CORTICAL STROBIC ACID CONCENTRATIONS IN EASTERN WHITE PINE RESISTANT AND SUSCEPTIBLE TO THE WHITE-PINE WEEVIL

by Ronald C. Wilkinson, research geneticist, USDA
Forest Service, Northeastern Forest Experiment
Station, Durham, New Hampshire 03824

ABSTRACT.--Resin acid concentrations in cortical oleoresin from 48 weevil-resistant eastern white pines and 40 susceptible trees were analyzed quantitatively by gas-liquid chromatography. The analyses revealed no significant differences between weevil-resistant and susceptible groups for any of the nine resin acids detected. Strobic acid concentration averaged 22.3 percent of total resin acids in resistant trees; 22.7 percent in susceptible trees. Mean strobic acid concentration was slightly lower in the 20 shortest susceptible trees than it was in the 24 tallest resistant trees. Of the 88 trees sampled, only 2 did not have strobic acid; and both of these trees were highly susceptible to weevil attack. Except for the two trees that had no strobic acid, resistant and suceptible trees were approximately equally distributed within the entire range of strobic acid concentration. This study suggests that eastern white pine with no or low concentrations of strobic acid are rare; and that even if such trees could be easily located, they may be no more resistant to the white-pine weevil than trees with high concentrations.

THE WHITE-PINE WEEVIL (*Pissodes strobi* Peck) is considered to be the greatest deterrent to effective management of eastern white pine (*Pinus strobus* L.) in the Northeast. Developing weevil-resistant white pine through genetic improvement by selection and hybridization is one method that has been proposed for solving this white-pine weevil problem. Numerous environmental factors that influence weevil behavior and the irregularity of natural infestations may prevent effective selection of resistant phenotypes from natural populations and the evaluation of progeny and species hybrids at an early age in test plantations. Under these conditions, indirect methods--selection for properties correlated with resistance--may be preferable and possibly necessary (Gerhold 1961 and 1966; Gerhold and Stroh 1963).

Numerous characteristics that are associated with resistance and that could be utilized in an integrated selection program have been identified for forest tree insects (Gerhold 1966). One characteristic implicated in resistance to the white-pine weevil is the rate of insect-induced resin crystallization. Non-crystallization of cortical oleoresins of white pines has been positively correlated with resistance to attack by the white-pine weevil (Santamour 1965; van Buijtenen and Santamour 1972). Recent studies by Santamour and Zinkel (1976a, 1977) have stimulated further interest by revealing that non-crystallization of resins from two horticultural cultivars of eastern white pine and Balkan pine (*Pinus peuce* Griseb) x eastern white pine hybrids is correlated with the absence of strobic acid in the resins as well as with weevil resistance.

I have been researching the relationship between host resin systems and white-pine weevil for several years and am quite interested in the possibility of utilizing chemical characteristics of white-pine resins as criteria for selecting weevil-resistant trees. Santamour and Zinkel (1977) have advocated screening populations and provenances of eastern white pine to find prospective parents with no strobic acid and non-crystallizing resin for use in a weevil-resistance breeding program. But, to my knowledge, no one has yet made an extensive study of the variation in strobic acid concentration among eastern white pines. The supposition that eastern white pine can be found with normal growth rate and form but no strobic acid is strictly conjecture and has not been tested. More important, no one has satisfactorily established a relationship between strobic acid concentration and observed weevil resistance in eastern white pine.

Before I began searching for trees with low concentrations of strobic acid to use in a resistance breeding program, I felt that these two points needed further investigation. In this study I have quantitatively analyzed the cortical resin acid compositions of weevil-resistant and susceptible eastern white pines.

MATERIALS AND METHODS

I obtained samples of oleoresin for gas-chromatographic analyses of resin acids from selected weevil-resistant and susceptible eastern white pines growing in a provenance test in southern Maine. The planting consists of 24 blocks; each block contains one tree from each of 27 geographic seed sources covering a major portion of the botanical range of white pine. Trees are completely randomized in each block.

The trees in the planting were 12 years old from seed and averaged about 9 feet in height when they were first exposed to weevil attack in 1968. Before 1968 the trees in the planting were sprayed each spring with insecticide to prevent weevil feeding. After the spring of 1967, this practice was discontinued and weevils entered the planting for the first time. The weevil population in both 1968 and 1969 was extremely high. Weevil damage was recorded in early August of 1968, 1969, and in 1970. The main stem terminal shoot of each tree was examined and the tree was then placed in one of three categories: (1) weeviled, leader dead; (2) weeviled, leaders not dead; and (3) no apparent weevil attack.

