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ENTOMOLOGY

Foliage Consumption by Late-Instar Birch Casebearer Larvae.—The birch casebearer [*Coleophora fuscedinella Zeller*] was accidentally introduced into Newfoundland in about 1953 and has become the most important pest of white birch [*Betula papyrifera* Marsh.] on the Island (Raske, Newfoundland For. Res. Centre, Info. Rep., N-X-108, 1974). Eggs are laid in July, larvae hatch in August, feed briefly on the host leaves, and overwinter as second instar larvae in a case attached to a twig or to the stem of the tree. Feeding by the third and fourth instar larvae during the following spring and early summer causes browning of the leaves, defoliation, and sometimes branch and tree mortality. Late-instar larvae attach their case to a leaf and feed by mining as far as they can reach. They then move and feed in another area and frequently on another leaf. Thus each larva feeds in several areas during its last two instars.

Previous studies on the life history and habits of this casebearer had indicated that the number of feeding areas by these latter instars appeared to be greater than would be expected from the number of larvae present. Consequently it was dificult to forecast damage based on larval number in the spring. Therefore, a study was initiated to determine the total amount of food consumed by these larvae, the number of feeding sites per larva, and the number of days spent in each instar.

In early May 1973, six white birch trees 0.9–1.2 m high, infested with casebearer, were potted and kept out-of-doors at Pasadena in western Newfoundland. On each tree the first 10 larvae to break winter dormancy were allowed to feed, and the remainder were removed. The number of feeding areas was recorded daily. At pupation, all feeding areas were enlarged by 100X optically and areas of the enlarged images measured with a dot-grid overlay and expressed as square millimeters per feeding area. The results reported here are the feeding history of only 26 larvae, because only these remained isolated from other larvae. The average feeding time was 12 days. Results are shown in Table 1.

TABLE 1
Leaf surface mined and number of feeding areas made by birch casebearer larvae during third and fourth instars on white birch*

Instar	Avg. size per feeding area (mm^2)	No. of feeding areas per larva	Total leaf surface mined per larva (mm^2)	Duration in days
3	3.4 ± 1.1	8.8 ± 4.0	29.2 ± 13.1	12.5 ± 1.6
4	10.6 ± 2.4	14.2 ± 5.0	149.8 ± 43.1	11.4 ± 2.5
Total	—	22.9 ± 4.6	178.9 ± 41.2	23.9 ± 4.2

* $X + S.D.$, No. of larvae = 26.

These results show the amount of foliage consumed by the larvae without considering the effect of population pressure on feeding. Therefore damage estimates that include the estimated number of insects and the above figure for consumption may be high. However, in estimating such defoliation it would also be necessary to consider the number and size of leaves available on the crowns of infested trees.—A. G. Raske, Newfoundland Forest Research Centre, St. John's, Nfld.

A Simple Method for Removing Pupae From Cocoons: *Malacosoma disstria* (Hbn.) and *Orgyia* spp.—Large numbers of forest tent caterpillar [*Malacosoma disstria* (Hbn.)] and tussock moths, *Orgyia leucostigma* (J. E. Smith), *O. definita* (Pack.), and *O. vetusta* (Boisduval) have been reared in recent years primarily for sex pheromone studies. The requirement for virgin adults of a known sex and age made it necessary to remove pupae from cocoons manually. Most workers eventually became allergic to the irritating larval hairs incorporated in the silk of tussock moth cocoons and, to a lesser extent, to the yellow powder found in the inner layer of forest tent caterpillar cocoons. Reactions included minor to severe irritation of exposed areas of skin and congestion of the respiratory tract. The use of a fume hood, surgical masks, and the soaking of cocoons in water helped to some extent, but the allergy problem persisted.

The problem was successfully resolved by using an inexpensive solution containing the household bleach "Javex", (containing 6% available sodium hypochlorite) mixed with an equal volume of tap water. This solution desolves the cocoon silk.

Forest tent caterpillar and tussock moths are reared in ¾ oz (2/ml) plastic creamer cups fitted with unwaxed cardboard lids. Several larvae are reared per cup on artificial food. When larvae are transferred to fresh cups of food the numbers of larvae per cup are reduced to avoid crowding. For the best quality pupae and adults, one last-instar forest tent caterpillar larva or three mid-instar tussock moth larvae are reared per cup. If cups are placed in an upright position cocoons are spun and pupation occurs on the underside of the lids.

Approximately 100 lids with attached cocoons are placed on a square piece of cheesecloth. The ends of the cloth are drawn together and tied with a plastic twister to form a loose bag. The bag of cocoons is immersed in the hypochlorite solution for approximately 30 seconds and then immediately, and thoroughly rinsed in a large bath of running tap water. The bag is then placed in another container of tap water where it is opened and the free-floating cardboard lids and pupae are collected. The pupae are then placed in a screened box and rinsed under running water to remove debris such as cast skins and larval excrement. The mesh size of the screen is just sufficient to retain the smallest pupae. Pupae are then set aside on a porous surface for drying and sexing.

