ODC 160.2:174.7 *Pinus strobus* L.:453--145.7x19.91 *Pissodes strobi* Peck.

OLEORESIN CRYSTALLIZATION IN EASTERN WHITE PINE:

Relationships with Chemical Components of Cortical Oleoresin and Resistance to the White-Pine Weevil

by Ronald C. Wilkinson

FOREST SERVICE RESEARCH PAPER NE-438
1979
FOREST SERVICE, U. S. DEPARTMENT OF AGRICULTURE
NORTHEASTERN FOREST EXPERIMENT STATION
370 REED ROAD, BROOMALL, PA. 19008

The Author

RONALD C. WILKINSON is a Research Plant Geneticist at the Northeastern Forest Experiment Station's Forestry Sciences Laboratory in Durham, New Hampshire.

MANUSCRIPT RECEIVED FOR PUBLICATION 22 DECEMBER 1978

OLEORESIN CRYSTALLIZATION IN EASTERN WHITE PINE:

Relationships with Chemical Components of Cortical Oleoresin and Resistance to the White-Pine Weevil

Abstract

Natural and weevil-larva-induced crystallization of oleoresin from 45 eastern white pine trees with known resin acid and monoterpene composition, and from 59 pairs of nonweeviled and heavily weeviled trees from the same seed sources, was examined in mid- and late spring. Very little difference was found between larva-induced and natural crystallization. Strobic acid—usually the major resin acid found in the cortical oleoresin of eastern white pine—was positively correlated with the rate and extent of crystallization. Crystallization of oleoresin from the same trees varied greatly between sampling dates, but oleoresin from white pines with concentrations of strobic acid of less than 5 percent did not crystallize even after 3 to 10 days from the time of collection. Trees with high concentrations of strobic acid had oleoresin that crystallized within 4 hours. Concentration of sandaracopimaric acid was also positively correlated with crystallization. Concentrations of abietic and neoabietic acids, when expressed as proportions of the total resin acids, were negatively correlated with crystallization. None of eight monoterpenes was consistently related to crystallization properties.

Variation in crystallization reactions is not useful as a selection criterion for weevil resistance. Although noncrystallizing oleoresin was found more frequently among heavily weeviled trees than in nonweeviled trees, the differences were small and not significant. Eastern white pine trees with oleoresin that does not crystallize are rare. Only eight trees had oleoresin that did not crystallize after 3 to 10 days from both samplng dates, and of these, six had been heavily weeviled. Most potential candidates for resistance breeding programs would not be selected because 58 percent of the trees that had resisted weevil attack had oleoresin that crystallized after 4 hours and 97 percent had oleoresin that crystallized after 3 days.

INTRODUCTION

FOR MANY YEARS, researchers attempting to breed eastern white pine trees (*Pinus strobus* L.) that are resistant to the white-pine weevil (*Pissodes strobi* Peck) have been searching for host characteristics, both morphological and biochemical, that are responsible for or correlated with resistance and could be used in an indirect selection program. As early as 1965, Santamour suggested that noncrystallization of cortical oleoresins of white pines may be correlated with resistance to attack by the white-pine weevil; a finding later substantiated by van Buijtenen and Santamour (1972).

Recently, Santamour and Zinkel (1976, 1977) have proposed an interesting hypothesis linking low concentrations or absence of strobic acid—one of the major resin acids found in the cortical oleoresin of eastern white pine—to noncrystallization of oleoresin, and consequently, to resistance to the white-pine weevil. They have advocated screening populations and provenances of eastern white pine to find prospective parents with no strobic acid for use in a program to breed weevil-resistant trees.

Santamour and Zinkel's proposal was greeted with enthusiasm by those concerned with the white-pine weevil problem, but in a recent study I have found that eastern white pines with no strobic acid are as susceptible to white-pine weevil attack as trees with high concentrations (Wilkinson 1979).

