Testing of Pinus pinea and P. pinaster Progenies for Resistance to Cronartium flaccidum

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ABSTRACT


Seedlings of 33 half-sib maritime pine (Pinus pinaster) families and 40 Italian stone pine (P. pinea) families were artificially inoculated with basidiospores of Cronartium flaccidum produced on rusted white swallowwort leaves. Italian stone pine was highly susceptible; 99.9% of tested seedlings had needle spot symptoms of the blister rust disease. No rust resistance was found in this species; 93% of the seedlings died. Maritime pine was less susceptible than the first species. Significant differences were observed among families for the amount of infection of the needles and for survival of seedlings with needle spots. In as many as 22% of the spotted seedlings the pathogen failed to become established in the stem. The heritability values for the needle infection character (0.124 ± 0.039) and the survival of "spotted-only" seedlings (0.156 ± 0.046) suggested that a breeding program for resistance of maritime pine to blister rust is feasible.

Additional key words: resistance mechanisms.

Blister rust of two-needle pines is caused by Cronartium flaccidum (Alb. et Schw.) Wint. and is widespread in all the European countries within the ranges of Pinus sylvestris L., P. pinaster Ait., P. nigra Arnold, P. pinea L., P. mugo Turra, and P. halepensis Mill. All are the hosts of this fungus (11). In central and southern Italy in the past 20 yr epidemic outbreaks of this disease have caused heavy damages in plantations of Italian stone pine (P. pinea L.), maritime pine (P. pinaster Ait.), and Austrian pine (P. nigra Arnold) (11). In response to increasing need for resistant planting stocks, breeding programs are in progress in our laboratory with Italian stone pine and maritime pine. In a trial to determine the relative susceptibilities of several European and American Pinus spp., the two- and three-needled American species had a very high degree of resistance to blister rust (12). Comparison of rust fungus isolates from three sources from different localities and tested for percentage of diseased seedlings and rates of disease development revealed variation in pathogenicity of C. flaccidum (10).

The primary objective of this study was to test the incidence and variation of the disease among families of maritime pine and Italian stone pine to determine the feasibility of developing a breeding program for resistance to blister rust.

MATERIALS AND METHODS

In 1973, 32 rust-free maritime pines and 39 rust-free Italian stone pines were selected as parents in heavily rust-infected stands. In addition to the rust-free parental trees, one susceptible tree was only observed among families for the amount of infection of the needles and for survival of seedlings with needle spots. In as many as 22% of the spotted seedlings the pathogen failed to become established in the stem. The heritability values for the needle infection character (0.124 ± 0.039) and the survival of "spotted-only" seedlings (0.156 ± 0.046) suggested that a breeding program for resistance of maritime pine to blister rust is feasible.

Additional key words: resistance mechanisms.

More replicates of each species were established in which the seedlings were randomized completely on an individual basis. In all, the experiment contained 6,600 seedlings of Italian stone pine (40 families × 11 replicates × 15 seedlings per replicate) and 5,280 seedlings of maritime pine (33 families × 10 replicates × 16 seedlings per replicate). Naturally infected leaves of white swallowwort (Vincetoxicum officinale Moench., the alternate host) were collected at San Rossore, in the habitat of the Italian stone pine. In the artificial inoculations, 15 infected leaves of white swallowwort bearing telia of the rust fungus were attached to cellophane tape placed 20 cm above the seedlings across the nursery bed on each row. Inoculations were made in July, August, and September of 1974. This was during the second growing season of the seedlings. At this time, Italian stone pine had only primary needles, and maritime pine had primary and secondary needles. The inoculated seedlings were kept for 1 wk under cool, moist conditions provided by a large, shaded polyethylene inoculation tent that covered each bed. As a control, 600 Italian stone pine seedlings and 528 maritime pine seedlings growing in a nursery bed within the inoculation tent were not inoculated.

Individual seedlings were inspected for the presence of needle spots during autumn 1974 and winter 1975, and for the presence of spermatonia and aecia during the 3 yr following inoculation. Moreover, 3 yr after inoculation data were taken on living seedlings which presented rust spots on the needles. They were subdivided in two classes: without fungus fruiting bodies on the stem ("spotted-only") and with fungus fruiting bodies on the stem.