By this method one person can separate approximately 2,000 tussock pupae from their cocoons in one-half hour. Adults from pupae treated in this manner show no adverse effects and subsequent sex pheromone testing revealed no behavioral difference between moths from treated or untreated pupae. No cases of allergy have occurred since pupae have been treated in this manner.—D. G. Grisdale, Insect Pathology Research Institute, Sault Ste-Marie, Ont.

Mortality of Overwintering Birch Casebearer Larvae.—The birch casebearer [*Coleophora fuscedinella* Zeller] is the most important pest of white birch [*Betula papyrifera* Marsh.] in Newfoundland. The larvae overwinter in the second instar in a case attached at branch crotches or at the base of buds on the host trees. This sedentary stage lasts from October to May and, consequently, provides a convenient stage for use for forecasting expected damage in the succeeding year and for planning appropriate control measures. Results of a preliminary study (Cochran, M.Sc. Thesis, McGill University, Que., 1974) indicated a reduction of larval numbers during the winter months in Newfoundland. A more intensive study was initiated to determine the annual and geographic variation of such losses in order that the numbers of overwintering

TABLE 1
Geographical and annual variation in percent losses, deaths and total mortality of overwintering birch casebearer larvae

Area	1971–72				1972–73				1973–74			
	Total Oct. number	% loss	% death	% Total mortality	Total Oct. number	% loss	% death	% Total mortality	Total Oct. number	% loss	% death	% Total mortality
Corner Brook	348	29.3	28.7	58.0	101	39.6	29.7	69.3	71	7.0	45.1	52.1
Wild Cove Point[a]	552	58.0	12.1	70.1	412	42.0	23.8	65.8	—	—	—	—
Cormack	1,100	26.1	31.3	57.4	452	27.2	35.0	62.2	531	59.7	11.7	71.4
Badger	532	53.2	6.9	60.1	699	39.0	20.8	59.8	818	42.1	10.5	52.6
Gambo[b]	—	—	—	—	—	—	—	—	508	47.2	4.9	52.1
Weighted Average		35.7	23.1	58.8		36.1	26.2	62.3		47.0	10.6	57.6

[a] Eliminated in '73-74 because the site became a public campground.
[b] An area newly infested by the birch case bearer.

larvae could be used in forecasting expected damage.

Study areas were located at Corner Brook, Wild Cove Point, and at Cormack in western Newfoundland; at Badger in central Newfoundland; and at Gambo in eastern Newfoundland. Four branch crotches were marked for sampling on each of 10 white birch trees, 3.7–5.5 m high, chosen at random in each study area by the nearest-tree bearing method with 15 m between trees. These sample crotches were chosen, one each per quarter of crown height, and at a position halfway between the stem and the crown perimeter. The number of cases were totalled for the 10 trees in each study area in October, and again in the succeeding May and June. Branch crotches containing more than 100 cases were not selected because experience indicated that such high numbers could not be counted accurately. Cases present in May contained both living and dead larvae, and any decrease from the October number was termed "overwintering losses". The number of cases present at the overwintering site in June, after live larvae had moved to the feeding sites, was termed "overwintering deaths". The sum of the "overwintering losses" and "overwintering deaths" was termed "overwintering mortality", and expressed as a percent of the October totals for each area. Data were transformed to arc sin $\sqrt{}$ percent before regression analyses.

The variation between areas within any given year ranged from 57–70% in 1971-72, from 60–69% in 1972–73, and from 52–71% in 1973–74. The average was about 60% in each year for all areas combined (Table 1). Regressian analysis showed no correlation between number of larvae and percent "overwintering losses" percent "overwintering deaths" or percent "overwintering mortality". Consequently it appears that population density had no influence on mortality of overwintering birch casebearer larvae.

There are no data available to determine the causes of "overwintering losses" and "overwintering deaths". However, bird predation may be a cause of "overwintering losses" and snow crystals, especially when driven by high-velocity winds, may contribute to both "overwintering losses" and "overwintering deaths". The results of the study indicate that estimates of fall population levels have some potential for use in forecasting casebearer damage in the succeeding year, because total overwintering mortality appears to be fairly constant. However, I have not tested the accuracy of such a forecasting system.—A. G. Raske, Newfoundland Forest Research Centre, St. John's, Nfld.

European Pine Shoot Moth on Ponderosa Pine in British Columbia.—*Rhyacionia buoliana* (Schiffermueller), reported from eastern North America in 1914, was first found in the west in 1926, at Victoria. It has since become established on ornamental pines throughout the lower Fraser Valley on the mainland, and was collected in the Okanagan Valley in 1962. Infested plants are the most common means of dispersal and, since 1964, several transfers of *R. buoliana* to Interior British Columbia have occurred. In each instance, the trees were destroyed or thoroughly pruned, sprayed and kept under observation. With the rapidly expanding market for pine and the increased use of plantation stock, quarantine regulations have been updated in an effort to prevent further distribution of *R. buoliana*. The climate of the southern Interior, particularly in the semi-arid areas of native ponderosa pine [*Pine ponderosa* Lawson], seems to permit survival of the pest, which can withstand air temperatures down to −29°C, and lower under snow cover.

