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Variation and Inheritance of Levopimaric Acid Content and its Relationship to Oleoresin Yield in Slash Pine

By A. E. SQUILLACE, G. W. HEDRICK, and A. J. GREEN[1]

Levopimaric acid is a potentially useful chemical occurring in moderately large quantities in the oleoresin of slash (Pinus elliottii ENGELM.) and longleaf (P. *palustris* MILL.) pines (HEDRICK et al., 1965). Procedures for isolating mixtures of resin acids rich in levopimaric acid were recently described by SUMMERS et al. (1963), and the process has a potential for commercial use (SUMMERS et al., 1965). Because these recent developments have a direct bearing on breeding for high oleoresin yield, an exploratory study was undertaken to investigate the feasibility of selection and breeding for high levopimaric acid content and to determine its relationship to oleoresin yield.

Procedure

A group of 22 trees representing 4 full-sib families and 1 half-sib family and a group of 25 rooted cuttings from 14 clones were sampled. The 18-year-old trees were growing in separate plantations at Olustee, Florida. Both plantations were chipped under commercial methods (BENGTSON and SCHOPMEYER, 1959) in 1964 and 1965 to obtain oleoresin yield. Oleoresin yield data were adjusted to a common base of 10 inches d.b.h. by regression (SCHOPMEYER and LARSON, 1955).

Oleoresin samples for analysis of levopimaric acid content were taken from the progenies on June 22, 1964, and from the clones on May 2, 1966. Eleven of the trees sampled on June 22, 1964, were resampled on December 14, 1964. Methods described by LLOYD and HEDRICK (1961) were used to determine levopimaric acid content, expreased as a percentage of the resin acids.

Results

Effect of sampling date. — In the trees sampled on two dates, average levopimaric acid content was 24.6 percent on June 22 and 26.2 percent on December 14. The difference was not statistically significant, and it was concluded that seasonal effects, if any, are minor.

Tree-to-tree variation *and* inheritance: —

Differences in levopimaric acid content between ramets of the same clone varied from as little as 0.4 to as much as 7.6 percent, and the clone means varied from 22 to 32 percent. Clonal differences were significant at the 0.05 level ($F = 3.78$, with 13 and 11 degrees of freedom for clones and within-clones, respectively). Broad-sense heritability was estimated to be 0.61 ± 0.34 with 90 percent confidence. The means of the five families varied from 22 to 26 percent, but the differences were not statistically significant.

Although the clonal data suggest the presence of genetic variation, more intensive sampling would be required for proof.

Relationship of levopimaric content to oleoresin yield: —

Phenotypic correlation coefficients (r) between levopimaric acid content and oleoresin yield were as follows:

	d.f.	r
Between clones	13	.29 NS
Within clones	10	.74**
Total	23	.32 NS
Between families	4	.47 NS
Within families	16	.56*
Total	20	.49*

NS = Nonsignificant
* = Significant at the .05 level
** = Significant at the .01 level

The results suggest that high oleoresin yielders tend to have high levopimaric acid content, at least when trees are compared within clones or within families.

Among the clones sampled for levopimaric content, 11 had been bred and progeny tested for oleoresin yield. Indices, expressing the oleoresin yielding ability of the progeny of each clone, were computed and compared against levopimaric acid content in a correlation analysis. The correlation coefficient was found to be .80 ($.48 < r < .93$, with 90 percent confidence), significant at the .01 level. This correlation suggests that the relationship between oleoresin yield and levopimaric acid content is partially genetic.

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Freezing Resistance of Conifers[1]

By A. SAKAI[2] and S. OKADA[3]

Introduction

Desiccation injury in winter and frost injury occuring in various seasons constitute the greatest limiting factors for growing forest trees individually or in combination. From an ecological point of view, these injuries are also known to be the most important factors affecting distribution of plants. However, no extensive survey concerning the freezing resistance of main coniferous species has been carried out.

To clarify the characteristics in freezing resistance of the main genera of conifer, and the relation between the freezing resistance and meteorological conditions of their native habitats, the freezing resistance of over 100 coniferous species native to the northern hemisphere was investigated.

