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PATHOLOGY

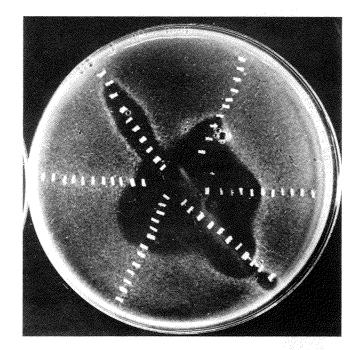
Distribution of MBC-Phosphate Injected into Elms for Protection against Dutch Elm Disease. — Water-soluble salts of methyl-2-benzimidazole-carbamate (MBC), particularly MBC-HC1 and MBC-P, have been used experimentally by several investigators as possible agents for control of Dutch elm disease (DED). In Canada, MBC-P (Lignasan BLP, — registered trade name of E.I. du Pont de Nemours & Co. Inc.), is registered for use by licensed applicators employing a closed system for injection through roots, root-flares, or trunks. In practice, root-flate injection has evolved the most acceptable method and was therefore used in Fredericton in 1974 to introduce MBC-P into the vascular systems of two healthy high-value elms, in an attempt to protect them against DED.

Early in the year following injection, the trees became severely infected with *Ceratocystis ulmi* (Buism.) C. Moreau, the casual fungus of DED. One was felled and burned, but the second tree, which expressed severe symptoms of the disease shortly after the first was destroyed, was felled and portions of the main stem and limbs were collected for detailed examination. The analysis revealed serious shortcomings in the distribution of MBC-P within the vascular system. Consequently, 10 additional trees were injected and analyzed during 1976 for further investigation of the problem of distribution. The results form the basis of this report.

The elms, *Ulmus americana* L., 10 to 20 cm dbh, were root-flareinjected during June and July with a 0.1% concentration (1,000 ppm) of MBC-P (Table 1). At least 1 L/2.5 cm dbh of the fungitoxicant was injected into the outermost two to four annual growth rings of six trees, by using a modified version of a pressure injector head described by Jones and Gregory (USDA Forest Serv. Res. Pap. NE-233, 1971). In the remaining four trees, the chemical was injected into the outermost 10 to 20 annual growth rings by a method similar to that described by Kondo and Huntley (Can. For. Serv. Inf. Rep. O-X-235, 1975). Injection into the xylem of all trees was done at a pressure of 1.78 kg/cm⁻² (10 psi), and injection points were spaced 10 to 15 cm apart.

The distribution of MBC-P within the elms was determined between 21 July and 15 Nov. After felling, disks about 0.5 cm thick were cut from each tree at several locations in the trunk and from all main branches. From these disks, tangential segments, about 1 mm in radial depth and 3 mm wide, were excised, beginning at the cambium and extending 1-3 cm (1-3 annual rings) into the xylem. Segments were excised from several positions around the circumference of each disk and arranged in a series in 14 cm petri dishes on the surface of 1.5% potato dextrose agar (PDA) freshly seeded with spores of C. ulmi (Fig. 1). Similar bioassays were also performed on wedges cut from the outer annual rings of the trunk. Split sections of twigs, 2-5 cm long, were cut from the current and preceding years' growth of main branches and similarly assayed. The cultures were allowed to grow about 48 h at 20 to 22°C. Any clear zones, indicating growth inhibition of C. ulmi, that occurred around the excised wood in the seeded-plate cultures were noted. After an additional 2 to 3 weeks' growth, all cultures were examined for the production of coremia, a fruiting stage of C. ulmi, on the disks and twigs. Growth of coremia was considered even more indicative than mycelial growth of an absence of the fungitoxicant, since coremia are reported to be extremely sensitive to MBC compounds (Smalley et al., Phytopathology 63:1239-1252, 1973). The distribution of MBC-P determined from the bioassays was plotted on cross-sectional diagrams of the trees (Fig. 2).

The two methods of injection ensured uptake of MBC-P in the current annual growth rings, but the apparent distribution of the fungitoxicant was



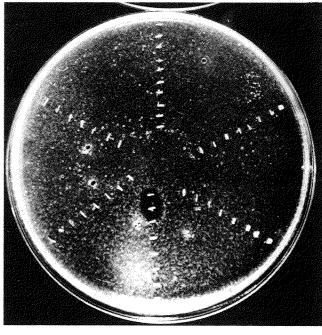


Figure 1. Three-day-old petri dish cultures of *Ceratocystis ulmi* containing tangential sections of elm wood excised from disks cut 0.3 and 3 m above the point of injection 12 weeks earlier, by the Jones and Gregory method. Rows show contiguous segments from the outer 1-3 growth rings taken from various points around the circumference of the tree.

far from complete. Bioassay of elms injected by the method of Jones and Gregorry revealed the presence of MBC-P in parts of annual growth rings from 1973 to 1976 at most levels in the main stem, but it was rarely found in complete rings of the current growth in disks excised from immediately below the crown or from main branches. In elms injected later in the summer by a method similar to that of Kondo and Huntley, the fungitoxicant was present in a greater number of growth rings and the circumferential distribution appeared to be a little more extensive than that shown by the other injection method. However, distribution within the current annual growth rings was little better than that previously described.



Figure 2. Diagrammatic representation of a tree injected in 1974 by the method of Kondo and Huntley with MBC-P and showing the distribution of fungistatic activity (dark areas) in the xylem. Disks about one-fifth actual size

TABLE I

Data on trees injected during 1976 with 1,000 ppm of MBC-P and felled and analyzed several weeks later

		Volume of			Date		
Tree no.	DBH (cm)	solution (L)	Injection method ¹	Hours of injection	Injected	Fellod	
50	15	6.0	J and G	24	28 May	15 Sept	
64	10	5.5	J and G	48	16 June	6 Sept	
65	10	5.0	J and G	48	18 June	6 Sept	
76	11	10.0	J and G	72	21 June	? Sept	
214	14	5.0	J and G	48	7 July	15 Nov	
238	1.1	24.0	K and H	24	26 July	7 Sept	
239	9	17.5	K and H	72	26 July	15 Nov	
240	13	24.0	K and H	48	28 July	13 Sept	
241	11	24.0	K and H	48	28 July	30 Aug	
2254	14	6.5	J and G	24	15 June	21 July	

¹ Methods of Jones and Gregory or Kondo and Huntley

The most significant finding, common to both methods of injection, was that the bioassays indicated that MBC-P was generally absent from the wood formed after injection in 1976 (Figs. 1, 2). Also, coremia were produced readily on most wood formed after injection. Therefore, it appeared that MBC-P did not move radially outward into xylem formed after injection. Consequently, after a relatively short period of tree growth, possibly 1 to 3 weeks, little or none of the chemical was present in the outermost sapwood, the area of beetle feeding and fungus activity.