Garrett (1972) has previously described weevil damage and resistance for each provenance in the planting. Differences between provenances in the amount of leader damage by weevils varied from 71 to 100 percent in 1970. Of 627 living trees in the planting, 57 trees had resisted weevil attack (category 2) in at least 1 of the 3 years of evaluation and had not been attacked in the other years. Ten of these trees resisted attack in 3 successive years. Another 22 trees were not attacked by the weevil (category 3), but these trees were all very slow growing. The remainder of the trees were placed in category 1 in at least 1 of the 3 years; 54 of these trees had their leaders killed by the weevil in all 3 years.

In May, 1976, these trees were re-examined prior to collection of oleoresin. Forty-eight of the original 57 trees still showed evidence of resistance, and these trees were selected for resin acid analyses. For comparison, 40 of the trees that had been attacked in each of 3 years and showed evidence of continued damage in the years from 1970 to 1976, were also sampled. The results of this study are described by Bridgen, et al. [1] The analyses showed no differences in mean resin acids between resistant and susceptible trees, but the data received some criticism because the Versamid 900 column did not separate strobic acid from dehydroabietic acid. Therefore I collected oleoresin samples again from the same trees in the early fall of 1977, using the same procedures. I put the samples in sealed vials with a nitrogen atmosphere and sent them to the Forest Products Laboratory for analysis with a different column system.[2]

The cortex oleoresin acids were methylated with diazomethane and the resulting methyl esters were analyzed by gas-liquid chromatography on a 10 percent EGSS-X column. Two of the oleoresin samples were separated into neutral and acidic (resin acid) fractions by the DEAE-Sephadex procedure of Zinkel and Rowe (1964) before methylation and analysis of the acidic fractions. The small effect that this procedure had on the quantification of strobic acid was judged to be of no significance in comparing resistant and susceptible trees, so it was not used for the remainder of the samples. The EGSS-X column readily separated strobic and dehydroabietic acids, and all of the data and results reported here are from these analyses.

The data for each resin acid are expressed as percentages of the total resin acids. I used a one-way analysis of variance to analyze the data statistically. Differences in mean resin acid composition between resistant and susceptible trees were tested for significance by the F test at the 5 percent level of probability.

RESULTS AND DISCUSSION

Nine identified resin acids were detected in the cortical oleoresin of eastern white pine (Table 1). These resin acids were the same as those reported for eastern white pine by Santamour and Zinkel (1976a, 1976b, 1977). Hanover (1975) also found pimaric acid, hut did not report the occurrence of communic acid. Pimaric acid was also detected by Bridgen, et al. in the first analyses on a Versamid 900 column, but its identification was only tentative as it was only based on retention time and co-chromatography with a pimaric acid standard. Two resin acids, levopimaric and palustric were not separated on either set of columns. Several unknowns were also detected, but they were not considered in making comparisons between weevil-resistant and susceptible trees.

As in the original analyses by Bridgen et al., none of the resin acids differed significantly between weevil-resistant and susceptible trees. Strobic acid concentration averaged 22.3 percent and ranged from 3.1 to 34.5 percent in the resistant trees; it averaged 22.7 percent with a range of 0.0 to 32.8 percent in susceptible trees.

Zinkel and Spalding (1971) reported that strobic acid made up 8 to 44 percent of the resin acids in cortex oleoresin from a small number of eastern white pine, and Santamour and Zinkel (1976a) reported a range of 0 to 35.3 percent in a sample of 24 trees. These concentrations for strobic acid, as well as the mean of 22.8 percent of the resin acids for dehydroabietic + strobic acid in 27 trees calculated from the data of Hanover (1975), agree closely with the means and ranges reported here.

Analyses made on the EGSS-X column showed that dehydroabietic acid is only a minor component in most of the trees analyzed. Once this was established, it then became clear that our earlier results were not seriously affected by the lack of separation between strobic acid and dehydroabietic acid. In fact, the correlation between strobic acid concentrations obtained from the EGSS-X column and dehydroabietic + strobic acid concentrations from the Versamid 900 column was quite high, and the conclusions based on the data from either analytical method are identical.

