RESIN ACIDS, RESIN CRYSTALLIZATION, AND WEEVILING IN BALKAN x EASTERN WHITE PINE HYBRIDS

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ABSTRACT. -- The cortical resins of some trees of *Pinus peuce* do not contain strobic acid and the resins do not crystallize in contact with crushed heads of white-pine weevil larvae. Grafting of P. peuce scions on P. *strobus* rootstocks did not alter the resin acid composition in cortex or needles of P. Deuce. Interspecific hybrids between P. *peuce* and trees of P. *strobus* that produced strobic acid also contained strobic acid and crystallizable resin. Crosses between P. *peuce* and non-strobic individuals of P. *strobus* produced trees whose cortical resins did not crystallize and that were apparently resistant to infestation by the white-pine weevil. Resin acid analyses of some other white pine species and hybrids are discussed and recommendations for breeding weevil-resistant white pines are given.

TWO RECENT STUDIES have focused new attention on the relationship between the cortical resin acids of white pine and the susceptibility of these trees to infestation by the white-pine weevil (*Pissodes strobi* Peck). Santamour and Zinkel (1976a) showed that individual trees of eastern white pine (*Pinus strobus L.*) could vary in the presence or absence of strobic acid in the cortical resins. The presence of strobic acid was positively correlated with the crystallization of resin as determined by the weevil larva test (Santamour 1965; van Buijtenen and Santamour 1972). In the second study, Santamour and Zinkel (1976b) found that strobic acid was inherited as a dominant characteristic in one interspecific hybrid combination (P. strobus x P. griffithii McClel.).

The present study was undertaken to extend these analyses to other white pine hybrids, notably with the Balkan white pine (P. peuce Griseb.). Heimburger (1967) demonstrated that P. peuce was more resistant to successful weevil attack rdeath of terminal leader) than P. strobus, and suggested that this resistance might be caused by a heavier resin flow. Santamour (1965) showed that the cortical resins from two trees of P. peuce were readily crystallized in the weevil larva test, although a single P. peuce x strobus tree (not from the "crystallizing" P. peuce parents) did resist crystallization. Thus, information on the insect-induced crystallization of P. peuce cortical resins was, at best, confusing. In light of the potential weevil resistance in P. peuce, and the new-found relationships between resin acid chemistry and weeviling, it was decided to investigate the resins of Balkan x eastern white pine hybrids, using available materials.

MATERIALS AND METHODS

The trees used in this study were the parents used and progenies developed in two of the older tree improvement and breeding projects dealing with eastern white pine.

Albert G. Johnson made a number of interspecific pine crosses while he was with the Maria Moors Cabot Foundation for Botanical Research of Harvard University (1948-1955). He was especially interested in a group of four trees of P. peuce (Bulgarian origin - 1929 seed - accession No. 16569-A to -D) growing at the Arnold Arboretum. All of the trees had apparently been weeviled, but one (tree "A") had recovered from the attack and maintained a straight trunk. In 1950, Johnson made reciprocal interspecific crosses between the best-formed of these Balkan pines (tree "A") and an un-numbered, bushy-crowned specimen of P. strobus on the Arboretum grounds.

He also grafted scions of both P. peuce tree "A" and tree "D" onto P. strobus seedling rootstock. The grafted trees were outplanted in Plantation XV at the Arboretum's Case Estates in Weston, Massachusetts in 1952. The hybrid progenies were outplanted in the same plantation in 1954. Spacing in the planting was four feet between rows and trees and no artificial thinning had been performed up to 1975.

Branch and needle material and cortical resin was collected from the three remaining trees of P. peuce at the Arnold Arboretum (16569-A, B, and D) and from the P. strobus parent. Collections from the Case Estates included two trees each of 16569-A and 16569-D grafted on P. strobus rootstocks, and four trees each of 16569-A x strobus and P. strobus x 16569-A progenies.

The other project from which material was used was the U.S. Forest Service breeding work done by Jonathan W. Wright while he was stationed at the Morris Arboretum of the University of Pennsylvania. The progenies we studied resulted from crosses made in 1948, utilizing a P. peuce (S-2779) on the grounds of Swarthmore College, Swarthmore, Pa. as the female parent. The male parent was a P. strobus (G-1639) located at the abandoned Andorra Nursery near Philadelphia.

