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ACID RAIN

Acid Precipitation and Vegetation Interaction in the Turkey Lake Forest Watershed

Concern about the effects of acid rain on forests and water has stimulated renewed interest in how forests and soils alter the quality of precipitation. This report, an interim examination of results from a continuing study of the biogeochemical cycling of elements within a sugar maple (*Acer saccharum* Marsh.) – yellow birch (*Betula alleghaniensis* Britton) forest, was presented at the 1982 conference on Great Lakes Research. The work is part of the Canadian Forestry Service acid rain program examining the influence of acid precipitation on the cycling of elements within three forest catchment basins at Kejimikujik National Park, N.S., Montmorency Forest, Que., and Turkey Lake Forest, Ont.

The magnitude of chemical changes in precipitation within a 140-year-old hardwood forest in the Turkey Lake Forest watershed, Algoma District, Ont., was ascertained by sampling precipitation, throughfall, and forest floor percolate. Precipitation and throughfall were collected in 20-cm-diameter continuously open (bulk) collectors, either plastic funnel-bottle sets or aluminum rain gauges. Forest floor percolate was collected below F horizons with plastic zero-tension lysimeters (Jordan, Soil Sci. 105:81–86, 1968). Three precipitation collectors were located in an open area adjacent to a 12-ha forested subbasin containing 36 throughfall and 24 percolate collectors distributed over twelve 10-m² plots within a 1-ha area. Thirty-two discrete storm events were sampled during leafout time (June to October, 1980 and 1981). An additional 23 samplings were made of separate rain or snow events during the leafless period until the snow pack was established, after which snow cores were taken from snowboards. Solutions were analyzed for K⁺ and Ca⁺⁺ with a flame emission-atomic absorption spectrophotometer, for SO₄⁻⁻ and NO₃⁻-N with a Technicon Autoanalyzer System II using the methyl-

thymol blue and cadmium reduction methods respectively, and for pH by glass electrode.

PRECIPITATION

Despite the remote location of the watershed in relation to anthropogenic emissions of SO₂ and NO_x, between 1978 and 1980 the weighted mean pH of precipitation was 4.5 (Barry and Sirois, AES Report: AQRB-82-003-7, 1982). In our study, during leaf-out, precipitation pH averaged 4.2 and ranged from 3.7 to 6.0. Sulphuric acid was the dominant acid in precipitation, accounting for about 65% of H⁺ input.

In general, throughfall and forest floor ionic concentrations were not well correlated with those in precipitation (Table 1). This fact suggests that the forest canopy and decaying vegetation significantly alter ion concentrations in precipitation. An exception was a significantly positive correlation between throughfall and precipitation [H⁺]. Throughfall H⁺ was also significantly positively correlated with precipitation volume.

THROUGHFALL

Throughfall was highly enriched in K⁺ and somewhat less enriched in Ca⁺⁺ during the leafout period (Table 2). Enrichment of throughfall is generally attributed to leaching of ions from within leaves. Wash-off of atmospheric dry deposits of K⁺ and Ca⁺⁺ on leaf surfaces may also contribute to elevated throughfall concentrations.

Ratios of concentrations in throughfall to those in precipitation calculated from the 23 events during the leafless period were 1.78, 1.05, and 1.13 for K⁺, Ca⁺⁺, and H⁺ respectively. Forest vegetation in the leafless period, therefore, generally produced only minor changes in precipitation ion concentrations.

Acid precipitation may accelerate leaching of cations from foliage because H⁺ exchange is a leaching mechanism. Mean [H⁺] in throughfall was lower than that in precipitation during the leafout period (Table

TABLE 1
Linear correlation coefficients for element concentrations of precipitation, throughfall, and forest floor during 32 storms between June and October

		Precipitation						Throughfall					Forest floor		
		Volume	H	Ca	K	S	N	H	Ca	K	S	N	H	Ca	K
Precipitation	H	0.695													
	Ca	-0.434	-0.300												
	K	-0.094	0.168	0.396											
	S	-0.037	0.219	0.602	0.417										
	N	0.090	0.220	0.294	0.146	0.597									
Throughfall	H	0.862"	0.785"	-0.381	-0.207	0.085	0.194								
	Ca	-0.331	-0.134	0.764"	0.415	0.493	0.064	-0.326							
	K	-0.240	-0.168	0.489	0.184	0.200	-0.275	-0.261	0.872"						
	S	-0.094	-0.008	0.457	0.422	0.508	-0.110	-0.200	0.649	0.586					
	N	-0.337	0.113	0.765"	0.441	0.907"	0.629	-0.149	0.572	-0.025	0.176				
Forest floor	H	0.045	0.047	-0.192	-0.444	-0.312	0.301	0.264	-0.018	0.199	-0.283	-0.077			
	Ca	-0.389	-0.184	0.521	0.431	0.306	-0.103	0.510	0.571	0.359	0.527	0.303	-0.404		
	K	-0.354	-0.236	0.493	0.397	0.274	-0.032	-0.379	0.644	0.510	0.449	0.412	0.093	0.351	
	N	-0.596	-0.156	0.716"	0.311	0.616	0.296	-0.444	0.611	-0.040	0.025	0.762"	-0.418	0.598	0.537

"Significantly correlated.

