Inoculation of Jack Pine with a Concentrated Basidiospore Spray of *Cronartium quercuum* f. sp. *banksianae*

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ABSTRACT

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A concentrated basidiospore spray was used to inoculate *Pinus banksiana* with *Cronartium quercuum* f. sp. *banksianae*. Purple stem lesions were observed 2.5 wk after inoculation and galls were observed 14 wk later. Histological examination of symptomatic seedlings 20 and 80 days after inoculation showed intercellular hyphae and abnormal and occluded cortical and xylary cells. This technique appears applicable for large-scale screening of jack pine for resistance to pine-oak rust.

Jack pine (*Pinus banksiana* Lamb.) is an important commercial species in the Lake States. A major disease of jack pine in this region is pine-oak rust, caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *banksianae* Burdsall & Snow (2). In a study of 30 jack pine seed sources in three Minnesota plantations, Hodson et al (4) found stem galls in 61% of the trees in one plantation. Of these, 36% had died by age 25. In two plantations, pine-oak rust was the largest single cause of mortality. In Lake States nurseries, as many as 60% of 2-yr-old seedlings had stem galls in some years and were culled (16). Effective control measures have not yet been developed.

Hodson et al (4) found significant variation in resistance to *C. quercuum* f. sp. *banksianae* among jack pine populations from 30 seed sources in the Lake States when trees were field-tested in high-hazard areas. Thus, selection and breeding of jack pine for resistance to pine-oak rust may be possible. Investigations of fusiform rust (*C. quercuum* f. sp. *fusiforme* Burdsall & Snow) on southern pines have shown that it is possible to select genetically resistant trees (8,9,12,15,18). The concentrated basidiospore spray (CBS) technique used to screen for fusiform rust resistance (11) may have potential for use with pine-oak rust. With each rust, purple stem lesions are visible on infected pine seedlings within 6 wk of inoculation, and galls can be seen after several months (1,11). Previous investigations have demonstrated the formation of purple lesions for both susceptible and resistant reactions (14,17). Susceptible reactions can be identified by holding the seedlings for 6–9 mo or by histological examination of lesions 3–4 wk after inoculation. This research was done to determine whether the CBS technique could be used to infect jack pine seedlings with *C. quercuum* f. sp. *banksianae*.

MATERIALS AND METHODS

Aeciospores were collected in June 1982 from three locations in Minnesota and stored for 1 yr in test tubes at 4 C. Greenhouse-grown northern red oak (*Quercus rubra* L.) seedlings (4 wk to 1 yr old, with leaves 4–14 days old) were inoculated with the aeciospores. The undersides of leaves were inoculated either by brushing on the dry aeciospores with a fine-bristled brush or by spraying with a water suspension. After inoculation, potted oaks were watered, sealed in plastic bags, and placed in a chamber at