Although ponderosa pine is attractive to European pine shoot moth under nursery, plantation and experimental conditions, only a single specimen was collected on naturally growing trees prior to 1974. During May 1974, a heavy infestation of European pine shoot moth was found on mountain pine [*P. mugo* Turra] and Austrian pine [*P. nigra* Arn.] at Castlegar, in the southwest section of the Kootenay region — a substantial eastward extension. Approximately 140 trees in a small landscape area were invloved. These were severely pruned and sprayed. An initial examination of nearby natural ponderosa pine of varying ages yielded six *R. buoliana* larvae. However, a later, more detailed examination of an area extending up to 3.2 km (2 miles) from the infested exotics yielded no trace of the pest. No evidence of old shoot moth damage was found on ponderosa pine even though the insect had apparently been active on the ornamentals since their planting in 1968. Annual minimum temperatures from 1960 to 1974 ranged from −12 to −18°C, and snowfall at the site was meagre. Possibly the vigorous growth of the trees at Castlegar created a natural insect control through their copious resin flow.

One of the six larvae collected from *P. ponderosa*, one was crushed, one preserved and four developed to pupae. The pupae were small and not robust, and although they appeared otherwise healthy, no adults emerged.

During June, the flight period, 35 hormone-attractant traps were set out at Castlegar among ornamental and nearby ponderosa pine stands. Only two moths were recovered: one at the Austrian pines and the other in a ponderosa area about 0.4 km north of where three larvae had been collected previously. This ponderosa pine site will be monitored to determine if a population of the pest develops. Meanwhile, the European pine shoot moth is not considered to be an economic threat to ponderosa pine in British Columbia.—D. Evans, Pacific Forest Research Centre, Victoria, B.C.

Disruption of Mating Behavior of the Spruce Budworm in Air Permeated by Synthetic Sex Attractant.—Lepidopterous sex attractants have two potential uses for the economic entomologist: as bait to lure males to traps, either to prevent mating and so regulate subsequent population levels, or to monitor population changes; and as agents for disrupting mating

behavior. The trapping of sufficient male spruce bedworm to cause a reduction in the number of fertilized females is impractical because the number of traps required to cover large forest areas is prohibitive. Experimentation has therefore concentrated on attempting to disrupt mating behavior, i.e. preventing males from locating females. Three chemicals have potential as disruptants, the synthetic attractant *trans*-11-tetradecenal (tdal), a major component of the spruce budworm pheromone communication system (Weatherstone *et al.*, Can. Ent. 103:1741-1747, 1971), and two related compounds that inhibit male response to the attractant, *trans*-11-tetradecenyl acetate (tdacet) and *trans*-11-tetradecenol (tdol) (Sandares *et al.*, Bi-mon. Res. Notes 28:9-10, 1972). The experiment reported here demonstrates that, in the field, male spruce budworm are prevented from locating a virgin female in air permeated with synthetic attractant, tdal, but that tdacet apparently has no effect.

The experiments were carried out north of Sault Ste. Marie, Ont. in spruce-fir stands supporting moderate populations of spruce budworm. The area was at the southwest edge of a current extensive outbreak. Four 40 m x 40 m plots were laid out, with the sides oriented N-S, E-W. The closest plots were 2.4 km apart, the fartherest 8.8 km apart. Nine 3-M brand XC-26 insect traps were hung in each plot as a 3 x 3 grid, one at each corner, one midway along each side and one in the center. Traps were approximately 2 m above the ground and each was baited with a virgin female budworm housed in a small screen-cage. The strategy was to determine the nightly catch of each female, and then to surround the center female with chemical dispensers to determine the effect on her catch. The dispensers were sheet metal stovepipe raincaps, located at the eight cardinal compass points around the center trap at a distance of 10 m. Thus no matter which way the wind blew, some chemical would be wafted past the center female.

The chemicals were made up in a solid poly-vinyl chloride formulation (Diamond PVC74) (Fitzgerald *et al.*, Environ. Ent. 2:607-610, 1973) containing 3% active chemical by weight. A strip of the plastic, 4 mm in diameter and 10 cm long, was hung in each dispenser, protected from sun and rain. Before the start of the experiment the strips were exposed in a fume hood at 21°C and an air speed of 50 cm/sec for 10 days to ensure that the release rate of the chemical had stabilized.

All traps were baited with a 1-day-old virgin female spruce budworm and left overnight, and the catch was recorded the following morning, Day 1. The traps were changed but were baited with the same female. After the traps had been replaced on Day 1 dispensers containing tdal were placed in Plot 1, tdacet in Plot 2. No chemicals were placed in plots 3 and 4. The following morning, Day 2, catches were again recorded, traps changed and chemicals transferred to plots 3 and 4, leaving plots 1 and 2 untreated. On Day 3 catches were again recorded, traps changed, and dispensers removed, leaving all plots untreated. After a final count on Day 4 the first experiment was terminated. On Day 7 traps were again baited with fresh, 1-day-old females and the experiment was repeated using a different sequence of treatments.

