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ENTOMOLOGY

Laboratory Tests of 1-(4-Chlorophenyl)-3(2,6 difluorobenzoyl) Urea on Survival of Western Hemlock Looper.—Because of the controversy over the effectiveness of this pesticide, we decided to test it against western hemlock looper larvae [*Lambdina fiscellaria lugubrosa* Hulst.]. The compound, a 25% a.i. wettable powder, is commonly called pH 60-40 or Dimilin. Dimilin disrupts the synthesis of chitin in arthropods and fungi. Insects that ingest Dimilin form a thin integument which bursts under turgor pressure during moulting, thus exposing the larvae to dessication and death. Furthermore its toxicity to higher life forms is low, e.g. LD50 for rats is 10,000 mg/kg.

The test material, used as a water suspension at various concentrations, was applied to drip point to 3- to 4-year-old potted hemlock trees as a fine spray. The trees had flushed before spraying and continued to grow thereafter. Ten second- or fourth-instar field-collected larvae were released on each tree after spraying. Each dose and larval instar were represented by five such replicate trees. The infested trees were kept in the laboratory. Larvae, parasitized or lost, were not included in the results. Thus, each dose was represented by 40-50 larvae.

Residual effectiveness of the spray, up to 3 weeks after application, was tested by spraying five replicate trees with a single dose at weekly intervals and releasing fourth-instar larvae on these trees 1 week after the last treatment; the larvae were subjected to 1-, 2- and 3-week-old spray. Trees were kept outside during the pre-infestation period.

Mortality of the larvae was recorded every 3-4 days. The experiment was terminated when all larvae had either died or pupated.

The results show that all doses caused total mortality of second-instar larvae, wheras some fourth-instar larvae exposed to low doses survived the moult to next larval instar and pupated (Table 1). Fourth-instar larvae appeared more vigorous than second-instar larvae following exposure to the test material. Duration of larval survival after treatment was not clearly related to dosage, except among the fourth-instar larvae. Longer average survival of fourth-instar larvae exposed to lower doses is due to the fact that some lived long enough to pupate, contributing to increased duration of average survival (Table 1). Difference in the average duration of survival between second- and fourth-instar larvae after treatment was related to the length of their stadid (8 and 16 days, respectively, at 21°C). Differences in defoliation between treated and controlled trees were not measured quantitatively, but they were visibly obvious.

TABLE 1

Effect of Dimilin on the survival of second- and fourth-instar western hemlock looper larvae

	2nd instar		4th instar	
Dose*	% Survival	Average days to death	% Survival	Average days to death
Control	31	22.3	63.6	18.2
5 PPM	–	–	5.0	16.6
10 PPM	0	7.1	4.3	13.2
40 PPM	0	7.1	0	10.5
160 PPM	0	6.6	0	10.1
640 PPM	0	6.3	–	–

* PPM active ingredient.

TABLE 2

Residual effectiveness of 10 PPM Dimilin against fourth-instar western hemlock looper larvae

Age of deposit	% Survival	Average days to death
1 week	0	14.0
2 weeks	0	12.6
3 weeks	0	15.1

Figure 1. Hemlock looper larva with burst integument due to Dimilin treatment.

Dimilin applied as a single dose of 10 ppm remained fully effective for 3 weeks and caused total mortality of the fourth-instar larvae (Table 2). Mortality of larvae resulted from bursting of their integument (Fig. 1). Usually the breaks in the integument are not as obvious as the one shown, but are easily located by the haemolymph that oozes out through the rupture.

Dimilin appears to be effective against western hemlock loopers. It is more desirable than commonly used pesticides because of its low toxicity to non-arthropod forms of life. Present results warrant field trials.—T. S. Sahota and R. F. Shepherd, Pacific Forest Research Centre, Victoria, B.C.

Aerial Application of a Nuclear Polyhedrosis Virus to Control European Pine Sawfly.—A nuclear polyhedrosis virus of the European pine sawfly [*Neodiprion sertifer* (Geoff.)] was imported into Canada from Sweden in 1949. The virus was field tested by spraying from ground equipment in 1950 and 1951 and from an aircraft in 1952 (Bird, Can. Entomol. 85: 437-446, 1953). Since then, the virus has been sprayed from the ground by the staff of the Canadian Forestry Service, Ontario Ministry of Natural Resources and private growers to control small infestations of European pine sawfly in plantations.