Although the pollinations were "controlled", in the sense that the female conelets were hand-pollinated, no bagging of the conelets, to prevent natural pollination, was performed. This technique was frequently used when there was little chance of unwanted pollination by surrounding trees of a compatible species. However, in 1948, there were two trees of P. strobus 'Fastigiata' within 60 feet of the Swarthmore P. peuce. These P. strobus trees were probably flowering in that year and might have influenced the seed crop on the P. peuce parent.

Branch and needle material and cortical resin was collected from P. peuce (S-2779), P. strobus (G-1639), and in addition, from the fastigiate P. strobus (S-2780) at Swarthmore.

Seedlings resulting from Wright's crosses were out-planted in 1953 in two plantations (GP-15-53 and GP-16-53) at the Hopkins Experimental Forest in Williamstown, Massachusetts. The Forest Service has since abandoned this tract and the entire area is presently under the jurisdiction of Williams College. A total of nine trees of progeny Pi-302 (peuce x strobus) were originally included in these plantings, but only four were still alive and relatively full-foliaged in 1976.

Some of these trees had been used by Santamour (1965) in his insect-crystallization tests, and there was some indication that trees that produced non-crystallizing cortical resin were resistant to damage by the white-pine weevil. Branches, needles, and cortical resin was collected from two weeviled trees and from two trees that did not exhibit weevil damage.

The collections of branches and needles were made at various times from March to July, 1976. Cortical resin was collected in July, 1976, at which time weevil-infested leaders of P. strobus were also collected at another site in Massachusetts. All tests of insect-induced crystallization were made at the National Arboretum within three days of resin collection.

RESULTS AND DISCUSSION

Cortical resin acids.-- The data on resin acids and insect-induced resin crystallization for both parent-progeny groups are presented in Table 1 and Table 2.

The data for only P. peuce "A" is included in Table 1 (for the sake of space) but trees "B" and "D" were also analyzed and gave results that were extremely similar to tree "A". Of special interest is the fact that the resin acid content of tree "A" scions grafted on P. strobus rootstocks was similar to that of the parent tree. This was also true of grafts of tree "D". The Arnold Arboretum specimens of P. peuce did not produce communic or strobic acid, nor did grafting on P. strobus alter the resin acid composition.

All of the Cabot hybrid trees had been weeviled in previous years, so only two trees of each progeny were originally selected for detailed analysis. There were no significant differences in resin acids between the reciprocal crosses and the hybrids tended to resemble P. strobus, especially in the presence of strobic acid, and to a lesser extent communic acid. Further sampling of two more trees in each hybrid progeny, mainly to check on seemingly abnormal distribution of needle resin acids, did not alter the pattern observed in cortical resin acids.

None of the parents of the U.S. Forest Service hybrids had been weeviled, but this might be expected in view of the infrequent occurrence of the white-pine weevil in the Philadelphia area. The plantings of hybrids at Williamstown, Massachusetts were subject to high weevil populations and, even though the hybrids did grow more slowly than eastern white pine, they were available to weevil attack before stand closure caused any crowding and shading of the trees.

There was, however, complete correlation between resin acid content and test crystallization. Cortical resins containing more than about 2 percent strobic acid were readily crystallized, while those with no strobic acid or low or questionable amounts failed to crystallize. Only those trees that produced crystallizing resin were damaged by the white-pine weevil.

Needle resin acids. -- The needle resin acids were analyzed as before (Santamour and Zinkel 1976b). P. peuce of both origins contained appreciable amounts (28 to 40%) of 3-acetyl-anticopalic acid and an unknown acid (20 to 30%) found previously in P. flexilis James and P. bungeana Zucc. (D.F. Zinkel, unpublished). The needles of the P. strobus trees used as parents contained mostly anticopalic acid (ca. 85%). Neither the P. peuce nor P. strobus parents contained any 3-keto-anticopalic acid. Analyses of eight hybrids of Cabot Foundation origin, four from each of the reciprocal crosses, proved to be extremely confusing at best. The hybrids all contained 3-keto-anticopalic acid (trace to 60%), which was not present in the parents, plus widely varying amounts of the other acids mentioned above; anticopalic (7 to 70%), 3-acetyl-anticopalic (trace to 20%), and the unknown (1 to 31%). Fluctuations in the proportions of some other resin acids were also noted but they were not as pronounced as those listed. There were no differences associated with the direction of the cross, and it must be concluded that inheritance of resin acids in the needles is far more complex than that in the cortex.

