PATHOLOGY

In Vitro Growth of Two Blue Stain Fungi into Resinous Compounds Produced during the Wound Response of Lodgepole Pine.—Lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) responds to infection by *Europhium clavigerum* Robinson and Davidson and *Ceratocystis montia* Rumb. by impregnating tissue surrounding the lesion with resinous substances (Shrimpton, Can. J. Bot. 51:527, 1973). These sapwood-invading fungi have been frequently, but not consistently, isolated from resin-soaked tissues adjacent to mountain pine beetle (*Dendroctonus ponderosae* Hopk.) galleries. They may, however, elicit extractive production by tree tissues and preferentially colonize tissues that accumulate lesser quantities of resin (Reid et al., Can. J. Bot 45:1115, 1967). Volatile and liquid fractions of this resinous accumulation are inhibitory, but not lethal, to the fungi (Shrimpton and Whitney, Can. J. Bot. 46:757, 1968). Resins harden with time, after which they seem to form a physical barrier to fungi (Lyr, Arch. Forstwes. 16:51, 1967). Growth of fungi in the presence of liquid resins is therefore an important aspect of the host-pathogen interaction. We now report an in vitro system that permits visualization of the fungus-resin interaction.

Resin-impregnated sapwood of lodgepole pine surrounding mountain pine beetle galleries was excised, ground, and extracted with acetone. The extract was evaporated to the consistency of thick oil in a rotary evaporator under a water pump vacuum. Detailed procedures, yields and composition of such extracts are given in Shrimpton (1973). Five milliliters of the concentrated extract were aseptically pipetted into each of six sterile 100 mm petri dishes and about 15 mL of melted potato marmite agar (PMA) (Shrimpton and Whitney, 1968) were added to each. The dishes were swirled until the oil extract and agar mixed. The dishes were then allowed to stand and, while the PMA was still liquid, the two phases separated, leaving a film of oil on the surface and extractive-rich zones scattered throughout the hardened medium. The surface film of oil was made discontinuous by the breaking of groups of small bubbles (Fig. 1).

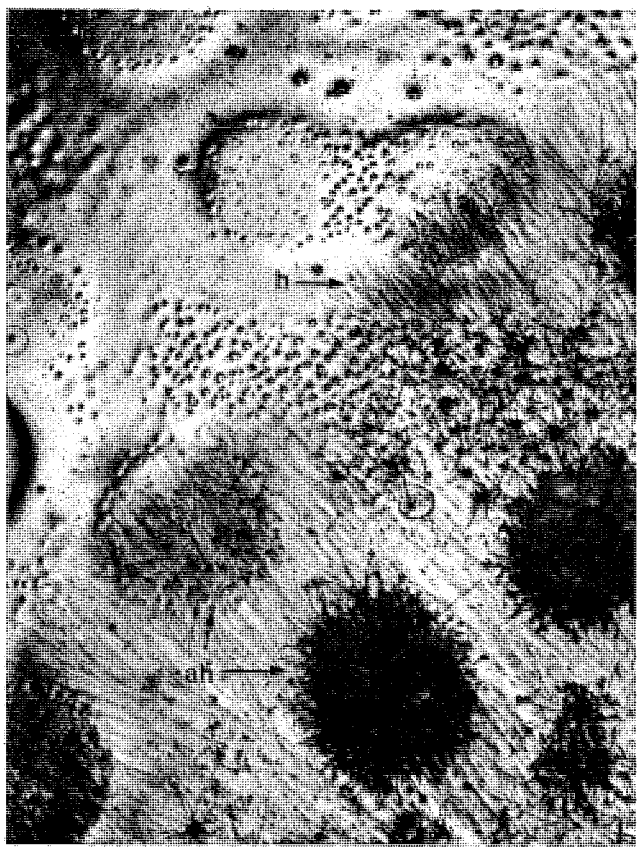

Figure 1. Hyphae (h) of *E. clavigerum* growing beneath the surface oil film and up through the discontinuities in the oil film (ah). x 20.

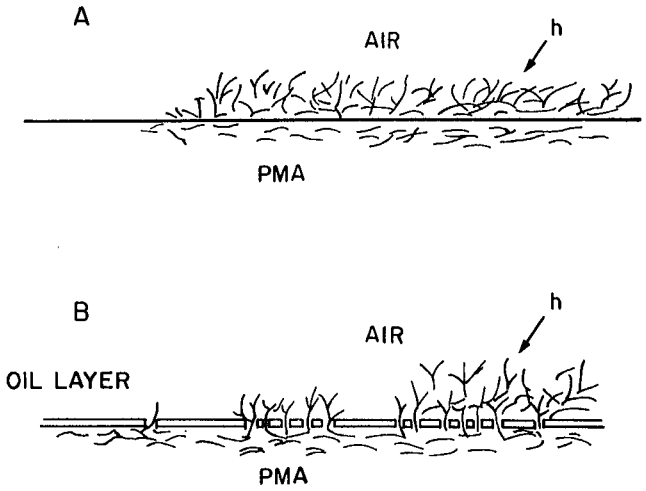

Figure 2. Sectional diagram showing the pattern of advancing hyphal (h) growth. A shows control with no oil and B growth beneath the surface film of oil (PMA — potato marmite agar).

