* (Dougl.) Lindl. seedlings were infested with aphids during mid-August according to Carrow and Betts (Can. J. For. Res. 3: 122-139, 1973). In October, eight selected trees were separated into two groups and placed in a growth room at 23 C day/17 C night, 66% day/56% night R.H. and 16 h photoperiod. For each group, the various aphid stages were counted and totalled. Aphids were then sprayed to the drip point on October 24 with a hand sprayer, with either 5% oleic acid emulsified in 0.1% Tween 20 (polyoxyethylene (20) sorbitan monolaurate) or water. A second set of eight infested seedlings was separated into two groups of 4 and, on November 27, sprayed with either 5% oleic acid in 0.1% Tween 20 or 0.1% Tween 20. For both trials, the aphids were counted after treatment and their development was observed for 1 month.

Oleic acid caused total mortality of first-, second- and third-instar aphids, as well as adults in both trials (Table 1). After treatment, the aphid wool lost its hydrophobic properties and collapsed. The aphids, however, remained attached to the stem, appeared bloated and black and showed no signs of activity when probed.

Periodic examination over the following month revealed no signs of life, although control aphids continued normal development. The emulsifier, Tween 20, also caused a certain amount of mortality, especially to the neosistens (Table 1).

Since oleic acid proved effective in killing the aphid, tests were initiated to analyze the effectiveness of various other fatty acids and their potassium (K) soaps. Even carbon-numbered, saturated fatty acids from caproic (C_6) to behenic (C_{22}), along with the unsaturated acids, oleic and linoleic ($C_{18:2}$), were made up as 1% solutions (w/v or v/v) and emulsified with 0.1% Tween 20 and using a Beckmann Polytron Disintegrator. Potassium soaps were made by neutralizing each fatty acid with 1N KOH and adding distilled water to make a 1% solution. Aphid-infested bark was obtained during winter from mature *A. grandis* in the field. The bark was separated into 2-cm diam plugs containing a minimum of 300 aphids and put into separate petri dishes on wet filter paper. One plug was used per treatment and each plug was sprayed to wetness at room temperature with the acid or soap using a hand sprayer. (Further tests are currently underway to ascertain the minimum lethal dose for the aphids.) Excess solution was drained off and plugs kept overnight in a growth room. The filter paper was kept wet to prevent dessication. On the following day, per cent mortality was determined by counting all living and dead aphids within a 0.8 cm^2 area.

Application of the 1% fatty acids caused varying degrees of mortality according to the type of compound used (Fig. 1). The short chain saturated fatty acids from caprylic (C_8) to palmitic (C_{16}) were the most toxic, causing in excess of 95% mortality, while the longer chain saturates, stearic (C_{18}) to behenic (C_{22}), were much less toxic. Oleic and linoleic, the two unsaturated C_{18} compounds, were among the most effective treatments and contrasted with the least effective saturated compound tested, stearic acid, which also has 18 carbons. Neutralization of the fatty acids to make soaps caused little change in their toxicity (Fig. 1), suggesting that the fatty acid component is the major cause of toxicity and not the potassium ion.

TABLE 1
Effect of topically applied oleic acid on balsam woolly aphid

	October Treatment: No. of live aphids before Spray Oct 24				No. of live aphids after Spray Nov 29			
	Instar I	Instar II & II	Adults	Eggs	Instar I	Instar II & II	Adults	Eggs
5% oleic acid	42	10	0	0	0	0	0	0
Control	11	28	4	13	41	26	4	0
	November Treatment: Before Spray Nov 27				After Spray Dec 18			
5% oleic acid	53	21	1	0	0	0	0	0
.1% Tween 20	18	17	0	0	1	17	1	6

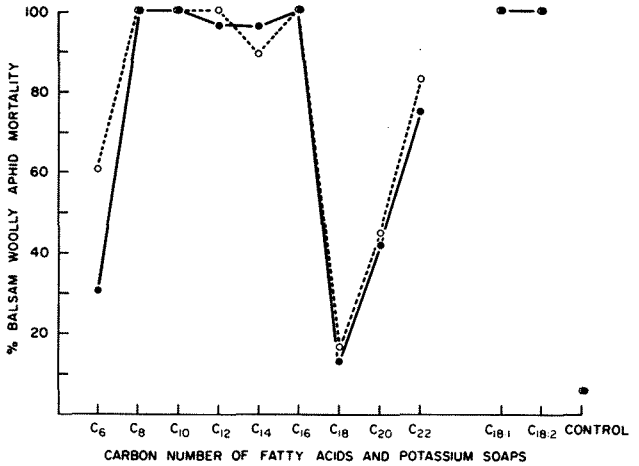

Figure 1. Effect of 1% fatty acids (—) and 1% potassium soaps (- - -) on mortality of the balsam woolly aphid (*Adelges piceae* (Ratz.)).