One fact is clear: no matter which type of analysis I used, there does not seem to be any association between strobic acid concentration and weevil resistance. Only 2 of the 88 trees sampled did not contain strobic acid. One of these trees was from a Georgia seed source (the southernmost seed source in the planting), and the other was from a northern Michigan source. It would appear that absence of strobic acid is very rare in eastern white pine, and that trees without strobic acid can occur anywhere within the range of the species. Moreover, Santamour and Zinkel (1976b, 1977) have determined that high concentrations of strobic acid are inherited as a dominant characteristic in interspecific hybrids made with eastern white pine. If the mode of inheritance is the same in eastern white pine, with a low frequency of recessive genotypes, then locating trees low in strobic acid would be quite difficult.

Probably the most important, and certainly the most unexpected, finding in this study is that the two trees that had no strobic acid were susceptible to the white-pine weevil and suffered leader mortality after attack in each of 3 successive years. This was totally surprising because Santamour and Zinkel (1976a) found that among trees with resins that did not crystallize naturally, only those trees with no strobic acid had resin that did not crystallize in the presence of crushed weevil larvae heads. At this time I do not know whether the resin from the 2 trees (without strobic acid) that I found crystallizes readily or not; but this certainly requires further investigation.

Hanover (1975) analyzed 27 western white pine (*Pinus monticola* Douglas) for resin acid content and apparently found little or no strobic acid in this species. Santamour and Zinkel (1977) found several *Pinus peuce* individuals that did not contain strobic acid, but they found no strobic acid in only two eastern white pine of several that they analyzed and these were both horticultural cultivars. Both *Pinus monticola* (Soles, Gerhold, and Palpant 1970; Garrett 1970) and *Pinus peuce* (Heimburger 1967) are more resistant to the white-pine weevil than is eastern white pine. My study suggests that these two species must derive their resistance from some factor other than strobic acid concentration in cortical oleoresin.

The resistant trees that I examined were resistant even to extremely high weevil populations. By 1970, 87.4 percent of all trees in the plantation had had their leaders killed by the weevil in at least one of three years of evaluation. These resistant trees, therefore, were ideal material on which to test the relationship between resistance and chemical composition. One factor, however, that must be considered in any comparisons involving the white-pine weevil and preferential host selection is growth rate. The weevil consistently attacks vigorous, fast-growing trees in preference to their slower growing neighbors. Very small trees within a group of larger trees frequently are not attacked at all. Smaller trees may also receive light weevil attacks without sustaining damage and may appear to be resistant, but this can often be attributed to their growth rate and associated morphological characteristics.

In the provenance test plantation that I sampled, the mean height of resistant trees was considerably shorter than that of susceptible trees; the 48 resistant trees averaged 5.9 feet tall at 10 years of age (2 years before the first weevil attack) and the susceptible trees averaged 7.7 feet. It is preferable to make comparisons between resistant and susceptible trees of equal height, or better still to compare tall resistant trees with short susceptible trees. Because of the height discrepency in the total sample, I examined resin acid concentration in subsamples of resistant and susceptible trees. The subsamples consisted of the tallest half (24 trees) of the resistant trees and the shortest half (20 trees) of the susceptible trees. These subsamples averaged 7.4 feet for resistant trees at 10 years of age and 6.2 feet for susceptible trees.

The story is the same for comparison of strobic acid in the subsamples as it was for the total sample. Strobic acid concentration was slightly lower in susceptible than in resistant trees, but the difference was not significant (Table 2). Moreover, the two trees that had no strobic acid were included in the short susceptible subsample although both were among the tallest trees in this group.

I further segregated the trees into five resistance categories based on height growth and examined the distribution of these trees within discreet classes of strobic acid concentration (Table 3). Three of the resistant trees were very short; they were placed in one category. The other four categories were tall resistant, resistant, susceptible, and short susceptible. Strobic acid concentration in the entire sample of 88 trees ranged from 0 to 34.5 percent of the total resin acids. Percentages of resistant and susceptible trees are nearly equal within each level of strobic acid concentration. Resistant trees are as likely to have high concentrations of strobic acid as they are to have low concentrations; the majority of the trees have a moderate amount. The same is true of the susceptible trees.