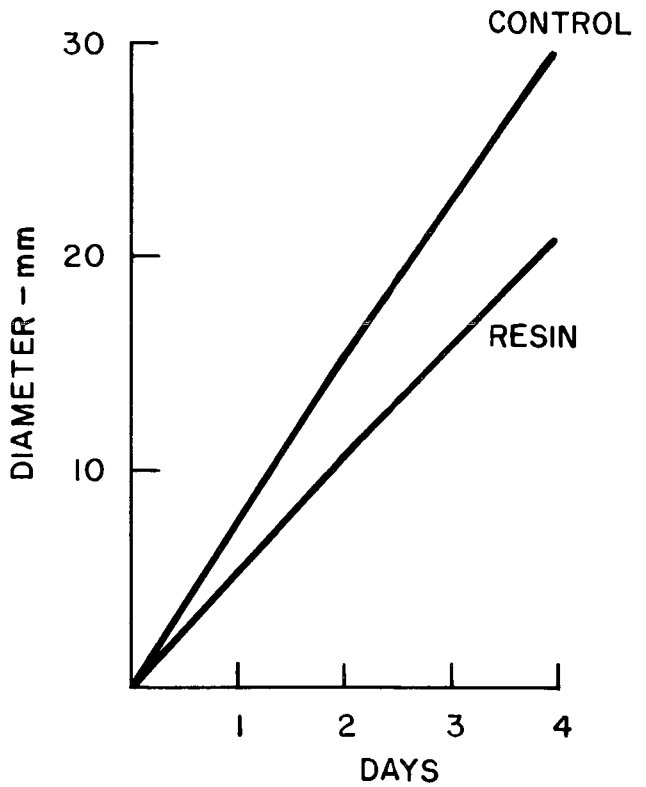

Figure 3. Linear growth of *C. montia* on a medium containing an oily wood extract. The values given are the average for three cultures.

C. montia and *E. clavigerum* were inoculated onto the medium and grown as described previously (Shrimpton and Whitney, 1968). Three cultures of each fungus were grown on PMA containing the oil and three on PMA alone. Cultures were kept at 25° C, examined daily until fully grown, measured 2 and 4 days after inoculation, and photographed after 5 days.

In the absence of oil, both species of fungi grew on the surface of the medium and developed a dense mat of aerial hyphae a few millimeters behind the advancing hyphal tips. On plates containing oil,

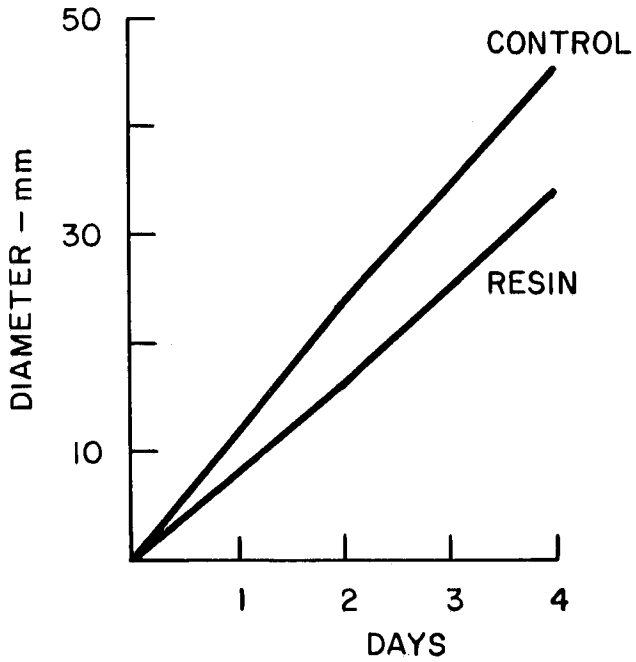

Figure 4. Linear growth of *E. clavigerum* on a medium containing an oily wood extract. The values given are the average for three cultures.

however, the advancing hyphae of both species grew in the medium just beneath the surface film of oil (Figs. 1 and 2) and avoided the embedded zones of oil. About 5-10 mm behind the advancing hyphal front, hyphae grew up through the groups of small holes that broke the otherwise continuous oil layer on the surface. Further behind the hyphal front, hyphae emerging from the small holes gradually developed a dense aerial mat and spread into the oil-containing areas within the medium. Linear growth of *C. montia* is shown in Fig. 3 and of *E. clavigerum* in Fig. 4.