Fatty acids and their soaps are effective insecticides for the balsam woolly aphid. These compounds are natural constituents of plants and animals, are relatively cheap, biodegradable and low in phytotoxicity. The unsaturated fatty acids, oleic and linoleic, are also readily oxidized and are low in toxicity to fish. Rogers (Pulp Pap. Mag. Can. 74: T303-T308, 1973 and personal comm.) found that oleic acid was about eight times, and linoleic about four times, less toxic to coho salmon than the resin acid, abietic, which has an incipient lethal level of 2.2 ppm. These compounds have also been shown at this laboratory to alter development of certain insects and to cause instant paralysis and death of other important forest pests, including the spruce budworm [*Choristoneura fumiferana* (Clem.)] (Puritch, unpublished results). The soaps and fatty acids thus hold promise as insecticides and agents to prevent insect development and may provide an alternative to the more toxic petro-chemicals. — George S. Puritch and M. Talmon de l'Armee, Pacific Forest Research Centre, Victoria, B.C.

FOREST PRODUCTS

Diazo Naphthalene-disulfonic Acid and Fungal Membrane Permeability. — Paradee and Watanabe (J. Bacteriol. 96:1049-1054, 1968) devised a reagent, diazo-7-amino-1,3-naphthalene-disulfonic acid (diazo-NDS) that inactivates enzymes and does not penetrate bacterial cytoplasmic membranes. This reagent is useful for determining on which side of the cell membrane a particular enzyme is located.

The site of cellulase (EC. 3.2.1.4), the main enzyme associated with wood rot, has not been definitely established for fungal species. An investigation was therefore begun and diazo-NDS used in the first tests.

Cell-free cellulase of *Myrothecium verrucaria* was shown to be inactivated by diazo-NDS. To determine this, freeze-dried culture supernatant obtained from growth of *M. verrucaria* on glucose in shake culture (Hulme and Stranks, J. Gen. Microbiol. 69: 145-155, 1971) was treated following the procedure of Day and Ingram (Can. J. Microbiol. 17: 1025-1028, 1971). After exposure for 1 hour at room temperature the cellulase preparation was 80% inactivated.

Bound cellulase, which is that remaining with the mycelium after washing, was then treated with the diazo-NDS reagent using the same procedure. After treatment the reagent was washed away, the mycelium resuspended in buffer (0.1M tris (hydroxymethyl) aminomethane HCl, pH 7.1), the whole disintegrated at 20 kHz, the debris centrifuged off, and the clear supernatant recovered. The cellulase activity of the supernatant was determined and then compared with that for an untreated control. Complete inactivation of the bound cellulase for 3-day-old and older culture material was observed (Table 1). If the membrane of this organism did not leak diazo-NDS under the test conditions, the bound cellulase could then be considered to be outside the membrane. To check for possible leakage, reaction of this reagent with fumarase, an

TABLE 1
Inactivation of cell-bound cellulase of *M. verrucaria* by diazo-nds

Growth Period (days)	Cellulase (units*/10 mg oven dry mycelium) Control	Treated	Cellulase Inactivation (%)
2	0.32	0.03	90.6
3	0.66	0.00	100.0
4	0.12	0.00	100.0
7	0.04	0.00	100.0

*(Stranks, Can. J. Microbiol. 19:1523-1526, 1973).

internally located enzyme (Lenaz *et al.* Biochem. Biophys. Res. Commun. 49: 536-542, 1972), was investigated.

Cell-free fumarase was recovered in the supernatant from fresh mycelial growth that had been washed and disintegrated in buffer. As with cellulase, fumarase was found to be readily inactivated (83%) by the standard 1-hour treatment with diazo-NDS. Racker's method for detecting fumarase (Biochem. Biophys. Acta, 4: 211-214, 1950) was used throughout.