The results are summarized in Table 1. When the center female was surrounded by tdal, catches were drastically reduced. In two instances (Plot 1, Day 2, and Plot 3, Day 3), the catches throughout the plot were reduced. Since catches in the other plots on the same day were not reduced it is concluded that the presence of the tdal confused the males. On only one occasion was there a substantial reduction in catch when the center trap was surrounded by tdacet (Plot 2, Day 2). It is therefore concluded, under these conditions, that tdacet is ineffective in preventing males from locating females. Evidently the tdal treatment was more effective in preventing males from locating females in the first experiment (Day 1-4) than in the second (Day 7-10). This could be due to two factors. First, it is possible that the quantity of tdal released per unit time in experiment 1 was greater than in experiment 2. However, the difference is unlikely to have been very great because laboratory studies indicated that weight loss would be relatively stable. Second, budworm population densities were much higher in the second experiment. At the relatively low densities in experiment 1 disruption of male orientation resulted in no males locating the center females. However, there is no reason to suppose that disruption was less effective at the

TABLE 1

Daily catches of male spruce budworm in traps baited with virgin females. Traps set out in a 3 x 3 grid, showng effects of surrounding center trap with *trans*-11-tetradecenal or *trans*-11-tetradecenyl acetate. See text for details.

		Experiment 1 Day				Experiment 2 Day			
		1	2	3	4	7	8	9	10
Plot 1	untreated traps*	21.5 ± 3.2	**1.0 ± 0.4**	28.9 ± 5.6	33.0 ± 6.9	29.3 ± 11.3	*48.7 ± 13.4*	74.7 ± 7.2	85.8 ± 5.6
	center trap	15	**0**	16	15	5	*61*	68	76
Plot 2	untreated traps*	24.9 ± 4.0	*22.2 ± 6.7*	24.2 ± 5.2	33.8 ± 5.4	72.0 ± 4.9	46.2 ± 6.7	*70.0 ± 6.2*	90.0 ± 10.2
	center trap	25	*7*	16	15	39	35	*31*	56
Plot 3	untreated traps*	17.6 ± 1.8	25.7 ± 4.7	**9.3 ± 1.7**	31.1 ± 3.5	67.2 ± 5.2	**50.9 ± 5.4**	55.2 ± 9.9	101.1 ± 9.3
	center trap	28	8	**0**	16	85	**8**	48	68
Plot 4	untreated traps*	18.4 ± 3.4	8.9 ± 2.0	*18.4 ± 3.3*	17.7 ± 3.1	63.1 ± 6.4	37.6 ± 6.0	**42.5 ± 9.0**	123.0 ± 12.1
	center trap	3	26	*26*	10	62	80	**2**	51

* Average of eight peripheral traps.
Bold type — Center trap surrounded by *trans*-11-tetradecenal.
Italic type — Center trap surround by *trans*-11-tetradecenyl acetate.

higher population density in experiment 2. Males caught in the center trap surrounded by tdal probably blundered in accidentally, but whether such accidental encounters between male and female would result in a successful mating is not known. In any event the results obtained with tdal are encouraging enough to warrant planning on aerial application of microencapsulated tdal in 1975. Tdol will also be evaluated in 1975 in a manner similar to that described here.—C. J. Sanders, Great Lakes Forest Research Centre, Canadian Forestry Service, Sault Ste. Marie, Ont.

Spruce Budworm Egg-Mass Sampling in Newfoundland.—Periodic outbreaks of the spruce budworm [*Choristoneura fumiferana* Clem.] are common and cause extensive damage, including tree mortality in eastern North America (Webb, Blais and Nash, Can. Entomol. 93:360-379, 1961). Previous outbreaks of this insect were of minor importance in Newfoundland, but populations have persisted at outbreak levels since 1971 and the current infestation now covers more than 7 million acres (2.8 million hectares) (nearly 50%) of forested land. Balsam fir on over 300,000 acres (121,000 hectares) have sustained (severe $>$ 75%) defoliation of new foliage for more than 2 years. Continuation of the infestation could lead to some tree mortality in 1975. To aid in a forecast of damage in 1975, we made an extensive egg-mass survey in September 1974.

In the absence of basic data for Newfoundland, a two-category sequential sampling system, developed for New Brunswick (Morris, Can. J. Zool. 32:302-313, 1954; Webb, Cameron and Macdonald, unpublished 1955-8, 1956) was used for our survey. This system was based in part on knowledge that spruce budworm egg-mass populations have a negative binomial distribution with parameter $k=2.394$.