Data for each family in this test are presented in the following categories: the total inoculated seedlings and the spotted seedlings; spotted and stem-diseased seedlings; spotted and infected but still alive, or "spotted-only" seedlings. If the value of the numerator of the proportion was 0 or equal to the denominator, adjustments were made according to Bartlett (1). The percentages data were arcsin-transformed √ % and family means were compared by LSD. test at P = 0.05 and 0.01. Heritability estimates for "all or nothing" traits present problems different than those involved in the calculation of the more common quantitative traits. We followed the method of calculating heritability, and also a rough estimate of its standard error, as proposed by Robertson and Lerner (14) and by Dempster and Lerner (2). This estimate includes a proportion of nonadditive variance; however, according to these
authors, the agreement between heritability (\(h^2_p\)) computed using their method and the narrow-sense heritability (\(h^2_n\)) is reasonably good.

### RESULTS

**Italian stone pine.** Infections, as indicated by the presence of spots on needles, took place on 99.93% of the Italian stone pine seedlings (Table 1). No apparent differences were detected among the three inoculation runs, families, or blocks. On 91% of the seedlings the fungus completed its life cycle as indicated by the occurrence of spermagonia and aecia. The remaining 9% died in the first stages of the development of the infection before spermagonia appeared. Only 7% of the seedlings were still alive and producing fruiting bodies of the fungus 3 yr after the inoculation. Probably these will die within the next few years. In the control bed only one seedling was infected.

The heritability (0.031 ± 0.004) for the development of the fungus with rust fruiting bodies was very low. The heritability for the survival of fruiting bodies 3 yr after the inoculation was 0.081 ± 0.002.

**Maritime pine.** Needle spots occurred on 83.36% of the inoculated maritime pine seedlings (Table 2). There were significant differences (\(P = 0.05\)) among families and inoculation runs.

### TABLE 1. Symptom development in Italian stone pine (Pinus pinea L.) seedlings inoculated with the blister rust fungus, Cronartium flaccidum

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Seedlings (no.)</th>
<th>Seedlings per family (no.)</th>
<th>Symptoms (%)</th>
<th>Heritability ((h^2))</th>
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<tbody>
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<td>-inoculated</td>
<td>5,985</td>
<td>148–150</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td>-needles spotted</td>
<td>5,981</td>
<td>148–150</td>
<td>99.93</td>
<td></td>
</tr>
<tr>
<td>-fruited bodies</td>
<td>5,440</td>
<td>125–143</td>
<td>90.95</td>
<td>0.031 ± 0.004</td>
</tr>
<tr>
<td>-alive 3 yr postinoculation</td>
<td>419</td>
<td>3–23</td>
<td>7.00</td>
<td>0.081 ± 0.002</td>
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*Forty half-sib families were tested.

### TABLE 2. Results of inoculation of maritime pine (Pinus pinaster Ait.) seedlings with the blister rust fungus, Cronartium flaccidum

<table>
<thead>
<tr>
<th>Rank</th>
<th>Family no.</th>
<th>Inoculated</th>
<th>Spotted</th>
<th>Arcsin ((%))</th>
<th>Family no.</th>
<th>Spotted-only</th>
<th>Arcsin ((%))</th>
<th>Family no.</th>
<th>With fungus fruiting bodies</th>
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Totals: 4,587 3,824 (83.36%) 2,399

Spotted seedlings (%) 100  62.73

LSD \(P = 0.05\) 9.50  9.80

\(F_{\text{families}}\) 2.45**  2.68**

\(F_{\text{runs}}\) 11.95**  9.14**

\(F_{\text{families} \times \text{runs}}\) ns

\(\hat{h}^2_p\) 0.124 ± 0.039

\(\hat{h}^2_n\) 0.156 ± 0.046

*ns = not statistically significant and ** = statistically significant F-values, \(P = 0.01\).
runs. The heritability for the presence of spots was 0.124 ± 0.039. The number of seedlings that produced fruiting bodies of fungus among those with needle spots also was significantly different according to families and to inoculation runs. A certain number of spotted seedlings (22%) did not show spermagonia or aecia in the 3 yr during which they were observed, and apparently were healthy at the end of that time. The number of “spotted-only” seedlings was significantly different among families, but not among inoculation runs. The heritability for this trait was 0.156 ± 0.046. On the other hand, several seedlings that did not show needle spots developed fungus fruiting bodies during the 3 yr. Therefore, the evaluation for disease incidence was conservative, and perhaps more than 22% of seedlings were able to survive the infection without fungus fruiting bodies on the stem. Three years after inoculation, 30% of the spotted seedlings with active cankers were still alive. There were significant differences for this character among inoculation runs but not among families. It is likely that the majority of these cankered seedlings will die. Therefore, it is too early to draw conclusions about the presence of tolerance to the disease or of recovery from cankerling. None of the control seedlings became infected.