Membrane impenetrability to diazo-NDS was tested by treating intact washed mycelium (obtained from 40-hour growth of *M. verrucaria*) with the reagent using the same procedures as for cellulase and noting the change in fumarase activity. A reduction in activity of 59.1% compared with untreated control material was observed (Fig. 1). This finding indicated that the membrane of this organism was indeed permeable to diazo-NDS. This reagent therefore is unsuitable for differentiating those enzymes of *M. verrucaria* which may be located outside the cytoplasmic membrane, — for example, cellulase.

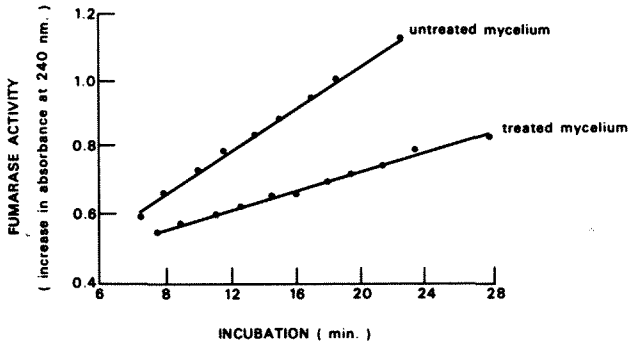

Figure 1. Effect of diazo-NDS on the intracellular enzyme fumarase. Mycelium lost 59.1% of its activity when treated with diazo-NDS calculated from difference in slope of the curves.

Penetration of the membrane by diazo-NDS was an unexpected find since the corresponding membranes of at least three different bacteria were found impermeable (Pardee and Watanabe; Day and Ingram, *loc. cit.*). As there may be something unique about the membrane of *M. verrucaria* or the test conditions under which the organism was treated which influenced the results, diazo-NDS should not be rejected for work with fungi in general until further studies have been made. On the other hand, if many fungal membranes are found to leak this reagent, supposed unable to penetrate membrane because of charged sulfonate groups, some fundamental physico-chemical or structural difference between bacterial and fungal membranse would be indicated. — D. W. Stranks, Eastern Forest Products Laboratory, Ottawa, Ont.

Polyethylene Vials as Slow-Release Containers for Volatile Fungicides. — One effective means for stopping internal decay in preservative-treated wood products is to inject high-vapor-pressure fungicides into the decaying area (Graham, For. Prod. J. 23(2): 35-38, 1973). The three soil fumigants of chloropicrin (trichloronitromethane), Vorlex (methyl isothiocyanate and chlorinated C_3 hydrocarbons) and Vapam (sodium N-methyl dithiocarbamate) have shown considerable promise as fungicides in field tests on Douglas-fir transmission poles. They not only stopped internal decay, but also provided residual protection against reinfection for several years (*ibid.*). Although these chemicals are presently applied effectively as free liquids, it is desirable to develop a method of controlling the release of their vapors to facilitate safe handling of the chemicals and to extend their duration of effectiveness in wood.

The two common methods for retarding pesticide vapor, adsorption of pesticide on inert carriers and solution of pesticide in a plastic monomer followed by polymerization of the plastic into solid pellets (Allan and Neogi, Int. Pest Control 14(4):21-27, 1972), were not considered. In each case, a large part of the formulation's total volume is taken up by inert substrate. Thus, samples of the three soil fumigants were heat sealed in three sizes of polyethylene vials (Table 1) and the rates of fumigant loss through the vial walls evaluated at 2, 22 and 32°C.

TABLE 1
Fumigant loss rates from polyethylene vials of capacities shown (mg/hr per cm² surface area per mm wall thickness)

Temp °C	Chloropicrin			Vorlex		
	6 dram	4 dram	2 dram	6 dram	4 dram	2 dram
2	0.020	0.014	0.011	0.025	0.019	0.015
22	0.124	0.098	0.064	0.151	0.129	0.100
32	0.329	0.325	0.206	0.432	0.356	0.210

Vapam was not able to diffuse through polyethylene, so is not shown in Table 1. Its fumigating action in soil involves reaction with water, after which it decomposes into volatile chemicals. It is probable that this decomposition cannot occur when Vapam is confined in vials. Vorlex diffuses through polyethylene faster than chloropicrin (Table 1). The loss rates remain constant at a given temperature until each vial is empty, indicating that all components of the Vorlex formulation permeate the walls at approximately the same rate.