A build-up of this sawfly was noted in Sandbanks Provincial Park, on Quinte Island, Prince Edward County, in 1974 and it was decided in consultations between Mr. K. B. Turner

of the Ontario Ministry of Natural Resources (OMNR) and staff of the Insect Pathology Research Institute and the Great Lakes Forest Research Centre to use a virus spray for control. In the Park, Scots pine, jack pine and underplanted red pine were all heavily infested. There were two areas to be sprayed; the first was 89 ha and the trees were 6 to 9 m tall; the second was a sand dune area of 36 ha where tree growth was poor and size varied from 0.3 to 4.5 m. Total area sprayed was 125 ha.

To formulate the spray, 0.26 g of pulverized, freeze-dried, virus-infected larvae, 30 g of IMC 90-001 UV protectant and 1.25 ml of Chevron® spray-sticker were added to each litre of water. This gave a virus concentration of 5.3 billion inclusion bodies per litre. The spray was applied on the morning (6:00 to 8:30) of May 23 at a rate of 9.4ℓ/ha using a Piper Super Cub fitted with a boom with 26 D7 nozzles and spraying a 41 m wide swathe. The deposit was monitored on Kromekote® cards exposed in open areas and also clipped to the foliage under colonies of sawflies selected for observation. The larger area was sprayed first, and the mean number of droplets per cm^2 was 15 ± 5; on the sand dune area it was 5 ± 2. The reduced deposit on the sand dune area was due to evaporation of the smaller droplets as the sun rose. In the first area the droplet diameters were mainly less than 100μ; in the second area the droplets were larger, few were less than 150μ and most were in the $200\text{-}700\mu$ range.

At the time of application most colonies of larvae were in the first instar with a very few second instar larvae present. For an assessment of the spray efficacy, 100 colonies were tagged and observed at weekly intervals following the spray. It was confirmed from spray cards that virus was deposited on all 100 colonies. Prior to the spray application 10 colonies were removed and reared outside the spray areas as a check for naturally occurring virus and five colonies were tagged at Cherry Valley, 5 km northeast of the Park as a further check.

Samples of larvae sent to IPRI for microscopic examination showed 90% virus infection 1 week following the spray. Two weeks post-spray, no healthy larvae remained in the 100 tagged colonies and 3 weeks after the spray, no European pine sawfly larvae could be found in the entire park. There was no measurable defoliation caused by the larvae prior to their death. No disease was found in the 15 check colonies, which were observed until the larvae were fully grown.

The operation was an unqualified success and, although the spray deposit was not considered to be particularly good, the virus was transmitted throughout the colonies giving rapid mortality (Bird, J. Insect Pathol. 3:352-380, 1961). The concentration of virus and volume of spray per hectare were both probably more than required and experimentation should be undertaken to establish a lower operational dosage. The timing of the operation was ideal and defoliation was prevented. Spraying with nuclear polyhedrosis virus as European pine sawfly larvae hatch was recommended by Griffiths *et al.* (Biological Control Programmes Against Insects and Weeds in Canada, 1959-1968. Tech. Commun. Commonw. Inst. Biol. Control 4:150-162, 1971). The above results strongly support their recommendations. We wish to thank OMNR staff at Outlet and Sandbanks Provincial Parks for their cooperation and assistance and Dr. F. T. Bird of IPRI for his advice and encouragement.—J. C. Cunningham, W. J. Kaupp and J. R. McPhee, Insect Pathology Research Institute and W. L. Sippell and C. A. Barnes, Great Lakes Forest Research Centre, Sault Ste. Marie, Ont.

PATHOLOGY

A Method of Tracing Fungi Inoculated into the Stems of Living Maple.—To understand the roles of decay-causing fungi and other fungi that invade and inhabit the wood in living trees, a knowledge of the relationship of the fungi to the host must be supplemented by a knowledge of the interaction between various fungus species within the host. Recent evidence has revealed that interactions between fungi observed in plate cultures or in wood blocks in the laboratory, though useful as indicators, do not always occur within intact, living trees.

In experimental inoculations of the stems of living trees with fungi, it is virtually impossible to be certain whether the subsequent reisolations represent the fungi from the deliberate introductions, or ones from natural infections. There are, of course, statistical ways of handling such situations. Wood pathology, however, involves laborious and difficult sampling procedures and, therefore, a large number of replicates are not easily obtained. Thus, a statistical approach rarely gives results that are completely unambiguous. An alternative approach would be to develop genetically marked strains of wood-inhabiting fungi, especially fungi likely to be of interest by virtue of their influence upon the principal decay organisms either as precursors or as antagonists.

