

Resistant Responses in Loblolly Pine to Spray and Injection Inoculations with *Cronartium quercuum* f. sp. *fusiforme*

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ABSTRACT

Kuhlman, E. G., Powers, H. R., and Pepper, W. D. 1990. Resistant responses in loblolly pine to spray and injection inoculations with *Cronartium quercuum* f. sp. *fusiforme*. Plant Dis. 74:1013-1015.

The responses of 6-wk-old loblolly pine seedlings from resistant families to concentrated basidiospore spray (CBS) system inoculations with *Cronartium quercuum* f. sp. *fusiforme* indicated the relative susceptibility of branches of those seedlings at age 2 yr to hypodermic inoculations with basidiospores. Seedlings with no stem symptoms or with symnos (purple stem spots with no stem swelling) after CBS inoculations were most resistant to hypodermic inoculations. Seedlings with no stem symptoms could be considered disease escapes, but they were as resistant to hypodermic inoculations as those with symnos. Seedlings with small or large galls after CBS inoculations were equally susceptible to hypodermic inoculations. Infection increased with an increasing inoculum density from 3×10^3 to 3×10^5 basidiospores per milliliter. However, an inoculum density of 3×10^6 basidiospores did not produce more infections of susceptible loblolly pines nor more frequent aecial sporulation than did 3×10^5 spores. Aecia occurred equally frequently in the 3×10^5 and 3×10^6 treatments but were more frequent after 2 yr than 1 yr.

Additional keywords: fusiform rust

Seedlings of loblolly pine (*Pinus taeda* L.) display a variety of symptoms after concentrated basidiospore spray (CBS) system inoculations with *Cronar-*

tium quercuum (Berk.) Miyabe ex Shirai f. sp. *fusiforme* (6). Susceptible seedlings develop galls. Resistant seedlings may develop symnos (purple stem spots with no stem swelling), purple needle bases, or no stem symptoms (needle spots as the only symptom or symptomless). Recently, Kuhlman (5) reported that all loblolly seedlings with no stem symptoms and most seedlings with symnos 9 mo

after inoculation still had no galls 24 mo after inoculation. About two-thirds of seedlings with small galls (<10 mm long) at 9 mo were symptomless at 24 mo, whereas most seedlings with large galls (>10 mm long) were still galled at 24 mo. Previously, Griggs et al (2) reported similar results for slash pine (*P. elliottii* Engelm. var. *elliottii*).

The susceptibility of loblolly seedlings in various symptom groups were tested with a hypodermic inoculation procedure (3,8) following CBS inoculations. Hypodermic inoculations bypass any resistance mechanisms at the stem surface, providing a severe test for resistance. In the greenhouse, 90% of 544 slash pine stems injected directly with basidiospores formed typical stem galls of fusiform rust, whereas no galls developed on 50 control plants (8). The objectives of this experiment were to determine if new growth flushes on loblolly pines in the field could be successfully inoculated by injection of basidiospores, if seedlings with different symptom types after CBS inoculations reacted differently to injection inoculations, and how inoculum concentration affected disease development. A second experiment was designed to determine the effect of inoculum con-

Accepted for publication 18 June 1990.

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centration on infection and sporulation of the rust as a means for increasing specific rust phenotypes. A higher inoculum density was used to determine if more frequent infection and sporulation occurred.

MATERIALS AND METHODS

Effect of symptom type. Open-pollinated loblolly pine seedlings from 156 putatively resistant families were inoculated in 1984 in the CBS system (6). Nine months after inoculation, seedlings from resistant families were separated into groups based on symptoms and established in row plots in raised nursery beds near Athens, Georgia (5). For the present study, four symptom types were recognized: no stem spots (either symptomless, needle spots only, or purple needle bases) with 65 seedlings; symnos (purple stem spot with no swelling) with 66 seedlings; small galls (<10 mm long 9 mo after inoculation) with 90 seedlings; and large galls (>10 mm long) with 67 seedlings. On 7 May 1986, these seedlings were approximately 2 yr old with a 15–30-cm-long first flush of growth. The needles on these shoots were about half their mature length.