Materials and Methods

Winter twigs taken from about 10 year old trees and saplings of 3 to 6 years of age which are grown in the Tokyo University Forest at Yamabe, Hokkaido, or the Hokkaido Branch of the Breeding Station of Forest Trees in Sapporo were mainly used. The mean January temperatures in Yamabe and Sapporo are about —7 and —5° C respectively. The snow cover in winter amounts to about 1 to 2 m at both stations.

Some coniferous species not wintering in Hokkaido were sent from Honshu, chiefly from the Nagano prefecture in late December to mid January. Thereafter, materials sent from Honshu were hardened at —3 and —5° C for 2 weeks in hardy species and at 0 and —3° C for 2 weeks in less hardy species respectively to enhance their freezing resistance maximum (SAKAI 1970 A).

In each experiment, 3 to 5 of saplings or 1-year-old winter twigs were used. Twigs or saplings were placed in a polyethylene bag. They were kept in a cold chamber at —5° C for one hour. Thereafter, they were transferred at 2 hours intervals to successively colder chambers varying by 5 to 10° C until the desired test temperatures were reached. These twigs or saplings were kept at the test temperatures for 16 hours, and then were thawed in air at 0° C. To determine the pre-freezing temperatures (SAKAI 1960, 1965) which are effective in maintaining the viability of the twigs, the twig pieces were bound with thread and pre-frozen for 6 hours at temperatures of —15 to —70° C. These pre-frozen twigs were then immersed directly in liquid nitrogen (—196° C) for 30 minutes. They were transferred to a chamber at —30° C for 2 hours before being exposed to 0° C. The frozen twigs and saplings place in polythylene bags, were kept at room temperature for 1 month. Thereafter, the freezing injury was determined by the degree of browning in each tissue. The degree of freezing resistance in twigs and saplings was represented by the lowest temperatures at which they survived freezing without injury.

Climatic zones in native habitats of conifers were divided into 7 zones based on the annual minimum temperatures (REHDER 1967). The average annual minimum of the temperatures of these zones are as follows: Zone I; exceeding —50° C, Zone II; —50 to —35°, Zone III; —35 to —20° C, Zone IV; —20 to —10° C, Zone V; —10 to —5° C Zone VI; —5 to 5° C, Zone VII; 5 to 10° C (REHDER 1967).

The results of plantation of conifers in Hokkaido were divided into 3 grades (good, bad, impossible) based on the growing tests at Yamabe and Sapporo for several years.

Results

1. Seasonal variations of freezing resistance in different tissues of Saghalien fir (Abies sachalinensis) and Cryptomeria (Cryptomeria japonica)

Seasonal variations of freezing resistance in different tissues of 5-year-old Saghalien fir native to Hokkaido were investigated. As shown in *Fig. 1,* in any season the freez-

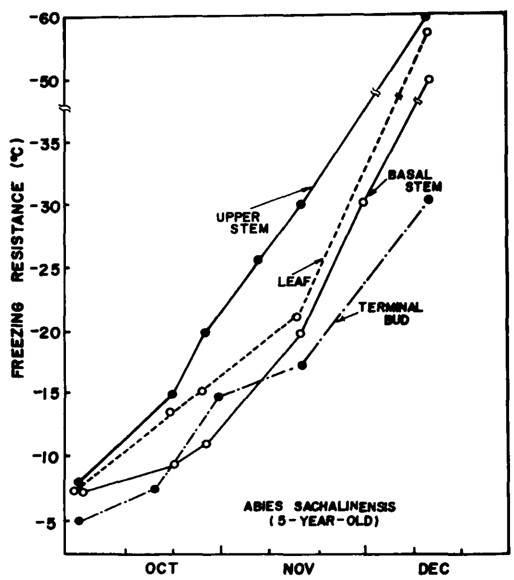

Fig. 1. — Seasonal variations of freezing resistance in different tissues of *Abies sachalinensis.*

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