INHERITANCE OF RESIN ACIDS IN AN INTERSPECIFIC WHITE PINE CROSS

Frank S. Santamour, Jr. and Duane F. Zinkel[1]

ABSTRACT

Hybrids between eastern white pine *(Pinus strobus)* and Himalayan white pine (P. *griffithii)* tended to resemble P. strobus (female parent) in resin acid composition of cortex and needles. The hybrids contained a high proportion of strobic acid in their cortical resins and the resins crystallized readily in the weevil larva-crystallization test. Lambertianic acid accounted for about 90% of the needle resin acids in P. *griffithii,* but was present in only trace amounts in the hybrids, where anticopalic acid was predominant, accompanied by a significant proportion of 3-keto-anticopalic acid and smaller amounts of strobic acid.

Lack of crystallization of cortical oleoresins of white pines has been correlated with resistance to attack by the white-pine weevil, *Pissodes strobi* Peck. (Santamour, 1965; van Buijtenen and Santamour, 1972). Resin acids, especially strobic acid in eastern white pine *(Pinus strobus* L.), may play a role in the crystallization reaction (Santamour and Zinkel, 1975). Therefore, we were interested in the inheritance of resin acids in hybrids between two white pine species that differ in resin acid composition and also in weevil resistance,

Eastern white pine may be generally considered as very susceptible to attack by the white pine weevil. Certainly, the provenance tests investigated by Garrett (1972), where weeviling ranged from 71-100 percent over a three-year period, demonstrated the widespread weevil

[1] Respectively, Research Geneticist, National Arboretum, Agricultural Research Service, U.S. Department of Agriculture, Washington, D.C. 20002 and, Research Chemist, Forest Products Laboratory, Forest Service, U.S. Department of Agriculture, Madison, Wisconsin 53705.

The authors wish to thank Gordon Brandes (Manager) and Gary L. Koller, (Curator) of the Morris Arboretum of the University of Pennsylvania for their assistance in this study.

susceptibility of this species. There is conflicting evidence as to the weevil susceptibility of the Himalayan white pine (P. _griffithiii_ McClel.).[2] MacAloney (1943) and Wright and Gabriel (1959) stated that this species was rarely attacked but Lemmien and Wright (1963) reported it as very susceptible in Michigan. Trees of two clones of P, _griffithiii_, grafted on P. _strobus_, suffered heavy weevil attack in Canada (Heimburger and Sullivan, 1972). Hybrids between P, _strobus_ and P. _griffithii_ apparently are very susceptible to weevil attack (Garrett, 1970; Heimburger and Sullivan, 1972), but these reports were based on hybrid scions grafted on P. _strobus_ rootstocks.

Cortical resin of P. _griffithii_ did not crystallize in the standard test with crushed heads of weevil larvae, but most P. _strobus_ and interspecific hybrids produced resins that crystallized readily (Santamour, 1965).

Analyses of the resin acids of P. _strobus_ were reported by Santamour and Zinkel (1975). Data on the resin acids of P. _griffithii_ presented by Santamour (1967) cannot be considered reliable, especially since the "unknown" acids of the two species were thought to be comparable. We now know that the major resin acid of P. _strobus_ cortical resin is strobic acid and that of P. _griffithii_ is lambertianic acid, Lambertianic acid was first isolated from the wood of sugar pine (P. _lambertiana_ Doug].) by Dauben and German (1968).

MATERIALS AND METHODS

The trees used in this study were all growing at the Morris Arboretum of the University of Pennsylvania in Philadelphia, The parent trees were part of the Arboretum's permanent collection and the hybrids resulted from controlled pollinations made in 1949 by Dr. Jonathan W. Wright when he was stationed at the Arboretum with the U.S. Forest Service. The female parent was P. _strobus_ M 7382. The pollen of P. _griffithii_ used by Wright was actually a mixture of pollens of one Arboretum tree (M 8604) and two trees at the old Andorra Nursery. Only the Arboretum tree was available for study in 1974. The _strobus_ x _griffithiii_ hybrids were represented by five trees (M 55-135-A, B, C. D, and E) from the 1949 cross.

