Research Note

UNITED STATES DEPARTMENT OF AGRICULTURE
FOREST SERVICE

INTERMOUNTAIN FOREST & RANGE EXPERIMENT STATION
OGDEN, UTAH 84401

USDA Forest Service
Research Note INT-124

November 1970

RESISTANCE TO *CRONARTIUM RIBICOLA* IN *PINUS MONTICOLA*: EARLY SHEDDING OF INFECTED NEEDLES

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ABSTRACT

Detailed analysis of symptom history provided information on the nature of resistance to Cronartium ribicola *J. C. Fisch. ex Rabenh. in* Pinus monticola *Dougl. seedlings. Two independent and sequential resistance factors appeared to be operative in seedling populations obtained from phenotypically resistant parents, but only one of these factors was present in seedling populations derived from phenotypically susceptible parents.*

The white pine blister rust disease, caused by *Cronartium ribicola* J. C. Fisch. ex Rabenh., has been a major forest management problem in the Western United States since the early 1920's (Ketcham, Wellner, and Evans 1968). A breeding program for development of resistance in *Pinus monticola* Dougl., western white pine, was initiated by the USDA Forest Service in 1950. The progress of this program was recently summarized by Bingham, Olson, Becker, and others (1969). The need for more detailed information on the mechanisms of resistance and their sites was stressed by Hoff and McDonald in August 1969 during a NATO-IUFRO Advanced Study Institute on Biology of Rust Resistance in Forest Trees at Moscow, Idaho (see Hoff and McDonald 1971).

The fungus enters both *Pinus strobus* L., eastern white pine, (Clinton and McCormick 1919; Patton 1967) and western white pine (Chapman 1934) through the stomata. It grows down the needle to become established in the bark within 1 to 2 years after penetrating the needle (Chapman 1934).

Any interference with the pathogen's growth down the needle or its penetration of the stem could prevent development of a canker, but not development of needle lesions. Occurrence of normal needle symptoms without the subsequent development of cankers (termed here "the needle-spots-only" reaction) has been observed in *P. strobus* (Riker, Kouba, Brener, and others. 1943; Hirt 1944); in white pine hybrids (Heimburger 1962; Patton 1966); and in *P. monticola* (Bingham 1954; Bingham, Squillace, and Wright 1960)

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Without elaborating, Riker, Kouba, Brener, and others (1943, p. 758) stated: "Many trees showed needle spots that were probably infections, but no stem cankers." Hirt (1944, p. 10) made one reference to the needle-spots-only phenomenon, when he said, "...occasionally needles had typical infection spots that failed to result in cankers." Heimburger (1962, p. 360) noted a moderately heavy needle infection in *P. griffithii* McClelland that "did not reach the stem before the needles were sloughed off." Patton (1966) reported that various hybrid white pine seedlings that had supported infected needles 1 year after inoculation were "disease-free" 2 years after inoculation.

Shedding of infected needles before the mycelium reaches the stem would be a discrete and effective mechanism of resistance. The needle-spots-only phenomenon suggests that such a system is operative in the white pines. This incomplete blister rust syndrome could also be explained in other ways; e.g., the mycelium could fail to reach or to penetrate the short shoot before characteristic shedding occurs or penetration of the short shoot could be followed by a reaction lethal to the fungus.

The objectives of the analysis presented here were to determine: (1) the percentage of seedlings supporting needle infections 9 months after inoculation; (2) the rate at which infected needles were shed; and (3) the effectiveness of shedding as a resistance mechanism.

MATERIALS AND METHODS

Disease-free western white pine were selected from areas exhibiting high levels of blister rust infection (Bingham, Olson, Becker, and others. 1969). These phenotypically resistant trees were subjected to a crossing program that produced many full-sib families. However, for purposes of this investigation, these families were combined into one group (termed here "resistant families").

Seed was obtained from several phenotypically susceptible, wind-pollinated trees or from squirrel caches in heavily infected stands. These seeds were bulked according to geographic source. Each of the 10 sources used was given a seed lot designation and handled as a full-sib family. Data from all control lots were combined into a second group ("susceptible families").

Seeds were planted at Moscow, Idaho, in early winter, 1964. The experimental design used was a randomized complete block consisting of 10 blocks, 475 plots per block, and 16 planting spots per plot. Each plot contained a full-sib family or a control. There was one replication (plot) per randomized block. Each block contained 383 separate full-sib families, 10 control lots, and some miscellaneous crosses. In order to make a manageable study, 116 full-sib families were chosen and only plots in blocks 4, 5, 6, and 7 were inspected. The 10 control lots in the same blocks also were examined. Two-year-old seedlings were inoculated between September 20 and 25, 1966 (Bingham, Olson, Becker, and others. 1969).