Of the 10 elms examined, one tree (no. 50) expressed symptoms of DED 7 weeks after injection. Isolation and bioassay results showed the fungus to be present in the outermost sapwood immediately adjacent and external to the fungitoxicant that had been injected into the sapwood at the time of treatment. On the basis of these results, it is apparent that very little protection against DED can be expected from a single root-flare injection with MCB-P. The lack of radial movement of the fungitoxicant into xylem formed after injection markedly reduces the potential of this compound as an effective and practical fungitoxic agent for control of DED. — M.A. Stillwell (deceased), Maritimes Forest Research Centre, Fredericton, N.B.

Evaluation of Surface Sterilants for Isolation of the Fungus Geniculodendron pyriforme from Sitka Spruce Seeds. — Geniculodendron pyriforme salt is an internally borne seed fungus that spreads among seeds and kills them during cold stratification, or in nursery seedbeds during cool, moist weather (Salt, Trans. Br. Mycol. Soc. 63:339-351, 1974). The fungus had been isolated from Picea and Pinus seeds that failed to germinate in Ontario forest nurseries (Epners, Can. J. Bot. 42:1589-1604, 1964) and from Sitka spruce, Picea sitchensis (Bong.) Carr., seeds imported into Britain from western North America (Salt, 1974). In 1976, the fungus was found in stored Sitka spruce seeds in British Columbia (Sutherland, Phytopathology 67, in press). Initially, we tried isolating the fungus by the method described by Epners (1964), which consists in removing the seed coat from suspected seeds and plating the contents on nutrient-agar medium; this procedure, however, was too time-consuming for large numbers of seeds. We then used Salt's (1974) technique in which intact seeds are surface-sterilized with 1% sodium hypochlorite (NaOCl), but bacterial and fungal contaminants frequently prevented accurate assessment of G. pyriforme incidence. Preliminary trials with concentrated (30%) hydrogen peroxide (H₂O₂), which has been used to surface-sterilize Sitka spruce seeds (Trappe, J. For. 59:828-829, 1961), showed promise for our work; thus, the present experiment was made to determine the best combination of concentration and treatment time of either H2O2 or NaOCl for surface sterilization of Sitka spruce seeds and subsequent isolation of G. pyriforme

Using Salt's (1974) procedure, we selected three Sitka spruce seedlots that had high, intermediate, and low incidence levels of G. pyriforme, i.e. with 26, 7, and 1.6% of the seeds infected with the pathogen. Unstratified seeds were surface-sterilized for 60, 30, and 5 min with three concentrations each of H_2O_2 (30, 6, and 1.2%) and NaOCl (5, 1, and 0.2%). Surface-sterilized seeds were washed with sterile, distilled water, plated on 2% water agar, and incubated at 15°C. Each treatment contained 250 seeds (25/petri dish). The incidence of G. pyriforme, other filamentous fungi, bacteria and yeasts, and seed germination (radicle twice as long as the seed coat) was determined, with a stereomicroscope, every 3 days for 15 days following plating. The cumulative data were transformed, when necessary, to correct for heterogeneity of variance and subjected to analysis of variance; the means were compared by the Student-Newman-Keuls test (Steel and Torrie, Principles and procedures of statistics, McGraw-Hill New York, 1960).

Overall, seeds surface-sterilized with H₂O₂ yielded significantly (P=.05) more *G. pyriforme* (12.5 vs. 10.2%) and less bacterial and yeast contaminants (0.5 vs. 2.1%) than NaOCl-treated seeds. There were fewer filamentous fungus contaminants on NaOCl than on H₂O₂-treated seeds (28 vs. 63%). Table 1 gives the results of the various treatments, concentrations, and treatment times. To conserve space, only the average effects of the three seedlots (with high, intermediate, and low *G. pyriforme* incidences) are shown; isolation percentages for the fungus were almost identical with the preexperiment determined levels. Also omitted are the seed germination data. Germination was significantly (P=.05) less for H₂O₂ than for NaOCl-treated seeds (24 vs. 27%), but differences within treatments were not significant. Percentage seeds yielding *G. pyriforme* tended to increase as H₂O₂, but not NaOCl, concentration and exposure

TABLE 1

Effects of surface sterilants, their concentrations, and duration of treatment on isolation of
Geniculodendron pyriforme and incidence of contaminants on Sirka spruce seeds

Parameters		Surface sterilants and their concentrations ^a									
measured and	H ₂ O ₂	H_2O_2	H ₂ O ₂	NaOCI	NaOCI	NaOCl					
times	30%	6%	1.2%	5%	1%	0.2%					
G. pyriforme,											
60 min	13.9 abc	11.6 abcd	17.9 a	8.4 cd	10.0 bcd	10.4 bcd					
30 m in	17.2 a	12.0 ab	10.8 bcd	12.1 bed	7.64	11.2 ed					
5 min	10.3 bcd	9.5 bcd	9.3 bed	11.1 bcd	11.6 bcd	9.6 bed					
Other filamen	tous										
fungi, %						1					
60 min	5.1 a	9.2 b	36.9 de	10.8 b	9.3 b	10.4 b					
30 min	68.8 f	85.9 g	88.6 gh	24.0 c	20.0 c	27.6 cd					
5 min	89.2 gh	90.5 g	90.0 gh	68.4 f	43.1 e	34.3 de					
Bacteria and											
yeasts, %											
60 min	θа	0.1 a	0.5 a	0.1 a	0.4 a	0.3 a					
30 min	1.3 a	0.5 a	0.5 a	0.4 a	1.1 a	0.3 a					
5 min	0.5 a	0 a	1.2 a	1.1 a	5.1 b	9.9 €					

^a Values are for the triple interaction of treatment (surface sterilant), concentration, and treatment duration (min); valid comparisons can be made only within each of the three parameters where all means followed by a letter in common are not significantly (P = 0.5) different. Values are cumulative data for the 15-day incubation period.

time increased (Table 1). The best treatments for G. pyriforme isolation were 30 and 1.2% H_2O_2 for 30 and 60 min respectively, i.e. the long exposure—lower concentration treatment was as good as the high concentration—short exposure treatment. Seeds treated with 30% H_2O_2 for 1 h had the fewest filamentous fungus contaminants, while the next best treatments were 6% H_2O_2 and 5, 1, or 0.2% NaOCl, all for 1 h. In general, lengthening the exposure period was more beneficial than increasing the concentration of the surface sterilant for reducing numbers of filamentous fungus contaminants. The numbers of bacterial and yeast contaminants were unaffected by any of the H_2O_2 treatments, but the numbers of these contaminants decreased as NaOCl concentration increased at the 5-min exposure period.