2). On the basis of a comparison of average amounts of K^+ , Ca^{++} , and H^+ in precipitation and throughfall, approximately one-third of the Ca^{++} and K^+ leaching from the forest canopy could be accounted for by H^+ exchange from precipitation. Throughfall $[K^+]$ and $[Ca^{++}]$ on an event-by-event comparison was non-significantly negatively correlated with precipitation $[H^+]$ (Table 1). Our observations thus suggest that H^+ exchange alone does not control the leaching of cations from hardwood foliage despite the low average pH of precipitation at Turkey Lake.

FOREST FLOOR

Water percolating through the forest floor was greatly enriched in K^+ and Ca^{++} (Table 2). Despite H^+ enrichment of water passing through the forest floor relative to throughfall, the average pH of the percolate, although it was still acidic, was higher than that of precipitation. Nitrate-N enrichment of the percolate suggests that nitrification of forest floor materials contributes to acidity. Of the four ions examined in the percolate, only NO_3^-N was significantly correlated with any precipitation or throughfall parameters. The $[NO_3^-N]$ was significantly correlated with throughfall $[NO_3^-N]$ and precipitation $[Ca^{++}]$ (Table 1). The strongly acid forest floor is composed of distinct L and F horizons with a pH in the range of 4.5–5.5. Calcium inputs to the ecosystem had a liming effect that helped counter the acidity of the forest floor and favored nitrification.

In summary, as precipitation passed through the forest canopy (during leafout) and the forest floor horizons, a moderate reduction in acidity and a consid-

TABLE 2
Weighted mean solution concentrations (ppm) during 32 storms between June and October and the ratios of concentration in solution to those in precipitation

Element	Precipitation	Throughfall	Ratio	Forest floor percolate	Ratio
H	0.071	0.052	0.73	0.059	.83
Ca	0.48	1.04	2.17	4.14	8.62
K	0.21	2.68	12.76	3.29	15.67
SO_4	3.7	6.6	1.78	—	—
NO_3-N	0.65	0.60	0.92	1.77	2.72

erable enrichment in bases and strong acid anions were observed. Throughfall $[H^+]$ and $[Ca^{++}]$ could be explained by variation of these elements in precipitation; $[K^+]$, $[SO_4^{--}]$, and $[NO_3^-N]$ could not. On the other hand, for K^+ , Ca^{++} , and H^+ , the composition of the forest floor percolate was more closely associated with the internal cycle of ions in the ecosystem than with external inputs of ions in precipitation. Several factors, other than those examined, significantly influenced ion concentrations in throughfall and forest floor percolate. A greater number of events are being studied to examine further how some of these factors, such as the size, intensity, and frequency of storms, the time of year, and vegetation chemistry, influence the washoff and leaching of ions from living and decaying vegetation and modify precipitation chemistry beneath a maple–birch canopy. — N.W. Foster and J.A. Nicolson, Great Lakes Forest Research Centre, Sault Ste. Marie, Ont.

ENTOMOLOGY

Flight Behavior of Ambrosia Beetles near Free-standing Sticky-screen Traps

Many aspects of ambrosia beetle research and population management employ flight barrier or interception traps for flight monitoring and mass trapping. Such traps are usually made from open-wire mesh. It is assumed that the physical nature of this material has little effect on the air flow pattern near the traps and that the poorly perceptible silhouettes of such traps do not cause the flying beetles to avoid them. Thus it is believed that flying beetles approach the traps and are intercepted by them in a random fashion. This report presents evidence from two experiments to demonstrate that the spatial distribution of beetles caught in these traps is not random. The implications of this finding are pointed out.

The experiments were carried out in a second-growth stand of Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, near Caycuse, B.C., during the spring and summer of 1979. In the first experiment, traps were made of 6 × 6 mm mesh hardware screening 60 cm high and 80 cm wide, treated with sticky material (Tanglefoot®), and stretched between two poles (5 × 5 cm) driven into the forest soil so that the bottom edge of the screen was approximately 60 cm above ground level. Only traps placed west (W), north (N), and south (S) in relation to the center of the plot, at distances of 75 m from each other, intercepted sufficient numbers of beetles for analysis. The screens were divided into 10 × 10 cm blocks and the trapped beetles were recorded by species and sex according to the block in which they landed (Table 1).