In the Newfoundland survey, time and manpower were available to sample 745 locations distributed as follows: 263 in stands containing trees with more than 75% current defoliation, 151 in stands with 25% to 75% defoliation, 239 in stands with up to 25% defoliation, and 92 in stands with no evidence of defoliation. Three branches were collected per location but only an average of 1.9 branches had to be counted to class a location as expected light ($<$ 25%) or severe ($>$ 75%) defoliation in 1975.

The survey results indicated that 4.5 million acres (1.8 million hectares) (27% of total forested land) may contain severely defoliated trees in 1975. The reliability of this forecast depends on the validity of using New Brunswick parameters in Newfoundland. One parameter, the relation of future defoliation to present population levels, can only be tested after a defoliation survey in 1975. The remainder of this note examines statistical parameters of the 1974 survey.

The number of sample locations in the 1974 survey was selected intuitively. To test sample size, seven areas, each containing forests in one defoliation class and averaging 500 sq mi (1300 km^2) in area, were selected. These contained an average of 63 sample trees from 33 sample locations. Ten sample locations were selected at random from each area and an analyses of variance of egg-mass counts (transformed to $\log_{10}(X+1)$) showede there were significant ($P \leq 0.05$) differences between locations in only two of the 10 areas (Table 1); the source of the difference is unknown. Based on the maximum variance obtained, 59 trees, in an area, would have been an adequate sample for a practical estimate of population level with 90% confidence in an interval of 0.739 at 95% probability. Based on a pooled estimate of the variance, $s^2 = 0.545591$, 30 trees would have been adequate. Although not equivalent, these calculations suggest that a suitable sample size in a sequential sampling system would be 15 locations per 500 sq mi (1300 km^2) of forested area, thereby indicating that sampling effort could be reduced by about 55% in future surveys.

The data (Table 1) also indicate that the variability of egg-mass numbers was not related to the amount of current defoliation, the average number of egg masses, or the proportion of balsam fir in the stand. Accordingly, analyses were made without stratification for species or defoliation.

Table 2 shows that the k-values are relatively constant at about 16 for population levels less than 400 masses/100 ft^2 (9.3 m^2), but that population levels decrease to 4.4 at 461 masses/100 ft^2 and to 2.8 at 595 masses/100 ft^2. These values suggest that spruce budworm egg masses tend to be dispersed at random at low to high population levels and that aggregation intensifies at the highest population levels. These results contradict those reported by Morris (*op. cit.*). Because of the low number of branches counted per sample location, it would be prudent to recalculate k from more intensive sampling in Newfoundland before further comparisons are made. Bliss (Proc. Xth. Intern. Congr. Entomol. 2:1015-1031, 1956) recommends that k be estimated from counts of more than two, preferably 10, branches per sample location.

TABLE 1
Mean and variance of egg masses/100 ft^2 (9.2 m^2) foliage in relation to current defoliation and proportion of balsam fir in the sample

Area	1974 defoliation	Mean (Log (X + 1))	Variance	No. trees	Proportion balsam fir
1	75%	2.0631	1.0512*	14	100%
2	75%	2.2736	0.2239	16	100%
3	25 — 75%	1.0952	1.1557*	20	50%
4	25 — 75%	2.4156	0.1354	16	81%
5	25%	2.4034	0.1039	22	95%
6	25%	1.6143	0.6433	23	91%
7	25%	1.7883	0.5982	19	84%

* Significant (P 0.05) differences within areas.

TABLE 2
Samples sizes, average and parameter k for successive classes of spruce budworm egg-mass population levels expressed as masses/100 ft^2 (9.2 m^2) of foliage in Newfoundland

Class	0.1-50[a]	51-100	101-200	201-300	301-400	401-500	>500
Average	30.2	73.8	144.3	244.8	350.6	460.9	595.0
Locations	48	25	25	27	20	9	4
Branches	100	58	65	68	45	20	9
Class k	15.65	14.82	16.53	19.53	12.64	4.40	2.83

[a] Locations with $\Sigma_i X_{i,} = 0$; $i = 1$, 2 and 3 were omitted.

The Island-wide unweighted and weighted k-values (Bliss op. cit.) were 4.161 and 15.250, respectively. Although these values differ from the one used for the New Brunswick sequential sampling table, they do not negate the results of the 1974 Newfoundland survey because k is a relative (Waters, J. Econ. Entomol. 52:1180-84, 1959) not an absolute measure of aggression in insect populations. Thus, the provisional $k = 4.1616$ obtained in Newfoundland, can be considered similar to the one used in the New Brunswick sequential tables. The weighted $k = 15.250$ was obviously influenced by population levels below 401 masses/100 ft^2 where individual k-values varied between 12.64 and 19.53 (Table 2). The low k value used to construct the sequential sample table was related to high population levels (Table 1) and consequent severe defoliation (Morris, op. cit.); the two factors of foremost concern in the Newfoundland 1974 egg-mass survey. Thus the results of the survey, which are based on New Brunswick standards, are acceptable as first estimates. However, refinements for Newfoundland conditions can be made with data from continuing annual egg-mass counts and defoliation assessments.—D. G. Bryant and R. C. Clark, Newfoundland Forest Research Centre, St. John, Nfld.