Cortical resin was collected in July, 1974, by severing the current season's growth on lateral branches and either allowing the resin to drip into a vial or "picking off" the accumulated resin droplets with a steel needle. In addition to the parent trees and hybrids, resin was collected from several other trees of both parental species, Samples of all resins were subjected to the standard crystallization test with crushed heads of weevil larvae within 24 hours after collection.

Needle samples were taken at the same time the cortical resin was collected. Resin acid analyses of pine needles have proved to be a valuable taxonomic tool in certain other instances.

This species is also known as P. _wallichiana_ A.B. Jackson and P. _excelsa_ Wall.

The cortex oleoresins and needle extracts were separated into neutral and acidic (resin acid) fractions by the DEAE-Sephadex procedure of Zinkel and Rowe (1964). The acidic fractions were then methylated with diazomethane and the resulting methyl esters analyzed by gas-liquid chromatography,

RESULTS AND DISCUSSION

Cortical Resins.--The identities and quantities of the various cortical resin acids (Table 1) found in P. *strobus* M 7382 were similar to normal trees of this species, with strobic acid being the major resin acid. Lambertianic acid was the major resin acid in P, *griffithii.* Small amounts of communic acid were found in P. *strobus* but the acid was absent in P. *griffithii* (M 8604 and others).

The most significant finding was that the production of strobic acid in cortical resins appeared to be dominant in the interspecific hybrids. The proportions of strobic acid in the hybrids was within the range found in a broader sampling of P. *strobus* (Santamour and Zinkel, 1975). Lambertianic acid was present in the hybrids in relatively small amounts.

In comparing cortical resin acid analyses among P. *strobus* that differed in the proportion of strobic acid, strobic acid appeared to be formed at the expense of abietadienoic (abietic, neoabietic, and levopimaric/palustric) acids (Santamour and Zinkel, 1975), However, in the hybrids between P. *strobus* and P. *griffithii,* the production of lambertianic acid was far below the level that might be expected if the two acids were inherited independently.

The hybrids also showed an increase in the proportions of abietic and neoabietic acids. The biosynthetic relationships among strobic, lambertianic, and the abietic acids are not known at present.

Cortical oleoresin of P. *griffithii* M 8604, or any other P. *griffithii,* did not crystallize when tested with crushed heads of weevil larvae. The resins of P. strobus M 7382 and all of the hybrids exhibited rapid and complete crystallization. If strobic acid is a major factor in the crystallization reaction and in weevil susceptibility, the inheritance pattern of this resin acid may explain why hybrids between eastern white pine and Himalayan white pine are susceptible to the white-pine weevil.

The possibility remains, however, that the apparent correlation among cortical resin acids, resin crystallization, and weevil resistance is spurious. Critical tests should be carried out by researchers working in areas where eastern white pine and the weevil are both native.

Needle Resins.--Data on the resin acids of needles from the parents and hybrids are presented in Table 2. Again, P. *strobus* and the hybrids contained strobic acid, while an extremely high proportion of the P. *griffithii* resin acids was lambertianic. The major resin acids in P. *strobus* were anticopalic and 3-keto-anticopalic (see footnote 4, Table 2),

Table 1.--Resin acids of cortical resin of Himalayan and eastern whie pines and their hybrids.

	P. strobus (M 7382)	P. griffithii (M 8604)	P. strobus x griffithii Average	P. strobus x griffithii Range
Resin Acids (% of oleoresin)	58.0	68.1	58.3	54.3-60.9
Individual Resin Acids[a]				
sandaracopimaric	10.5[b]	2.5	6.1	5.0-6.9
communic/levopimaric/palustric[c]	9.3	23.9	20.7	17.7-22.2
isopimaric	21.9	14.1	13.3	8.9-17.2
strobic	40.0	--	22.6	16.5-28.7
abietic	7.4	5.6	9.4	6.0-11.7
neoabietic	10.9	20.9	24.9	21.4-27.3
lambertianic	--	33.0	3.0	1.2-5.3

[a] Analysis by gas chromatography of the methyl esters on 10% sitar 10C. Communate is not resolved from levopimarate/palustrate, and dehydroabietate is not resolved from neoabietate.

[b] All figures for individual resin acids are percent of total.

