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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-HCl 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-flare-injected 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 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.

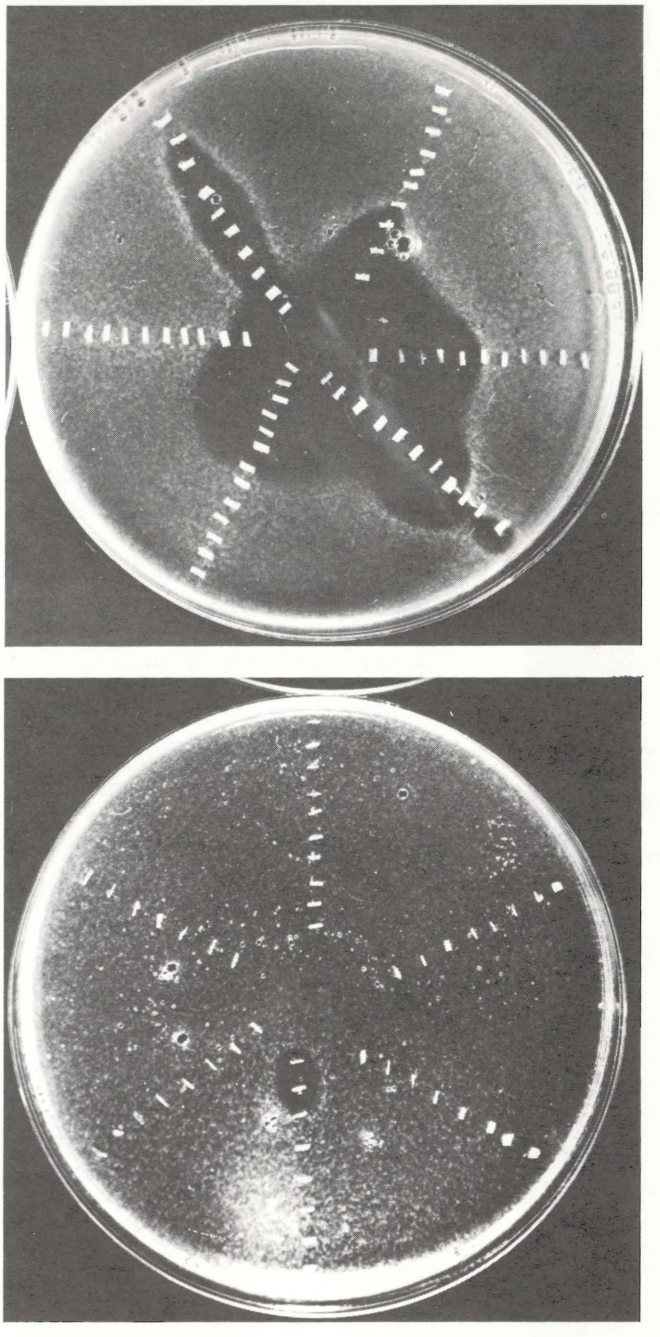

Figure 1. Three-day-old petri dish cultures of *Ceratocystis ulmi* containing tangential sections of elm wood excised from disks cut 0.3 and 3 m above the point of injection 12 weeks earlier, by the Jones and Gregory method. Rows show contiguous segments from the outer 1-3 growth rings taken from various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

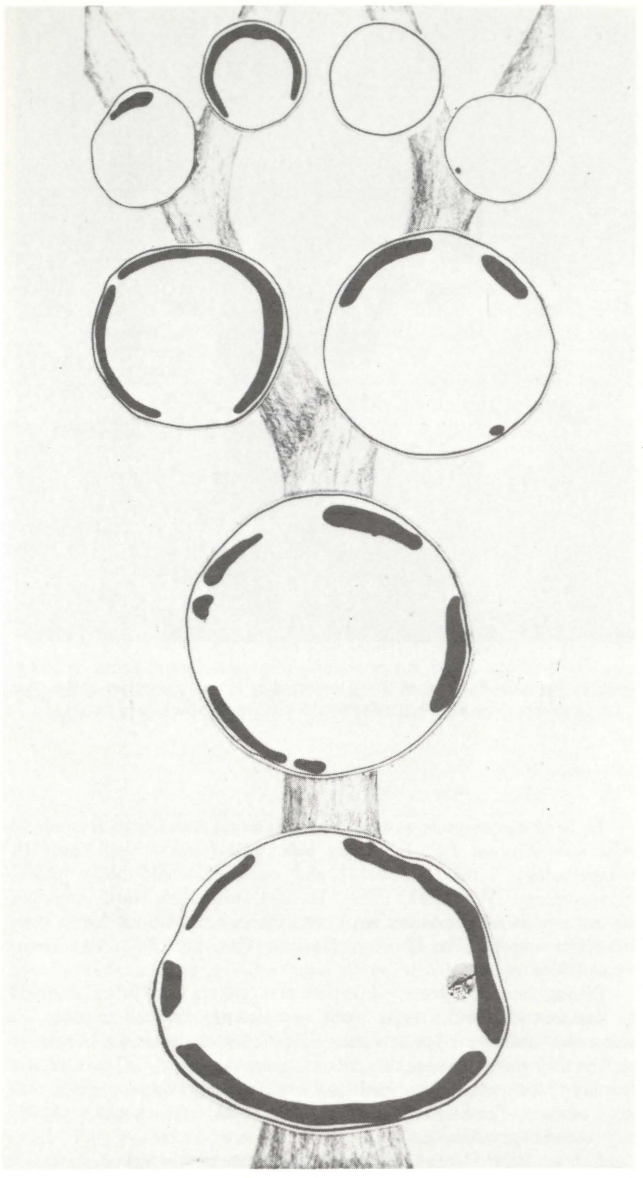

Figure 2. Diagrammatic representation of a tree injected in 1974 by the method of Kondo and Huntley with MBC-P and showing the distribution of fungistatic activity (dark areas) in the xylem. Disks about one-fifth actual size.

TABLE 1

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

Tree no.	DBH (cm)	Volume of solution (L)	Injection method[1]	Hours of injection	Date Injected	Date Felled
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	11	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 (H_2O_2), 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 H_2O_2 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 H_2O_2 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 H_2O_2-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 H_2O_2 than for NaOCl-treated seeds (24 vs. 27%), but differences within treatments were not significant. Percentage seeds yielding *G. pyriforme* tended to increase as H_2O_2, but not NaOCl, concentration and exposure