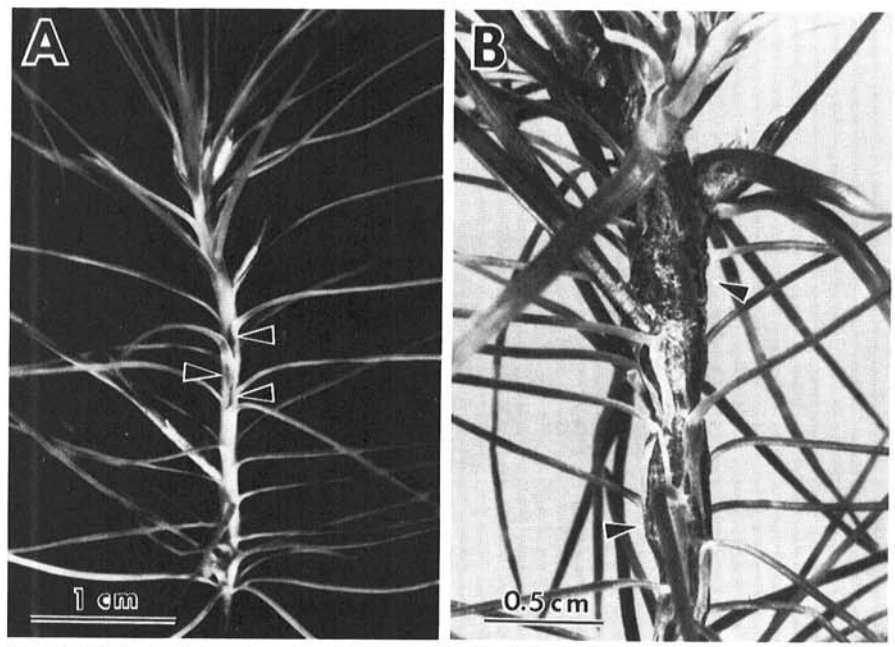

Fig. 1. (A) Purple lesion (arrow) on jack pine seedling 2.5 wk after inoculation with *Cronartium quercuum* f. sp. *banksianae*. **(B)** Galls (arrows) on jack pine seedling 14 wk after inoculation.

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18 C and relative humidity greater than 95% for 48 hr. After incubation, seedlings were removed from the bags and placed on greenhouse benches at 21–28 C.

Urediospores were produced on most oak leaves 1 wk after inoculation. Telia began to form after 9–10 days and were allowed to elongate for 2–3 wk before basidiospore collection. The procedure for collection of basidiospores was similar to that of Matthews and Rowan (11). Oak leaves were stapled to filter paper disks that were moistened and placed on the undersides of petri dish covers over distilled water adjusted to pH 3.0 with 1 N HCl. The petri dishes were incubated in darkness for 48 hr. Cast basidiospores were concentrated on Millipore filter disks and stored at 5 C in petri dishes lined with moistened filter paper (12).

The jack pine seed used in these tests was a commercial bulk lot selected for rapid growth from a seed orchard in western Minnesota. For experiment 1, five flats of 30 seedlings per flat were inoculated 12 wk after planting. Inoculum was prepared immediately before inoculation by mixing stored basidiospores with distilled water to a concentration of 3×10^5 per milliliter as determined with a hemacytometer. One flat of 30 seedlings was included as an unsprayed control. A Power Pak aerosol sprayer was used to distribute 15 ml of the basidiospore suspension over the seedlings in each flat. Each inoculated flat was placed in a plastic bag that was misted with water, sealed, and placed in a chamber at 15 C