Seedlings were inspected three times over a 2-year period. The first inspection was begun June 15, 1967, and completed 15 days later. Five seedlings in each plot (family) selected from blocks 4, 5, 6, and 7 were examined for needle lesions caused by *C. ribicola*. The principal criterion was the yellow coloration typical of blister rust.

The second inspection was carried out between August 11 and September 28, 1967; blocks 5 and 6 were examined from August 11 to August 21 (the 11th-month measurement) and blocks 4 and 7, from August 22 to September 28 (the 12th-month measurement). Any evidence of *C. ribicola's* reaching the stem was recorded at the time of inspection. Yellow to orange discoloration, pycnia, and fusiform swelling were typical signs and symptoms encountered. Atypical symptoms, such as roughening of the bark, scaling of small bark patches at the base of the short shoot, and presence of small necrotic areas

at the base of the short shoot, were also observed. If these atypical symptoms appeared on wood formed during the year of inoculation, they were interpreted as positive evidence that the fungus had reached the stem. All the above signs and symptoms associated with the stem were considered in judging whether or not the rust had penetrated the short shoot.

The third inspection was carried out between September 15 and October 31, 1968, 24 months after inoculation, to determine whether or not stem penetration had occurred. Seedlings were examined for typical symptoms and signs (swelling, discoloration, and pycnial scars) and atypical symptoms (swollen and roughened, necrotic and sunken, scaly, and deeply fissured bark). All indications of penetrations were labeled simply "stem symptoms."

Seedlings were classed according to parental phenotype and the presence or absence of stem symptoms 24 months after inoculation:

Class	Parental phenotype	Stem symptoms
I	Susceptible	Absent
II	Susceptible	Present
III	Resistant	Absent
IV	Resistant	Present

Seedlings were also classed according to the date of inspection; so each of the above classes consisted of seedlings inspected during the 9th, 11th, and 12th month after inoculation. The percentage of seedlings with needle infections was calculated for each class each of these inspection periods. These data provided a means of evaluating the relationships between the appearance of stem symptoms and the rate of needle shedding in susceptible and in resistant families. However, this arrangement does not provide information as to the effectiveness of shedding as a resistance mechanism. The effectiveness of shedding was evaluated by classifying the seedlings inspected during the 11th and 12th months after inoculation on the basis of the presence or absence of infected needles rather than on the presence or absence of stem symptoms. Chi-square values were calculated according to Snedecor (1956).

RESULTS

Results of the 9th-month inspection showed that from 97.4 to 100 percent of the seedlings supported needle infections (table 1); so it was assumed that none of the seedlings had escaped infection.

Performances of seedling classes II and IV were compared on the basis of the needle shedding rates of seedlings that developed stem symptoms within 2 years of inoculation. The population of seedlings from phenotypically resistant parents exhibited a faster shedding rate than the population of seedlings from phenotypically susceptible parents (table 1).

Comparison of the shedding rates of seedlings that exhibited the needle-spots-only reaction (no stem symptoms 24 months after inoculation) gave a different result. Seedling classes I and III showed the same shedding rate (table 1).

If infected needles had been shed by the end of the 11th month, contingency table chi-square analysis showed that parental phenotype had no significant effect on the percentage of seedlings in the needle-spots-only class (table 2). These data also indicated that *early shedding of infected needles was an efficient predictor of resistance* since 65 percent of the seedlings remained disease-free 13 months after their needles were shed.

Table 1.--Percentage of P. monticola *seedlings supporting* C. ribicola *needle lesions 9-12 months after inoculation*

Stem symptoms 24 months after inoculation	Parental phenotype	Seedlings inspected and inspection time					
		9 months		11 months		12 months	
		Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Present	Resistant (Class IV)[1]	1,636	99.8	2,338	97.1	2,255	84.2
	Susceptible (Class II)	180	100.0	310	98.7	271	93.4
Absent	Resistant (Class III)	465	97.4	558	74.6	801	47.2
	Susceptible (Class I)	13	92.3	35	77.1	17	47.1

[1]Seedling class in parentheses.