Apparently, the three-way relationship involving strobic acid content, oleoresin crystallization, and weevil resistance is still open to question. In this study the relationships between oleoresin crystallization with concentrations of cortical resin acids and monoterpenes and the occurrence of weeviling in eastern white pine have been reexamined.

MATERIALS AND METHODS

The trees usd for this study are all growing in three geographic seed source tests planted in 1960 on the Massabesic Experimental Forest, Alfred, Maine—Plantations 1, 2, and 3. The plantations contain 24 to 28 geographic seed sources of eastern white pine originating from throughout the species range. Each seed source is represented in Plantation 1 by one-tree plots and in Plantation 2 by two-tree plots located in 24 randomized blocks. Plantation 3 has four-tree plots in 12 blocks. The trees were all 22 years old when the study was conducted.

All three plantations are adjacent and there is a high weevil population in the immediate area. Initially all three plantations were protected from the white-pine weevil with insecticide. This was discontinued in 1967 for Plantation 1 and in 1972 for Plantations 2 and 3. Each tree in each plantation was evaluated for weevil damage; Plantation 1 initially in 1970 and again in 1976, and Plantations 2 and 3 in 1976. In each case each tree was rated as heavily, lightly, or not weeviled. Heavily weeviled was defined as having leader mortality in more than two years; lightly weeviled as having leader mortality in 1 or 2 years; and nonweeviled as being free of attack or leader damage in at least 3 years.

Natural crystallization of oleoresin was tested in mid-May and late June, 1978. During the June test larva-induced crystallization was examined using both weevil larvae reared on white pine stems in the laboratory and ones collected from naturally infested stems. Crushed white-pine weevil larva heads and other parts are reported to induce crystallization in white pine cortical oleoresin (Santamour 1965).

The procedure for analyzing oleoresin crystallization largely followed that described by Santamour (1965) and van Buijtenen and Santamour (1972). The experimental procedure was as follows: Lateral branches were cut from near the top of the crown of each tree and transported to a laboratory. Small incisions were then made into the cortex of 1-year-old internodes of each branch and two 10-microliter samples of the exuded oleoresin were drawn into calibrated microcapillary tubes and then transferred to glass microscope slides. In June, a third oleoresin sample was collected from each tree and a weevil larva head was crushed into that oleoresin droplet.

Crystallization of the oleoresins at 21° to 27°C was observed and scored by observation of the droplets through a binocular dissecting scope at 4 hours, 8 hours, 24 hours, and at irregular intervals for 3 to 10 days after collection; at which time crystallization remained unchanged for all trees. Each oleoresin sample was scored on a numerical scale based on the area crystallized at each time period. The scoring system was the same as that used by van Buijtenen and Santamour (1972):

0	No crystals
1	Few crystals
2	¼ crystallized
3	½ crystallized
4	¾ crystallized
5	Crystallization complete

Oleoresin crystallization properties in eastern white pine were examined in two concurrent studies.

Study 1: Resin acid concentration and crystallization

The cortical resin acids of 88 trees from Plantation 1 were quantitatively analyzed by gas-liquid chromatography in 1977 (Wilkinson 1979). The monoterpene composition of the cortical oleoresin of these same trees had been determined previously (Bridgen et al. In press). The 88-tree sample consisted of 48 nonweeviled trees and 40 heavily weeviled trees. Strobic acid concentration in these trees ranged from 0.0 to 34.5 percent of the resin acids. Crystallization was studied in the 10 trees with the highest and the 10 trees with the lowest concentrations of strobic acid, plus 1 of each 3 trees from among those with intermediate amounts for a total of 45 trees.