[c] GLC analyses on 10% EGSS-X of a second set of samples showed communic acid contents of 7.1% for P. strobus (M 7382), none in P. griffithii, and a range of 1.9- 2.8% in the hybrids. Dehydroabietic acid content for all samples was in the range 0.9-2.6%.

Table 2.--Resin acids of needles of Himalayan and eastern white pine and their hybrids.

	P. strobus (M 7382)	*P. griffithii* (M 8604)	*P. strobus* x *griffithii* Average	Range
Resin Acids[a]				
sandaracopimaric	2.5[b]	1.4	2.0	1.7-2.3
communic/levopimaric/palustric	2.6	0.6	2.2	1.2-5.0
isopimaric[c]	--	1.9	--	---
anticopalic	59.8	--	56.9	46.7-62.1
strobic	6.2	--	4.4	3.2-6.4
3-keto-anticopalic[d]	21.4	--	27.7	26.0-28.9
abietic	2.5	0.9	1.8	0.7-3.7
neoabietic	5.0	5.3	3.0	1.3-4.6
lambertianic	--	89.9	Trace	Trace

[a] Analysis by gas chromatography of the methyl esters on 10% Silar 10C. Communate is not resolved from levopimariate/palustrate, and dehydroabietate is not resolved from neoabietate.

[b] All figures are percent of total resin acids.

[c] Needles of P. strobus and hybrids may contain small amounts of isopimarate but it is difficult to resolve in the presence of anticopalate.

[d] Isolation and identification, D. F. Zinkel and D. A. Foster (Unpublished) both of which were absent from P. griffithii. The hybrids tended to resemble P. strobus in needle resin acids as they did in cortical resin acids.

More extensive analyses (D. F. Zinkel, unpublished), of P. strobus needles has indicated that there is considerable variation among trees (and provenances?) in needle resin acids. Some trees may produce anticopalic acid primarily, while in others 3-keto-anticopalic may be the predominant resin acid.

On the other hand, variation in resin acid composition of cortical resins appear to be much more restricted; the wide variations seen in the needle resin acids of individual trees and provenances were not reflected in the cortex composition, Only the two cultivars of P, strobus noted previously (Santamour and Zinkel, 1975) have thus far been shown to contain no strobic acid in the cortex.

LITERATURE CITED

Dauben, W. G. and V, F, German. 1968. The structure of lambertianic acid. Tetrahedron 22:679-683,

Garrett, Peter W. 1970, Early evidence of weevil resistance in some clones and hybrids of white pine. USDA Forest Service Research Note NE-117, 4 pp.

Garrett, Peter W. 1972, Resistance of eastern white pine (*Pinus strobus* L.) provenances to the white-pine weevil (*Pissodes strobi* Peck.). Silvae Genet, 21:119-121.

Heimburger, C. C, and C. R. Sullivan. 1972. Screening of Haploxylon pines for resistance to the white pine weevil. II, *Pinus strobus* and other species and hybrids grafted on white pine. Silvae Genet. 21:210-215,

Lemmien, Walter and Jonathan W. Wright, 1963. Himalayan white pine in southern Michigan. Mich. Agr. Expt, Sta. Quart. Bull. 45:618-621.

MacAloney, H. J. 1943, The white pine weevil. U. S. Dept. Agri. Circ. 221, 31 pp.

Santamour, Frank S., Jr. 1965. Insect-induced crystallization of white pine resins. I, White-pine weevil. U. S. Forest Service Research Note NE-38, 8 pp.

Santamour, Frank S., Jr. 1967, Notes on pine resins and pest resistance. Univ. Penna. Morris Arb. Bull. 18:8286.

Santamour, Frank S., Jr. and Duane F. Zinkel. 1975. Weevil-induced resin crystallization related to resin acids in eastern white pine. Proc. Northeast. Forest Tree lmpr. Conf. (in press).

van Buijtenen, J. P. and Frank S. Santamour, Jr. 1972. Resin crystallization related to weevil resistance in white pine (*Pinus strobus*).Can. Ent. 104:215-219.

Wright, Jonathan W, and William J. Gabriel. 1959. Possibilities of breeding weevil-resistant white pine strains. U. S. Forest Serv, Northeast. Forest Expt. Sta. Paper 115, 35 pp.

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.