In a second experiment, similar sticky-screen traps were used with a 20 × 40 cm piece cut from the lower center so as to fit over a log section of Douglas-fir (Fig. 1). Four log sections, each 75 cm long and 35 cm in diameter, were cut from trees felled in November 1978. The log sections, each fitted with a trap, were placed in two pairs. Within each pair the log sections were placed 5 m apart and the distance between the pairs was at least 10 m. In both experiments, beetle collections were made during May through July on days following flight activity. The sticky screens were divided into 10 × 10 cm blocks and the number of beetles caught in each block was recorded. Depending upon their position, these blocks were assigned to the center, intermediate, or the outside 10-cm-wide zones on the surface of the sticky screen. These zones respectively contained 8, 16, and 24 such blocks in the first experiment and 8, 12, and 20 such blocks in the second experiment. For a valid comparison, the average number of beetles caught per

10 × 10 cm block in three zones was calculated and the results subjected to analysis of variance. An examination of cumulative numbers of trapped *Trypodendron lineatum* (Oliv.) in the 10-cm-wide zones revealed an increased density of beetles toward the center of the screen (Table 1). Results for individual collection days showed the same pattern, as did those for each sex and also for *Gnathotrichus sulcatus* (Lec.). The major flight activity was delayed for about 2 weeks around the logs fitted with sticky traps. Once attack started, the distribution pattern of the beetles caught on the traps was found to be similar to that observed in the first experiment.

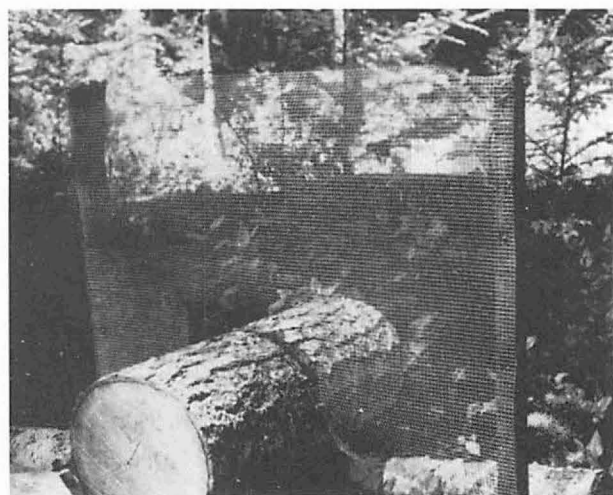


Figure 1. Sticky-screen trap positioned over log section.

TABLE 1
Trypodendron lineatum caught on three concentric zones (center, intermediate, and outside) of sticky-screen traps in the first experiment

Zone*	No. of beetles	Avg no. of beetles per 10 × 10 cm block**	Zone avg	
			Outside zone avg	
Trap W	Center (8)	313	39.1 ^a	2.3
	Intermediate (16)	514	32.1 ^a	1.9
	Outside (24)	412	17.2 ^b	1.0
Trap N	Center (8)	145	18.1 ^a	2.0
	Intermediate (16)	217	13.6 ^a	1.5
	Outside (24)	218	9.1 ^b	1.0
Trap S	Center (8)	50	6.3 ^a	2.3
	Intermediate (16)	97	6.1 ^a	2.2
	Outside (24)	66	2.8 ^b	1.0

* Number of 10 × 10 cm blocks per zone are in parentheses.

** Means for each trap followed by the same letter are not significantly different ($p > 0.05$).

Without knowledge about the beetles that avoided the traps, any explanations of the results become highly speculative. The probability that the beetles flying near the trap perimeter were carried away by air currents seems less than that their optical acuity prompted them to avoid the trap altogether. Hypotheses that the screen affords little impediment to the air flow and that the beetles observe an obstruction only at close range are supported by the increased likelihood of the beetles being caught at the screen's center. Under such circumstances, the beetles encountering the trap near its peripheral parts have a greater probability of escaping by active avoidance. Subsampling of large screens to obtain total beetle catches can be misleading if these catch patterns are not taken into consideration. Practical testing of different screen dimensions for providing optimum efficiency in mass trapping is recommended. — W.W. Nijholt, Pacific Forest Research Centre, Victoria, B.C.