Simple correlations between crystallization scores at each observation period and concentrations of all resin acid and monoterpene components were calculated. Crystallization scores in May and June were treated separately as they were quite variable within the same trees. Replicate samples within a collection period, May or June, had identical or similar scores; and means of the two replications were used for calculating correlations. Larva-induced crystallization scores were similar to scores obtained for natural crystallization, and with only one exception, correlations with resin acid and monoterpene components were nearly identical to those obtained with natural crystallization. Addition of crushed adult weevil heads and heads of horsefly larvae to several additional oleoresin samples resulted in crystallization reactions identical to natural crystallization. Therefore, all correlations reported here are for natural oleoresin crystallization.

Study 2: Crystallization and weevil resistance

Crystallization was studied in nonweeviled trees that exceeded the plantation mean in height and diameter in each of the three plantations, and in a corresponding heavily weeviled tree from the same seed source. Only fast-growing nonweeviled trees were used to reduce the chance that selected trees had escaped weevil attack because of low vigor. In Plantation 1 there were 15 nonweeviled trees that exceeded the mean growth rate at 10 years; 2 years before the first weevil attack. In Plantations 2 and 3 there were 24 and 20 trees, respectively, that exceeded their mean plantation growth rate at 17 years; 1 year after first exposure to weevil attack. In all, crystallization was studied in 59 nonweeviled and 59 heavily weeviled trees for a total of 118.

Seed-source pairs of crystallization scores for nonweeviled and heavily weeviled trees were compared. A sign test was made to determine statistical significance. Crystallization differences between nonweeviled and heavily weeviled trees were also tested by 2 × 5 contingency tables and chi-square test for independence with 4 degrees of freedom. A further test was made by categorizing each tree as having noncrystallizing (score 0) or crystallizing (score 1-5) oleoresin and using a 2 × 2 table with 1 degree of freedom. Comparisons were made for each observation period: 4, 8, and 24 hours and 3 to 10 days; for each sampling period, May and June; and for each plantation separately and all three plantations combined. A series of analyses of variance was also used to test for seed source differences in oleoresin crystallization.

RESULTS AND DISCUSSION

The hypothesis attributing noncrystallization of oleoresin to the absence of strobic acid in eastern white pine had not, until this time, been extensively tested. Studies of oleoresin crystallization reactions, coupled with quantitative gas-chromatographic analyses of resin acids, have involved only a relatively few white pines and white pine hybrids. On the other hand, the reported relationship between noncrystallization of oleoresin and weevil resistance has generally been accepted without question. Surprisingly, this study reveals that there is a clear relationship between crystallization and strobic acid, but that there is no relationship between crystallization and weevil resistance or susceptibility.

Study I: Strobic acid concentration and crystallization

The rate and extent of oleoresin crystallization varied considerably from one tree to another. Oleoresin from white pines with little or no strobic acid did not crystallize readily, even after several days (Table 1). Only 1 tree among 10 with low concentrations of strobic acid had oleoresin that was half crystallized after 3 to 10 days, and this tree had the highest concentration of strobic acid within the group. Oleoresin from four trees with less than 5 percent strobic acid did not crystallize naturally in May or in June; nor did oleoresin from these trees crystallize in the presence of weevil larva heads.

Trees with high concentrations of strobic acid had oleoresin that was half crystallized after 8 hours, and all 10 trees had some crystals forming in the oleoresin after only 4 hours. In May, two trees with 32.8 and 34.5 percent strobic acid had oleoresin that was completely crystallized after 4 hours, although crystallization was slower in these same trees in June.

Resin crystallization is probably dependent on specific resin acids rather than on total resin acid content of the oleoresin (Santamour 1967). It has generally been suggested that any resin acid present in the oleoresin at concentrations greater than 30 percent can trigger the crystallization reaction. Interestingly enough, of the four trees that had low concen-

Table 1.—Mean oleoresin crystallization scores (May and June) in 10 eastern white pine with the lowest and the 10 with the highest strobic acid concentrations