Because the inoculation of living trees with genetically marked strains of fungi was a new approach, we decided to thoroughly test a fungus consistently encountered in the stems of living sugar maple [*Acer saccharum* Marsh.] to determine the feasibility of the entire concept. Isolates of several such fungi were sent to Dr. F. Cooke at Queen's University, Kingston, Ont. After several months of testing, Dr. Cooke concluded that *Coniochaeta velutina* (Fckl.) Munk was the most amenable to laboratory manipulation and gene analysis. (Cooke *et al.* Can. J. Bot. 47-1019-1026).

Our laboratory tests involved studies of the rate of spread of several of the *C. velutina* mutants and of the wild type in blocks of naturally decaying sugar maple wood, approximating the natural habitat. Some of the mutant strains were clearly altered in that they showed significantly slower rates of spread than the wild type. The majority of the mutants, on the other hand, showed rates of spread nearly identical to those of the wild *C. velutina* strains.

Several of the more suitable mutants were tested in living sugar maple trees growing under normal stand conditions. Five *C. velutina* mutants, which could be identified by their growth on selective media, were used for testing on the basis of their genetic stability and their ecological behavior being similar to that of the wild type in the laboratory.

For this study, 20 mature sugar maple trees that had been wounded on two sides of the trunk 5 years earlier by the removal of patches of bark with a drawknife were used. In August 1970, four of these trees were selected for each of the five mutants, and sugar maple dowels (roughly 3.4 cm long and 9.5 mm in diameter) colonized with the mutants were inserted in holes drilled in the center of the wounds. Thus eight inoculations were made for each mutant. The inserted dowels were carefully sealed with asphalt compound immediately after inoculation to guard against contamination.

Five trees per year, each inoculated with one of the mutants, were sampled and analyzed in 1972, 1973 and 1974. The remaining five trees were sampled in 1975 but have yet to be analyzed. In addition, 20 unwounded sugar maple were inoculated in 1973, each with both a *C. velutina* mutant and the wild type. These trees are to be sampled in 1976 and 1977. This is a preliminary report on the study based on the results available from the first 15 trees sampled.

In all, 50 isolation attempts were made from the wood adjacent to each dowel in a predetermined pattern, primarily directly above and below the dowel for a distance of 28 cm. These include two attempts made from the core stain surrounding the pith, on the opposite side of the pith from the dowel, and 12 made in the wood above and below the center of the dowel but approximately 13 cm on either side of the vertical

plane running lengthwise through the dowel. No isolates of the *C. velutina* mutants resulted from any of these attempts; all reisolations being obtained from wood located in or very close to the vertical plane directly above and below the dowels. This plane was the site of 36 isolation attempts per dowel, for a total of 1080 attempts in all 15 trees sampled.

C. velutina was isolated with steadily diminishing frequency as the distance above or below the inoculation dowels increased (Table 1).

TABLE 1

Occurrence of *C. velutina* mutant isolates at varying distances above and below inoculation dowels[a]

Distance from edge of dowel	No. of *C. velutina* isolates			% of total *C. velutina* isolates
	Above dowel	Below dowel	Total	
1.27 cm	32	33	65	34.0
2.54 cm	27	27	54	28.3
3.81 cm	25	19	44	23.0
6.35 cm	11	13	24	12.6
11.90 cm	1	3	4	2.1
22.00 cm	0	0	0	0.0
Total	96	95	191	

[a] For each dowel the same number of isolation attempts was made at each of the six distances. An equal number of attempts was made above and below the dowel.

The 180 isolation attempts made 1.27 cm from the dowels yielded 65 isolates of *C. velutina*, whereas the same number of isolation attempts made 22 cm away yielded none. This strongly suggests that the 191 isolates of *C. velutina* originated from the dowels. To confirm this, two or three *C. velutina* isolates from wood adjacent to each dowel were tested for the biochemical characteristics exhibited by the mutant strains inoculated in 1970. Without exception, all isolates tested were found to be the mutants and not the wild type of *C. velutina*.

All five mutant strains of *C. velutina* were isolated from the wood of inoculated trees above and below the dowels (Table 2). Two mutants, C and E, were not obtained from the trees sampled in 1972; however the five trees sampled in both 1973 and 1974 yielded all of the mutants. Two mutants, B and D, were isolated from the wood above and below five of the six dowels tested. Mutant C, on the other hand, was isolated from only two of the six inoculation zones.