A Comparison of the Pathogenicity of Two Baculoviruses to the Spruce Budworm and the Western Spruce Budworm

Baculoviruses that infect the spruce budworm, *Choristoneura fumiferana* (Clem.), also infect the western spruce budworm, *C. occidentalis* Free. Extensive field trials have been conducted since 1971 to regulate the spruce budworm with viruses (Cunningham, pages 355–386, in Kurstak, ed. Microbial and Viral Pesticides, Marcel Dekker, New York and Basel, 1982) and two small field trials were conducted in 1976 and 1978 with nuclear polyhedrosis virus on the western spruce budworm (Hodgkinson et al., B.C. Min. For. – Can. For. Serv. Joint Rep. No. 10, 1979; Cunningham 1982; Shepherd et al., Can. Entomol. 114:281–282, 1982). It was noted during routine propagation that both a nuclear polyhedrosis virus (NPV) and a granulosis virus (GV) appeared more pathogenic to the western spruce budworm than to the spruce budworm. The NPV was a nondiapausing strain originally obtained from Dr. Jacqueline L. Robertson, USDA Forest Service, Berkeley, Calif. To quantify these observations, the viruses were bioassayed in both spruce budworm and western spruce budworm larvae.

Suspensions of NPV originally isolated from the spruce budworm and GV originally isolated from the western spruce budworm were standardized using a dry-counting technique (Wigley, pages 29–35 in Kalmakoff and Longworth, eds. Microbial Control of Insect Pests, N.Z. Dep. Sci. Ind. Res. Bull. 228, 1980) and seven 10-fold dilutions were prepared from these stock suspensions. Plastic cream cups (18 mL)

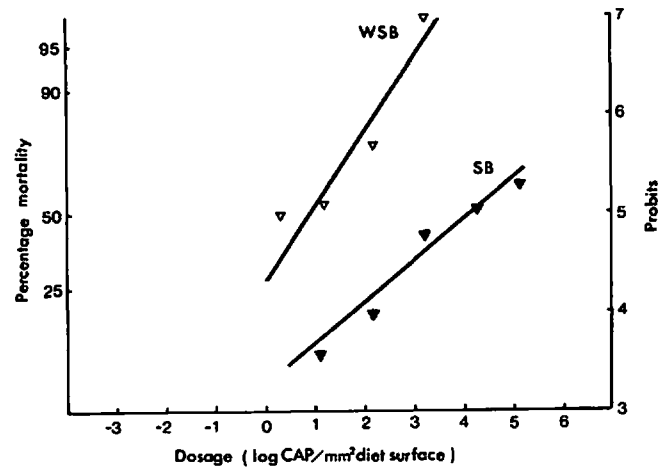


Figure 1. Concentration-mortality regressions for granulosis virus in fifth-instar spruce budworm (SB) and western spruce budworm (WSB).

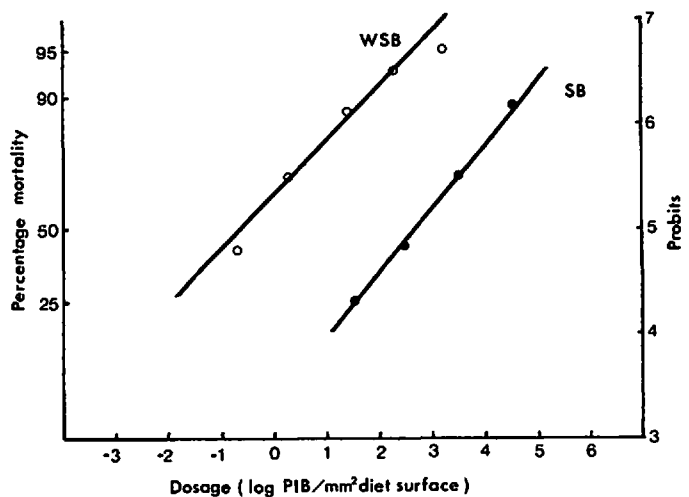


Figure 2. Concentration-mortality regressions for nuclear polyhedrosis virus in fifth-instar spruce budworm (SB) and western spruce budworm (WSB).

were half filled with artificial diet (McMorran, Can. Entomol. 97:58–62, 1965). The surface of the diet (700 mm²) was covered with 0.2 mL of virus suspension with 20 cups per dilution and allowed to air-dry. Five fifth-instar larvae were placed in each cup and 10 cups were set up for each budworm, giving 50 larvae of each species per dilution. A further 100 larvae of each species were reared as controls on untreated diet. All larvae were reared at 26°C, 50% R.H. until pupation or death occurred. The experiment was performed twice. Mortality results were processed using probit and regression analysis and are shown in Figures 1 and 2. Dosages that give 100% or 0% mor-

tality cannot be used in probit analysis. Both viruses were much more lethal to the western spruce budworm than to the spruce budworm. No mortality was recorded in most controls and the highest figure in any control test was 4%. The LC_{50} (concentration that kills 50% of the test insects), with fiducial limits, for GV, expressed as capsules (CAP) per mm^2 of diet, was 8.46 (22.95–2.70) for the western spruce budworm and 14 652 (167 951–3277) for the spruce budworm. The LC_{50} of NPV, expressed as polyhedral inclusion bodies (PIB) per mm^2 of diet, was 0.22 (1.13–0.0045) for the western spruce budworm and 483 (805–278) for the spruce budworm.