Aeciospores collected in 1974 from several galls on loblolly pines in Clarke County, Georgia (composite collection 2-74), were used to inoculate immature leaves of northern red oak (*Quercus rubra* L.) (7). Basidiospores were harvested from telia 3 wk after aeciospore inoculation. Basidiospore concentrations in water were adjusted to inoculum densities (ID) of 3×10^3 , 3×10^4 , and 3×10^5 spores per milliliter with a Coulter particle counter (Coulter Electronics, Inc. Hialeah, FL). Germination of these basidiospores on 1.5% agar was 97%.

Three lateral branches on each seedling were inoculated with each branch randomly assigned to one of the three IDs. Basidiospores were injected within 10 cm of the distal end of the young

shoot. Inoculum (0.1 ml) was injected with a No. 27 hypodermic needle to form a water-soaked area in the cortical tissue. The entire nursery bed of seedlings was covered with shade cloth for 1 wk after inoculation. Temperatures during this week ranged from 18 to 29 C. Approximately one-half of the seedlings were removed in June 1987 to reduce crowding.

Data on the presence of galls and aecial sporulation were taken after 1 and 2 yr (May 1987 and 1988). Presence or absence of a gall on each branch was recorded as a dichotomous variable. This resulted in three correlated responses for each seedling. Thus, inoculum density was treated as a repeated measure factor and analyzed accordingly to account for correlation. SAS procedure CATMOD was used to fit a linear model to functions of categorical response frequencies. Independent variables were symptom type and ID (10). Sample proportions were used to compute test statistics asymptotically distributed as chi-square variables. The null hypothesis for the main effect of symptom type and ID was that marginal proportions of these main effects were homogeneous, implying that response frequencies were not dependent on these factors. When main effects were statistically significant, contrasts among all possible factor levels were tested.

Effect of inoculum concentration on susceptible saplings. Saplings in a 3-yr-old stand of silviculturally improved loblolly pine (rust susceptible) growing in Baldwin County, Georgia, were selected for this test. The trees were severely infested with tip moth (*Rhyacionia* spp.) but symptoms of fusiform rust were infrequent. Sixty trees in each of four adjacent rows were selected on 21 May 1987. Trees selected for inoculation had at least four non-tip moth infested branchlets with good vigor and the crown position to assure continued rapid growth. Selected branches were at a height of 1.2–2.1 m. All inoculations were into the second flush of growth, which had needles less than 1.2 cm long. Some shoots had just started to elongate.

Table 2. Gall development and aecial formation 2 yr after injection inoculations of branches of 3-yr-old, rust-susceptible loblolly pine saplings^y in Baldwin County, GA

Inoculum density	Branches with galls (%)	Galls with aecia (%)
3×10^4	30 b ^z	20
3×10^5	51 a	36
3×10^6	55 a	36

^y Number of saplings = 240. Saplings were not previously inoculated.

^z Data for 3×10^3 ID were not included in the analysis. Increasing ID from the intermediate to the highest level had no significant effect ($P = 0.2852$). However, the other two contrasts were significant ($P < 0.017$), providing an upper limit of 0.05 on experimentwise error rate (11).

Injections were made with a No. 22 hypodermic needle inserted roughly parallel to the long axis of the branch for approximately 1 cm. Each of the four branches received about 0.1 ml of inoculum in the wound.

In order to obtain enough basidiospores, inoculum came from five aeciospore sources: two composite collections from Clarke County (1-73 and 2-74); a composite collection from Houston County (Houston); and collections composed of either multiple fusoid (collection 861) or globose (collection 862) galls on separate trees in Barnwell County, South Carolina. Roughly equal quantities of basidiospores were produced from each aeciospore collection and combined into a five-source composite.

Four IDs were used: 3×10^3 , 3×10^4 , 3×10^5 , and 3×10^6 spores per milliliter. Four lateral branches on each sapling were inoculated with each branch receiving a different ID, according to a random assignment. Temperatures ranged from 21 to 30 C on 21 and 22 May 1987 when inoculations were made.

