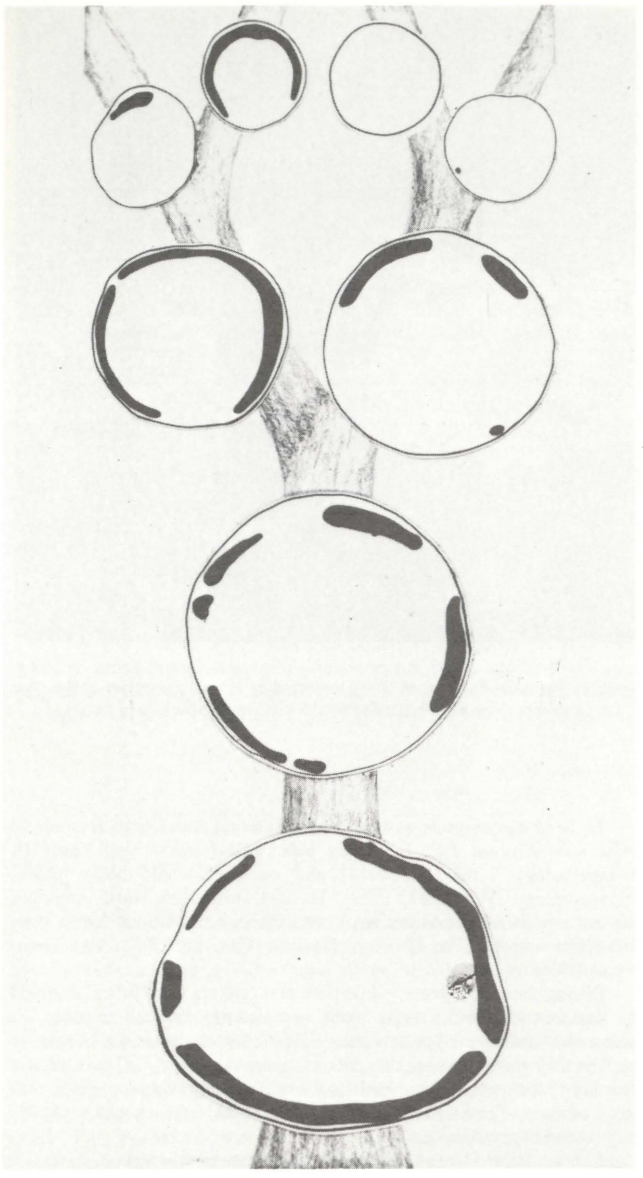

Figure 2. Diagrammatic representation of a tree injected in 1974 by the method of Kondo and Huntley with MBC-P and showing the distribution of fungistatic activity (dark areas) in the xylem. Disks about one-fifth actual size.

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

TABLE 1

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

| Parameters measured and treatment times | Surface sterilants and their concentrations[a] | | | | | |
|---|---|---|---|---|---|---|
| | $H_2O_2$ 30% | $H_2O_2$ 6% | $H_2O_2$ 1.2% | NaOCl 5% | NaOCl 1% | NaOCl 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 min | 17.2 a | 12.0 ab | 10.8 bcd | 12.1 bcd | 7.6 d | 11.2 cd |
| 5 min | 10.3 bcd | 9.5 bcd | 9.3 bcd | 11.1 bcd | 11.6 bcd | 9.6 bcd |
| Other filamentous fungi, % | | | | | | |
| 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 | 0 a | 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 c |

[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% $H_2O_2$ 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 $H_2O_2$ reduced Sitka spruce seed germination, but this is of no concern to those interested in determining *G. pyriforme* incidence. Although various concentrations of $H_2O_2$ 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 $H_2O_2$ 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% $H_2O_2$ 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 molluscs 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).

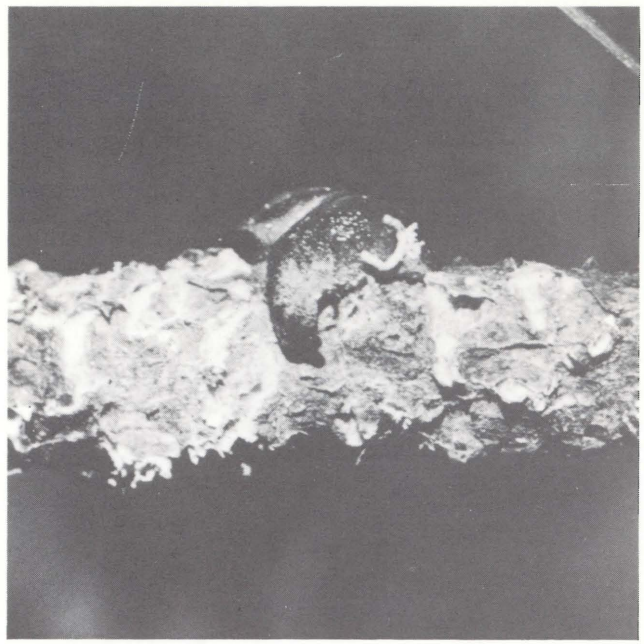

Figure 1. The slug *Prophysaon andersoni* feeding on a *Cronartium comptoniae* canker. Note the tendril of egested aeciospores on the side of the slug.

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.