On the basis of these results, two recommendations can be made. First, that the use of the western spruce budworm as the host for virus propagation in the laboratory should be investigated because less inoculum would be required and there would be less loss to pupation than with the spruce budworm. Secondly, that the feasibility of regulating the western spruce budworm in the field with GV be examined and that NPV be retested. A point that requires clarification is whether the nondiapausing stock of western spruce budworm used in this experiment has the same susceptibility to virus infection as wild stocks of this species. — **J.C. Cunningham, W.J. Kaupp, and J.R. McPhee, Forest Pest Management Institute, Sault Ste. Marie, Ont.**

Ground Spray Trials with Two Baculoviruses on Western Spruce Budworm

A nuclear polyhedrosis virus (NPV) and a granulosis virus (GV) were found to be highly pathogenic to western spruce budworm, *Choristoneura occidentalis* Free., larvae when tested in the laboratory (Cunning-

ham et al., Can. For. Serv. Res. Notes 3:9–10, 1983) and a trial was undertaken to compare the efficacy of these two viruses on ground-treated, small, naturally infested trees.

Plots of 1–2 m tall regeneration Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, were selected near Cache Creek, south central British Columbia. The average area of six treated and two check plots was 0.0057 ha and there was an average of 22 trees per plot. At the time of spray application on 29 May, 1981, the development of western spruce budworm larvae was as follows: 15% in the second instar, 63% in the third instar, and 22% in the fourth instar.

Both virus preparations consisted of lyophilized, virus-infected spruce budworm, *C. fumiferana* Clem., larvae ground to a fine powder. The NPV and GV contents were compared on the basis of weight of powder, with 0.5 g of each powder suspended in 4.44 L of water to give the highest dosage. A 10-fold dilution was prepared by adding 400 mL of the highest concentration to 3.6 L of water and a 100-fold dilution by adding 40 mL to 3.96 L of water. The actual number of virus inclusion bodies in each suspension was established using a dry-counting technique (Wigley, pages 29–35 in Kalmakoff and Longworth, eds. Microbial Control of Insect Pests, N.Z. Dep. Sci. Ind. Res. Bull. 228, 1980). The highest dosage of NPV contained 3.67×10^5 polyhedral inclusion bodies (PIB) per mL and the highest dosage of GV contained 2.16×10^8 capsules (CAP) per mL. A hand-held pressure sprayer was used to apply 4 L of virus suspension to each of the six treated plots — an application volume estimated to be 700 L/ha. The three dosage rates on a per hectare basis for NPV and GV are given in Table 1, along with the number of larvae that were reared, infected with virus, and harvested to produce these dosages.

At 14 days postspray, collections of foliage containing larvae were obtained from the six treated and

TABLE 1
Nuclear polyhedrosis and granulosis virus treatments on western spruce budworm near Cache Creek, B.C., in 1981

Plot No.	Virus	Dosage (Inclusion bodies per ha)	No. of larvae for a 1-ha dosage	No. of larvae reared	% Parasites emerged	% Dead larvae and pupae	% Emerged adults	% Population reduction due to treatment
1	NPV	2.5×10^{11}	2500	129	12.4	83.7	3.9	91.7*
2	NPV	2.5×10^{10}	250	168	30.9	53.6	15.5	67.2*
3	NPV	2.5×10^9	25	146	23.9	31.5	44.5	5.7
4	GV	1.5×10^{14}	2500	113	9.7	83.2	7.1	86.0*
5	GV	1.5×10^{13}	250	163	20.2	66.3	13.5	71.4*
6	GV	1.5×10^{12}	25	159	15.1	64.2	20.8	55.9*
7	Check	—	—	197	18.8	39.1	42.1	—
8	Check	—	—	206	25.7	21.8	52.4	—

* $P > 99.999$ that survival in treated areas is significantly different from that in check areas.

two check plots and shipped to the Forest Pest Management Institute where the larvae were picked off the foliage, placed individually in 18-mL plastic cream cups containing artificial diet (McMorran, Can. Entomol. 97:58–62, 1965) and reared at 26°C, 50% R.H. until death or adult emergence. The number of larvae reared from each plot is recorded in Table 1 along with the results obtained. Numbers of emerged adult parasites ranged from 9.7 to 30.9% in the eight plots and this figure was scored separately from death caused by virus infection alone and from other causes. The percent population reduction as a result of treatment was estimated from the laboratory-reared larvae using a modified Abbott's formula (Retnakaran and Tomkins, Can. For. Serv. Res. Notes 2:5–6, 1982). The mean of the two check plots was used in these calculations.

