Inheritance of Yellow Oleoresin and Virescent Foliage in Slash Pine

By John F. Kraus and A. E. Squillace

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Simply inherited, qualitative traits, although often not of economic importance, can be valuable aids in studies of reproductive systems, cytogenetical analyses, population studies, and others. Such "gene markers" have been used extensively in animal and crop plant genetics, and results of these studies have been basic to breeding work. In forest trees, where life spans are relatively long, qualitative traits are especially useful when their expression occurs early in the life of the plant.

The occurrence of two such characters in slash pine (Pinus elliottii Engelm.) is described in this report. One of these is yellow oleoresin, and the other is a type of chlorophyll deficiency, "virescent foliage". They were found incidentally in a program designed to breed for high oleoresin yield (Mitchell et al., 1942). A third qualitative character occurring in slash pine, albinism, was reported upon earlier (Squillace and Kraus, 1965).

Yellow Oleoresin

Normal slash pines yield an oleoresin that is transparent and colorless when fresh. Occasionally deviants from this type have been found. One, identified as tree 29, yields a golden yellow oleoresin. Analysis of the turpentine fraction of this oleoresin by gas chromatography has revealed no difference in chemical composition which would account for the unusual color. However, the \( \beta \)-phellandrene content was unusually high, 405 percent in contrast to 7 percent or less in commercial turpentine reported by Fisher et al. (1957). Turpentine yield as a percent of the oleoresin weight was relatively low, 15 percent, compared with the normal yield of about 22 percent given by Minov (1961).\(^5\)

 CROSSES WERE MADE IN THE SPRING OF 1960 (TABLE 1). AT THE TIME OF CONE COLLECTION, CONES POLLENALSO WERE COLLECTED. SEED WERE EXTRACTED, AND WINGS REMOVED. SOUND AND UNSOUND SEED WERE SEPARATED IN AN AIR COLUMN SEED SEPARATOR. SEED WERE SOWN IN THE SPRING OF 1962 IN ROW PLOTS RANDOMIZED AND REPLICATED THREE TIMES.

In November 1962 a narrow strip of bunk and wood approximately 1 inch long was cut from the stem of each seedling. Within 2 hours enough oleoresin had exuded from the wounded surface to permit discrimination of oleoresin color.

Table 1. - Results of crosses on slash pine 29 producing yellow oleoresin.

<table>
<thead>
<tr>
<th>Mating</th>
<th>Seedlings producing yellow oleoresin</th>
<th>Total seedlings examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>29 X 29</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>29 X (1960 mix(^1))</td>
<td>0</td>
<td>271</td>
</tr>
<tr>
<td>29 X wind</td>
<td>1</td>
<td>140</td>
</tr>
</tbody>
</table>

\(^{1}\) The "1960 mix" consisted of equal volumes of pollen from 21 trees.

All of the selfed seedlings produced yellow oleoresin. All of those from outcrosses produced normal colorless oleoresin (table 1). One seedling among the wind-pollinated progeny produced yellow oleoresin. This probably resulted from natural self-pollination.

These results indicate that tree 29 is homozygous for a recessive gene or genes governing production of yellow oleoresin. Further breeding by selfing or crossing trees

\(^5\) Thanks are due Mr. Gordon S. Fisher of the U. S. D. A. Agricultural Research Service, Naval Stores Laboratory, Olustee, Florida, for his analysis and interpretation of the oleoresin composition.
heterozygous for this trait would be necessary to determine the number of genes involved.

**Virescent Foliage**

Chlorophyll deficiencies of various types have been described for a large number of plant species. Included are Scotch pine (*P. sylvestris* L.) (Eichh., 1955); eastern white pine (*P. strobus* L.) (Johnson, 1945); and Austrian pine (*P. nigra* var. *austriaca* [Hoess] Aschers. and Graebn.) (Johnson, 1948).

Three chlorophyll-deficient trees were located in a 17-year-old plantation of slash pine progeny being tested for oleoresin yield. All are from the wind-pollinated progeny of the same mother tree. One of them has been described previously (Downs, 1949). The trees are similar in type, but they differ in the intensity of their chlorophyll deficiency. They exhibit the trait most strongly during dry summers. Tree 193 is the most deficient; its new needles each year show an alternating pattern of bright yellow and green. At the other extreme, tree 194 exhibits its deficient character only during the driest summers and then the bright yellow is restricted to the basal one-fourth of the new needles. In late fall all three trees develop a normal green color. The needles do not show the trait during their second year. This type of chlorophyll deficiency is probably best classified as *virdoalbina* (Gustafsson, 1942), or in the terminology of maize genetics as a virescent (Demerec, 1935).

Controlled pollinations were made on two of the virescent phenotypes, 193 and 194, in 1959 and 1960 (table 2). Cone collection and subsequent procedures were as previously described.

Chlorophyll-deficient seedlings were detected 4 weeks after sowing. The cotyledons were normally pigmented, but the cluster of primary needles appeared yellow in the center of the cotyledons. As the primary needles developed, the seedlings gradually assumed a normal green color. A similar sequence of development by chlorophyll-deficient seedlings has been reported for Austrian pine (Johnson, 1948).