Other white pines. -- Resin acid analyses of cortical resins were also performed on a few other white pine species and hybrids.

Japanese white pine (P. parviflora Sieb. i Zucc.) may be crossed with most of the important white pine species. We tested a specimen at the Morris Arboretum (M-1793) and found both strobic acid (4.2%) and lambertianic acid (21.2%) to be present. A putative natural hybrid between Japanese white pine and P. griffithii (Santamour 1962) produced 8.0% strobic acid and 24.3% lambertianic acid. The only surviving U.S. Forest Service hybrid (in a New Jersey planting) between Japanese and eastern white pines produced 17.1% strobic acid and only 3.1% lambertianic acid.

A specimen of P. pumila Reg. produced no unusual resin acids and no strobic acid but the percentage of neoabietic acid in the cortex resins (66.1%) and in the needles (94.8%) was higher than most pines. A specimen of P. strobiformis Engelm. did not produce any new or unknown resin acids, but the rather high amount of isopimaric acid (47.4%) in the cortex and of sandaracopimaric acid (23.8%) in the needles may be unusual. It could be significant that the major unknown resin acid of P. flexilis (and P. Deuce) needles was not found in P. strobiformis which is supposedly closely related to P. flexilis. Both of the above trees were growing at the Case Estates of the Arnold Arboretum.

Strobic acid was likewise not present in the cortical resins of P. monticola Dougl. (Zinkel and Spalding 1972) or P. armandii Franck.

Santamour and Zinkel (1976a) mentioned that certain new crosses were being made at the National Arboretum to test resin acid inheritance. A limited number of inter- and intraspecific hybrids were developed (in collaboration with Dr. John B. Genys, University of Maryland) and are presently growing in containers at the Arboretum. Analyses of cortical resins of three seedlings of P. Ariffithii x. P. strobus 'Pendula' parentage did not produce strobic acid. Since neither parent contained strobic acid, these results are not surprising in view of our hypotheses regarding inheritance. Two seedlings resulting from pollination of P. strobus 'Fastigiata' (non-strobic) with a "normal" P. strobus produced an average of 45.3% strobic acid.

CONCLUSIONS

The cortical resins of the P. Deuce trees studied did not contain strobic acid nor did they crystallize in the presence of crushed weevil heads. Neither the chemical composition nor reactivity of these resins was altered by grafting on P. strobus. Strobic acid was inherited as a dominant character in the interspecific hybrids between P. Deuce and P. strobus regardless of which species was used as the male or female parent. The limited observations on weeviling permitted by the few trees available for study indicated a complete correlation between presence of strobic acid and damage by white-pine weevil.

The available information regarding the occurrence, inheritance, and reactivity of strobic acid in the white pines suggests several possible lines of activity in research to develop weevil-resistant pine progenies. First, it is likely that individual trees of P. strobus, of normal growth rate and habit, and lacking strobic acid, probably do exist. Thus we can screen populations and provenances to find more non-strobic, non-crystallizing prospective parents, select and propagate outstanding individuals, create seed orchards, test combining abilities, and breed superior P. strobus progenies that also lack strobic acid.

Second, it is probable that the majority of individuals of P. griffithii, P. monticola and P. peuce presently available in arboreta or test plantings do not contain strobic acid in their cortical resins. The use of these species in a multi-species breeding pool should, however, be accompanied by chemical or crystallization tests. Pines labeled as a particular exotic species, especially in arboreta, may in fact be hybrids with P. strobus. All of the species mentioned above have been reported by one person or another, at one time or another, as being more weevil-resistant than P. strobus. However, poor growth rate, lack of cold hardiness, or lack of general adaptability to Eastern climatic conditions may limit the widespread utility of interspecific hybrids.