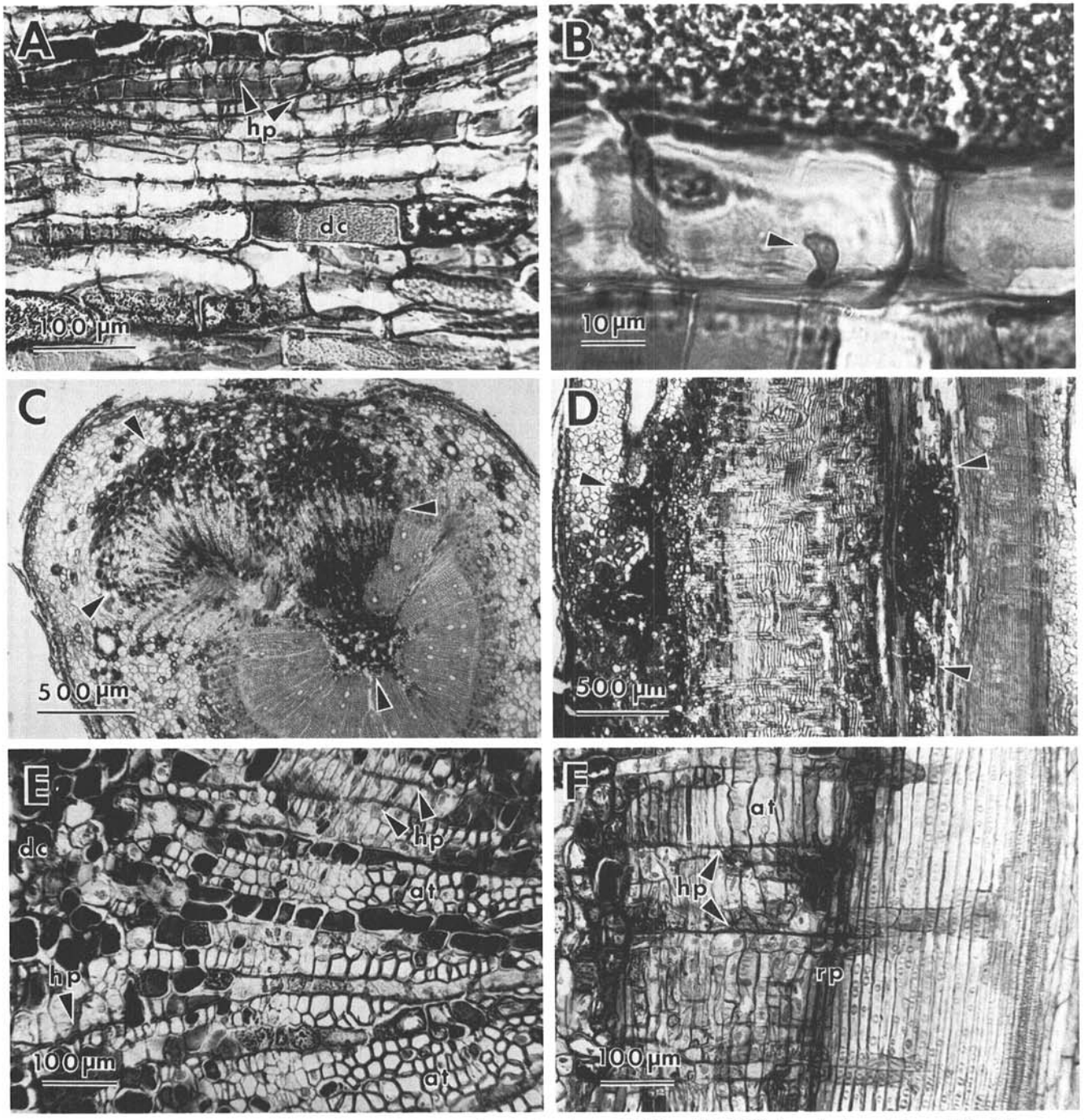

Fig. 2. Histological sections of infected tissue from jack pine seedlings collected **(A and B)** 20 and **(C–F)** 80 days after inoculation with basidiospores of *Cronartium quercuum* f. sp. *banksianae*. **(A)** Radial section with distorted cortical cells (dc) and hyphae (hp) spreading through the cortex. **(B)** Normal haustorium (arrow) in a cortical cell. **(C and D)** Transverse and radial sections, respectively, with infection and host response from cortex cells to the pith (arrows delimit infected area). **(E)** Transverse section with distorted cortical cells (dc), abnormal trachieds (at), and hyphae (hp) in the cortex and along ray parenchyma cells in the xylem. **(F)** Radial section from the margin of infected xylem with reaction parenchyma (rp), abnormal trachieds (at), and hyphae (hp) along ray parenchyma cells.

Table 1. Percentage (±SE) of jack pine seedlings infected[a] 2.5, 14, and 33 wk after inoculation with *Cronartium quercuum* f. sp. *banksianae*

Experiment	Lesions (2.5 wk)	All symptoms (14 wk)	Galls (14 wk)	Galls (33 wk)
1	74.7 ± 9.6	76.7 ± 10.3	64.7 ± 11.7	63.5 ± 12.5
2	73.8 ± 4.2	94.6 ± 6.9	80.0 ± 10.9	83.2 ± 9.5

[a] Percentage infected is computed by averaging the percentages in five flats within an experiment, all flats weighted equally.

and relative humidity greater than 95% for 24 hr. Flats were then removed from the plastic bags and placed on a greenhouse bench where temperatures ranged from 21–32 C. For experiment 2, an additional set of five flats of seedlings from the same seed source was inoculated as before with freshly prepared inoculum 11 wk after planting.

Seedlings with respresentative symptoms were selected for histological examination 20 and 80 days after inoculation. Seedlings were fixed in formalin-acetic acid-alcohol and dehydrated with tertiary butyl alcohol (7). The material was embedded in paraffin and sectioned serially at 12–15 μ on a rotary microtome. Sections were stained with periodic acid-Schiff reagent (3) and mounted in Permount.