TABLE 2

The occurrence of successful inoculations, and of *C. velutina* mutant isolates, in sugar maple trees sampled over a 3-year period[a]

C. velutina mutant	Number of dowels associated with stem infection, followed by total number of *C. velutina* mutant isolates recovered			
	1972	1973	1974	Total
A	1, 2	1, 14	1, 9	3, 25
B	1, 15	2, 22	2, 13	5, 50
C	0, 0	1, 5	1, 14	2, 19
D	1, 5	2, 21	2, 22	5, 48
E	0, 0	1, 24	2, 25	3, 49
Total	3, 22	7, 86	8, 83	18, 191

[a] Based on two inoculation attempt assessments per year, or a total of six, for each mutant. Ten inoculation attempts were assessed each year, for an overall total of 30.

These results show that biochemical mutant strains of *C. velutina* can be introduced into the stems of living sugar maple where they spread for distances up to 12 cm over a 5-year period, and that they can be reisolated and identified as the original inoculum. Although all five mutants showed equal rates of spread within maple blocks *in vitro*, they apparently differ appreciably in the rate at which they colonize the wood of living sugar maple trees. Analyzes will be carried out to determine whether these differences are related to the particular wound microflora the mutants encountered in the vicinity of the inoculation dowels. The unwounded maple inoculated in 1973 will perhaps give a better indication of the relative stem-colonizing ability of the five mutants, and should permit a valid comparison of rate of spread and wood-altering capacity of wild type and mutant within living trees. Results to date suggest that this method shows promise as a new experimental approach to the study of wood-inhabiting fungi in living trees, and possibly as a technique for future research on the biological control of stem decays.

We thank Dr. F. Cooke for the development of the stable biochemical mutants of *C. velutina* used in this study, and Miss M. Tucker of A. D. Revill Associates for confirming the identity of isolated mutant strains.—J. T. Basham, Great Lakes Forest Research Centre, Sault Ste-Marie, Ont. and H. M. Good, Department of Biology, Queen's University, Kingston, Ont.

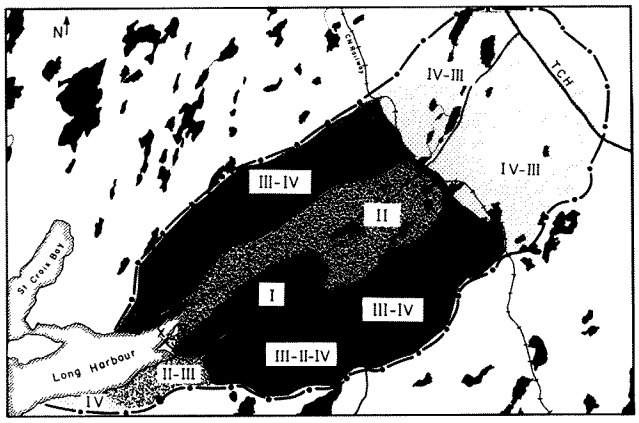
Damage to Vegetation in the Vicinity of a Phosphorus Plant, Long Harbour, Newfoundland.—The phosphorus plant at Long Harbour (47°26′N, 53°47′W) commenced operation in 1968 and produces elemental phosphorus by the electrical reduction of phosphate rock rich in calcium phosphate and calcium fluoride. The details of chemical reactions involved and by-products obtained, are given by Idler (In: Jangaard, Atlantic Regional Office, Fish. Res. Board Can., Circ. No. 2, 1972: 1-6).

In the process most of the gaseous by-products are removed by lime treatment; however, some untrapped carbon monoxide (CO), hydrogen fluoride (HF) and silicon tetrafluoride (SiF_4) are released as gases into the air. Sulphur dioxide (SO_2) is also released during pelletizing process by the combustion of bunker C oil. In addition to these gaseous emissions, phosphate-rock dust is generated and vegetation near the industrial plant is exposed to these emissions. Of these emissions, HF and SiF_4 constitute the major concern.

In 1970, vegetation in vicinity of the plant had symptoms of fume damage, tip burn and margin burn. Since 1970 this damage has worsened. In 1973, on request from the Newfoundland Government, intensive research was undertaken by the Newfoundland Forest Research Centre, Canadian Forestry Service, to assess the problem. This note summarizes the extent and intensity of fume damage presented as a map of vegetation damage until fall of 1973, the concentration values for total fluoride (F) in foliage, and the levels of available fluoride (F) in soil-humus from damaged and control plots.

The forest inventory maps (Nfld. & Labrador Forest Service, Nfld. & Labrador For. Inventory, 1:50,000 Forest Type Maps, Nos. 7-153 and 7-145) and our ground checks showed that the study area was 25% productive forest (6-9 m high balsam fir and black spruce), 20% non-productive scrub forest (3-5 m high, soft and hardwoods), 33% open bog, soil barrens and rock barrens, and 22% fresh and salt water.