PATHOLOGY

Identification of *Ceratocystis ulmi*, Based on Production of Coremia in Vials.—Culturing large numbers of samples suspected of containing *Ceratocystis ulmi* (Buisman) C. Moreau, the causal fungus of Dutch elm disease, is time-consuming. The surface sterilized twig sections are pealed, then small wood chips are aseptically removed and transferred to petri dishes containing potato dextrose agar. Plant protection instructions (unpublished, late 1940's) call for the incubation of 15 chips from each of six twigs in a sample. About 10 years ago, at our laboratory, we reduced the number of chips to five per twig but even with this reduction it takes 20 to 30 minutes to culture each sample, depending on how easily the bark can be removed. Also, some training in aseptic techniques is required because this work is usually performed by summer assistants and they change almost yearly.

C. ulmi produces coremia in moist chambers on infected pieces of wood (R. Campana, University of Maine, Orono, Maine, personal communications) and we found this to be true also, on infected, debarked pieces of twigs. After some experimentation the following method was chosen and tested for reliability before being adopted as a routine laboratory technique for determining *C. ulmi* from samples suspected of Dutch elm disease.

A twig selected from a sample is surface sterilized by dipping in 70% alcohol which is removed by flaming. After the aseptic removal of the bark, the end 2–3 cm section of the twig is cut off to remove the portion of the twig into which alcohol may have penetrated. The cut is made at about 45° to give a larger cut surface. The next cut is made about 3–4 cm from the first and the section is put into a screw-cap vial (about 20 ml capacity, 20 x 50 mm) about half-filled with sterile distilled water. The closed vial is shaken vigorously for about 30 seconds, then the water is aseptically decanted, the cap loosely replaced, and the "moisture chamber" incubated at room temperature either in light or darkness. The average time for sample preparation is 3 to 5 minutes.

After 4 to 5 days incubation, without removing the twig from the vial, it is examined under a dissecting microscope for the presence of coremia of *C. ulmi*, and is checked periodically. If, after 2 weeks incubation there are no coremia found, the sample which has been kept in "cold storage" during this period, is cultured by the conventional method. Sometimes I find that even when coremia are absent there is frequently enough mycelia growing on the surface of the twig to ascertain the presence and identity of *C. ulmi* from its *Cephalosporium* stage. However, by plating the "negative" vial samples, the "misses" can be picked up, true negative samples confirmed, and other fungi causing symptoms on the tree, can be identified.

The Table shows the results obtained with two groups of samples received for identification in two different years.

Only 29 of the 243 samples required culturing on artificial medium, and in both years over 95% of the samples positive for *C. ulmi* were identified by the vital technique. In the first year, over 60% of the positive samples produced coremia within the first week of incubation.

TABLE 1
Results obtained with the vial technique for identifying Dutch elm disease

	Number of samples	
	1973	1974
Total samples tested by vial method	114	129
Coremia produced within 1 week	67	not recorded
Coremia produced within 2 weeks	106	108
C. ulmi identified by plating	5	3
Sterile, bacteria, other fungi	3	18
Efficiency of coremia determination	95.5%	97.3%

The advantages of the described technique include: (1) reduction in culturing time, resulting in up to ten-fold increase in the number of samples that can be handled by persons with minimum training in culture techniques; (2) reduction in media preparation, saving both time and culture medium; (3) reduction in the number of contaminated samples because no media other than the natural wood section is involved; (4) reduction in examination time, due to the reduction in the need for microscopic mount preparation; and (5) maintenance of the reliability of the chip plating technique.

The vial technique of *C. ulmi* determination could be carried one step further by supplying persons collecting suspect-samples with a small bottle of alcohol and tightly closed vials containing sterile water, and requesting that a vial preparation of the twig be made in the field and included with the sample. Assuming no more than 4 days in transit, the vials would reach the laboratory at the time when coremia development usually starts, thereby shortening the necessary in-laboratory incubation period and also shortening the interval between collection and identification. The latter has practical implications when control measures against *C. ulmi* are anticipated.—Laszlo P. Magasi, Maritimes Forest Research Centre, Fredericton, N.B.

Cellulolytic Enzymes Enhance Wound Response in Lodgepole Pine.—The bark beetle, *Dendroctonus ponderosae* Hopk., carries spores of blue stain fungi into the stems of lodgepole pine [*Pinus contorta* Dougl. var. *latifolia* Englm.] where it colonizes the living cells of the inner bark and sapwood. The tree responds by synthesizing resins and aromatic compounds that impregnate the tissues around the wound (Shrimpton, Can. J. Bot. 57: 527, 1973). Visible symptoms and changes at the cellular level were described by Reid *et al.* (Can. J. Bot. 45:1115, 1967). Although triggered by a physical wound, the response is enhanced by the blue stain fungi. This note presents evidence to suggest that a similar response may be triggered by enzymes of the type secreted by sapwood invading fungi.