RESULTS

Purple lesions (Fig. 1A) occurred on 74.7% of inoculated seedlings in experiment 1 and on 73.8% of those in experiment 2 within 2.5 wk of inoculation (Table 1). Lesions ranged from 1 to 10 mm long and occupied up to one-third of the stem circumference. Occasional lesions were observed on needles.

After 14 wk, the percentage of seedlings with lesions increased to 76.7% in experiment 1 and 94.6% in experiment 2. In addition to lesions, 64.7% of the seedlings in experiment 1 and 80.3% of the seedlings in experiment 2 had galls (Fig. 1B). Galls usually occurred on seedlings that had lesions after 2.5 wk and purple lesions were still visible on or surrounding the galls. Twelve percent of the seedlings in experiment 1 and 14.2% in experiment 2 showed lesions but no galls, and 23.3 and 5.4%, respectively, were asymptomatic. The number of seedlings with galls at 33 wk was not significantly different from the number observed at 14 wk (Table 1).

Experiment 2 had higher rates of infection at 14 and 33 wk, more evenly distributed infection within flats, and less variability between flats than experiment 1. In experiment 2, there were no more than two contiguous uninfected seedlings, whereas in experiment 1, there were as many as 11 in one flat of 30. The ends and edges of flats in experiment 1 had an unusually high proportion of uninfected seedlings.

Histological examination of seedlings with purple lesions collected 20 days after inoculation showed intercellular hyphae in the cortex (Fig. 2A). Cortical cells were enlarged and often occluded with dark staining material. Haustoria, in parenchyma cells of infected tissue, appeared normal, not encrusted, distorted, or granular (Fig. 2B).

Sections from seedlings collected 80 days after inoculation had extensive hyphal growth and host response from the cortex to the pith (Fig. 2C,D). Hyphae were present among ray parenchyma cells in the xylem (Fig. 2E,F). The area of infection in the xylem was bordered by occluded vertical parenchyma cells, or reaction parenchyma (6). Trachieds were shorter and wider and rays were more numerous in infected xylem than in uninfected xylem. Cells of infected pith were enlarged and occluded.

DISCUSSION

The CBS technique, modified slightly from that used for fusiform rust (11), was used successfully to infect jack pine seedlings with *C. quercuum* f. sp. *banksianae*. Early symptoms were similar to those of pine-oak rust on nursery seedlings (1) and also similar to fusiform rust on slash and loblolly pines. Purple lesions were visible on stems about 18 days after inoculation, and histological examination revealed that they resulted from infection and colonization. Needle lesions, as reported by Lundquist and Luttrell (10), were observed occasionally. Most infections developed into galls within 33 wk. A small percentage of lesions, however, did not develop into galls. Miller et al (14) demonstrated that red pigment production may also be produced as a resistance response. In our study, 12–14% of seedlings showed red pigmentation without subsequent gall formation. This may have resulted from a resistance response in some of the seedlings resulting from genetic variability in the bulk seed source. Additional studies are needed to ascertain the relationship between various types of early symptom expression and subsequent gall formation for pine-oak rust on jack pine.

Colonization of jack pine seedlings by the pine-oak rust fungus and subsequent host response were similar to those described for slash pine infected with fusiform rust (5,6,13,14). Extensive mycelial growth and haustorial development, distorted and occluded cortical cells, abnormal rays, trachieds and pith cells, and reaction parenchyma occur in both host-pathogen combinations. In fusiform rust of slash pine, some types of resistant reactions resulted in stem lesions that were superficially identical to those resulting from a susceptible reaction (14). These hypersensitive-type reactions were characterized by the formation of a periderm (17) that limited hyphae to the cortex near the point of infection. Haustoria were present in these infection zones but were encrusted, distorted, or granular (14). In our study, no periderm limited the spread of rust mycelium in any of the seedlings examined, and all haustoria appeared normal.

The CBS technique appears suitable for large-scale screening of jack pine for resistance to pine-oak rust. With increasing intensity of jack pine planting and management in the Lake States, screening for rust resistance may have a major impact on commercial production.

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