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

P. Raddi, L. Mittempergher, and F. Moriondo

Geneticists, Centro di studio per la Patologia delle specie legnose montane, Consiglio Nazionale delle Richerche, Presso l'Instituto di Patologia Forestale e Agraria, delle Università, Piazzale delle Cascine, 28, 50144 Firenze (Florence), Italy; and associate professor of Forest Pathology, University of Florence, 50144 Florence, Italy; respectively.

The technical assistance of A. Fagnani is acknowledged.

Accepted for publication 18 January 1979.

ABSTRACT

RADDI, P., L. MITTEMPERGHER, and F. MORIONDO. 1979. Testing of *Pinus pinea* and *P. pinaster* progenies for resistance to *Cronartium flaccidum*. Phytopathology 69: 679-681.

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 (*Pinus 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 chosen for each species among heavily infected individuals. Seeds produced by open pollination from the chosen parents were sown in Paperpots 315 (Nippon Beet Sugar Manufacturing Co., Japan), treated paper containers 3 cm in diameter and in 15 cm in height, in April 1973 and transplanted to adjacent parallel seedbeds in a nursery. Each seedbed was regarded as one replicate which was subdivided into two parts, one for each species. Each replicate contained 15 seedlings for each of the 40 Italian stone pine families and 16 seedlings for each of the 33 maritime pine families. The experimental design was a randomized complete block with six replicates of Italian stone pine and five of maritime pine in which the seedlings were distributed in family plots. Five 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 spermagonia 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 $\sqrt{\%}$ 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

00031-949X/79/000122$03.00/0

authors, the agreement between heritability ($\hat{h}_p^2$) computed using their method and the narrow-sense heritability ($\hat{h}_{pa}^2$) is reasonably good.

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

Symptoms	Seedlings (no.)	Seedlings per family (no.)	Symptoms (%)	Heritability ($\hat{h}_p^2$)
Seedlings:				
-inoculated	5,985	148–150[a]	100.00	
-needles spotted	5,981	148–150	99.93	
-with rust fruiting bodies	5,440	125–143	90.95	0.031 ± 0.004
-alive 3 yr postinoculation	419	3–23	7.00	0.081 ± 0.002

[a]Forty half-sib families were tested.

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 fruiting bodies was very low. The heritability for the survival of infected seedlings at the 3rd yr 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

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

	Seedlings (no.)				Seedlings with fungus fruiting bodies			Living seedlings 3 yr after inoculation					
									Spotted-only			With fungus fruiting bodies	
Rank	Family no.	Inoculated	Spotted	Arcsin ($\sqrt{\%}$)	Family no.	No.	Arcsin ($\sqrt{\%}$)	Family no.	No.	Arcsin ($\sqrt{\%}$)	Family no.	No.	Arcsin ($\sqrt{\%}$)
1	12	131	84	53.19	16	37	39.62	20	41	41.32	19	49	38.41
2	20	142	94	54.45	20	43	42.53	27	51	40.45	10	45	38.12
3	23	139	92	54.45	27	57	43.34	16	36	38.98	29	46	37.88
4	18	140	101	58.31	17	49	43.62	23	33	36.78	2	44	36.75
5	16	124	91	58.95	14	61	44.54	14	40	36.42	26	45	36.69
6	17	140	103	59.08	23	46	45.00	18	34	35.45	6	43	36.57
7	8	131	100	60.87	12	43	45.63	12	28	35.24	21	41	36.30
8	30	137	108	62.58	3	62	47.75	15	39	33.65	18	35	36.06
9	24	141	113	63.51	15	76	50.65	24	33	32.71	32	41	35.76
10	21	142	117	65.20	24	68	50.89	10	33	31.92	9	41	35.61
11	1	134	113	66.66	30	65	50.89	8	26	30.66	7	35	35.24
12	27	143	121	66.89	18	62	51.59	19	34	30.51	13	40	34.60
13	6	143	121	66.89	19	79	52.06	17	26	30.16	11	41	34.33
14	10	139	118	67.13	7	73	52.19	31	27	28.45	5	41	34.14
15	31	140	119	67.21	33	75	52.24	26	28	28.11	15	40	34.14
16	3	133	113	67.21	8	63	52.54	6	26	27.62	28	37	33.90
17	33	141	120	67.29	22	79	52.95	21	25	27.56	3	35	33.83
18	28	139	119	67.70	4	83	53.31	3	22	26.21	27	37	33.58
19	32	140	120	67.82	5	84	53.49	9	23	25.84	1	33	32.71
20	9	140	121	68.36	10	77	53.88	28	22	25.47	22	36	32.60
21	7	135	117	68.57	31	78	54.03	5	24	25.47	Susc.	38	32.17
22	22	143	124	68.61	26	83	54.27	22	22	24.88	17	29	32.04
23	2	141	123	69.04	28	80	55.06	Susc.	23	24.47	20	26	31.72
24	13	142	124	69.12	Susc.	91	55.49	32	19	23.42	24	31	31.56
25	26	143	126	69.82	1	77	55.61	11	19	22.54	30	29	31.22
26	14	140	124	70.27	11	90	56.76	2	18	22.46	31	31	30.69
27	15	143	127	70.45	2	88	57.73	13	18	22.38	23	23	30.00
28	19	142	127	71.00	13	89	57.92	4	18	21.92	4	32	29.87
29	29	136	122	71.28	6	87	57.99	33	15	21.56	14	30	29.47
30	11	142	129	72.34	21	86	59.02	7	15	20.96	8	23	28.66
31	4	141	129	73.05	9	89	59.06	30	13	20.27	16	19	27.20
32	Susc.	142	134	76.19	29	90	59.21	29	14	19.82	12	17	26.71
33	5	138	132	77.96	32	89	59.47	1	12	19.00	33	23	19.16
Totals		4,587	3,824	(83.36%)		2,399			857			1,160	
Spotted seedlings (%)		100				62.73			22.41			30.33	
LSD $P = 0.05$				9.50			9.80			9.29			
$P = 0.01$				12.49			12.88			12.21			
$F_{families}$				2.45**[a]			2.68**			4.55**			ns
F_{runs}				11.95**			9.14**			ns			12.67**
$F_{families \times runs}$				ns[a]			ns			ns			ns
$\hat{h}_p^2$				0.124 ± 0.039						0.156 ± 0.046			