Predictably, temperature has a large effect on the permeation rates of Vorlex and chloropicrin. At higher temperatures, the vapor pressure of the chemicals is higher, resulting in a higher concentration gradient and, therefore, a greater rate of permeation across the walls. Also, the diffusion process is inherently temperature dependent, with higher diffusion coefficients at higher temperatures. Thus, storage of vials at low temperatures between manufacture and use will prevent significant losses of chemicals.

According to permeation theory for the diffusion of substances through plane membranes, the movement of chemical should be directly related to the total surface area and inversely related to the membrane thickness (Barrer, Diffusion In and Through Solids, Cambridge, England, 1941. p62). However, in our study, the loss rates were lower for smaller vials, even after corrections for total surface area and wall thickness. Thus, it is not possible to design polyethylene slow-release vials to generate vapor at a desired rate by simply designating the required wall thickness and surface area. However, the results show the general magnitude of loss rate that can be expected with polyethylene containers. — P. A. Cooper, Western Forest Products Laboratory, Vancouver, B.C.

SILVICULTURE

Greenhouse and Field Trials of Gramoxone and PP493 Herbicides on Boreal Forest Vegetation of Northeastern Ontario. — Ground vegetation ties up considerable quantities of nutrients that might be used by trees in wood production. This nutrient tie-up may play an important role in determining the effectiveness of applied fertilizers.

During forest fertilization studies it was deemed necessary to evaluate the effects of vegetation removal on the ability of trees to increase their nutrient uptake and, consequently, their growth. A preparatory study was established to assess the effectiveness of two herbicides in controlling ground vegetation common to sandy jack pine [*Pinus banksiana* Lamb.] sites and peatland black spruce [*Picea mariana* [Mill.] B.S.P.] sites in northeastern boreal Ontario.

This paper reports results of a greenhouse study and a field trial to evaluate the effectiveness of two herbicides: Gramoxone®, with the active ingredient paraquat, and PP493, supplied as an aqueous solution (JF 2408) of the potassium salt.

Gramaxone® is a contact herbicide that kills vegetation rapidly by interfering with the oxidation-reduction reactions within plant tissues (Costen, pap. presented at Ont. Prof. For. Assoc., Herbic. Semin., Toronto, Ont., 11 p., 1968). The active ingredient is claimed to lose its effectiveness on contact with soil (Anon., Chipman Chem. Ltd., Hamilton, Ont., 3 p., n. d.).

PP493, a herbicide not commercially available, has undergone limited testing under agricultural conditions but no information is available on its effects on forest vegetation species. It is reported to be both a soil-acting and a contact herbicide that interferes with the formation of chlorophyll. The manufacturer's preliminary studies have indicated that, in the several soil types tested, herbicidal activity following application of 5.5 kg/ha (5.0 lb.-acre) ceased after 2-3 months (Anon., Imp. Chem. Ind. Ltd., Agric. Div., Jealott's Hill Res. Stn., Bracknell, Berks., England, Tech. Data Sheet, 10 p., 1969). These studies also showed that PP493 in combination with Gramoxone® enhanced the activity of the latter by delaying the rate of desiccation.

For the greenhouse trial, intact samples of the upper 15 cm (5.85 in.) of a podzol soil (H, Ah, Ae and upper B horizons), including the dormant ground vegetation, were collected in October from a 45-yr-old jack pine stand near Chapleau, Ontario. The samples, placed in plastic tubs 30 x 33 x 25 cm (11.70 x 12.87 x 9.75 in.) were overwintered in a greenhouse at 21°C (69.8°F) and watered regularly. By March the following plant species had emerged and were flowering: *Cornus canadensis*, *Diervilla lonicera*, *Epilobium angustifolium* and *Vaccinium angustifolium*. Newly flushed 2-yr-old black spruce and 22-wk-old jack pine seedlings were planted in the soil samples at this time.

Herbicide treatments (not replicated) were applied in late March under room conditions at the following rates: 1) control, no herbicide; 2) Gramoxone®, 0.56 kg (0.50 lb.) a.i. in 336 *l* of water/ha (30 gal/acre); 3) PP493, 0.56 kg a.i. in 336 *l* of water/ha; 4) combination of Gramoxone® and PP493 at 0.56 kg a.i. each. These applications thoroughly wetted the surface of both the vegetation and the soil. Oat seeds were drilled into the soil of each treatment 9, 47 and 97 days after the herbicide application to test the residual effects of the treatments.