CONCLUSION

When I initiated this study I had hoped that I could substantiate the theories of Santamour and Zinkel (1976a, 1977), and that I could use concentration of strobic acid as a selection criterion for a weevil resistance breeding program. At this time, however, there does not appear to be any relationship between strobic acid concentration and white-pine weevil resistance in eastern white pine.

There is a remote possibility that the resistant trees I examined owe their resistance to some factor other than low strobic acid levels and that the absence of this resin acid may confer resistance in other trees. However, finding that the only two trees that had no strobic acid were also highly susceptible to white-pine weevil attack is certainly not encouraging.

Until this relationship is finally clarified to everyone's satisfaction, I think that the correlation between strobic acid concentration and resin crystallization should be re-examined. A good place to start would be to test both natural and insect-induced crystallization in the two eastern white pines that I have located that do not have any strobic acid. Depending on the results of these studies, it may also be time to take a new look at the correlation between resin crystallization and white-pine weevil resistance.

LITERATURE CITED

Garrett, P. W.
1970. EARLY EVIDENCE OF WEEVIL RESISTANCE IN SOME CLONES AND HYBRIDS OF WHITE PINE. U.S. Dep. Agric. For. Serv. Res. Note NE-117. 4 p.

Garrett, P. W.
1972. RESISTANCE OF EASTERN WHITE PINE (PINUS STROBUS L.) PROVENANCES TO THE WHITE-PINE WEEVIL (PISSODES STROBI PECK.). Silvae Genet. 21(3-4):119-121.

Gerhold, H. D.
1961. TESTING WHITE PINE FOR WEEVIL RESISTANCE. Northeast For. Tree Improv. Conf. 9:44-50 (1960).

Gerhold, H. D.
1966. IN QUEST OF INSECT RESISTANT FOREST TREES. *In* Breeding pest resistant trees, H. D. Gerhold, E. J. Schreiner, R. E. McDermott, J. A. Winieski, eds. Pergamon Press, New York. p. 305-318.

Gerhold, H. D. and R. C. Stroh.
1963. INTEGRATED SELECTIONS FOR WHITE PINE WEEVIL RESISTANCE AND ITS COMPONENTS. Proc. World Consult. For. Genet. and Tree Improv. Stockholm FAO/FORGEN 63-6b/1. 7 p.

Hanover, J. W.
1975. COMPARATIVE PHYSIOLOGY OF EASTERN AND WESTERN WHITE PINES: OLEORESIN COMPOSITION AND VISCOSITY. For. Sci. 22(3):214-221.

Heimburger, C.
1967. SOME OBSERVATIONS ON WEEVIL RESISTANCE OF *PINUS PEUCE.* Northeast. For. Tree Improv. Conf. 14:64-69 (1966).

Santamour, F. S., Jr.
1965. INSECT-INDUCED CRYSTALLIZATION OF WHITE PINE RESINS. I. WHITE-PINE WEEVIL. U.S. Dep. Agric. For. Serv. Res. Note NE-38. 8 p.

Santamour, F. S., Jr. and D. F. Zinkel.
1976a. WEEVIL-INDUCED RESIN CRYSTALLIZATION RELATED TO RESIN ACIDS IN EASTERN WHITE PINE. Northeast. For. Tree Improv. Conf. 23:52-56 (1975).

Santamour, F. S., Jr. and D. F. Zinkel.
1976b. INHERITANCE OF RESIN ACIDS IN AN INTERSPECIFIC WHITE PINE CROSS. Northeast. For. Tree Improv. Conf. 23:46-51 (1975).

Santamour, F. S., Jr. and D. F. Zinkel.
1977. RESIN ACIDS, RESIN CRYSTALLIZATION, AND WEEVILING IN BALKAN X EASTERN WHITE PINE HYBRIDS. Northeast. For. Tree Improv. Conf. 25:164-175 (1977).

Soles, R. L., H. D. Gerhold and E. H. Palpant.
1970. RESISTANCE OF WESTERN WHITE PINE TO WHITE-PINE WEEVIL. J. For. 68:766-768.

van Buijtenen, J. P. and F. S. Santamour, Jr.
1972. RESIN CRYSTALLIZATION RELATED TO WEEVIL RESISTANCE IN WHITE PINE *(PINUS STROBUS).* Can. Entomol. 104:215-219.