Table 2.--Percentage of P. monticola *seedlings exhibiting needle-spots-only reaction 24 months after inoculation, when needle infection is measured 11 months after inoculation*

Infected needles	Parent phenotype	Seedlings			Seedlings with needle-spots-only	χ^2	Probability of larger χ^2
		Stem symptoms present	Stem symptoms absent	Total			
		Number			*Percent*		
Absent	Resistant	67	124	191	65		
	Susceptible	4	8	12	67		
Total		71	132	203		.034	.60
Present	Resistant	2,271	416	2,687	15		
	Susceptible	306	27	333	8		
Total		2,577	443	3,020		43.46	.0005

On the other hand, the same analysis performed with seedlings retaining infected needles through the 11th month indicated that the effect of parental phenotype was highly significant and that low percentages of the seedlings remained free of stem symptoms (table 2).

The above analysis applied to seedlings from the same parents *but inspected 1 month later* had a very different outcome; *parental phenotype had no effect* if infected needles had been shed within 11 months of inoculation, but *a significant effect* if infected needles were not shed by that time (table 3, Infected needles, Absent). When the infected needles were retained through the 12th month (table 3, Infected needles, Present), the resistant families performed about the same as previously, but the susceptible families showed a decrease in the percentage of seedlings in the needle-spots-only class.

DISCUSSION

The results shown in table 1 have a particular bearing on the interpretation of those data. The high percentage of seedlings (all populations) supporting needle infections in June is the basis for an important assumption. Escape from and/or resistance to needle infection were so low that they could be disregarded and 100 percent infection assumed. The remote possibility that needle spots were caused by an agent other than *C. ribicola* was eliminated when histological examination revealed elements of the rust in 99 percent of 22,000 spots.[2]

This high level of needle infections in June also suggests a second important assumption. Seedlings that had needle spots in June but no needle spots in the 11th and 12th months must have shed diseased needles. Ninety-eight percent of the seedlings inspected had at least a few needles on the stem internode of the inoculation year.

The authors' interpretation of the relationship between the formation of stem symptoms and premature shedding of infected needles is based on the above assumptions and on the observation that all infected needles *eventually* are shed. At Moscow, Idaho, complete shedding generally occurs by the end of the second growing season, 22-23 months after inoculation in the nursery beds.

The reason for the increased rate of shedding exhibited by seedling class III (see page 3) is not immediately obvious. Early shedding may be related to a stem resistance factor because offspring of resistant parents have higher levels of stem-based resistance than those of susceptible parents (Bingham, Olson, Becker, and others. 1969). Also worth mentioning is the fact that seedlings were located in different blocks in the nursery. Consequently, the difference in shedding rates could be due to a block effect.

The equal needle shedding rates exhibited by seedlings that failed to develop stem symptoms may indicate that seedlings from resistant parents and those from susceptible parents were exhibiting the same resistance mechanism. Recessive genes controlling resistance could be present in both parental populations.

The data showed that seedlings expressing the needle-spots-only reaction tended to shed infected needles earlier than those that developed stem symptoms (table 1). Since some seedlings that had shed needles prematurely also showed stem symptoms and others retained infected needles into the 12th month but failed to produce stem symptoms, the effectiveness of early needle casting as a predictor of resistance was interpreted another way.

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[2]G. I. McDonald and R. J. Hoff. Variation in size and color of blister rust lesions on needles of western white pine. USDA Forest Serv., Intermountain Forest and Range Experiment Station, Ogden, Utah. (In preparation.)

Table 3.--*Percentage of* P. monticola *seedlings exhibiting needle-spots-only reaction 24 months after* C. ribicola *inoculation, when needle infection is measured 12 months after inoculation.*

Infected needles	Parent phenotype	Seedlings: Stem symptoms present	Seedlings: Stem symptoms absent	Seedlings: Total	Seedlings with needle-spots-only	χ^2	Probability of larger χ^2
		Number			*Percent*		
Absent	Resistant	357	423	780	54		
	Susceptible	18	8	26	33		
Total		375	431	806		5.56	.025
Present	Resistant	1,898	378	2,276	17		
	Susceptible	253	9	262	3		
Total		2,151	387	2,538		31.32	.0005

The fate of every infected needle is death or casting. Hence, if premature casting prevents the formation of stem symptoms, then all or a high proportion of seedlings that exhibit premature needle shedding should fall into the needle-spots-only category. Furthermore, the size of this category should be much smaller proportionately at a later inspection time, because susceptible seedlings characteristically would swell the needles-absent category. Using this approach, the authors reinterpreted the data (tables 2 and 3).