This study has shown that surface sterilization with 30% H₂O₂ for 1 h is the best overall treatment for isolating G. pyriforme from diseased seeds and for reducing filamentous fungus and bacterial and yeast contamination (Table 1). This procedure yields more G. pyriforme and fewer contaminants than does surface sterilization with 1% NaOCl for 5 min (Table 1) as recommended by Salt (1974). Reduction of contamination facilitates detection of the characteristic mycelium of G. pyriforme and allows the pathogen, whose growth is frequently inhibited by seed coat microorganisms, to grow from diseased seeds. Strong H₂O₂ reduced Sitka spruce seed germination, but this is of no concern to those interested in determining G. pyriforme incidence. Although various concentrations of H₂O₂ have been used to stimulate seed germination or to reduce or eliminate seed coat microflora (e.g. Barnett, Tree Plant. Notes 27:17-19, 24, 1976; Ching and Parker, Forest Sci. 4:128-134, 1958; Riffle and Springfield, Forest Sci. 14:96-101, 1968; and Trappe, Forest Sci. 13:121-130, 1967) or for both, this is the first report of H₂O₂ being used to surface-sterilize seeds for isolating a pathogenic fungus. Recently, we have isolated G. pyriforme from stored P. glauca (Moench) Voss, P. engelmannii Parry, Abies grandis (Dougl.) Lindl. and Pseudotsuga menziesii (Mirb.) Franco seeds after surface sterilization with 30% H₂O₂ for 1 h. - Jack R. Sutherland, T.A.D. Woods, W. Lock, and Denis A. Gaudet, Pacific Forest Research Centre, Victoria, B.C.

Slugs Feeding on Cronartium in British Columbia. — Slugs (Gasteropoda pulmonata) are land molluses that have evolved from snails by reduction or loss of their shells. Common in the humid Pacific Northwest, they are primarily vegetarians, feeding on fungi, fruit, and foliage of herbaceous plants during the night or on overcast days (Kozloff, Plants and Animals of the Pacific Northwest, J.J. Douglas Ltd., Vancouver, 1976).

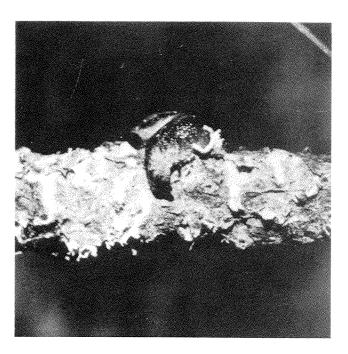


Figure 1. The slug *Prophysaon andersoni* feeding on a *Cronartium comptoniae* canker. Note the tendril of egested aeciospores on the side of the slug.

Early observations in eastern North America noted slugs feeding on *Cronartium ribicola* J.C. Fisch. ex Rab. telia (Gravatt and Marshall, Phytopathology 7:368-373, 1917) and on pycnia and aecia (Snell, Phytopathology 19:269-283, 1929). To date, in western North America, only arthropods and mammals have been associated with coniferous stem rusts (Hiratsuka and Powell, Dep. Environ. Can. For. Serv. For. Tech. Rep. 4, 1976).

During the past 3 years, while pine rust cankers were being observed on Vancouver Island, slugs were occasionally noticed feeding on aeciospores and infected bark tissues. They fed in characteristic patches or trails as they moved across the cankers, removing nearly all aeciospores from individual aecia. Slime trails and tendrils of egested bleached spores were common. Feeding wounds on infected bark were characteristically shallow and bore radula marks.

Slugs collected were identified as Prophysaon andersoni (Cooper) on Endocronartium harknessii (J. P. Moore) Y. Hirat. and Cronartium comptoniae Arth. (Fig.1), Ariolimax columbianus (Gould) and, tentatively, as Hemphillia glanulosa (Bland and Binney) on C. ribicola. (They were identified by D. Rollo, of the Department of Plant Science, University of British Columbia.) Also, P. andersoni was observed feeding on the secondary fungus Tuberculina maxima Rostr. on a C. comptoniae canker, and T. maxima on a C. ribicola canker had been partially consumed by an unknown slug.

In a lodgepole pine plantation, 10 or more slugs were frequently observed feeding on individual C. comptoniae cankers, but only in the early mornings or on wet days. During warm days, slugs were found under the duff or, occasionally, under the exfoliating bark of E. harknessii galls.

The spores of some species apparently do not pass through slugs intact, while others are still capable of germination (Wolf and Wolf, Bull. Torrey Bot. Club 66:1-5, 1939). Snell (1929) hinted that fecal aeciospores were nonviable. However, in water droplets on slides and on water agar. I obtained germination of *E. harknessii* and *C. comptoniae* aeciospores egested by *P. andersoni*, and *C. ribicola* aeciospores egested by an unknown slug.

Slugs probably reduce the inoculum potential of rusts, for tendrils of aeciospores are unlikely to be airborne, and their feeding wounds possibly provide infection courts for secondary fungi, which may further limit rust development. — Richard S. Hunt, Pacific Forest Research Centre, Victoria, B. C.

SILVICULTURE

Fertilizing after Thinning 70-year-old Lodgepole Pine (Pinus contorta Dougl. var. latifolia Engelm.) in Alberta. — This study explored the effect of fertilization and thinning shortly before harvest on the growth of crop trees and on the ingrowth of smaller trees into merchantable size classes on medium-quality sites. This note presents growth response 7 years after treatment.

The stands in the study area (lat. 52°2', long. 115°8', elevation, 1 280 m) belong to the Foothills Section of the Boreal Forest Region, B.19a (Rowe, Forest Regions of Canada, Can. For. Serv. Publ. 1300, 1972). They are growing on Podzolized Gray Luvisol (Peters and Bowser, Alta. Soil Surv. Rep. 19, Univ. Alta., 1960) developed on level-to-undulating coarse outwash material with an alluvial veneer. The soil has good drainage and a fresh moisture regime. Table 1 summarizes the analysis of soil samples collected on each plot in 1975.