Six lodgepole pin logs, about 25x180 cm, were cut on the Kananaskis Forest Experiment Station, Alberta, and their cut surfaces sealed immediately with wax. Loose bark scales were brushed off but living tissues were not exposed. Commercial enzyme preparations, to be injected into the logs, were pectinase – 40 mg/ml dissolved in 1/10 N phosphate buffer, pH 40; – cellulase 40 mg/ml dissolved in 1/10 N phosphate buffer, pH 4.0; hemicellulase – 40 mg/ml dissolved in 1/10 N phosphate buffer, pH 4.5. Enzyme solutions were sterilized by filtration through a 0.3 mμ millipore filter. Prior to inoculation the bark was rinsed with methanol. Holes 1.5 cm deep were punched about 30 cm from the basal end of the log with a sterilized nail. A sterilized, 1 ml syringe fitted with a needle was filled, positioned in the hole, and sealed in place with wax before the contents were injected. Each log received five treatments spaced equally around the circumference: one each of the three enzymes, one of sterilized water and one control, the nail hole alone.

Wounds were examined after 18 days. A chip of wood, removed asceptically from sapwood adjacent to the hole, was placed on water agar to check for microorganisms. The appearance of wood and phloem affected by the wound and the extent of resin soaking was noted. Criteria of Reid *et al.* (*op. cit.*) were used. Treatments in which microorganisms were found were not included in results.

The changes around each wound were of the same shape and general appearance as those observed in wounded, living trees (Reid *et al., op. cit.*). A white streak extended vertically

for the length of the log, and resin had accumulated in all holes. Each hole was surrounded by an elliptical zone of resin-soaked tissue that extended through the phloem to a few annual rings into the sapwood. The size of the resin-soaked zone, measured in the sapwood, varied within each treatment from log to log, but was consistently larger for enzyme treatments than for controls. Sizes are given in Table 1. There was no difference in appearance and little in the size of the resin-soaked zone for the pectinase, cellulase or hemicellulase treatments.

TABLE 1
Sizes of the resin soaked area from a physical wound and from cellulolytic enzymes free of microorganisms

Treatment	Hole only	Water	Pectinase	Cellulase	Hemicellulase
Number	3	2	2	4	3
Sizes of resin-soaked area	9x6 5x4 4x5	8x5 5x5	79x5 31x5	41x7 48x6 36x7 52x9	78x6 70x5 35x8
Average Size*	6x5	7x5	55x5	44x7	44x6

* Length x width in mm.

The enzymes used in this investigation have at least two effects upon the sapwood. First, the walls of the ray cells are dissolved and, because protoplasts of these cellss are interconnected, this damage will be sensed by neighboring cells. Second, the bordered pits are damaged by the enzymes (Meyer, Wood Sci. 6:220, 1974), which alters the local moisture regime. Previous work on the response of lodgepole pine to wounding has shown that the resin-soaked zone arising from wounds involving blue stain fungi is much larger than that from a mechanically inflicted wound from which fungi were excluded. Also, a zone of dry sapwood usually develops behind open wounds and resin soaking occurs within this zone (Reid *et al., op. cit.*). These results suggest that the development of resin soaking in lodgepole pine in response to a wound is promoted either by the action of fungi as they invade ray cells or by a changing moisture regime. Because moisture movement is minimal in a log, disruption of ray cells seems more probable.—D. M. Shirmpton, Pacific Forest Research Centre, Victoria, B.C.

SILVICULTURE

Influence of Bacterial Inoculations on Growth of Containerized Douglas-fir Seedlings.—Early work with bacterial inoculations of *Azotobacter* sp. or *Bacillus megaterium* var. *phosphaticum* to stimulate growth of agricultural and vegetable crops in field and pot experiments has had limited success (Brown *et al.*, Plant Soil, 20:194-214, 1964). With arboreal plants, there is only one report of a stimulatory influence, *i.e.* when oak and ash grown in sand culture were inoculated with *Azotobacter chroococcum* (Akhromeiko and Shestakova, International Conference on the Peaceful Uses of Atomic Energy, 2nd Geneva. 1958. pp 193-199).

In a nutritional trial with container-grown Douglas-fir in 1972, we superimposed a mixed bacterial inoculation upon the nutrient treatments. A coastal Douglas-fir seedlot (B.C. Forest Service #315), from 460 m elevation that had been stratified prior to seeding, was sown in "Styroblock 2" containers (Matthews, Can. For. Serv. Info. Rep. BC-X-58, 1971) on 25 April 1972. The soil was a 3:1 (v/v) unsterilized peat vermiculite mix adjusted to pH 4.8 with 3 kg dolomite lime per m^3. Nutrient solutions of 28:14:14 (Plant Products Ltd., Port Credit, Ont.), at a concentration of 156 mg/liter, were applied to field capacity twice weekly throughout the experimental period, with extra watering as required. Half the plants were leached with additional twice-weekly watering to field capacity and then rewatering until water flowed freely through the soil volume.

