WOLFGANG LANGNER die Aufwartung zum 70. Geburtstag gemacht wird, so geschieht dies mit Dankbarkeit für seine Arbeit und in Hochachtung seiner Leistung sowie zugleich in der Hoffnung, daß Prof. LANCNER neben zahlreichen anderen Arbeiten auch sein neu angelegtes Privat-Arboretum in Ritzerau, unweit Schmalenbeck, noch lange gesund und mit viel Freude betreuen möge. Gleichzeitig möge die „Silvae Genetica" als eines seiner Lebenswerke weiterhin ihre Aufgabe zum Wohle der Forstgenetik und Forstpflanzenzüchtung in aller Welt erfüllen und sich weiter entwickeln.

HELGE JOHNSSON

Geographic Variation of Monoterpene in Cortical Oleoresin of Slash Pine

By CH. R. GANSEL and A. E. SQUILLACE[1])

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Introduction

Geographic variation in monoterpene composition has been studied in several species and appreciable differences have usually been found, especially where a large portion of the species range was covered. In sampling only a limited portion of the range of slash pine (*Pinus elliottii* ENGELM.), SQUILLACE and FISHER (1966) found evidence of clinal variation. The present study was designed to sample the entire range of this species and also to determine effects of planting site on monoterpene composition.

Materials and Methods

Samples for this study were obtained from slash pines in 9-year-old provenance planlations establishcd in 1962 at Macon, Georgia; Olustee, Florida; and Fort Myers, Florida. These plantings were established from seed collected from trees at each of 54 locations scattered throughout the species range. A detailed description of the study was given by SQUILLACE (1966).

To study seed source effects, up to 3 individuals in each family in the Olustee plantation were sampled (725 trees). To study planting site effects and interaction between planting site and seed source, 5 individuals in each of 2 families from each of 5 seed sources at each of the 3 plantations were used (150 trees).

Oleoresin samples were obtained from cortical tissue by excising lower crown branch buds ½ inch from the tip. Exuded oleoresin was placed in small screw-cap vials, sealed and stored in a refrigerator until analyzed. All samples were collected in the fall to minimize any seasonal effects. Samples were analyzed within a few weeks of collection to reduce effects of storage (SQUILLACE, 1971). The monoterpene composition of each sample was determined by gas-liquid chromatography on a 20-foot, 3/16 inch, 60/80 mesh chromosorb W eolumn packed with 20 percent carbowax 20 M. The relative amount of each monoterpene was expressed as a percentage of total monoterpenes.

Results

Individual tree variation and classification

Ac expected from earlier studies of variation in monoterpene composition of slash pine (SQUILLACE and FISHER, 1966 and SQUILLACE, 1971), much individual tree variation was found (*Table 1*).

Table 1. — Mean and range of monoterpene composition of cortical oleoresin at Olustee plantation.*

Monoterpene	Mean	Range
	percent	
α-pinene	30.8	3-95
ß-pinene	44.0	0-76
myrcene	7.0	0-60
limonene	7.1	0-81
β-phellandrene	11.0	0-69

* Small or trace amounts of camphene and α-phellandrene were also found in some samples.

Frequency distributions of 4 of the 5 major chemicals, F-pinene, myrcene, limonene, and β-phellandrene, were bimodal. This was expected because SQUILLACE (1971), using progenies of known parentage, had previously shown single-gene inheritance for presence of high β-pinene and myrcene. Also, more recent progeny data (to be published separately) suggested that limonene and β-phellandrene were also controlled by single genes. The only other major chemical, α-pinene, showed some indication of bimodality, but single-gene control could not be shown for it on the basis of control-pollinated progeny data.

In order to check further on the distribution of a-pinene and also to develop a refined classification scheme, all available data on the composition of monoterpenes in slash pine were combined with the present seed source study data. A total of 2292 trees were included.