The stand of essentially pure lodgepole pine was 72 years old when treated in 1968. Before thinning, it support 2,500 trees/ha; basal areas was 33.4 m²/ha and average dbh 13 cm. Average site index for the sample plots was 17.4 m (reference age 70 years; Kirby, Can. For. Serv. Inf. Rep. NOR-X-142, 1975), a little below medium site class for lodgepole pine in west-central Alberta.

The thinning was a commercial fence-post-cutting operation intermediate in both intensity and character. It released vigorous large trees by removing smaller ones. The number of trees was reduced by about 66%, basal area was reduced by about 55%, and average dbh was increased by 16%.

The fertilization study was established as a randomized but incomplete factorial experiment, with three levels of N, P, and S. (Soils in this region are generally well supplied with K.) Treatments were replicated three times in thinned stands only, and included the following (in kg/ha):

	N:0		N:112			N:673		
	S:0	\$:0	S:28	S:84	S:0	S:28		
P:0	X	X			X			
P:56 P:168	X	X	X		X	X		
P:168	X	X	X	X	X	X		

Urea (45-0-0), concentrated superphosphate (0-45-0), ammonium phosphate (11-48-0), and ammonium phosphate sulphate (16-20-0-14) were broadcast by hand on the plots, according to treatment specifications, in October 1968.

Forty-two 0.04 ha sample plots with 4 m buffer zones were established in August 1968. All trees were tagged, and dbh was measured to the nearest 0.25 cm. A sample of heights (approximately 10) of a range of tree sizes was also measured on each plot. A remeasurement in October 1975 included a dbh tally and height measurements of the same sample trees. Two increment cores were taken at breast height (1.37 m) from 6 to 10 of these sample trees. Because of the lack of unthinned control plots, 20 sample trees of a range of sizes were selected in the adjacent unthinned stand in 1975, measured, and bored. From the core data, diameters were estimated at the end of the 1963, 1966, 1968, 1970, 1972, and 1975 growing seasons.

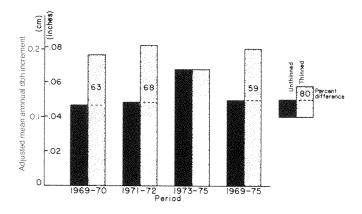


Figure 1. Adjusted mean annual dbh increments for four growth periods in thinned and unthinned stands.

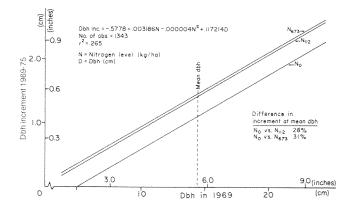


Figure 2. Seven-year dbh increment trends for three levels of N (derived from response surface analysis).

Response was evaluated from individual tree data by using covariance and response surface analyses, at the (0.05) significance level. This approach was chosen because (1) the experiment did not fulfill formal ANOVA design criteria and (2) this approach helped remove some of the pretreatment variation in the experimental material.

The results of the analysis (Fig. 1) show a mean increase of about 60% in dbh increment in the first 7 years after thinning. The response was largely in the first 4 years; the response from 1973 to 1975 was not statistically significant. There was an additional 30% increase in mean dbh

TABLE 1
Soil chemical and physical properties in 1975 from 42 plots^a

Mineral horizon	Values	pН ^b	Cond° pHb dS/m 25°C	Organic matter	PPM Exch.			CEC [†]	C	Particle size (%) by weight		
(cm)				%	NO ₃ -N	NH ₄ -N	p^d	Κ¢	meq/100 g	Sand	Silt	Clay
0-30	Average Minimum	5.3 4.7	.13	1.3	0	3.40	19.4	134,0	15.3	34.3	42.9	22.8
	Maximum	5.8	.23	2.5	0	7.5	43	64 253	21.5	19 49	21 58	31
30-60	Average Minimum Maximum	5.9 4.9	.27	.8	0	2.27 1,0	10.6	143.4 96 193	20.7 16.1 26.7	40.2 16	27 15	32.7 12

Nutrient levels in 1975 showed no correlation with treatment levels.

^b Saturated paste.

^e Specific conductivity on saturated extract, 1 dS/m = 1 mmho/cm.

^{6 0.5} M (pH 8.5) NaHCO₅ extractable * N (pH 7.0) NH₄OAc extractable.

¹ Cation exchange capacity

increment during the first 7 years following N fertilization after thinning (Fig. 2). The amount and duration of response were similar for both levels of N, although the higher level of N was six times that of the lower (673 kg/ha vs. 112 kg/ha). As was the case with thinning, the analysis also showed that most of the response occurred in the first 4 years after treatment; no significant effect could be detected in the ensuing 3 years.

Gross volume increments for the two levels of N application were very close in terms of both total (32.0 and 32.4 m³/ha) and merchantable (31.2 and 32.4 m³) volume. However, plots that received N fertilizers surpassed plots that did not, on the average by 27% in total (32.2 vs. 25.4 m³/ha) and 31% in merchantable (31.8 vs. 24.3 m³/ha) volume. This means an absolute increase in gross production of 6.8 m³/ha in total and 7.5 m³/ha in merchantable volume that may be attributed to application of N fertilizers in the 7-year period. Net increment values were variable owing to mortality caused mainly by wind damage, and on the average were slightly under 80% of gross volume increment.

Tree growth was unaffected by P and S application, nor was there any significant interaction between P and N or between P and S. It seems that P concentrations found in the soil (Table 1) are sufficient to ensure adequate growth of lodgepole pine (Wilde, J. For. 64:389-391, 1966).

Although this study showed a moderate response in diameter increment to thinning, such treatment also increased the likelihood of wind damage in these stands. Thus thinning in similar stands at this late age would be unjustified unless the value of the material removed exceeded thinning costs, or there was sufficient increase in final merchantable yield to cover treatment cost. These results showed very limited response to fertilization, in contrast to the Swedish experience (e.g. Hagner, McMillan Lectures, Univ. B.C., 1967). As these stands are now near rotation age and may be harvested within a few years, little further improvement can be expected. Although results from an exploratory study like this require verification, they indicate little scope for operational use of similar treatments in lodgepole pine management in Alberta's Foothills, particularly in view of the recent escalation in cost of chemical fertilizers. — I.E. Bella, Northern Forest Research Centre, Edmonton, Alta.