Results show that the trait is heritable (table 2). Both parents produced a large proportion of virescent seedlings when selfed or when mated to each other, and they produced none or few when outcrossed. The data clearly indicate the heritable nature of the trait, but some latitude is possible in interpretation. If we accept the low frequency of virescents in the outcross progenies as indicating that trees 193 and 194 are homozygous recessive for one or more genes regulating chlorophyll development, then the normal seedlings among the selfed progenies require explanation. These could be the result of contamination by outcross pollen or of classification errors at the time the seedlings were examined. Soil moisture conditions seem to exercise some control over the phenotypic expression of the trait in larger trees, and variations in microclimatic conditions in the nursery possibly could have caused classification errors.

Because the existing data are subject to such diverse explanations, all involving questionable assumptions, it seems reasonable at this time to state only that a heritable chlorophyll deficiency exists in slash pine. We must wait for the results of further breeding to clarify the mode of inheritance.

**Discussion**

The potential usefulness of these aberrant genotypes lies in the ease of identification at an early age and in the manner of difference physiologically and biochemically from normal seedlings. The yellow oleoresin genotype seems to possess characteristics useful in studies of the biosynthesis and transport of oleoresin. Chlorophyll-deficient mutants in other crops have found extensive use in studies of plastid development (Gustafsson, 1942), mutation rates (Eichh., 1955), and pollination studies (Cuany, 1958), to mention only a few.

Presently being considered is the inclusion of yellow oleoresin, virescent foliage, and albino carrier genotypes in slash pine seed orchards of clones selected for economic traits. The frequency of aberrant seedlings produced under these conditions should provide valuable information on the occurrence of selfing in the orchards. Clones containing marker traits could be rogued at a relatively early age in the life of the orchard to prevent contamination.

**Summary**

A single slash pine tree known to produce yellow oleoresin was selfed and outcrossed. All of the selfed seedlings produced yellow oleoresin, but none of the outcrossed ones did. The trait was carried as a homozygous recessive, but the number of genes involved has yet to be determined. Two other slash pines with virescent foliage were selfed, outcrossed, and crossed with one another. The seedlings produced showed the heritable nature of the chlorophyll deficiency, but clarification of the mode of inheritance will require further breeding. The potential usefulness of these aberrant genotypes in forest genetics and physiology is discussed.

**Résumé**

Tite de l'article: *Hérédité du caractère résine jaune et feuillage panaché chez Pinus elliottii*.

Un individu de *Pinus elliottii* connu pour produire une résine jaune fut autofécondé et soumis à des croisements contrôlés. Tous les semis autofécondés ont produit une résine jaune, par contre aucun des semis résultant des croisements contrôlés. Ce caractère est transmis comme homozygote récessif, mais le nombre de gènes qui le commandent doit encore être déterminé. Deux autres pins avec feuillage panaché furent autofécondés, soumis à croisements contrôlés et croisés entre eux. Les semis produits ont montré le caractère héréditaire de la déficience chlorophyllienne, mais l'explication du mode d'hérédité demeure de nouvelles recherches. On discute les possibilités d'utilisation de tels génotypes aberrants en génétique et physiologie forestière.
Zusammenfassung

Titel der Arbeit: Über die Vererbung von gelbem Oleoresin und vireszenten Benadelung bei Pinus elliottii.


Literature Cited


Sex Ratio and Sex-Related Characteristics in Eastern Cottonwood

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This paper deals with sex ratio and secondary sex characteristics in a Mississippi Valley cottonwood (Populus deltoides Bartr.) population. Early empirical observations in natural populations of Populus species (P. tremuloides, P. tremula, P. deltoides) revealed a preponderance of males (1, 8, 9). More recently Paulus and Mennel (7) have reported a sex ratio of 3:1 (male : female) in a P. tremuloides population, while Enspar (8) has published data on P. tremuloides showing no significant departure from a 1:1 ratio. Several studies have pointed to the possibility of male superiority in vigor and form (4, 5, 6). Variance in reported sex ratios in aspen and lack of data on eastern cottonwood provided the stimulus for the study described below.

Methods

In August 1962, ten 30-foot-wide transects were established in natural, even-aged cottonwood stands in the flood plain of the Mississippi River between Vicksburg and Clarksdale, Mississippi. The first 60 dominant and codominant trees intersected by each transect were numbered from 1 to 60 with white marking paint. In December 1962, after leaves had fallen, each tree was sexed on the basis of the external characteristics of its flowerbuds (Fig. 1) as seen from the ground with 7 X 50 binoculars. Trees were visited again during flowering in March 1963, when positive sex identification was established and the first five males and first five females on each transect were measured for age, d. b. h., height, form class, stem straightness, and degree of branching. Specific gravity determinations based upon large-core (11.3 mm. diameter) samples from each tree were made on four transects. Two cores were removed from each tree, and six wood samples were obtained by taking one 2-cm. section from inner, middle, and outer portions of each core.

Results

A total of 551 trees flowered out of the 600 marked; 54 percent of these were males and 46 percent females (table 1). A chi square test revealed no significant departure from a 1:1 sex ratio on any of the transects or in the pooled sample. Male trees predominated on 7 out of 10 transects.

1) The author is stationed at the Southern Hardwoods Laboratory, which is maintained at Stoneville, Mississippi, by the Southern Forest Experiment Station in cooperation with the Mississippi Agricultural Experiment Station and the Southern Hardwood Forest Research Group.

Fig. 1. Twigs of male and female cottonwoods bearing dormant flowerbuds. Male flowerbuds (right) average 1 inch in length and typically have a curved shape. Female flowerbuds are smaller and similar to leaf buds in external appearance.