For the field trial, a twice-replicated series of 9.1-m (30-ft)-diam circular plots was established in late June in a 45-yr-old jack pine stand in Nimitz Twp near Chapleau, Ontario (Section B.7, Rowe, Can. For. Serv., Publ. No. 1300, 172 p., 1972). Similar plots were established in early August in both a 60-yr-old jack pine stand in Calvert Twp and a poor black spruce peatland area in Hanna Twp south of Cochrane, Ontario (Section B.4, Rowe, Can. For. Serv., Publ. No. 1300, 172 p., 1972). Most shrubs, herbaceous annuals and mosses typical of these sites were present in all plots.

At the time of plot establishment herbicides were applied with a mist blower to the ground vegetation at the following rates: 1) PP493, 0, 0.56, 1.12, 2.24 and 3.36 kg/ha (0, 0.5, 1.0, 2.0 and 3.0 lb.-acre); 2) Gramoxone®, 0, 0.56, 1.12 and 2.24 kg/ha; 3) combined treatments of PP493 and Gramoxone®, respectively, 0.56+0.56, 1.12+0.56, and 2.24+0.56 kg/ha. All chemicals were applied as aqueous solutions dissolved in 336 *l*/ha of water. Vegetation was assessed daily for the first 2 wk and periodically thereafter to determine the effectiveness of the herbicide treatments in killing vegetation and controlling its regrowth.

In the greenhouse trial Gramoxone® killed all plants (including tree seedlings) within 6 days, PP493 required 40 days, and the combined treatment required 12 days. During the 140-day test period all vegetation flourished in the control treatment. Approximately 50 days after the Gramoxone® treatment, *V. angustifolium*, *C. canadensis* and *D. lonicera* began to re-emerge. In the PP493 treatment *C. canadensis* and *V. angustifolium* re-emerged about 85 days after herbicide treatment, but these plants subsequently blanched and died. No re-emergence of vegetation was observed in the combined treatment for the duration of the test. No damage occurred to young oat shoots in the control or Gramoxone® treatments and all shoots grew well, matured and produced viable seed. In both the PP493 and combined treatments, oat seeds germinated but blanched and died shortly after emergence. These results were consistent for all three dates of oat sowing.

In all of the Gramoxone®-treated field plots, annuals such as *C. canadensis*, *M. canadense*, *D. lonicera*, *Fragaria* spp. and *Rubus chamaemorus* were dead within 6 days, and did not regreen. *Carex* spp. partially browned but were not killed. In the pine areas *V. angustifolium*, *Salix* spp. and *Rosa acicularis* browned, but regreened within 30 days. In the peatland areas *Andromeda glaucophylla*, *Ledum groenlandicum*, *Chamaedaphne calyculata* and *V. angustifolium* browned within 9 days. Only *A. glaucophylla* regreened the following spring. Gramoxone® did not have much effect on the brown and feather mosses, but did blanch some of the *Sphagnum* species. The lower branches of both jack pine and black spruce trees, and black spruce seedling regeneration, showed needle tip-burn and some needle loss as a result of spray drift.

In the PP493 field treatments *C. canadensis*, *M. canadense*, *D. lonicera*, *R. chamaemorus*, *Carex* spp., *V. angustifolium*, *Salix* spp., *R. acicularis*, *L. groenlandicum*, *C. calyculata*, *A. glaucophylla* and the mosses *Dicranum* spp. and *Pleurozium schreberi* browned within 13 days and were dead within 18 days. However, in the 0.56 kg/ha treatment *V. angustifolium*, *A. glaucophylla*, *R. acicularis*, *Salix* spp. and the mosses *Dicranum* spp. and *P. schreberi* regreened after 30 days. The stems of *L. groenlandicum* and *C. calyculata* were killed, but regrowth did occur the following spring at the 0.56 kg/ha treatment level. Considerable needle tip-burn on the lower branches of mature jack pine and black spruce on the plot was present with all concentrations.

Plants listed for the PP493 treatment also occurred in the plots where combinations of the two herbicides were used. All species were killed in 12 days rather than 18 days by combinations of the two herbicides. In the pine areas reflushing of most ericaceous and woody shrubs and mosses occurred after 30 days at the lowest combined concentration (0.56+0.56 kg/ha). *Comptonia peregrina*, *Prumus pensylvanica*, *Alnus rugosa* and *Populus tremuloides* present in the Cochrane area jack pine plots, but not elsewhere, were killed by even the lowest concentrations of the combined herbicides. The heavier dosages effectively controlled all species throughout the first season, but new growth appeared the following spring.