Preliminary criteria for classifying concentrations of each chemical as "high" or "low" were first chosen on the basis of the modes found in the frequency distributions for all trees. Criteria were set for each of the 4 major chemicals with obvious bimodal distributions. Then all trees were grouped into the 16 possible phenotypes that trees can exhibit (high or low for 4 chemicals). Frequency distributions of all 5 major chemicals, within each phenotype, were then examined individually.

The bimodal tendency for a-pinene (*Fig. 1* C) in the distribution of all trees is caused by a strong negative correlation between concentrations of this chemical and myrcene. Trees having high myrcene concentrations had low α-pinene (*Fig.* 1 A), while trees having low myrcene had high α-pinene (*Fig. 1 B*). There was no clear evidence of bimodality in *Fiyures 1* A and *1 B*. The small mode on the left of *Figure* 1 *B* and the extended tails on the right of

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both figures were shown to be largely due to an interaction with limonene — trees with high limonene tended to have low α-pinene. Thus, the tendency for bimodality of α-pinene was taken to be false, and this chemical was omitted from further analyses.

Frequency distributions for the other 4 monoterpenes, within the 16 categories, revealed nothing to reject the bimodal hypotheses *(Figs. 2 to 5)*. However, the results led to refinements in the classification schemes for β-pinene and myrcene. Among trees having high limonene, the low β-pinene group ranged from about 0 to 4 percent *(Fig. 2 A)*,

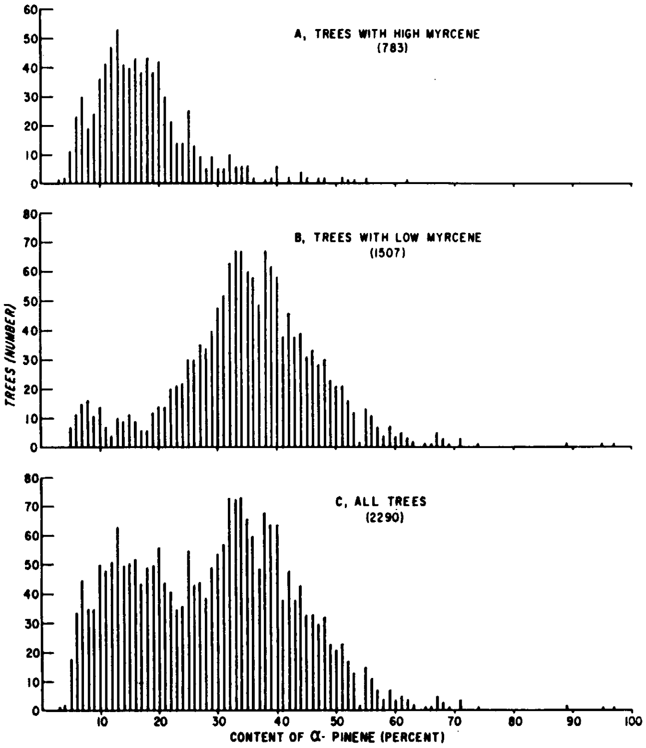

Figure 1. — Frequency distributions for α-pinene.

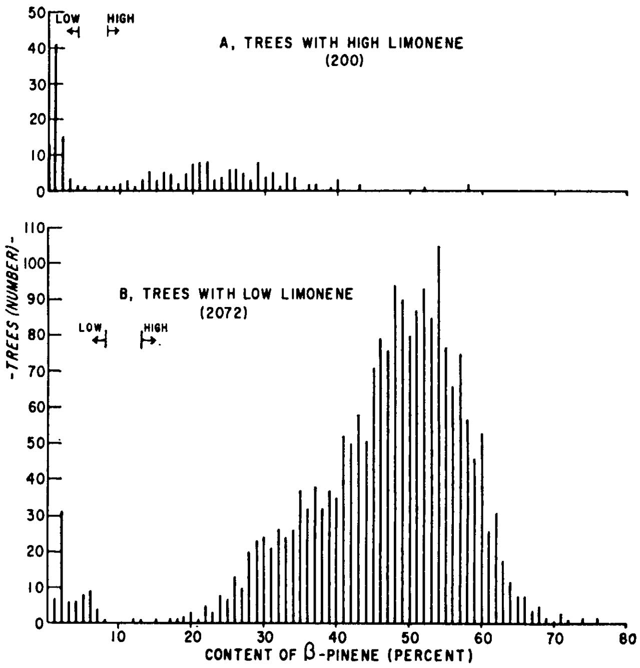

Figure 2. — Frequency distributions for β-pinene.