Strobic acid concentration (Percent of resin acids)	Crystallization score after			
	4 hours	8 hours	24 hours	3-10 days
0.0	0	0	0	0
0.0	0	0	0	0
3.1	0	0	0	0
4.1	0	0	0	0
10.1	0	0	1	1
11.6	0	0	0	1
13.0	0	1	1	2
13.0	0	0	1	1
13.5	0	0	1	1
13.5	0	1	2	3
28.8	1	2	2	3
29.0	1	3	3	5
29.0	1	2	3	4
29.2	1	1	2	3
29.5	3	3	4	5
29.8	1	1	1	3
31.4	1	3	3	5
32.8	3	3	3	4
33.3	1	2	3	5
34.5	3	3	3	4

trations of strobic acid and oleoresin that did not crystallize, two had concentrations of neoabietic acid greater than 30 percent and the other two had more than 27 percent.

Oleoresin crystallization differed between the two sampling dates in May and June as well as between individual trees. Twenty-five of 140 trees from both studies had oleoresin that crystallized readily at one sampling time but did not crystallize at all at the other. Correlations between crystallization scores in May and June were only .188 at 4 hours, .262 at 8 hours, .239 at 24 hours, and .393 at 3 to 10 days.

Correlations between strobic acid concentration and crystallization were highly significant for both sampling dates (Table 2). Oleoresin with either high or low concentrations of strobic acid crystallized rapidly or not at all in both May and June, accounting for the similarity in correlation coefficients for the two dates.

Somewhat surprisingly, three other resin acids are also significantly correlated with crystallization; and the coefficients are as large as, or larger than, those for strobic acid. Like strobic acid, sandaracopimaric acid is positively correlated with crystallization. Abietic acid and neoabietic acid are both negatively correlated with crystallization. Correlation coefficients were generally progressively higher from 4 hours to 3 to 10 days for all four resin acids that are significantly correlated with crystallization. But all four resin acids were significantly correlated with crystallization scores after only 4 hours.

The correlations between sandaracopimaric, abietic, and neoabietic acids and crystallization may simply be due to correlations between these resin acids and strobic acid. However, only two trees with intermediate amounts of strobic acid (20 percent) had oleoresin that crystallized readily after only 4 hours. These two trees had the lowest abietic acid (7.2 per-

Table 2.—Simple correlations (r) between oleoresin crystallization and relative concentration of cortical oleoresin components in eastern white pine

Oleoresin components	May sample				June sample			
	Crystallization after:				Crystallization after:			
	4 hr	8 hr	24 hr	3-10 days	4 hr	8 hr	24 hr	3-10 days
Resin acids								
Sandaracopimaric	0.393**	0.408**	0.495**	0.527**	0.372**	0.401**	0.437**	0.601**
Communic	-.134	-.151	-.097	-.081	.095	.150	.156	-.054
Levopimaric/palustric	.053	.027	-.121	-.168	-.126	-.112	-.133	-.282
Isopimaric	-.337*	-.255	-.116	-.075	-.060	-.041	-.029	-.119
Strobic	.425**	.491**	.564**	.599**	.424**	.462**	.487**	.663**
Dehydroabietic	-.184	.038	-.064	-.037	-.058	-.023	-.024	-.061
Abietic	-.466**	-.610**	-.678**	-.674**	-.413**	-.454**	-.498**	-.575**
Neoabietic	-.357*	-.376**	-.406**	-.451**	-.447**	-.465**	-.507**	-.567**
Monoterpenes								
Alpha-pinene	-.076	-.196	-.196	-.193	-.011	.024	.018	.064
Camphene	-.215	-.438**	-.388**	-.358*	-.040	.037	.029	-.034
Beta-pinene	.052	-.100	-.084	-.209	-.165	-.284	-.274	-.210
3-carene	.043	.193	.122	.234	.112	.170	.101	.132
Myrcene	.057	.036	.020	-.059	-.024	-.077	.049	-.102
Limonene	.008	.058	.117	.083	-.092	-.033	.002	-.055
Beta-phellandrene	-.161	.040	.056	.134	.240	.243	.196	.162
Terpinolene	.056	.176	.127	.248	.184	.199	.154	.157
Total monoterpene	-.006	.009	-.049	-.044	.002	.021	-.045	.012

*Significantly different at the .05 level of probability.
**Significantly different at the .01 level of probability.

cent) of any of the 45 trees examined. It may be that crystallization of oleoresin is retarded by high concentrations of some resin acids. Santamour (1967) has cited several instances where this proved to be true.