The results obtained during the greenhouse and field trials support the already published information on the effectiveness of Gramoxone® and PP493 herbicides in controlling vegetation in agricultural areas. Annual plants seem very susceptible to Gramoxone®, which was found to be fast acting with no apparent residual effect on regrowth. The lack of immediate re-emergence (greenhouse and field) of forest vegetation and the early death of seeded oats in the greenhouse study attest to the residual effectiveness of PP493 used either alone or in combination with Gramoxone®.While greenhouse and field results with Gramoxone® were similar, PP493 indicated faster herbicidal activity under field conditions. Control of regrowth by the 0.56 kg/ha concentration of PP493 was not as effective in the field as in the greenhouse, but both herbicides may be beneficial for specific forestry purposes. — D. A. Winston and V. F. Haavisto, Great Lakes Forest Research Centre, Sault Ste. Marie, Ont.

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15 **Angus, T. A. 1974.** Microbiological control of insects: Bacterial pathogens. Proc. Summer Inst. Biol. Control Plant Insect Disease. Miss. State Univ. June 1972. pp.532-540.

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10 **Bella, I. E. and J. P. DeFranceschi. 1974.** Analysis of jack pine thinning experiments, Manitoba and Saskatchewan. *Can. For. Serv.* Pub. 1338. 21 p.

15 **Bird, F. T. 1974.** The development of spindle inclusions of *Choristoneura fumiferana* (Lepidoptera: Tortricidae) infected with entomopox virus. *J. Invertebrate Pathol. 23*:325-332.

6 **Bouzane, J. 1974.** Some ERTS observations in Newfoundland. *Can. Surv. 28*:162-163.

13 **Calvert, W. W. and A. M. Garlicki. 1974.** The use of ring barkers at low temperatures. *Can. For. Serv.* Pub. 1334. 24 p.

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Addresses of the Canadian Forestry Service

Requests for additional information should be addressed as shown by the code.

Enquiries Centre, 1
Department of the Environment,
Ottawa, Ontario
K1A 0H3.

Forest Fire Research Institute, 2
Department of the Environment,
Nicol Building,
331 Cooper Street,
Ottawa, Ontario
K1A 0W2

Forest Management Institute, 3
Department of the Environment,
Majestic Building,
396 Cooper Street,
Ottawa, Ontario
K1A 0W2

Forest Economics Institute, 4
Department of the Environment,
Nicol Building,
331 Cooper Street,
Ottawa, Ontario
K1A 0W2

Chemical Control Research Institute, 5
Department of the Environment,
25 Pickering Place,
Ottawa, Ontario
K1A 0W3

Newfoundland Forest Research Centre, 6
Department of the Environment,
Bldg. 304, Pleasantville,
P.O. Box 6028,
St. John's, Newfoundland
A1C 5X8

Maritimes Forest Research Centre, 7
Department of the Environment,
P.O. Box 4000,
Fredericton, New Brunswick
E3B 5G4

Laurentian Forest Research Centre, 8
Department of the Environment,
1080 Route du Vallon, P.O. Box 3800,
Ste. Foy, Quebec
G1V 4C7

Great Lakes Forest Research Centre, 9
Department of the Environment,
P.O. Box 490, 1189 Queen St. E.
Sault Ste. Marie, Ontario
P6A 5M7

Northern Forest Research Centre, 10
Department of the Environment,
5320 - 122nd Street,
Edmonton, Alberta
T6H 3S5

Pacific Forest Research Centre, 11
Department of the Environment,
506 West Burnside Road,
Victoria, British Columbia
V8Z 1M5

Western Forest Products Laboratory, 12
Department of the Environment,
6620 N.W. Marine Drive,
Vancouver, British Columbia
V6T 1X2

Eastern Forest Products Laboratory, 13
Department of the Environment,
Montreal Road,
Ottawa, Ontario
K1A 0W5

Petawawa Forest Experiment Station, 14
Department of the Environment,
Chalk River, Ontario
K0J 1J0

Insect Pathology Research Institute, 15
Department of the Environment,
P.O. Box 490, 1195 Queen St. E.,
Sault Ste. Marie, Ontario
P6A 5M7