Preliminary field observations showed that the most severe damage to vegetation had occurred NE to E of the emission source (map). Interpretation of aerial photographs (black and white, false color infrared, natural color) of the study area followed by ground checks indicated that an area of 77,2 km^2 had vegetation damage attributable to fluoride. The severity of this damage varied from complete death of the overstory trees to mild tip-burns of conifer needles (balsam fir, white spruce, black spruce) and leaf-margin burns in broad leaf species (alder, white birch, fireweed, blueberry). Percentage crown damage was determined with a dot-grid and 1973 aerial photographs. The damage area was divided into four zones of increasing damage based on the degree of fume damage to the crowns of dominant trees. Extensive ground checks were made to confirm the accuracy of these zones. Ten trees at each of 104 locations were checked for percent tree mortality



Damage zones in the Long Harbour area. The X indicates the source of the emissions. Black areas outside of Zone I are fresh water.

and percent defoliation. Data are summarized in Table 1. The boundaries of the damage zones are not discrete because of the matrix nature of the vegetation, height of trees, degree of exposure of individual trees to wind, topography, and elevation. The most severe damage occurred to the tallest trees on either exposed slopes or in valleys closest to the emission source. The protected undergrowth showed the least damage. Severe damage (Zones I and II) to vegetation occurred up to a distance of 9,6 km NE to E from the emission source, but damage could still be observed as far as 18, 5 km to the NE.

To study the levels of fluoride concentrations in plants and soil, 54 balsam fir foliage (1973 growth) samples and 25 soil-humus samples were collected at various distances and directions from the industrial plant and within the boundaries of the damage zones. Control samples of foliage and soil-humus were collected from similar sites located 48-80 km from the emission source (St. John's, Goobies, Logy Bay). Samples from Long Harbour as well as control areas were treated in exactly the same way. The foliage samples were oven-dried at 70°C and ground to pass through a 40-mesh sieve. The plant samples were analysed for total fluoride by a semi-automatic method (Buck and Reusmann, Fluoride 4:5-15, 1971). The soil-humus samples were oven-dried at 35°C and ground to pass through a 20-mesh sieve. For each soil sample 5 g of soil-humus was shaken with 50 ml of distilled water and 50 ml of TISAB (Orion Res. Inc.) and concentrations of available fluoride determined with an ion-specific electrode, as for foliar analyses.

TABLE 1

Concentrations of total fluoride in foliage and available fluoride in soil humus samples from 4 damage zones near a phosphorus plant, Long Harbour.

Damage zone	Observed damage to dominant trees	Fluoride concentrations (F^-, ppm/dry wt.) Total in foliage (balsam fir) range	average	Available in soils humus range	average
Zone I	80-95% trees dead; severe to complete defoliation	171-900*	281	47.4-64.6	58.0
Zone II	40-60% trees dead; severe to complete defoliation on windward side; tip burn all through the crown	96-230	141	8.8-81.0	27.0
Zone III	20-30% trees dead; partial defoliation on windward side; severe tip burn on windward side, light or no tip burn on leeward side or sheltered windward side	57-152	91	5.6-22.6	15.0
Zone IV	No dead trees; little or no defoliation; light tip burn on the needles in the top crown, mostly on windward side	24- 88	44	1.8- 6.4	3.8
CONTROL	No damage	5- 11	7	1.9- 2.7	2.4

* Foliage samples for Zone I were from sheltered trees only.

The severity of damage was found to be correlated with the fluoride in the needles and soil-humus (Table 1). Foliar samples from Zone I showed values of 171-230 ppm (dry weight basis) in 80% of the samples, the remaining 20% showing values of 280-900 ppm. In Zone II concentrations were 91-160 ppm in 70% of the samples, over 190 ppm in 20% and below ppm in 10% of the samples. In Zone III, 75% of samples fell in the range 51-100 ppm, the other 25% being above 121 ppm. In Zone IV, values were 21-60 ppm in 90% of the samples and 70-90 ppm in the remaining 10%. All control samples had a concentration of less than 10 ppm. Both severity of damage and foliar fluoride concentrations were inversely correlated with the distance from the phosphorus plant. The fluoride concentrations in soil-humus showed similar correlations with the severity of damage and distance from the emission source (Table 1). The concentrations were highest in Zone I, closest to the source, and the lowest in Zone IV, farthest from the source.

Permanent samples plots were established and studies on the progression of fluoride damage symptoms and patterns of seasonal accumulation of fluorides in several plant species are in progress.—S. S. Sidhu and B. A. Roberts, Newfoundland Forest Research Centre, St. John's, Nfld.

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