The bacterial inoculum was prepared by collected 10 5-cm soil cores from a 28-year-old Douglas-fir stand (elevation 330 m) and thoroughly mixing them before removing a 5 g subsample and placing it in 1 liter of medium. The medium had the following composition: K_2HPO_4, glucose, avicel (TG104), and soluble starch at 0.1% concentration; $MgSO_4 \cdot 7H_2O$, 0.22%; $CaCl_2$ and NaCl, 0.01%; $FeCl_3$, 0.001% and actidione at 60 ppm. The actidione was incorporated to prevent fungal development in the inoculum. This solution was incubated on an orbital shaker (200 rpm) for 4 days at 13-15°C, and 10 ml was removed and inoculated to fresh medium. The subsampling and re-inoculation was repeated three times before 2 ml of the mixture was inoculated to each seedling cavity on 18 May 1972. Uninoculated blocks were similarly treated with 2 ml of the heat sterilized mixture. The plants were kept in a shadehouse until sampled in March 1973. Measurements of a 20-plant sample from each quarter block included stem diameter (at cotyledons); height (from cotyledons to tip +2.5 cm); total dry weight (120 hr at 70°C), and concentration of nitrogen, phosphorous, potassium, iron, copper, zinc, boron, manganese, calcium and magnesium in the whole plant.

Table 1 demonstrates the difference in plant size between control and leached treatments. As expected, the leached treatment, which provided a lower nutrient supply, produced smaller plants. This difference in nutrient supply is, however, not reflected in the plant analysis, where the concentration of all elements monitored is greater in leached than in control treatments. This would suggest that an element, not measured but very mobile in solution, may be the limiting factor (*i.e.* sulfur). Another possibility is that the higher copper content in the leached plants have lead to a copper-induced iron deficiency (Dykeman and de Sousa, Can. J. Bot. 44: 971-978, 1966), thus reducing growth.

TABLE 1
Modifying influences of bacterial inoculation of seedling growth and nutrient content

Factors	Control		Leached	
	Uninoculated	Inoculated[1]	Uninoculated	Inoculated[1]
Stem Diameter (mm)	2.5	104	1.9	110*
Height (cm)	12.6	102	7.3	122*
Whole Plant Dry Weight (g)	1.349	111	0.721	127
Nutrient Concentrations				
Nitrogen %	1.10	116*	1.37	70*
Potassium %	.44	98	.48	116*
Magnesium %	.18	95	.24	110*
Copper (ppm)	17.3	110*	35.2	109*
Iron (ppm)	61.9	110	67.5	132*
Zinc (ppm)	26.2	69*	44.5	69*
Manganese (ppm)	83.8	112	125	65*
Boron (ppm)	23.8	106	29.0	83*
Calcium (%)	.28	104	.40	69*
Phosphorous (%)	.35	95	.34	76*

* Significantly different from the uninoculated ($P = 0.05$)
[1] Expressed as a % of uninoculated plants.

Visual examination of plants, 5 weeks after bacterial inoculation, indicated that inoculated plants were slightly smaller and weakly chlorotic. This was gradually overcome and, in 13 weeks, the inoculated plants were visibly larger. Table 1 indicates the positive bacterial inoculation influence on growth. There is a significant increase in stem diameter of plants grown under both nutritional regimes and a significant increase in height with the leached treatment. In addition, there were increases of 11 and 27% in the dry weight of plants of the two treatments, respectively, as a result of bacterial inoculation. This difference in growth is not mycor-

rhizal in origin as all treatments exhibited extensive mycorrhizae of *Thelephora terrestris*.

One explanation for the observed stimulatory effect is that the inoculation with bacteria isolated from a Douglas-fir litter layer lead to the establishment of a more typical rhizosphere than developed in the absence of inoculation (Williamson and Wyn Jones, Soil Biol. Chem. 5: 569-575, 1973). Such organisms could bring about the improved growth either through increased nutrient supply (Miller and Chau, Plant Soil, 32: 146-160, 1970) or through production of various growth hormones (*Brown et al. loc. cit.*).

A significant influence of bacterial inoculation on the uptake of both macro and micro nutrients is shown in Table 1. Inoculation of leached plants lead to significant increases in K, Mg, Fe and Cu. Potassium was below the concentration ranges normally reported for Douglas-fir nursery stock (Kruger, U.S. Forest Service Research Paper PNW-45, 1967; van den Driessche, Research Note 47, B.C. Forest Service) and could have accounted for some of the increased growth. Conversely, in the control plants, only nitrogen and copper were significantly increased. Notrogen was also below the level considered adequate for optimal growth.

While these observations are of a preliminary nature, they do indicate the potential importance of the root associated bacterial flora in the growth and nutrition of Douglas-fir seedlings and the need for continuing efforts to understand and control this association.—A. K. Parker and J. A. Dangerfield, Pacific Forest Research Centre, Victoria, B.C.

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