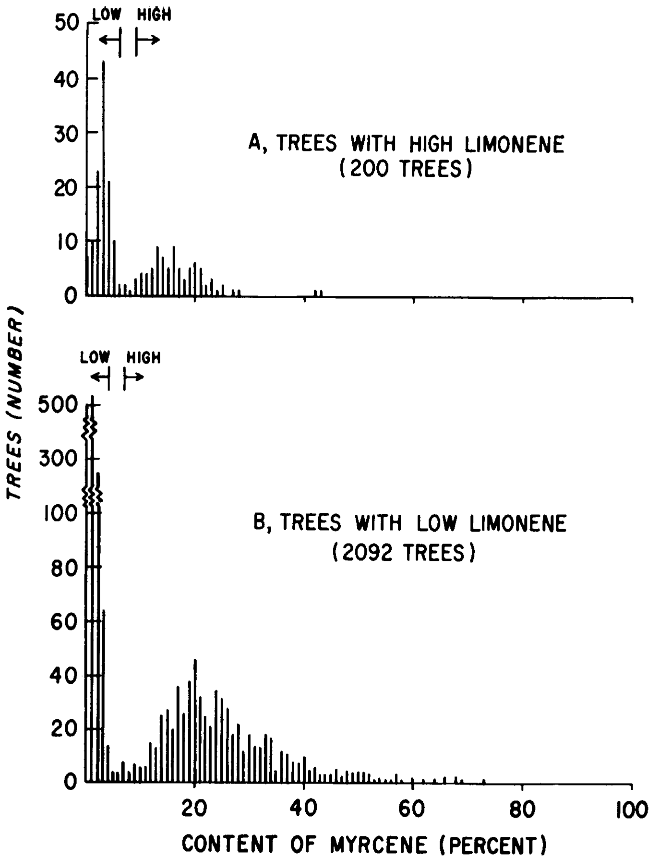

Figure 3. — Frequency distributions for myrcene.

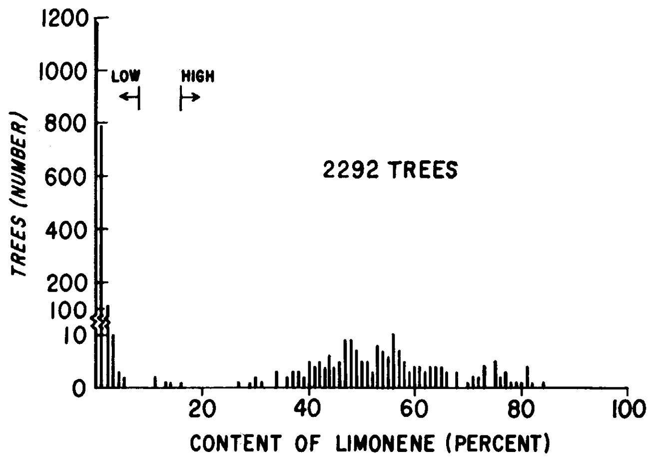

Figure 4. — Frequency distribution for limonene.