[a]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 cankering. 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

1. BARTLETT, M. S. 1947. The use of transformations. Biometrics 3:39-52.
2. DEMPSTER, E. R., and I. M. LERNER. 1950. Heritability of threshold characters. Genetics 35:212-236.
3. HOFF, R. J., and G. I. McDONALD. 1971. Resistance to Cronartium ribicola in Pinus monticola: short shoot fungicidal reaction. Can. J. Bot. 49:1235-1239.
4. HOFF, R. J., G. I. McDONALD, and R. T. BINGHAM. 1976. Mass selection for blister rust resistance: a method for natural regeneration of western white pine. U.S. Dep. Agric., For. Serv., Res. Note INT-202. 11 pp.
5. McCLUSKEY, R. A. 1972. Computerized mapping of blister rust epidemics in nursery seed beds. Pages 393-395 *in* U.S. Dep. Agric., For. Serv., Misc. Publ. 1221.
6. McDONALD, G. I., and R. J. HOFF. 1970. Resistance to Cronartium ribicola in Pinus monticola: early shedding of infected needles. U.S. Dep. Agric., For. Serv., Res. Note INT-124. 8 pp.
7. McDONALD, G. I., and R. J. HOFF. 1971. Resistance to Cronartium ribicola in Pinus monticola: genetic control of needle-spots-only resistance factors. Can. J. For. Res. 1:197-202.
8. MILLER, T. 1973. Mechanism of resistance in slash and loblolly pines to Cronartium fusiforme. Abstract 0229 *in* Abstracts of Papers, 2nd Int. Congr. Plant Pathol., 7-12 Sept 1973, Minneapolis, MN.
9. MILLER, T., E. B. COWLING, H. R. POWERS, and T. E. BLALOCK. 1976. Types of resistance and compatibility in slash pine seedlings infected by Cronartium fusiforme. Phytopathology 66:1229-1235.
10. MITTEMPERGHER, L., and P. RADDI. 1977. Variation of diverse sources of Cronartium flaccidum. Eur. J. For. Pathol. 7:93-98.
11. MORIONDO, F. 1975. Caratteristiche epidemiche della ruggine vescicolosa del pino, Cronartium flaccidum (Alb. et Schw.) Wint. in Italia. (Epidemic characteristics of pine blister rust by C. flaccidum in Italy). Annali Accad. Ital. Sci. For. 24:331-406.
12. RADDI, P., and A. FAGNANI. 1978. Relative susceptibility to blister rust caused by Cronartium flaccidum of several species of pine. Eur. J. For. Pathol. 8:58-61.
13. RADDI, P., and A. FAGNANI. 1978. Miglioramento genetico del pino per la resistenza alla ruggine vescicolosa: controllo ed efficacia della densità di inoculo. (Blister rust resistance in maritime and Italian stone pine: effects of the inoculum density level in resistance testing). Phytopathol. Mediterr. 17:8-13.
14. ROBERTSON, A., and M. I. LERNER. 1949. The heritability of all or none traits: viability of poultry. Genetics 34:395-411.