**DISCUSSION**

**Italian stone pine.** It is apparent that seedlings of this species at the primary needle stage are highly susceptible to the blister rust fungus under conditions of artificial inoculation. Practically all the seedlings became infected; without any evidence of host resistance to rust. The experiment was performed on 2-yr-old seedlings because Italian stone pines retain primary needles until 4-5 yr of age. No data are available regarding susceptibility of trees with only secondary needles. Even under conditions of relatively low inoculum density this species appeared both highly and uniformly susceptible (13).

The uniform susceptibility of the Italian stone pine seedlings to rust may be due to the fact that the parental trees were chosen from plantations. Italian stone pine was introduced in Tuscany many centuries ago and it is now naturalized in restricted areas along the coast. The data we obtained on infection makes it apparent that there is little basis for continuing a breeding program for resistance among these selected trees. Apparently Italian stone pines survive in the area of Tuscany that was examined because the proper conditions for the onset of the disease seldom occur (11).

**Maritime pine.** This species was less susceptible than Italian stone pine in our tests. This is confirmed by the results with the artificial inoculation test using uniform numbers of basidiospores of *C. flaccidum* (13). Significant differences were detected among families for percent infection, percent spotted seedlings bearing fruiting bodies of *C. flaccidum*, and percent of “spotted-only” seedlings. Because of the large number of parental trees tested and of seedlings used in each family, of the two experimental distributions used (family and one seedling plot) and of the high percentage of spotted seedlings, we consider it reasonable to estimate the variability among families for the presence of spots on needles. In the past, researchers working with white pine blister rust (caused by *Cronartium ribicola* J. C. Fisch. ex Rabenh.) considered nonspotted seedlings to have escaped infection, but the absence of needle spots may indicate another mechanism of resistance (R. J. Hoff, personal communication). The significance of the variability of this character among families seems to indicate the existence of mechanisms in the maritime pine seedlings which stop the pathogen in the first stages of the infection process. This seems reasonable because the complete randomization on an individual basis should also avoid the principal effects of biases in inoculum distribution, as suggested by McCluskey (5). It is interesting that the families with the lower percentages of spotted seedlings tended to have a higher number of “spotted-only” seedlings at the end of the test.

The fact that as many as 22% of the spotted seedlings remained without fungus fruiting bodies indicates the presence of a mechanism of resistance in the host which stops pathogen development after needle infection has taken place. In Western white pines, resistance mechanisms were described which operate in the needles by shedding the infected leaves (6) or by stopping the fungus growth (7). Another mechanism in the short shoot causes host cell necrosis and death of the fungus (3). A similar mechanism of stopping the fungus growth in the needles was found in slash pine seedlings attacked by the fusiform rust fungus (8). Normal canker development may also be prevented by resistance mechanisms which work when the pathogen has already entered the stem. In this case, the host isolates the pathogen within tissue reactions and walls the fungus off, as indicated by Miller et al. (9) on slash pine seedlings infected by the fusiform rust fungus. We also observed some individuals with this type of reaction in maritime pine, which accounts for part of the “spotted-only” seedlings. The remaining “spotted-only” seedlings owe their resistance to mechanisms present in the needles.

In agreement with the work of Hoff et al. (4), it seems possible to find tolerance to infection or other mechanisms of resistance, such as a host reaction killing the fungus or slowing its growth in the stem. However, these mechanisms cannot be determined in the first 3 yr following inoculation. It is, therefore, very likely that the percentage of the resistant trees actually could be more than the 22% than we indicated.

The values of the heritability estimates for infection of needles and for survival of “spotted-only” seedlings indicate that it should be possible to establish a program of breeding maritime pine for resistance to blister rust through controlled crosses among the best candidates for those traits.

**LITERATURE CITED**