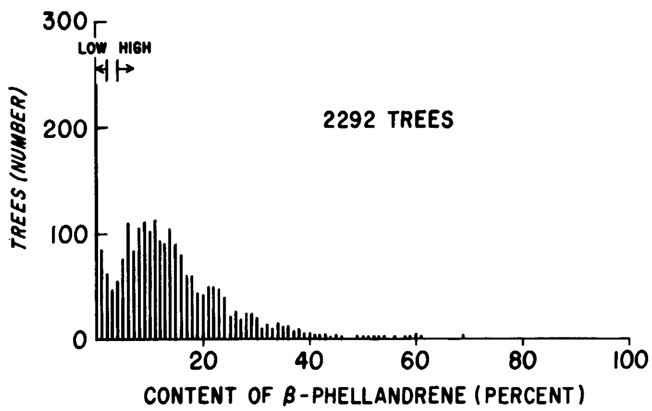

Figure 5. — Frequency distribution for β-phellandrene.

while among trees having low limonene the low β-pinene group ranged from 0 to 8 percent *(Fig. 2 B)*. The modes for myrcene were also affected by the presence of limonene, but in an opposite direction — in trees with high limonene, the mode for low myrcene was higher than in trees with low limonene *(Fig. 3)*.

These findings were considered in developing the final classification criteria *(Table 2)*. All trees were then classified by these criteria. Trees with data falling in the indeterminate categories were not used in subsequent analyses. For example, high limonene trees having 5 to 7 percent β-pinene were not classified with respect to β-pinene. Because on non-normality and heterogeneity of the data, chi-square methods rather than analyses of variance were used in subsequent tests of significance of planting site and seed source effects.

Table 2. — Criteria used for classifying trees as having high or low amounts of each monoterpene.

Monoterpene	*Low* (percent)	*High* (percent)	*Constraint condition*
β-pinene	0-4	8+	high limonene
	0-8	12+	low limonene
myrcene	0-6	9+	high limonene
	0-4	7+	low limonene
limonene	0-8	16+	none
β-phellandrene	0-2	4+	none

We should note here that considerable variance still occurs within each of the 16 phenotypic classes delineated. Further analyses with this data, along with data from families produced by controlled pollination, suggest that a part of this variance is due to incomplete dominance for some of the monoterpenes. This evidence of incomplete dominance, which may permit more refined studies (such as tests of panmixia), will be published elsewhere.

Planting site effects

Planting site and planting site by seed source interaction were found to have negligible effects *(Table 3)*. Squillace and Fisher (1966) reported that neither differences in location nor type of propagation had any appreciable effect on turpentine composition in slash pine. Hanover (1966) reported that genetically identical western white pines *(Pinus monticola* Dougl.) growing at three Idaho sites with diverse nutritional conditions revealed practically no differences in monoterpene composition associated with site.

Seed source effects

Seed source effects on composition were found to be highly significant for each of the 4 major constituents tested. The percentages of "high" trees at each geographic origin, when plotted on maps, revealed distinctive patterns, but the seed source data alone were rather scanty in some regions. For example, no seed source data were available for the Florida Keys region. To strengthen the basis for drawing isograms, we utilized data from 348 trees in other studies. Samples for these had been taken in the same manner as in the seed source study, and the geographic origins were known. Trees originating from the same area were grouped to make a total of 54 origins with from 6 to 88 trees per origin and 1223 trees in all. The procedure was considered justified in view of the lack of planting site effects noted earlier.

Percentages of trees having high β-pinene increased gradually from none in extreme southern Florida to 100 percent near the Florida-Georgia boundary and remained high to the north and west *(Fig. 6)*. Percentages of trees having high myrcene were high in south Florida and generally decreased to the north and west, with a trend reversal in the vicinity of Baldwin County, Alabama *(Fig. 7)*. Percentages of trees with high limonene were high in Brevard County, Florida, decreased to the southwest and north in Florida, with a "flat area" of mostly 0 percent in the remaining region *(Fig. 8)*. The pattern for β-phellandrene was the least clear of those studied — but it showed a gradual decrease from south to north in peninsular Florida, with several trend reversals in the remaining regions *(Fig. 9)*.