Efficiency of Nitrogen Recovery from Plastic-coated Urea in a White Spruce Plantation. — Growth responses to fertilizer nitrogen have been reported from many forests in Canada (Weetman et al., Can. For. Serv. For. Tech. Rep. 16, 1976). However, generally poor rates of recovery of applied nitrogen have been encountered in the northern coniferous forest. Although nitrogen uptake by conifers has been shown to continue through the growth season (Salonius, Soil Sci. Soc. Am. J. 41:136-139, 1977), the availability of formulations normally applied to amend forest soils is usually highest in the short period immediately after application. A new formulation, plastic-coated urea, has been suggested (Salonius and Adams, For. Chron. 48:96-97, 1972) to extend the period of dissolution and diminish losses through volatilization, leaching, and microbial and chemical immobilization. One of these, volatilization, has been shown to be partly inhibited by the new formulation (Mahendrappa and Salonius, Soil Sci. 117:117-119, 1974).

In 1973, a field study to compare plastic-coated urea with conventional urea was initiated in a 30-year-old white spruce planation on the Acadia Forest Experiment Station in New Brunswick. This plantation suffered variable losses of current foliage from spruce budworm feeding during the years the study was under way. This was a single-tree experiment with five sample trees chosen at random throughout the plantation for each treatment. Current foliage samples were taken from the top third of the live crowns in April 1973, by means of pole pruners. On 30 May, 1973, when the trees had just started shoot elongation, the fertilizer was applied uniformly around the trees within a radius of 1.6 m. Both forestry-grade urea and the material that had been plastic-coated were applied at the rate of 225 kg N/ha. The soil was saturated from very heavy rains during the month before fertilizer application. Approximately 25 mm of rain fell between 9 June and 14 June, 1973, and the rest of the month was unusually wet.

Visual inspection of plastic-coated urea during July showed that none of the particles had completely dissolved but that concave-shaped erosion zones had developed close to weak spots in the coating in approximately 50% of the units. In October 1973, at the time of the first of four annual current foliage samplings from the control and treated trees, about 60% of the plastic-coated-urea particles were empty, 20% showed concave

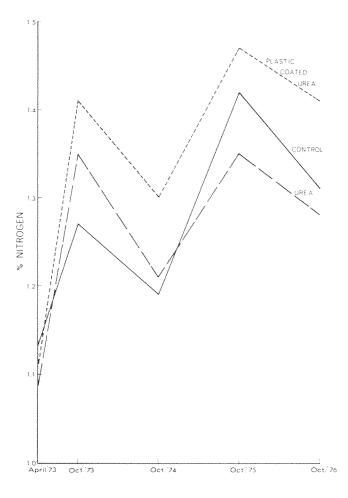


Figure 1. Nitrogen concentration in current foliage (% ovendry weight).

dissolution zones, and 20% showed no sign of dissolution. By October 1974, all of the plastic-coated-urea particles were empty. The conventional forestry-grade urea was completely dissolved within 3 days after application.

Current-foliage samples were oven-dried for 24 h at 70°C and analyzed for nitrogen concentration (MacDonald, Can. For. Serv. Inf. Rep. M-X-28, 1972). Triplicate samples of 100 needles from each tree for each collection were weighed to assess possible changes in foliar weight due to treatment. Foliar weights were stable over the period of sampling and were not affected by treatment. The results of foliar analysis for nitrogen are shown in Fig. 1. Year-to-year foliar nitrogen levels varied considerably, control and treated trees showing similar-sized shifts in the same direction in each year. No detailed defoliation studies were done in this plantation during the years of foliage collection, but, in general, years during which a large proportion of the current foliage was destroyed by budworm feeding were characterized by relatively high nitrogen levels, and years with limited budworm feeding by lower nitrogen levels distributed within the greater surviving current foliage mass.

The nitrogen concentrations of foliage from control and urea-treated trees were not significantly different in the posttreatment period (t-test), whereas foliar nitrogen concentrations from trees treated with plastic-coated urea were significantly greater than concentrations in both control and urea-treated trees. Both urea and plastic-coated urea raised foliar nitrogen concentrations above those in the control in the first 2 years after treatment; plastic-coated urea produced the highest concentrations. The advantage of plastic-coated urea in the early treatment period probably lies in its resistance to leaching and volatilization losses. Also its rate of dissolution more closely corresponds to the rate of uptake shown by trees of fertilizer nitrogen over time (Salonius, 1977). Conventional urea, which

dissolves immediately, is made available rapidly to immobilization mechanisms that can remove a significant portion of the nitrogen from the available soil pool (Salonius, Soil Sci. 114:12-19, 1972). By the third fall after treatment, the nitrogen concentrations of foliage from trees fertilized with urea had resumed their pretreatment levels relative to the controls. Lee (Can. For. Serv. Inf. Rep. BC-X-55, 1971) has shown that similar declines in foliar nitrogen to pretreatment levels occur rapidly with conventional urea.

The extent of mass mortality and darkening of the humus layer, both due to the fertilizer treatments, suggested that slow dissolution of the plastic-coated urea affected only a limited surface area around each particle, whereas treatment with conventional urea affected a much larger area of the forest floor. The larger mass of soil effectively treated by the same amount of conventional urea may expose this form to greater microbial and chemical immobilization (Salonius, 1972).

The relatively greater efficiency of plastic-coated urea, in first producing and then maintaining higher foliar nitrogen levels than conventional urea, appears to be more marked at each succeeding annual sampling. This extended recovery may be related to the smaller volume of soil treated by the same amount of fertilizer as of plastic-coated urea and the consequently lesser immobilization of nitrogen from this source. Further studies are required to show whether there are differences in the type of immobilization (microbial or chemical) to which the two sources of nitrogen are subjected in the field. — P.O. Salonius, Maritimes Forest Research Centre, Fredericton, N.B.

Relationship between Duration of Initial Growing Period and Subsequent Growth of Greenhouse-reared White Spruce Seedlings. — This note reports height growth of transplanted white spruce (Picea glauca [Moench] Voss) seedlings 1, 2, and 3 years after initial growing periods of 8 to 15 weeks' accelerated growth in a controlled environment greenhouse (22 ± 2°C, 16-h photoperiod). While the size of seedlings is only one of several criteria for early survival, there is sufficient evidence to assume that within certain limits larger seedlings fare better after planting (Dobbs, Can. For. Serv. Rep. BC-X-149, 1976). In greenhouse nurseries, development of seedlings and their suitability for planting are often judged from height; and, in early-flushing species such as white spruce, height may be critical to survival, since it will determine the position of young tissues within cold air layers near the ground in spring.