Only one monoterpene component of the oleoresin was significantly correlated with natural crystallization, and that correlation was not consistent. Camphene was significantly negatively correlated with crystallization in May but not in June. In May, oleoresin crystallization was negatively correlated, although not quite significantly, with oleoresin viscosity. Oleoresin droplets that tended to spread out on the glass slides crystallized more readily than those that remained as small-diameter droplets. Camphene was positively correlated with viscosity in these same trees (Bridgen et al. In press). In June, differences in viscosity, determined by measuring the diameter of oleoresin droplets, between trees were noticeably less pronounced than they were in May. Most samples were less viscous in June, possibly because of higher ambient temperatures at the time of collection.

Monoterpene concentrations in the oleoresin (including that of limonene, the only monoterpene related to weevil resistance by Bridgen et al.) is probably not directly related to crystallization. However, concentration of beta-phellandrene was significantly positively correlated with crystallization when crushed weevil larva heads were introduced into oleoresin samples. All other correlations between larva-induced crystallization and chemical components were nearly identical to those obtained with natural crystallization, and for that reason I did not report them here. Close inspection of oleoresin samples with introduced larva heads revealed that in trees with high beta-phellandrene a cloudy white precipitate could possibly be mistaken for crystallization. This precipitate was most noticeable at 4 hours, but tended to be obscured by normal crystallization as time passed. In fact, correlations between beta-phellandrene and crystallization were highest at 4 hours and were nonsignificant at 3 to 10 days. The precipitate was not found when natural crystallization was observed in trees with high concentrations of beta-phellandrene.

Table 3.—Simple correlations (r) between oleoresin crystallization and resin acid composition as a percentage of the oleoresin in eastern white pine

Resin acids	May sample				June sample			
	Crystallization after:				Crystallization after:			
	4 hr	8 hr	24 hr	3-10 days	4 hr	8 hr	24 hr	3-10 days
Sandaracopimaric	0.337*	0.399**	0.417**	0.449**	0.475**	0.494**	0.527**	0.644**
Communic	−.059	.042	.038	.085	.325*	.399**	.427**	.282
Levopimaric/palustric	.106	.184	.043	.027	.203	.146	.175	.077
Isopimaric	−.206	−.036	.020	.084	.217	.258	.280	.222
Strobic	.367*	.462**	.480**	.509**	.518**	.535**	.558**	.676**
Dehydroabietic	−.158	−.033	−.042	−.014	.002	−.058	−.050	−.138
Abietic	−.403**	−.449**	−.545**	−.514**	−.178	−.199	−.220	−.226
Neoabietic	−.210	−.080	−.156	−.142	.009	.021	.009	−.008
Total resin acids	.041	.167	.111	.146	.378**	.385**	.396**	.380**

*Significantly different at the .05 level of probability.
**Significantly different at the .01 level of probability.

In all, four resin acids were significantly correlated with crystallization scores in both May and June. The correlations in Table 2 are based on resin acid concentrations expressed as a percentage of the total resin acids (relative basis) and correlations due to constraint are present. Correlations in Table 3 are based on resin acid concentrations expressed as a percentage of the oleoresin volume analyzed. Correlations between crystallization and neoabietic acid concentration on an absolute volume basis are close to zero. The correlations between abietic acid and crystallization are also much lower than they are on a relative basis; in fact, they are nonsignificant in the June tests. A significant positive correlation between crystallization and communic acid was revealed by expressing resin acid concentration on an absolute basis, but only in the June tests. The positive correlations between crystallization and sandaracopimaric acid, and most importantly strobic acid, are consistently high regardless of how the data are expressed. Apparently the oleoresin crystallization reaction involves complex interactions between several resin acids. Multiple correlation analyses might have been useful to sort out these relationships, but were beyond the scope of this study.