Discussion

The use of segregation data for analyzing and expressing differences in monoterpene composition among seed origins is rather new, being employed previously by Juvonen and Hiltunen (1972). Normally in studies of this nature the average content of each chemical is determined for each seed origin. In a preliminary step we used this older procedure. However, the resulting patterns were not nearly as clear

Table 3. — Percentage of trees having high amounts of each of 4 monoterpenes from 5 seed sources at each of 3 plantations.*

Plantation	Seed source: Laurens Co., GA	Atkinson Co., GA	Baker Co., FL	Alachua Co., FL	Citrus Co., FL	Plantation average
			β-pinene			
Macon, GA	100	100	100	100	100	100
Olustee, FL	100	100	100	100	100	100
Fort Myers, FL	100	100	100	100	100	100
			Myrcene			
Macon, GA	0	0	20	20	50	18
Olustee, FL	10	0	10	20	40	16
Fort Myers, FL	0	0	12	11	10	7
			Limonene			
Macon, GA	0	0	0	0	30	6
Olustee, FL	0	0	0	10	0	2
Fort Myers, FL	0	0	0	0	20	4
			β-phellandrene			
Macon, GA	50	56	60	78	100	69
Olustee, FL	56	89	50	80	80	71
Fort Myers, FL	50	80	44	90	70	67

* Based on 8 to 10 trees sampled per source at each location.

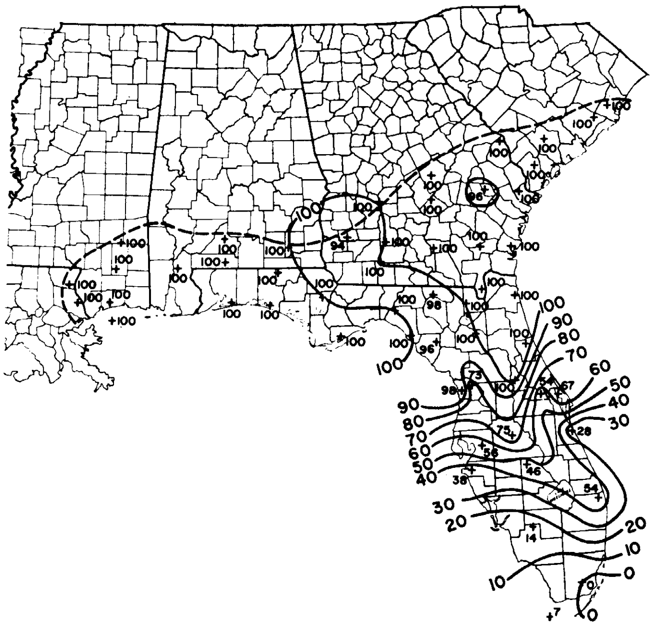

Figure 6. — Percent of trees having high β-pinene.

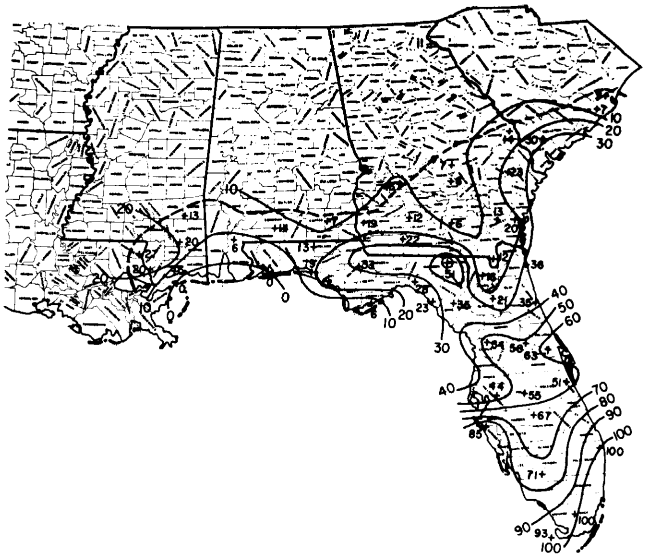

Figure 7. — Percent of trees having high myrcene.