At the highest dosage levels, there was little difference in mortality between NPV and GV treatments. However, at the lowest dosage level, the NPV had no detectable effect with more adults emerging than from check 1, but there was 55.9% mortality attributed to the GV treatment of 1.5×10^{12} capsules per ha that represents virus obtained from only 25 infected larvae. These results indicate that GV may be a better candidate than NPV for initiating and maintaining an epizootic in populations of the western spruce budworm. — **J.C. Cunningham, W.J. Kaupp, and J.R. McPhee, Forest Pest Management Institute, Sault Ste. Marie, Ont.; and R.F. Shepherd, Pacific Forest Research Centre, Victoria, B.C.**

PATHOLOGY

Mortality of Juvenile Yellow Birch

Unexplained mortality has been reported frequently in yellow birch, *Betula alleghaniensis* Britton, at all stages of growth from seedlings to mature trees. The disease syndrome described herein does not entirely match any previously described symptom patterns although some parallels have been noted.

During the past 12 years, large tracts of hardwood land in central New Brunswick have been clearcut for pulpwood. Most of these areas have regenerated well and some of them support fairly dense stands of yellow birch. In an attempt to shorten rotation ages and maximize yields, many of these young stands are being spaced to about 2000 stems per ha with brush saws (Higgs, Pulp and Pap. Can. 82(7):26–32, 1981). Growth response to spacing appears to have been satisfactory.

Dead and dying yellow birch saplings were first noticed by the author during the summer of 1980 in regenerating cutovers near Fredericton. The apparent cause of mortality was a rapidly spreading diffuse canker that originated above ground. The cambium and phloem had a brownish discoloration and in some instances the sapwood was stained a grayish color. Externally, there was often a slight flattening of the stem and a purplish discoloration of the bark. Wounds of various kinds were evident on most trees, but cankers were not always associated with them and no obvious point of origin could be found for many of the cankers. Cankers were found on all sides of affected trees.

Crown symptoms usually appeared only after the cankers had almost completely girdled the trees and most often appeared as a chlorosis of all foliage above the canker followed by browning and shrivelling of the leaves. When girdling occurred in the early spring, either the buds failed to open or leaf size was reduced. After the sapling died, numerous fungi fruited on the canker face and the underlying wood deteriorated rapidly.

The early stages of the disease could be found only by peeling the bark of apparently healthy trees growing in locations where the disease was prevalent. In incipient cankers, the area of discolored phloem exceeded the corresponding area of discolored cambium and a slight depression was often evident in the cambium. In one tree, a small elliptical patch of discolored phloem matched a corresponding patch of sunken cambium that had no discoloration. Isolations from the affected cambium and phloem yielded no cultures of microorganisms capable of growing on malt extract or nutrient dextrose agars.

Numerous attempts, using a variety of agar media, to isolate microorganisms from the bark and wood of more advanced cankers have yielded many species of fungi and bacteria as well as sterile cultures. During the summer, a bacterial slime was evident between the cambium and phloem of affected tissues. The common canker fungi of yellow birch, *Nectria galligena* Bres. and *Diaporthe alleghaniensis* R.H. Arn., have seldom been recovered. Healthy trees growing in the Dunbar area were inoculated with representative fungus and bacterial isolates in the late summer of 1980, in the spring and summer of 1981, and in the summer of 1982. No cankers or mortality had resulted from these inoculations as of November 1982 and we have tentatively concluded that neither fungi nor bacteria cause this disease.

To measure disease incidence in young yellow birch stands, a survey was conducted in July 1981 in the Dunbar and Pokiok areas of southwestern New Brunswick. Beginning 50–100 m from roadsides

(where disease incidence appeared to be greatest), 10 circular 40-m² plots at 10-m intervals were examined along transects perpendicular to the roads. Stands that had been thinned were compared with nearby unthinned stands. Results, summarized in Table 1, indicated no difference between the Dunbar and Pokiok areas that are about 40 km apart. In thinned stands, 7% of the yellow birch had died or showed chlorotic symptoms whereas, in nearby unthinned areas, only 1% were affected. Within the thinned areas, there was a

TABLE 1
Incidence of dead and dying yellow birch in 40-m² plots in two areas in southwestern New Brunswick, July 1981