My study confirms a strong relationship between strobic acid concentrations and crystallization of cortical oleoresin from eastern white pine. Concentration of strobic acid could be useful for predicting crystallization. If trees were located that have less than 5 percent strobic acid in their oleoresin, it would be fairly certain that oleoresin from those trees would not crystallize. But several trees with intermediate and even relatively high concentrations of strobic acid had oleoresin that crystallized in May but did not crystallize in June. The reverse pattern also occurred frequently.

At this time it is not known whether the observed variation in crystallization is seasonal or whether large differences can occur in oleoresin obtained from different branches on the same tree. The degree of within-tree stability of resin acid concentration on a seasonal and year-to-year basis in eastern white pine is largely unknown. The measurements of strobic acid concentration and concentrations of the other resin acids that I made were not made at the same time as evaluations of crystallization. Changes in the concentrations of resin acids may be involved in observed differences in oleoresin crystallization at different times.

Weevil larva-induced crystallization. Crystallization induced by crushing insect larva heads into oleoresin is reported to be more rapid than natural crystallization (Yates 1962, Santamour 1965). In this study I found very little difference in crystallization between oleoresin samples treated with crushed weevil larva heads and those allowed to crystallize naturally. Crystallization scores for natural and larva-induced crystallization did not differ by more than 2 points in samples from the same tree. Larva-induced crystallization had higher scores in only 6 samples after 4 hours, 8 samples after 8 and 24 hours, and 12 samples after 3 to 10 days. Correlations between natural crystallization scores and larva-induced crystallization scores in the same trees ranged from a low of .606 at 4 hours to a high of .792 after 3 to 10 days. Most important, introduction of larva heads into oleoresin samples from trees with low concentrations of strobic acid and naturally noncrystallizing oleoresin did not result in crystallization of those samples.

The 10-microliter samples that I used were ideal for testing natural crystallization, but they may have been too small to function properly in larva-induced crystallization tests and that could be one reason why I found little difference between the two methods. Necessary but extensive spreading of the oleoresin droplets on the glass slides when crushing the larvae head can retard crystallization; the sample probably dries before crystallization can occur. Large samples would not dry as quickly.

These results do suggest, however, that the degree of larva-induced crystallization is closely related to natural crystallization. Van Buijtenen and Santamour (1972) induced crystallization in 89 percent of their samples after only 4 hours. Natural crystallization in my study was slower, but it was more rapid than the 4 to 12 days required for stored shoot resins to crystallize reported by Santamour (1965). In the combined sample of 140 trees from both studies, 57 percent had oleoresin that was crystallized at 4 hours, 72 percent at 8 hours, and 87 percent at 24 hours. Only two trees had oleoresin that did not form crystals until 3 days after collection, and crystallization scores for all but a few trees did not change between 3 and 10 days.

Natural crystallization appears to be as useful as larva-induced crystallization for discriminating between trees with crystallizing and noncrystallizing oleoresin. Overall, only 6.8 percent of the oleoresin samples from the trees tested did not crystallize naturally. Van Buijtenen and Santamour (1972) found 9.6 percent noncrystallizers among trees from the same seed sources. Using weevil larva heads to induce crystallization seems to have no special advantage, except shortening analysis time, over natural crystallization for testing oleoresin crystallization properties. Requirements for large oleoresin volumes, ease of contamination of samples with extraneous materials that could inadvertently trigger crystallization, and the precipitate formed in samples with high beta-phellandrene in the oleoresin could be serious disadvantages.

