

degree of M. Sc. in Forest Science. Portions were presented at Session 30 — Biology — of the 36th Annual Meeting of the Forest Products Research Society, June 24, 1982 in New Orleans, LA.

The authors would like to thank the Forest Development Research Trust of the Alberta Department of Energy and Natural Resources, and Natural Sciences and Engineering Research Council of Canada (NSERC A 1281, NSERC-SPF 00291) for financial support.

Literature Cited

- ANDERSON, V. L. and McLEAN, R. A.: Design of experiments. Marcel Dekker Inc., New York. 418 p. (1974). — BARNES, B. V.: The clonal growth habit of american aspens. *Ecol.* **47**: 439–447 (1966). — BARNES, B. V.: Natural variation and delineation of clones of *Populus tremuloides* and *P. grandidentata* in northern lower Michigan. *Silvae Genet.* **18** (4): 130–141 (1969). — BROWN, I. R.: Clonal variation in specific gravity in *Populus tremuloides*. M. Sc. thesis. State University College of Forestry at Syracuse Univ., Syracuse, New York (1961). — BROWN, I. R. and VALENTINE, F. A.: Natural variation in specific gravity and fibre length in *Populus tremuloides* clones. 10th. Northeastern For. tree imp. conf. proc. 25–41 (1963). — EINSPAHR, D. W., VAN BUIJTENEN, J. P. and PECKHAM, J. R.: Natural variation and heritability in triploid aspen. *Silvae Genet.* **12**: 51–58 (1963). — EINSPAHR, D. W. and BENSON, M. K.: Geographic variation of quaking aspen in Wisconsin and upper Michigan. *Silvae Genet.* **15**: 106–112 (1966). — EINSPAHR, D. W., BENSON, M. K. and PECKHAM, J. R.: Variation and heritability of wood and growth characteristics of five-year old quaking aspen. *Genetics and Phys. Notes. Institute of Paper Chem. Appleton Wisc. No.1* (1967). — FALCONER, D. S.: Introduction to quantitative genetics. 2nd Ed. Longman Inc. New York. 315 p. (1981). — GOGGANS, J. F.: The interplay of environment and heredity as factors controlling wood properties in conifers; with special emphasis on their effect on specific gravity. N. C. State Univ. Sch. For., Tree Improv. Tech. Rep. 11. 56pp. Raleigh (1961). — HARVEY, W. R.: Mixed model least-squares and maximum likelihood computer program. Dept. Dairy Science. Ohio State Univ., Columbus, Ohio (1977). — ISEBRANDS, J.: The proportion of wood elements within eastern cottonwood. *Wood Sci.* **5** (2): 139–146 (1972). — KENNEDY, R. W.: Fibre length of fast- and slow-grown black cottonwood. *For. Chron.* **33**: 46–50 (1957). — KENNEDY, R. W.: Anatomy and fundamental wood properties of aspen. In: Growth and utilization of poplars in Canada. (J. S. Maini and J. H. Cayford. Dept. Eds.) For. and Rural Development. For Branch. Dept. Publication No. 1205 (1968). — KENNEDY, R. W. and J. H. G. SMITH: The effect of some genetic and environmental factors on wood quality in poplar. *Pulp Pap. Mag. Can.* **59** (2): 37–38 (1959). — LEDIG, F. T., ZOBEL, B. J. and MATHIAS, M. F.: Geoclimatic patterns in specific gravity and tracheid length in wood of pitch pine. *Can. J. For. Res.* **5**: 318–329 (1975). — MICKO, M. M., YANCHUK, A. D., WANG, E. I. C. and TAYLOR, F. W.: Computerized measurement of fibre length. *IAWA Bull.* **3** (2): 111–113 (1982). — NAMKOONG, G. and A. E. SQUILLAGE: Problems in estimating genetic variance by Shrikhande's method. *Silvae Genet.* **19**: 74–77 (1968). — SEARLE, S. R.: Topics in variance component estimation. *Biomet.* **27**: 1–76 (1971). — SMITH, D.: Maximum moisture content method for determining specific gravity of small wood samples. USDA For. Serv. For. Prod. Lab. Madison, Wisc. No. 2014 (1955). — SMITH, J. H. G. and RUMMA, U.: Specific gravity and fibre length of hybrid poplar. *J. For.* **69**: 34 (1971). — SPURR, S. H. and HYVARINEN, M. J.: Wood fibre length as related to position in tree and growth. *Bot. Rev.* **20**: 561–575 (1954). — TAYLOR, F. W.: Fibre length measurements— an accurate inexpensive technique. *Tappi.* **58** (12): 126–127 (1975). — VALENTINE, F. A.: Natural variation in *Populus tremuloides* in New York. 9th North Eastern For. Tree. Imp. 17–34 (1962). — VAN BUIJTENEN, J. P., EINSPAHR, D. W. and JORANSON, P. N.: Natural variation in *Populus tremuloides* (MICHX.). *Tappi* **42**: 812–823 (1959). — YANCHUK, A. D.: Clonal variation of wood density and fibre length of trembling aspen (*Populus tremuloides* MICHX.) in north-central Alberta. M. Sc. thesis. Dept. of Forest Science. University of Alberta. Edmonton (1981). — YANCHUK, A. D., DANCIG, B. P., and MICKO, M. M.: Intra-clonal variation in wood density of trembling aspen in Alberta. *Wood and Fiber Sci.* **15**: 387–394 (1983). — ZOBEL, B.: The inheritance of wood properties in conifers. *Silvae Genet.* **10**: 65–70 (1961).