Zinkel, D. F. and J. W. Rowe.
1964. A RAPID METHOD FOR THE QUANTITATIVE SEPARATION WITHOUT ALTERATION OF ETHER-SOLUBLE ACIDIC AND NEUTRAL MATERIALS. Anal. Chem. 36:1160-1161.

Zinkel, D. F. and B. P. Spalding.
1971. STROBIC ACID, A NEW RESIN ACID FROM _PINUS STROBUS._ Tetrahedron Letters No. 27. p. 2459-2462.

FOOTNOTES

[1] Bridgen, M. R., J. W. Hanover, R. C. Wilkinson. Oleoresin characteristics of eastern white pine seed sources and relationship to weevil resistance (manuscript in preparation).

[2] Gas-chromatographic analyses were made by Duane F. Zinkel, Research Chemist, Forest Products Laboratory, Forest Service, U.S. Department of Agriculture, Madison, Wisconsin.

Table 1.--Means, standard deviations, and ranges of cortical resin acids from 48 eastern white pine resistant and 40 susceptible to the white-pine weevil.

Resin acid methyl esters	Weeviling category				F-test for chemical differences
	Resistant trees		Susceptible trees		
	Mean and S.D.	Range	Mean and S.D.	Range	
	- - - - - Percent of total resin acids			- - - - -	F-value*
Sandaracopimarate	7.3±1.8	3.0-10.3	7.1±1.7	2.4-10.4	.22
Communate	5.9±1.4	0-9.8	6.1±1.0	3.5-8.0	.63
Levopimarate/palustrate[a]	18.0±3.8	9.5-28.4	18.0±4.4	11.7-31.9	.02
Isopimarate	9.0±2.4	4.8-16.4	10.1±3.1	3.8-17.2	3.80
Strobate	22.3±6.7	3.1-34.5	22.7±7.2	0-32.8	.20
Dehydroabietate	1.6±0.9	0-4.8	1.7±1.2	0-5.7	.53
Abietate	13.2±5.0	4.8-22.4	12.6±4.3	5.7-26.1	.43
Neoabietate	21.3±3.9	14.2-30.2	21.5±4.3	13.8-31.6	.04

*Probability < 0.05 at 3.96.

[a] Levopimarate and palustrate are not separated on EGSS-X columns.

Table 2.--Means, standard deviations, and ranges of cortical resin acids from the 24 tallest weevil-resistant and 20 shortest weevil-susceptible eastern white pine.

Resin acid methyl esters	Weeviling category: Resistant trees: Mean and S.D.	Resistant trees: Range	Susceptible trees: Mean and S.D.	Susceptible trees: Range	F-test for chemical differences
	Percent of total resin acids				F-value*
Sandaracopimarate	7.4±1.6	4.4-10.2	6.8±1.5	2.4-8.8	1.34
Communate	5.8±1.6	0-7.8	6.0±1.2	3.5-8.0	.18
Levopimarate/palustrate[a/]	17.3±4.3	9.5-26.9	18.2±4.8	11.7-31.9	.46
Isopimarate	10.3±2.3	6.9-16.4	10.3±3.0	3.8-16.4	.00
Strobate	22.6±5.6	11.6-33.3	20.9±8.6	0-32.8	.62
Dehydroabietate	1.3±0.8	0-3.0	1.8±1.4	0-5.4	2.05
Abietate	12.9±4.7	5.8-20.7	13.0±5.8	5.7-26.1	.00
Neoabietate	21.3±3.5	15.0-27.9	22.0±4.9	13.8-31.6	.38

*Probability < 0.05 at 4.07.

[a/] Levopimarate and palustrate are not separated on EGSS-X columns.

Table 3.--Distribution of weevil-resistant and weevil-susceptible eastern white pine trees by classes of strobic acid concentration.

Weevil resistance category	Strobic acid concentration (in percent)						Total number of trees
	0.13.8	14.1-19.9	20.0-23.4	23.6-25.9	26.0-28.8	29.0-34.5	
	Number of trees						
Tall resistant	2	4	8	2	5	3	24
Resistant	4	2	2	6	6	1	21
Short resistant	1	0	1	0	0	1	3
Susceptible	2	4	4	5	3	2	20
Short susceptible	2	5	4	3	4	2	20
Totals	11	15	19	16	18	9	88