Treatment and location	Number of plots	Number of trees		Dead and dying yellow birch	
		All species	Yellow birch	Number of trees	Number of plots
Thinned, 1978–81					
Dunbar	60	586	262	16	11
Pokiok	60	476	247	20	15
Total	120	1062	509	36	26
Unthinned controls					
Dunbar	60	1460	620	9	8
Pokiok	50	1418	614	5	4
Total	110	2878	1234	14	12
Associated species (thinned stands only)					
Sugar maple					
– beech	70	695	336	14	12
Red maple					
– paper birch	50	367	173	22	14

higher incidence in red maple – paper birch – yellow birch stands (13%) than in sugar maple – beech – yellow birch stands (4%). In the Dunbar area, incidence was positively correlated with red maple ($r = 0.83$) and in the Pokiok area it was positively correlated with paper birch ($r = 0.88$). The disease, therefore, appears to be more prevalent on marginal sites than on the better-quality sites and could be an important factor limiting the density and distribution of yellow birch. — **R.E. Wall, Maritimes Forest Research Centre, Fredericton, N.B.**

ERRATUM

On page 24, column 2, line 7 in vol. 2, no. 4 (October–December 1982) the phrase “potential volume” should read “calculated volume.”

TREE PHYSIOLOGY AND ANATOMY

Origin and Early Development of Roots in Plantlets Derived from Embryo Sections of *Larix laricina* *in vitro*

Most *in vitro* propagation of conifers is achieved by rooting of adventitious shoots that formed in cultures of embryo or young seedling sections. Often this rooting is more difficult to achieve than the induction of the shoots and has focussed attention on the physiology and morphogenesis of roots. In the present study we investigated the histological origin and development of roots on adventitious shoots of *Larix laricina* (Du Roi) K. Koch that were produced from embryo sections cultured *in vitro*.

The rooted shoots were obtained as follows. Seeds were sterilized in 3% hydrogen peroxide for 24 h and germinated in petri dishes on damp sterile filter paper.

The germinating embryos were excised from the seeds as soon as the radicle tip became visible. The radicle was removed and the remainder of the embryo (cotyledons, plumule, and hypocotyl) was transferred to the nutrient medium. A total of 280 derooted embryos was cultured. The nutrient medium used was Campbell and Durzan's (Campbell and Durzan, Can. J. Bot. 53:1652–1657, 1975), with benzylaminopurine (BAP) at 1 mg/L and no auxin. The cultures were kept on 15 mL of nutrient in 18 × 150 mm test tubes closed with “Morton” stainless steel caps, and were maintained at 21°C, under about 50 $\mu\text{Em}^{-2}\text{sec}^{-1}$ Gro-Lux illumination for 16 h daily.

The explants were transferred to cytokinin- and auxin-free medium as soon as buds or shoots appeared. Subsequent transfers to fresh auxin- and cytokinin-free medium were made every 3 weeks. After two to four transfers, the 60 longest (>1 cm) shoots were separated from the explants. Before placing these shoots on fresh auxin- and cytokinin-free medium, the base of the shoot was dipped in an indolebutyric acid containing rooting powder (Hare, Can. J. Forest Res. 4:101–106, 1974). Once the shoots had produced roots about 1 cm long, most were planted in an autoclaved peatmoss – calcined clay (1:1) mix, wetted with the macro-elements of the nutrient medium. Later, the plantlets were planted outdoors in loam soil where they rapidly grew into plants that were indistinguishable from sexually produced ones.

To determine the origin of the first-formed roots, a few of the newly rooted shoots were embedded in wax, sectioned at 10 μ , and stained in rose-bengal (Graham, Phytopathology 54:1433, 1964). Most roots arose from primordia deep inside the callus at the base