RECOMMENDATIONS

We would suggest, as a first step, an intensive survey of P. strobus plantations in areas of high weevil density for those trees that have not been successfully attacked. Provenance tests or plantations of known seed origin would be ideal, but any plantations of apparently well-adapted trees would suffice. Why plantations? For one thing, the trees, up to 15-20 years old, are still young enough so that a weeviling history can probably be reconstructed by careful observations. Secondly, the trees are easily accessible for resin collection, controlled pollination, caging of weevils on branches, or any other test or manipulation the researcher would care to perform. Thirdly, since the progenies we intend to produce will be grown under plantation conditions, it would seem advisable to select the parents under similar growing conditions. This concept may be new to forest-tree improvement research, and may not even be applicable. We seldom have a wide range of choice of plantation sites and the variation among sites is frequently a significant factor. Still, there are lessons to be learned from horticulture and agriculture and the more closely the conditions under which the parents (or ortets) are selected approximate the conditions under which the progenies (or ramets) are to be grown, the greater will be the chances of successful culture.

Following an obvious elimination of trees of less than average growth rate, the remaining trees should be screened for potential weevil resistance by the simple weevil larva-resin crystallization test (Santamour 1965a; van Buijtenen and Santamour 1972). Lacking equipment, expertise, money, or a friendly chemist, this test may be the only basis for selection. However, if possible, it would be best to obtain a critical chemical analysis of trees that pass the crystallization test to make sure they do not contain strobic acid.

Vegetative propagation of selections, and controlled pollination among these selections can be carried on concurrently, and as soon as possible. There is no need to go into detail on the creation and management of seed orchards, or progeny testing, since these are lessons that the forest geneticist has been taught well--much better than the authors.

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Table 1. Cortical resin acids and resin crystallization reaction of parents, grafts, and hybrid progenies of _Pinus peace_ and P. _strobus_ of Cabot Foundation origin.

	peuce "A"	_peuce_ "A" /_strobus_ a/	_peuce_ "A" x _strobus_ b/	_strobus_ x _peuce_ "A" b/	_strobus_
Resin Acids c/					
sandaracopimaric	2.6 d/	3.5	5.0	5.7	5.1
communic	---	---	4.1	3.8	5.1
levopimaric/ palustric	16.4	20.0	20.2	23.8	21.1
isopimaric	33.2	32.3	17.2	13.4	20.0
strobic	----	----	17.6	16.6	20.1
abietic	8.2	6.4	13.2	14.4	12.3
dehydroabietic	2.5	1.0	1.6	2.1	2.1
neoabietic	37.1	36.8	21.1	20.2	14.2
Crystallization Reaction	0	0,0	+,+	+,+	+

a/ Average of two trees
b/ Average of four trees
c/ Analysis by gas chromatography of the methyl esters on a 10% EGSS-X column at 200 ^{0} C
d/ All figures are percent of total resin acids

Table 2. Cortical resin acids and resin crystallization reaction of putative parents and hybrid progeny of _Pinus peuce_ and P. _strobus_ of U.S. Forest Service origin.

	peuce S-2779	peuce x strobus weeviled a/	peuce x strobus non-weeviled a/	strobus G-1639	strobus 'Fastigiata' S-2780
Resin Acids b/					
sandaracopimaric	2.3 c/	4.8	2.5	5.7	1.8
communic	4.5	5.5	6.0	2.4	7.4
levopimaric/palustric	11.0	23.3	28.6	15.4	6.7
isopimaric	34.0	18.6	21.6	22.7	22.0
strobic	----	21.7	1.5 d/	25.0	trace
abietic	16.7	7.8	11.3	6.9	29.8
dehydroabietic	3.7	1.7	5.2	2.8	3.5
neoabietic	26.8	16.6	23.3	19.1	23.0
unknown	1.0	----	----	----	5.9
Crystallization Reaction	0	+,+	0,0	+	0

a/ Average of two trees

b/ Analysis by gas chromatography of the methyl esters on 10% EGSS-X and SE-30/EGiP columns

2/ c/ All figures are percent of total resin acids

d/ Neutrals were not separated in hybrid analyses. Therefore the amount of strobate may be less, but not more, than the figure indicated -- or may be absent entirely.