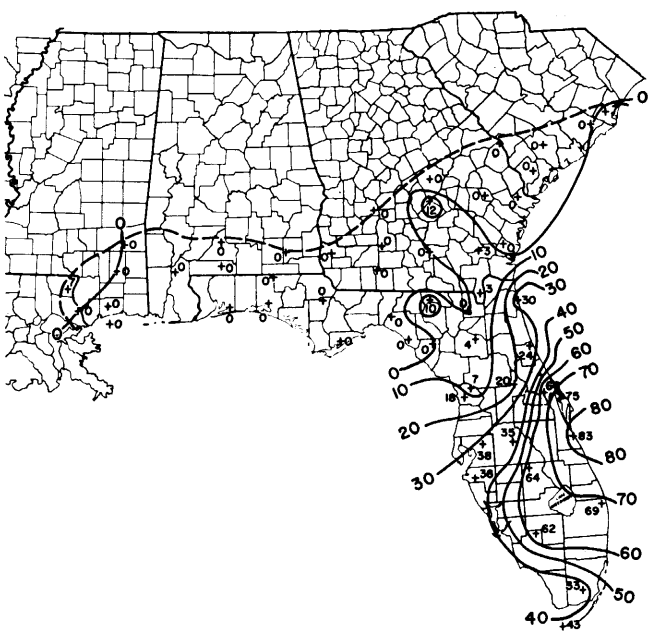

Figure 8. — Percent of trees having high limonene.

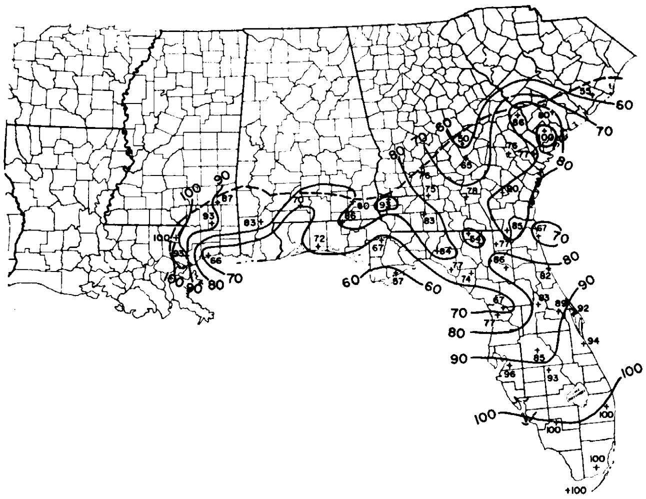

Figure 9. — Percent of trees having high β-phellandrene.

as when the percent of trees having high amounts of each chemical was used. Thus, where clear evidence of simple inheritance is at hand, use of segregation data is highly reccommended.

The patterns of variation found here agree with earlier studies of geographic variation in other traits (SQUILLACE 1966 and GANSEL *et al.* 1971) in that there is no distinct boundary between north and south Florida slash pine (*P. elliottii* var. *densa* LITTLE and DORMAN). Most traits show clinal variation latitudinally through Florida. For some traits, the gradients are steep in central Florida especially between Brevard and Lake Counties. Distinct varieties may have occurred in the distant past but, if so, the boundary between them has apparently been obscured by subsequent natural selection and introgression. In a study of monoterpene composition in stem xylem oleoresin of slash pine, MIROV *et al.* (1965) did not question the division of slash pine into varieties. However, they sampled a mixture of oleoresin from trees growing in a single location in north Florida and compared it against a similar sample from trees in a south Florida locality. Obviously, the presence of high variation within varieties and the gradients extending over both varieties found in the present study preclude the possibility of drawing conclusions from such limited sampling.

We believe that patterns of variation found afford good opportunities for identifying the seed origin of plantations. To do so, one could analyze monoterpene composition for a randomly selected group of trees in the plantation of unknown origin to obtain the percent of trees having high amounts of β-pinene, myrcene, limonene, and β-phellandrene. Then, by reference to *Figures 6, 7, 8, and 9*, he could judge the approximate seed origin. The results also suggest that monoterpene composition could be used as a means of checking the purported origin of seed lots in seed certification.