Greenhouse nurseries equipped with environmental control offer the means for rapid production of planting stock or transplants. However, operation of such systems is expensive, particularly at each end of the normal growing season. The forester must weigh possible benefits of extending the initial growing period against these operational costs. The minimum duration of the greenhouse phase is about 8 weeks. In this experiment, seedlings were grown for 8 to 15 weeks in a controlled-environment greenhouse; effects of the various extensions on subsequent height growth were recorded after 1, 2, and 3 years in an outdoor nursery.

Seedling crops to be reared during late winter in a heated greenhouse $(22\pm 2^{\circ}\mathrm{C})$ were sown at eight weekly intervals beginning 30 January 1975 in Styroblocks (41 mL cavities) filled with a peat-vermiculite (3:1) mix. Seedlings were irrigated daily with a nutrient solution (Ingestad, pages 265-269 in XIV Congr. Int. Union Forest Res. Organ. München III, 1967). The photoperiod was extended to 16 h at 18 000 lux, with fluorescent lamps.

Fifteen weeks after the initial sowing (8 weeks after the final sowing), seedlings were hardened through transfer to 8-h photoperiods for 9 weeks. At the end of this short-day treatment (18 July 1975), all seedlings were fully dormant and had completed bud development (Pollard, Can. J. Bot. 52:1569-1571, 1974). Twenty-four seedlings from each sowing date were then planted in a nursery bed of sandy loam. Seedlings remained dormant until the spring of 1976 (a second series received 5 weeks' chilling in July-August 1975 and flushed on planting in August; because of the lateness of planting, all seedlings were damaged by early frost in September). Heights of seedlings were measured in the fall of 1975, 1976, and 1977. The results presented in Fig. 1 indicate considerable height growth in all treatments. It should be noted, however, that seedlings were reared initially under conditions more favorable than usually encountered in greenhouses. Greenhouse-grown seedlings normally attain somewhat lesser heights in the periods stated (e.g. Scarrat, Can. For. Serv. Rep. O-X-168, 1972).

First-year differences in height growth that developed during the initial greenhouse phase were amplified substantially in subsequent years;

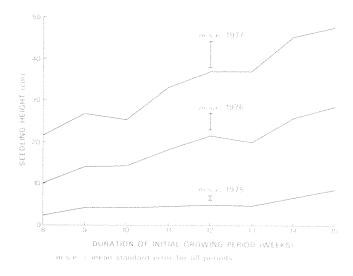


Figure 1. Seedling height 1, 2, and 3 years after the initial greenhouse phase of different periods.

thus an extension in initial growth period from 8 to 15 weeks conferred additional height increment of 5 cm for 1975, 17.3 cm for 1976, and 25.8 cm for 1977. Even a modest 3 weeks' additional growth, from 8 to 11 weeks, led to an increase of more than to 50% in height in the third year. Such advantages could be very significant in the seedlings' ability to compete under plantation conditions. The extension of the initial growing period may also offer opportunities for reducing the outdoor nursery phase, in which greenhouse-grown seedlings are produced as transplant stock. There remains, of course, the choice between growing two batches of large seedlings or three batches of small seedlings within the economical season of greenhouse operations. Data from this exriment suggest that a 36-week season (March through October) could produce three batches of seedlings capable of attaining 20 cm in height by the fall of the following year. The seedlings produced in late fall could be readily hardened under the natural short daylengths and placed in cold storage until spring. - D.F.W. Pollard, Petawawa Forest Experiment Station, Chalk River, Ont.

INSECT PATHOLOGY

Endogenous Light Action in Germination of Entomorphthora aphidis Resting Spores in Vitro. — After publication of our findings (Wallace et al., Can. J. Bot. 54(13):1410-1418, 1976) showing that light exposure for 14 h or more per day is a necessary condition for obtaining good germination of Entomorphinora aphidis resting spores in vitro, it was suggested to us that the enhanced germination may not result from action of the light upon the spores themselves but from an activator produced photochemically from some component of the agar, the liquid in which the spores are suspended for plating, or contaminant organisms in the spore suspension not killed by the mercuric chloride/gentamicin treatment. We have tested for these possibilities and report our results here.

Methods of spore production, plating, and incubation were as described previously. A resting-spore suspension was prepared in the usual fashion; then the spores were spun down and the supernatant was retained. Sixty 1% water agar plates were prepared: 20 received no additional material, 20 had 0.1 mL of supernatant spread over the surface in the same manner as would have occurred if spores were supended in it, and 20 had 0.1 mL of supernatant that had been passed through a mocrobiological membrane filter (0.22 μ m pore size) spread over the medium. One half of the plates (10) in each lot were wrapped in several layers of aluminum foil to exclude light. Then all the plates were incubated at $20 \pm .5$ °C with 16 h d-1 cool white fluorescent lighting at approximately 1 000 lux. Ambient RH in the incubator was 50-60%. After 31 days' incubation, the plates were all seeded in the usual fashion with a freshly prepared resting-spore suspension. Half of the plates from each of the three basic treatments that had been preincubated in the dark were rewrapped after seeding for incubation in darkness, and half were incubated under the light conditions

TARLE 1

Germination of E. uphidis testing spores at 20 ± .5°C and darkness or 16 h d³ fight on 1% water agar preincubated in darkness or light for 31 days before seeding; same with spore suspension supernatant added before preincubation; same with filtered supernatant added before preincubation. (Means of five replicates. Standard errors range from 0.1421 to 1.0241.)

			Germination	, iş					
		Spores incubated in 16 h d+ light							
Incubation	Agar		Agar + si	ipernatiant	Agar ÷ filtered supermatant				
time.	Preinc.	Preinc.	Preinc.	Preinc.	Preinc.	Preinc.			
days	light	dark	light	dark	light	dark			
3	2.10	2.86	2.09	2.34	1.58	1.94			
7	4.48	4.10	4.31	4.37	3.44	3.96			
11	19.45	18.59	17.59	17.07	17.37	17.16			
18	32.34	32.22	31.12	29.28	30.56	30.66			
25	46.80	47.02	46.62	44.73	46.95	46.54			
3.2	59.24	59.70	59.65	58.79	59.65	59.84			
41	64.46	65.56	64.84	63.73	64.03	64.41			
		Spor	res incubated in	ı darkness					
3	1.37	2.72	1.33	2.(39	1.56	1.89			
7	3.01	2.51	1.75	2.34	1.56	2.62			
11	3.11	3,27	2.13	2.49	2.29	2.48			
18	3.27	3.46	2.67	2.76	2.67	2.78			
25	3.63	3.88	3.28	3.26	3.25	3.49			
3.2	3.87	3.84	3.60	3.45	3.49	3.63			
41	4.21	4.48	4.29	4.64	4.28	4.38			

already indicated. Similarly, half of the plates that had been preincubated in the light were wrapped after seeding, and half were left to be exposed to light in the second or true incubation period. This procedure resulted in 12 combinations of treatments with five replicates in each.