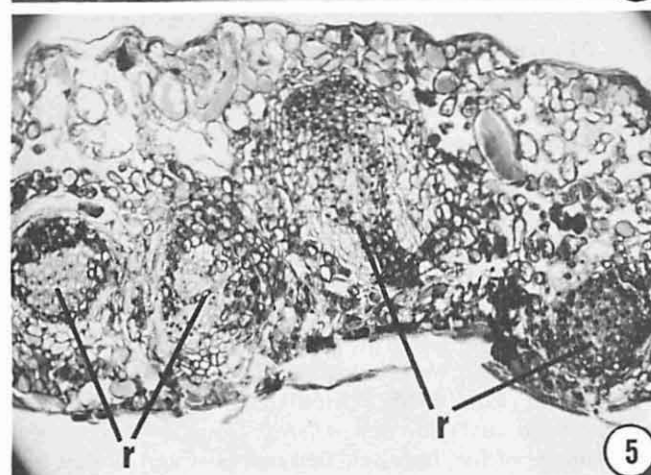
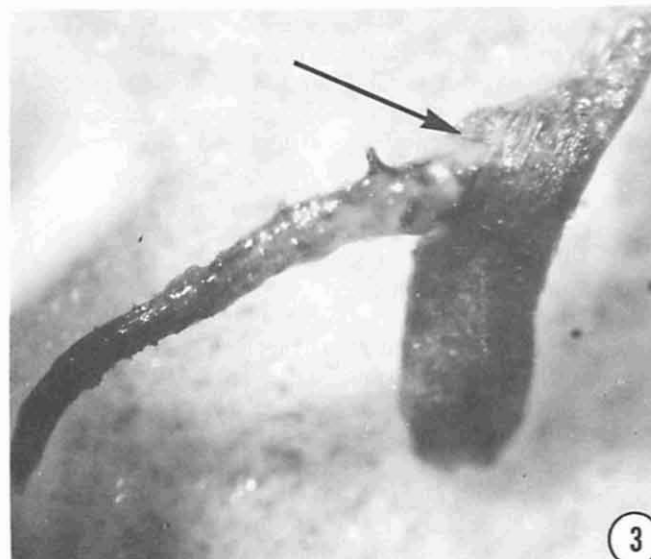
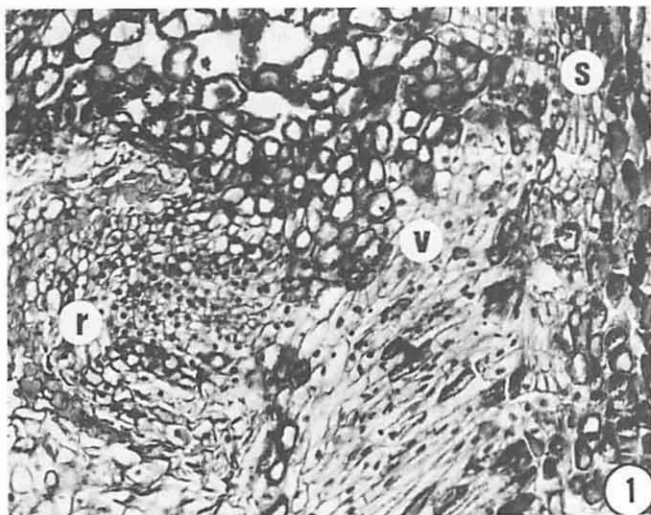


Figure 1. Stem with basal callus with a root primordium (r). Vascular connections (v) have been established between the root primordium and the stem vascular system (s). $\times 100$.

Figure 3. A root protruding through a split (arrow) in the needle epidermis and hypodermis. $\times 12$.

Figure 5. Several root primordia (r) in one needle. $\times 70$.

Figure 2. A shoot with a root arising from the stem between the needles. The most proximal portion of the root has grown upward, the middle section horizontally, and the most distal part down. Note root hairs. $\times 8$.

Figure 4. Two roots protruding from one needle. $\times 15$.

Figure 6. A root primordium (r) at a very early stage of development, originating between the endodermis and xylem. $\times 500$.

of the shoots. Once these primordia started elongating into roots, strands of tracheids developed in the callus between the roots and the vascular system of the stem (Fig. 1). The roots were either positively geotropic immediately or they grew horizontally for a few millimetres and then started to grow down. Other roots appeared between the needles on the elongating stem. These did not arise from callus, but from internal stem tissues. Initially they were geotropically negative, i.e. they grew, like the needles, obliquely upwards. After they had grown upwards a few millimetres, they started to grow horizontally, and finally turned downwards (Fig. 2).

Some roots originated from needle tissues, mostly in the proximal half of the needle. Since root formation on needles is unusual, the origin of these roots will be described in some detail. The first indication of their appearance was a 1- to 2-mm-long longitudinal split in the epidermis and underlying tissues through which a root tip emerged (Fig. 3). These root tips elongated into roots up to 15 mm long. Most needles that formed roots had one root per needle (Fig. 3); a few needles formed two roots (Fig. 4).

Sections of the needles that had formed roots revealed from one to six root primordia per needle (Fig. 5). Since more than two roots were never seen to emerge from a needle, most of the primordia did not grow enough to penetrate through the needle surface. As far as could be determined, most, if not all root primordia originated from transfusion parenchyma cells between the endodermis and the xylem (Fig. 6). As the root primordia grew, they formed their own vascular system that eventually linked up with that of the needle.

The fact that conifer needles *in vitro* will form shoots (David, in Bonga and Durzan, eds. *Tissue Culture in Forestry*, Martinus Nijhoff, The Hague, 1982), and sometimes roots (Bornman in Symposium on Clonal Forestry, Uppsala, Sweden, 1981), shows that young needles have a wide ranging organogenetic capability that is of interest in propagation research. — **J.M. Bonga, and A.H. McInnis, Maritimes Forest Research Centre, Fredericton, N.B.**

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