Study II: Crystallization and weevil resistance

Santamour (1965) showed that trees containing oleoresin that would not crystallize in response to contact with crushed heads of weevil larvae appeared to be more resistant to weevil attack, but exceptions did occur. Van Buijtenen and Santamour (1972) also found noncrystallization associated with resistance to weevil attack; but some trees containing noncrystallizing resin were heavily attacked, and some trees containing readily crystallizing resin were attacked unsuccessfully.

Analyses of resin acid composition in 88 eastern white pine revealed only two trees that had no strobic acid (Wilkinson 1979). Both of these trees were heavily weeviled. Now I have found that the oleoresin from both of these trees does not crystallize even after several weeks in storage. I have also found in this study that mean crystallization scores of oleoresin from nonweeviled and heavily weeviled white pine from three plantations, sampled in May and again in June, and observed after 4, 8, and 24 hours and 3 to 10 days, are very similar (Table 4). In many

Table 4.—Mean resin crystallization scores in nonweeviled and heavily weeviled eastern white pine in three plantations in May and in June

Crystallization after:	Weeviling category	Plantation 1			Plantation 2			Plantation 3		
		May	June	May and June	May	June	May and June	May	June	May and June
4 hours	Nonweeviled	0.3	0.8	0.7	1.0	1.0	1.1	0.5	0.8	0.8
	Heavily weeviled	.5	.4	.5	1.2	1.2	1.2	.7	1.0	1.0
8 hours	Nonweeviled	1.1	1.2	1.1	2.0	1.2	1.8	.9	1.2	1.0
	Heavily weeviled	1.1	.7	.9	2.0	1.4	1.7	1.1	1.4	1.4
24 hours	Nonweeviled	1.8	1.6	1.7	2.5	1.7	2.2	1.3	1.6	1.6
	Heavily weeviled	2.0	1.0	1.7	2.6	1.8	2.3	1.6	1.7	1.8
3-10 days	Nonweeviled	2.8	2.6	2.8	3.8	2.7	3.4	2.5	2.4	2.6
	Heavily weeviled	2.9	2.0	2.7	3.6	2.6	3.3	2.9	2.2	2.7

cases mean crystallization scores are higher in nonweeviled trees.

Sign tests for paired comparisons of crystallization scores in nonweeviled and heavily weeviled trees of the same seed sources in each plantation and all three plantations combined were nonsignificant in all cases. Analyses of variance revealed that there were no significant seed source differences in oleoresin crystallization and that seed source pairing was not necessary.

A 2 × 5 table and chi-square test were used to test differences in numbers of nonweeviled and heavily weeviled trees within each crystallization score. There were no significant differences in any of the three plantations, in either May or June, nor at any observation time from 4 hours to 3 to 10 days. Many of the cells contained only a few observations, and the data were combined into two groups. Chi-square tests for independence showed that there were no differences in numbers of trees with crystallizing and noncrystallizing oleoresin between the nonweeviled and heavily weeviled groups (Table 5).

In combined May and June tests more than 50 percent of both nonweeviled and heavily weeviled white pine had oleoresin that crystallized after 4 hours and more than 70 percent of both had oleoresin that crystallized after 8 hours. The number of trees with noncrystallizing oleoresin was much greater in June than in May, and of these trees more than 50 percent were heavily weeviled. At each period except 4 hours in May the heavily weeviled group had more trees with noncrystallizing oleoresin than did the nonweeviled group. Only eight trees had oleoresin that did not crystallize after 3 to 10 days in both May and June. Only two of these trees were nonweeviled; the other six were heavily weeviled. These 8 trees represent 6.8 percent of the total trees sampled, which is very close to the 4.5 percent of 88 trees that had very low concentrations of strobic acid.