Germination counts were made starting at Day 3 after seeding and continued at about weekly intervals until Day 41. Resting spores were tallied as germinated or ungerminated in randomly selected microscope fields until 100+ spores were recorded on each plate at each examination date. The results are shown in Table 1. It is clear without statistical analysis that neither the level nor the rate of germination was affected by any of the plate pretreatments. The action of light for 31 days (long enough to produce more than 50% germination in this test when spores were present) on the medium alone, on the medium with supernatant, or on the medium with filtered supernatant did not in any way alter the germination of spores subsequently incubated on the plates. The only effects evident in the results are those of light and time during the actual spore incubation. These effects were as expected from our previous work. We conclude that the light acts upon the spores themselves during incubation. — D.R. Wallace, Great Lakes Forest Research Centre, Sault Ste. Marie, Ont., and D.M. MacLeod and A.J. De Lyzer, Forest Pest Management Institute, Sault Ste. Marie, Ont.

Insect Virus Application with a Cold Fog Generator. — Cold fog generators have been used successfully for the application of the bacterium *Bacillus thuringiensis* for insect control (Falcon et al. Calif. Agric. 28:11-13, 1974; Frye et al. USDA Forest Serv. Note RM-315, 1976). A nuclear polyhedrosis virus has been used to control redheaded pine sawfly, *Neodiprion lecontei* (Fitch), and, to date, it has been applied with mist blowers (Anon., Can. For. Serv. Inf. Rep. DPC-X-1, 1970) and from aircraft (Kaupp and Cunningham, Can. For. Serv. Inf. Rep. IP-X-14, 1977). It was decided to conduct a trial with a cold fogger and ascertain the feasibility of applying a nuclear polyhedrosis virus on small plantation trees with this equipment.

The trial was conducted in a mixed red and jack pine plantation (trees 1 to 1.5 m high) located in Lot 17, Concession VIII, Rideau Twp., south of Ottawa, on the morning of 15 July, 1977, when larvae were mainly in the fourth instar. The population density of the insect was estimated at 132 colonies per 100 trees.

A Leco ULV cold fog generator Model HD (Figs. 1 and 2) was used and 7.5 L of virus formulation, containing 4.4 × 10⁹ polyhedra, 3.75 L water, 3.75 L Volck^R oil (Chevron Chemical [Canada] Ltd.), and sufficient rhodamine B dye to monitor the deposit, were disseminated. Both sides of a 100 m roadway were treated and, with an effective dispersal range of 25 m, about 0.25 ha was covered. The droplet



Figure 1. Leco ULV cold fog generator Model HD.



Figure 2. The generator in action.

spectrum, as analyzed on Kromekote^R cards, revealed sizes in the 15μ to 30μ range with a density of more than 5 $000/\text{cm}^2$ 2 m from the nozzle of the fogger and $390/\text{cm}^2$ 22 m from the nozzle.

Larvae were collected 6 days after the application from the area treated with the fog generator and from an untreated check plot. Microscopic examination of their guts revealed 43% of the larvae from the treated area had visible nuclear polyhedrosis virus infection. No virus was found in larvae from the check plot. As a further measure of the efficacy, 100 larvae were collected from each of the plots and reared in the laboratory until either pupation or death occurred. Mortality was recorded as 72% in the sample from the fogged plot and 9% in that from the check plot.

At fourth instar, larval development may have been too advanced to obtain complete control of the sawfly with the dosage of virus applied. However, on the basis of observations made in previous years, it was considered that sufficient virus was introduced into the insect population to cause a virus epizootic and to eradicate this sawfly from the treated area the following year.

These results indicate that cold fogging with an oil/water emulsion is a practical method of disseminating nuclear polyhedrosis virus in a plantation and that the coverage and swath width are impressive. The compatibility of Volck^R oil and nuclear polyhedrosis virus has not been tested in the laboratory, but the foregoing results indicate that there was no inhibitory effect.

Warren T. Johnson was a visiting scientist from Cornell University, Ithaca, N.Y., on subbatical at the Forest Pest Management Institute,

Ottawa. — W.T. Johnson, J.C. Cunningham, W.J. Kaupp, and J.C. Edwards, Forest Pest Management Institute, Sault Ste. Marie and Ottawa, Ont.

BOTANY

Variation in Fascicles, Primordia, and Phyllotaxy of Lodgepole Pine, Pinus contorta Dougl. var. latifolia Seedlings after Frost Damage. — Pine is often frost-damaged with resulting stem canker, bud injury, or needle browning (Zalasky, Can. For. Serv. Inf. Rep. NOR-X-190, 1977). The tissues most affected by frost are the bark and cambium of the stem (Zalasky, Can. J. Bot. 53:1888-1898, 1975) and the needles and primordia. This paper describes variable changes in growth characteristics of primordia, fascicles, and phyllotaxy of frost-damaged field-grown and overwintered containerized lodgepole pine seedlings.

Containerized seedlings were reared in the greenhouse by using Spencer-Lemaire containers, peat, a recommended fertilizer regime (Environ. Can., North. Forest Res. Cent. For. Rep. 5(4):6, 1976) and a 20-h photoperiod. In experiment 1, 16-week-old containerized seedlings were outplanted on two sites in late May 1976, 80 km south of Grande Prairie, Alta., to determine the occurrence and effects of frost damage in field plantings. Daily weather records were kept from May to October. In experiment 2, 17-week-old seedlings in containers were put outside in the nursery at Edmonton, Alta., during December 1976 and maintained under a 15 cm cover of perlite and snow until 20 April 1977. They had been reared for 10 weeks in the greenhouse and 7 weeks in the growth chamber,



Figure 1. A short shoot with 12 secondary needles. A, vestigial needles; B, variable-length needles; C, flat, broad, needle base, or occasionally a whole needle. The leading shoot above and all the primary needles were frost-killed; primary needles droop.