Even though the advancing margin of hyphal growth was in areas of the agar with no apparent oil content, there was an effect from the oil; growth rates were lessened. The advancing hyphae were prevented from growing on the agar surface by the surface film of oil and avoided the oil-rich zones within the medium. However, later growth into the oil-containing areas shows that these oily extractives are not a permanent barrier to hyphal growth. Our observation that oil-rich areas in the medium are colonized after the fungi are in direct contact with the atmosphere suggests that an increase in oxygen is required if hyphae are to grow in contact with the resinous substances produced by lodgepole pine in response to wounding. The growth inhibition, the tendency for hyphae to avoid resins, and the possibly increased requirement for oxygen before growth through oil-containing areas may explain why fungi can be isolated from resin-soaked tree tissues but grow slowly through such tissue.—D.M. Shrimpton and H.S. Whitney, Pacific Forest Research Centre, Victoria, B.C.

A Survey of Ontario Forestry Nurseries for the Presence of *Cylindrocladium floridanum*.—*Cylindrocladium floridanum* Sob. and Seymour, a cause of root rot in forestry nurseries, was found for the first time in Ontario in 1974 (Myren et al., Bi-mon Res. Notes 31:34, 1975). The fungus was isolated from the roots of black spruce (*Picea mariana* [Mill.] B.S.P.) seedlings grown at the Provincial Forest Nursery at Midhurst, Ont. The Forest Insect and Disease Survey Unit of the Great Lakes Forest Research Centre subsequently conducted surveys in a number of forest nurseries in Ontario to determine the distribution of this pathogen.

Soil samples were taken from four nurseries in 1975 and from six in 1976. In the larger nurseries, nine soil samples were collected from each of 15 randomly selected compartments and from up to 14 additional compartments if seedling mortality was present. At small nurseries, all compartments were sampled. The soil was tested for the presence of *C. floridanum* by means of the spot plate technique (Thies and Patton, Phytopathology 56:1116-1117, 1966). The results of the survey are presented in Table 1.

TABLE 1
Compartments yielding *Cylindrocladium floridanum* from soil samples collected at 10 forestry nurseries in Ontario

Nursery location	Compartments sampled (no.)	Compartments yielding *C. floridanum* (%)
Chapleau	6	0
Dryden	17	0
Gogama	5	0
Kemptville	23	13
Longlac[1]	9	0
Midhurst	29	14
Orono	28	4
St. Williams	25	20
Swastika	17	0
Thunder Bay	18	0

[1]Nursery owned by Kimberly-Clark of Canada Ltd.

Cylindrocladium floridanum was found in the four nurseries of southern Ontario — at Kemptville, Midhurst, Orono, and St. Williams — but was not found in any of the six northern nurseries. Although the survey failed to reveal *C. floridanum* in the northern nurseries, it was subsequently isolated from roots of declining black spruce from nurseries at Thunder Bay and Kirkwood (east of Sault Ste. Marie). The latter northern nursery was not included in the general survey. Also, after the survey, *C. floridanum* was found in the Provincial Forest Nursery at Kemptville in a compartment in which it had not been found in the original study. In addition, it was isolated from roots of eastern white pine (*Pinus strobus* L.) and eastern white cedar (*Thuja occidentalis* L.) from the Provincial Forest Nursery at St. Williams.

Thus far, damage caused by this fungus in Ontario forestry nurseries has been fairly light. Results of the special survey indicate that *C. floridanum* is present in southern Ontario nurseries, albeit at fairly low population levels. Failure to detect the fungus in soil samples from the northern nurseries indicated either its absence or a population level too low to detect with the survey technique used.—D.T. Myren, H.L. Gross, and E.B. Dorworth, Great Lakes Forest Research Centre, Sault Ste. Marie, Ont.

SILVICULTURE

Effect of Seed Weight and Germination Rate on the Initial Growth of Japanese Larch.—Recent interest in the potential of *Larix* species for high yield, short rotation crops has led to trials of various populations of Japanese larch, *Larix leptolepis* (Sieb. and Zucc.) Gordon. In 1978, a field test of seed from 88 sources was initiated at Petawawa National Forestry Institute in cooperation with the Ontario Ministry of Natural Resources. A complementary controlled-environment test revealed wide variation in initial size of seedlings. This report examines the relationship of seed weight and germination rate (time required for germination) to seedling size in the first few weeks of growth.

Twelve seeds of each of the 88 seedlots under investigation were weighed individually, stratified for 32 days at 2°C, and sown in BC/CFS Styroblock 2 containers (one seed per cavity) filled with a 3:1 mixture of peat and vermiculite. The Styroblocks were placed in a greenhouse at 18-25°C and soaked three times daily with Ingestad's nutrient solution (Ingestad, pages 265-269 *in* Proc. XIV IUFRO Congress, München III, 1967). Supplementary fluorescent lighting provided a 16-h photoperiod.

Germination began on the fourth day and was recorded daily for 6 wk. The rate of germination was expressed as days from sowing, and was subsequently used to determine individual seedling age. The population was sampled in two ways: for one sample, a single seedling