The data also show that one should avoid characterizing a species by sampling trees in only a few portions of the species range. With as much variation as is found in slash pine, results could be very misleading.

Abstract

Oleoresin obtained from cortical tissue was analyzed for monoterpene composition in slash pines throughout the natural range of the species. Composition varied greatly among individual trees, families and seed sources. Trees were classified as having high or low levels of each monoterpene. Frequency distributions for 4 of the 5 major constituents, β-pinene, myrcene, limonene, and β-phellandrene, were bimodal, but the distribution for α-pinene was not. Percentages of trees having high amounts of each monoterpene varied greatly among seed origins and revealed distinctive geographic patterns. Plantation effects were negligible, suggesting that wild trees in their native habitats can be used for studying geographic variation in monoterpene composition. Results suggest the possibility of using monoterpene composition in seed certification and in identifying the seed origin of plantations of unknown origin.

Key words: Pinus elliottii Engelm. races, geographic variation, clinal variation, turpentine composition, essential oils.

Zusammenfassung

Die Untersuchung der Rinde von *Pinus elliottii* Engelm. an 54 Herkünften aus dem natürlichen Verbreitungsgebiet (Provenienzversuch) ergab zwischen den Individuen, Familien und Herkünften eine z. T. erhebliche Variation, was die Monoterpen-Zusammensetzung betrifft. Die Frequenzverteilungen waren für 4 der 5 Hauptbestandteile, β-Pinen, Myrcen, Limonen und β-Phellandren bimodal, für α-Pinen dagegen nicht. Die auf den Standort und die Kultur der aus Samen gezogenen Pflanzen zurückzuführenden Effekte hatten nur einen geringen Einfluß, so daß die Untersuchung der Monoterpenzusammensetzung zur Herkunftsbestimmung geeignet erscheint.

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Summary results of the IUFRO 1938 Norway spruce (Picea abies (L.) Karst.) provenance experiment. Height growth*)

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Introduction

There have been several attempts to summarize the results of the IUFRO 1938 experiment on Norway spruce (Vincent and Vincent 1964, Gøhrn 1960, Baldwin 1967, Lines 1974). Vincent and Vincent (1964) have divided the provenances into latitudinal groups and discussed them jointly. They concluded on the basis of French, Belgian, Swedish and Czechoslovak results that only limited northward transfers are justifiable. Gøhrn's (1966) was a descriptive approach consisting of a consecutive evaluation of results on each site followed by a summary comment about some of the provenances. Baldwin (1967) looked only at the recommendations arrising from each study and considered the degree of their concurrence. Lines (1974) tabulated jointly all data on height for each site and provenance and calculated by three different methods the percentage deviation of each provenance from the mean at a given site. From these data he calculated the average percentage deviation for each provenance over all the sites. In this way he presented an estimate of the average quality of each provenance. This approach has two defects arrising from the existence of interactions and lack of orthogonality. One, admitted by the author, is that for an experimental site with relatively more of the good provenances the percentage estimates will be generally undervalued while for another with more of the poor ones the bias will be in the other direction. This difficulty could not be overcome even if a standard provenance appeared on all experimental sites, because the standard's value relative to other provenances need not be the same on all sites. Thus the difficulty will remain with us, it need not be exaggerated however, because the choice of provenances for each site can be considered as random (since no data about the possible value of the provenances were available in 1938) and therefore the averages comparable.

The other bias Lines' approach is that the overall percentage height estimates are based on different numbers of sites. This would be of no consequence it there were no provenance × site interactions, as has been frequently claimed for Norway spruce, (Gøhrn 1966, Baldwin 1967, Lines 1974), but as it will be shown this is not always the case.

Lines' (1974) review is by far the most comprehensive, yet it does not easily allow a simultaneous look at the performance of a provenance on all the sites where it was

* Dedicated to Professor Langner on the occasion of his 70th birthday.