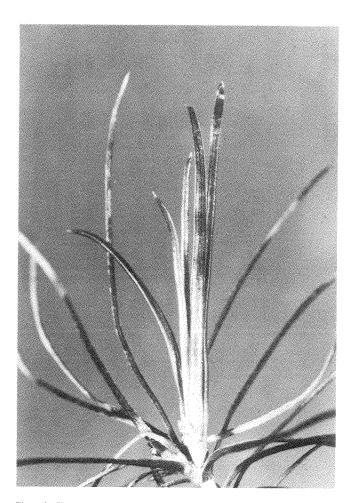


Figure 2. Short shoot showing five needles with broad flat bases. The leading long shoot bears healthy primary needles.

of which 4 weeks were at 20°C, 2 weeks were at 6°C, and 1 week was at 1.6°C. Surviving damaged seedlings from experiment 2 were then maintained in the greenhouse through the summer to determine their growth characteristics. Observations were made daily over a period of two growing seasons in experiment 1 and one growing season in experiment 2. Frost damage was recorded after an early or late frost in experiment 1, or after a spring thaw in experiments 1 and 2.

In a healthy seedling the primary needles are followed by secondary needle production on short shoots produced in the axils of primary needles. The primary needles are single and the secondary needles are in fascicles of two. Secondary needle production is maintained thereafter from apical and lateral buds or leading shoots. Seedlings of experiments 1 and 2 had primary needles and rudiments of short shoots and terminal buds at various stages of development when the experiment was started.

In most of the surviving seedlings from both experiments, frost damage resulted in dead, drooping, primary needles (Fig. 1); a few also had dead buds and small dead areas of bark. New needles of most surviving seedlings developed from existing lateral and terminal buds capable of producing candles of predetermined bicyclic growth (Bollmann and Sweet, N.Z.J. For. Sci. 6:376-392, 1976; Sweet and Bollmann, *ibid.*, 393-396, 1976). Some undamaged existing buds had a sterile needle zone at the base. The buds without a sterile needle zone were of two categories, those with uniformly developed needle primordia and those without. Those without uniform development usually produced the first needles in the lower midsection. Those with a sterile needle zone were subtended by a few tiers of advanced needle primordia followed by gradually smaller primordia toward the apex of the bud. In buds having two sterile zones, the advanced needle primordial zone was interrupted by a short sterile zone

followed by smaller needle primordia toward the apex. Newly proliferated adventitious buds were produced in seedlings only if existing buds were permanently damaged, failed to break, or broke belatedly.

Sterile zones in candles of damaged seedlings were entirely or intermittently leafless to the subapical area of the new shoot and measured up to 5 cm in length. Variable changes occurred in the number, shape, and size of secondary needles per fascicle as a result of sterility, fusion, or proliferation of tissues at the primordium. Occasionally a pair of secondary needles was fused, and frequently one was missing or vestigial (Fig. 1,A). Needles of abnormal fascicles were of diverse length (Fig. 1,B), ranging from a few millimeters to 14 cm. The variable-length needles tended to be tapered in the upper part, but thicker, flatter, and broader toward the base (Fig. 1,C). Short shoots that developed during May on the more vigorous plants often contained three, four, or five (Fig. 2) usually uniformly long needles per fascicle rather than the normal two measuring only 3-5 cm in length. Stunted seedlings developed new foliage belatedly only from adventitious proliferated buds, which produced stunted or very long needles. The stunted ones were usually primary and the long ones secondary needles. Distortion in shape and thickness was greatest in the primary needles. Stunted seedlings had very few short shoots, usually one, bearing up to 12 needles (Fig. 1).

Phyllotaxy of new shoots in frost-damaged pine seedlings varied in compactness and order of primary and secondary needles on first and second cycles of shoot growth. Variation was also accentuated by the presence or absence of June buds and scales at the end of the first cycle and the production of secondary needles in the absence of new primary needles. Thus, primaries were occasionally followed by a repeat flush of new primaries produced by a June bud. Secondaries, or primaries and secondaries, were often interrupted by a scale-scar region to be followed by compactly arranged secondaries, or primaries and secondaries. Or, lateral buds and a second-order scale-scar zone intervened between two compactly arranged tufts of secondaries. These examples of repeat flushes in frost-damaged pine were observed to arise from June buds in both experiment 1 and experiment 2. Repeat flushes are usually characterized by short shoots with two- and three-needle fascicles similar to those reported to occur in frost-damaged red pine (Kienholtz, J. For. 31:392-399, 1933); the needles of the earliest flushed short shoots are longest. — Harry Zalasky, Northern Forest Research Centre, Edmonton, Alta.

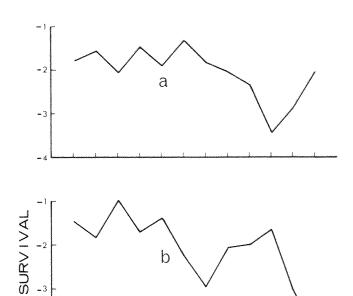
ERRATA

In the table on page 8 (vol. 34, no. 2) the symbols for male and female were incorrectly presented. The corrected table is as follows:

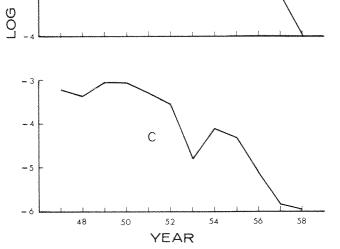
TABLE 1 Trap catches of Dendroctonus rufipennis (Kirby) at empty control cages and test cages containing spruce bolts, Naver Forest, 1975

Test	Trapping	Numbers of D. rufipennis caught							
area	days	Contro (em	leages	Test cages (bolts)					
		ै	Ç	3 + 2	♂	\$	3+9		
A	35	0	0	0	4	5	9		
		1	3	4	7	7	14		
		0	0	0	5	5	10		
		0	0	0	3	3	6		
		0	0	0	7	7	14		
В	31	0	0	Ó.	2	6	8		
		Ú.	()	0	3	5	8		
		0	1	1	į	3	4		
Totals			4	5	32	41	73		

On page 9, col. 1, line 9 (vol. 34, no. 2) reviewed should read viewed. In the figure on the same page all numbers on the vertical axis should be prefixed by a minus sign, as shown in the reproduction that follows.



b



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