Data on the presence of galls and aecial sporulation were taken after 2 yr in May 1989. The dichotomous variable, gall present or absent, was defined for each of the four lateral branches. As in the symptom type study, these four responses were treated as repeated measures and analyzed accordingly. SAS procedure CATMOD was used to fit a linear model with one independent variable (ID) to functions of categorical response frequencies (10).

RESULTS AND DISCUSSION

Symptom type. One year after injection inoculations, the presence of galls was dependent on both ID and the earlier symptom type in the CBS inoculations. Preliminary computations were performed to obtain proportions of seedlings with galls in a two-way contingency table of symptom type \times ID. The proportion with galls in the 3×10^3 treatment was so small (<10%) that the data did not meet the requirements for the CATMOD analysis with it included. Accordingly, the linear model fit by SAS procedure CATMOD contained symptom type with four levels, ID with two levels, and symptom type \times ID interaction. Chi-square tests on 1 yr of data showed that interaction was nonsignificant ($P > 0.05$), but main effects were both highly significant ($P < 0.001$). Seedlings with galls in the CBS inoculation were most susceptible to infection from injection inoculations (Table 1). Among seedlings with large galls after CBS inoculations, 63% developed galls on at least one branch, whereas 46% of those with small galls after CBS developed galls on at least one branch. Seedlings with symnos or no stem symptoms after CBS inoculations were more resistant to the injection inoculation.

Table 1. Fusiform rust gall development on at least one branch 1 yr after injection inoculation of basidiospores at two inoculum densities^y into branches of 2-yr-old loblolly pine seedlings that had been inoculated with the concentrated basidiospore spray (CBS) at age 4 wk

Response to CBS inoculation	Seedlings inoculated (no.)	Seedlings with galls (%)
No stem symptoms	65	25 a ^z
Symnos	66	30 ab
Small galls	90	46 bc
Large galls	67	63 c

^y Data for an inoculum density of 3×10^3 basidiospores per milliliter not included.

^z Proportions with the same letters are not significantly different at the 0.0085 level, which provides an upper bound of 0.05 for the experimentwise error rate (11).

Results confirm that some responses of seedlings to CBS inoculations are strong indicators of their resistance. Previous work has shown that slash pine seedlings without galls after inoculation in the greenhouse have less infection in the field than do noninoculated seedlings (1). Also, loblolly and slash pine seedlings without galls after CBS inoculations from more resistant families are less frequently infected in the field than are similar seedlings from less resistant families (9). We thought seedlings with no stem symptoms would include many disease escapes, whereas all seedlings with symnos had resisted invasion by the fungus. Our data indicate that disease escapes were not more frequent in the no stem symptom group, because seedlings with no stem symptoms and those with symnos were equally resistant to infection from hypodermic injections. In the injection inoculations at the lower ID, the lower rate of infection may be attributable in part to the reduced probability of having rust genotypes with virulence capable of overcoming resistance as suggested by Griggs et al (2) for slash pine. Field tests of the relative resistance of seedlings with various symptoms after CBS inoculation are currently under way.

The highest inoculum density (3×10^5) produced the highest average frequency

of galls, 35%, compared to 19% at 3×10^4 and 9% at 3×10^3 . At the highest ID, aecia occurred on 17% of the galls 1 yr after inoculation. One year later, the percentage of seedlings with galls at the highest ID increased slightly to 43%, and the proportion of galls with aecia almost doubled (33%). There was no effect of CBS symptom type on aecial sporulation. Hedgcock and Siggers (4) reported that some infections produced aecia within 1 yr but that, more often, 2 yr were required. The relatively rapid production of aecia suggested that injection inoculations at higher IDs might facilitate propagation of unique genomes.

Inoculum concentration. An ID of 3×10^6 basidiospores did not increase the percentage of branches with galls or of galls with aecia above that produced by 3×10^5 basidiospores even in a susceptible pine population (Table 2). The 3×10^3 ID produced so few galls (<6%) that it was dropped from the analysis. At the lowest ID, infection and sporulation were similar to those in the previous experiment.

We thought injection inoculations might be useful for producing aeciospores of isolates with unique virulence characteristics for preservation. However, even at the highest ID, the galls produced were relatively small (<8 cm

long) and aecial sporulation occurred on less than half of the galls after 2 yr.

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