This study clearly shows that there is no relationship between crystallization and resistance, but van Buijtenen and Santamour (1972) found that 20 of 209 trees had noncrystallizing oleoresin and that only 3 of these 20 trees had been successfully attacked by the weevil. These differences in results could conceivably be caused by differences in technique: natural versus larva-induced crystallization. However, the differences may be only a consequence of sampling scheme. All 59 nonweeviled trees that I studied had been attacked by the weevil in at least 1 of 3 years, but had survived the attack without leader mortality. In the other years they were free of attack. Most of the trees had, in fact, survived attack in 2 or 3 years. Only 3 of the 17 unsuccessfully attacked trees tested by van Buijtenen and Santamour (1972) were comparable. The other 14 trees had not been attacked at all. Therefore, even in their study, equal numbers of trees that had been successfully attacked and ones that had not been successfully attacked had noncrystallizing resin. Moreover, 11 of their trees that had not been successfully attacked had resin that crystallized.

Eastern white pine trees with oleoresin that does not crystallize are rare. Early selection, before the onset of weevil attack, or selection in lightly weeviled stands and plantations, of

Table 5.—Percentage of nonweeviled and heavily weeviled eastern white pines from which resin crystallized in 4, 8, 24, and 72+ hours

Hours	Date sampled					
	May		June		May and June	
	Nonweeviled	Weeviled	Nonweeviled	Weeviled	Nonweeviled	Weeviled
4	34	44	49	41	58	56
8	69	66	54	49	73	69
24	83	81	64	61	88	85
72+	90	86	81	68	97	90

None of the differences between weeviled and nonweeviled trees are statistically significant.

trees with noncrystallizing oleoresin would produce no more potentially resistant trees than would a completely random selection. Much more important, many potential candidates for resistance breeding programs would not be selected. My studies show that from 48 to 57 white pine from among 59 trees that had resisted weevil attack would not be selected because these trees all had oleoresin that crystallized.

LITERATURE CITED

Bridgen, M. R., J. W. Hanover and R. C. Wilkinson.
(In press). **Oleoresin characteristics of eastern white pine seed sources and relationship to weevil resistance. For. Sci.**

Santamour, F. S., Jr.
1965. **Insect-induced crystallization of white pine resins.** I. White-pine weevil. USDA For. Serv. Res. Note NE-38, 8 p.

Santamour, F. S., Jr.
1967. **Notes on pine resins and pest resistance.** Morris Arbor. Bull. 18(4):82-86.

Santamour, F. S., Jr., and D. F. Zinkel.
1976. **Weevil-induced resin crystallization related to resin acids in eastern white pine.** Northeast. For. Tree Improv. Conf. Proc. 23: 52-56 (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. Proc. 25:164-175 (1977).

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.

Wilkinson, R. C.
1979. **Cortical strobic acid concentrations in eastern white pine resistant and susceptible to the white-pine weevil.** Northeast. For. Tree Improv. Conf. Proc. 26 (1978).

Yates, Harry O., III.
1962. **Influence of tip moth larvae on oleoresin crystallization of southern pines.** USDA For. Serv. Res. Note SE-174, 2 p.

☆U.S. GOVERNMENT PRINTING OFFICE: 1979—603-011/14

Headquarters of the Northeastern Forest Experiment Station are in Broomall, Pa. Field laboratories and research units are maintained at:

- Beltsville, Maryland.
- Berea, Kentucky, in cooperation with Berea College.
- Burlington, Vermont, in cooperation with the University of Vermont.
- Delaware, Ohio.
- Durham, New Hampshire, in cooperation with the University of New Hampshire.
- Hamden, Connecticut, in cooperation with Yale University.
- Kingston, Pennsylvania.
- Morgantown, West Virginia, in cooperation with West Virginia University, Morgantown.
- Orono, Maine, in cooperation with the University of Maine, Orono.
- Parsons, West Virginia.
- Princeton, West Virginia.
- Syracuse, New York, in cooperation with the State University of New York College of Environmental Sciences and Forestry at Syracuse University, Syracuse.
- University Park, Pennsylvania.
- Warren, Pennsylvania.