The results presented in table 2 can be viewed in the following manner. Needle casting began some time during the 11th month. Of the seedlings that shed their infected needles during the 11th month, 65 percent were free of stem symptoms 24 months after inoculation *regardless of their parental phenotype*. Consequently, early dropping of infected needles appeared to be associated with resistance. A nonsignificant contingency table chi-square supports this conclusion, but the low number of observations in the seedling population obtained from phenotypically susceptible parents sheds some doubt on the statistical decision. Nevertheless, since a close decision was not involved, the statistical test should be valid (Snedecor 1956). On the other hand, if infected needles were retained through the 11th month, parental phenotype and appearance of stem symptoms were not independent (table 2).

When the same comparison was made from data collected during the 12th month after inoculation, an entirely different picture emerged. Parental phenotype and stem symptoms no longer were independent factors regardless of whether or not infected needles dropped (table 3). The statistical tests given in both tables should be valid since the correction for continuity was made and all cells contained more than five observations (Snedecor 1956).

The dependence on parental phenotype suggests: (1) that a second resistance mechanism is indicated by the tendency of some resistant seedlings to retain infected needles and (2) that susceptible families apparently did not possess this resistance mechanism, since only 3 percent of the control lot seedlings remained free of stem symptoms if infected needles were retained through the 12th month after inoculation (table 3).

CONCLUSIONS

The following generalizations can be made from this interpretation of the data. Early shedding of infected needles is implicated in resistance demonstrated by certain seedlings from both phenotypically resistant and susceptible parents. Seedlings from susceptible parents have only the mechanism for early shedding of infected needles. The data suggest that the needle-spots-only phenomenon is the result of two resistance mechanisms; the first is premature shedding of infected needles (needle-cast resistance), the second is failure to produce stem symptoms despite retention of infected needles 12 months after inoculation (needle-retention resistance). These mechanisms appear to be independent of one another and to function in a sequential manner.

LITERATURE CITED

Bingham, R. T.
1954. Development of rust resistant white pine, 1954. 4 p., in U.S. Dep. Agr., Forest Serv., Region One, Blister Rust Control Annu. Rep. (n.p.).

_____, R. J. Olson, W. A. Becker, and M. A. Marsden.
1969. Breeding blister rust resistant western white pine. V. Estimates of heritability, combining ability, and genetic advance based on tester matings. Silvae Genet. 18(1-2): 28-38.

_____, A. E. Squillace, and J. W. Wright.
1960. Breeding blister rust resistant western white pine. II. First results of progeny tests including preliminary estimates of heritability and rate of improvement. Silvae Genet. 9: 33-41.

Chapman, C. M.
1934. A study of the movement of the mycelium of *Cronartium ribicola* Fischer during the incubation period in *Pinus monticola* Doug. Pp. 293-298, in Blister Rust Work in the Far West. U.S. Dep. Agr., Div. Plant Dis. Contr.

Clinton, C. P., and F. A. McCormick.
1919. Infection experiments of *Pinus strobus* with *Cronartium ribicola*. Conn. Agr. Exp. Sta. Bull. 214: 428-459.

Heimburger, C.
1962. Breeding of disease resistance in forest trees. Forest. Chron. 38: 356-362.

Hirt, R. R.
1944. Distribution of blister-rust cankers on eastern white pine according to age of needle-bearing wood at time of infection. J. Forestry 42: 9-14.

Hoff, R. J., and G. I. McDonald.
1971. Stem rusts of conifers and the balance of nature. In R. T. Bingham, R. J. Hoff, G. I. McDonald (eds.), Biology of rust resistance in forest trees. New York: Gordon and Breach.

Ketcham, D. E., C. A. Wellner, and S. S. Evans, Jr.
1968. Western white pine management programs realigned on northern Rocky Mountain national forests. J. Forestry 66: 329-332.

Patton, R. F.
1966. Interspecific hybridization in breeding for white pine blister rust resistance. Pp. 367-376, in H. D. Gerhold, et al. (ed.), Breeding pest-resistant trees. Oxford: Pergamon Press.

_____,
1967. Factors in white pine blister rust resistance, Vol. 3. Pp. 876-890, in 14th IUFRO Congr. Proc., Munich, 1967. DVFFA, Munich.

Riker, A. J., T. F. Kouba, W. H. Brener, and L. E. Byam.
1943. White pine selections tested for resistance to blister rust. J. Forestry 41: 753-760.

Snedecor, G. W.
1956. Statistical methods applied to experiments in agriculture and biology. Ames: Iowa State College Press. 534 p.