Foliar terpene heritability in *Pinus contorta*²⁾

By E. E. WHITE¹⁾ and J.-E. NILSSON

Department of Forest Genetics and Plant Physiology,
Swedish University of Agricultural Sciences,
S-901 83 Umeå, Sweden

(Received 1st March 1983)

1. Abstract

Terpenes were analyzed in ten clones and ten corresponding open-pollinated families of lodgepole pine (*Pinus contorta* DOUGL.). Statistical descriptions of the genetic properties of this sample indicate strong genetic control of monoterpene levels by multiple genes acting on the factors which control terpene metabolic steady-state.

Heritabilities are discussed in regard to grafting effects, the use of different bases for chemical data, and the distribution of data based on amounts of secondary plant products.

Key words: *Pinus contorta*, Pinaceae, Lodgepole pine, genetics, terpenes, heritability.

Zusammenfassung

An 10 Pflanzklonen und 10 korrespondierenden, frei abgeblühten Familien von *Pinus contorta* DOUGL. wurden Terpen-Analysen durchgeführt. Nach statistischer Auswertung

ergab sich eine strenge genetische Kontrolle auf den Monoterpen-Niveaus durch multiple Genaktivität bei den Faktoren, welche metabolisch den Terpen-Gleichgewichtszustand kontrollieren.

Unter Berücksichtigung der Auswirkungen, welche aus der Pfropfung resultieren, werden die Heritabilitäten hinsichtlich verschiedener Grundlagen für die chemischen Daten und die Verteilung der Daten auf der Basis einer Wertung als sekundäre Pflanzenprodukte diskutiert.

2. Introduction

Terpene composition has been used as a taxonomic character to study geographic variation within populations of lodgepole pine (VON RUDLOFF and NYLAND 1978, PAULY and VON RUDLOFF 1971), introgression between lodgepole and Jack pine (*P. banksiana*) (MIROV 1956), and to identify sources of lodgepole plantations (FORREST 1980, 1981). The assessment of the taxonomic weight to be given to a terpene depends on how it is genetically controlled, but the genetics of terpene production in conifers is generally inadequately known (HUNT and VON RUDLOFF 1977).

Suggestions that levels of individual terpenes may be

¹⁾ On leave from Pacific Forest Research Centre, 506 W. Burnside Rd., Victoria, B. C., V8Z 1M5, Canada.

²⁾ This work was supported in part by a grant from the JACOB WALLENBERG fund.

controlled by one or a few genes in various conifers have been made based on the modality of their frequency distributions in large samples (HUNT and VON RUDLOFF 1977, GANSEL and SQUILLACE 1976, BERNARD-DAGAN *et al.* 1971, WILKINSON *et al.* 1971, ZAVARIN *et al.* 1970). Detailed studies of the genetics of terpene production in conifers include both tests of models of genetic control (segregation tests) and statistical descriptions of the degree of genetic control (heritability estimates). Segregation of progeny of controlled crosses into classes appropriate to one locus control with two alleles showing varying degrees of dominance were reported for 3-carene, myrcene, caryophyllene and longifolene in maritime pine (*P. pinaster*) (BARADAT *et al.* 1972, 1975, MARPEAU *et al.* 1975), for 3-carene in Scots pine (*P. sylvestris*) (HILTUNEN *et al.* 1975), for β -pinene and myrcene in slash pine (*P. elliottii*) (SQUILLACE 1971), for myrcene, limonene, β -pinene and β -phellandrene in loblolly pine (*P. taeda*) (SQUILLACE *et al.* 1980, ROCKWOOD 1973), for 3-carene in western white pine (*P. monticola*) (HANOVER 1966a), and for α -pinene in *P. radiata* \times *attenuata* hybrids (FORDE 1964). The generality of this simple model was questioned however. Wide ranges within dominant and recessive phenotypes suggested effects by modifying genes or multiple alleles. BARADAT *et al.* (1972) stated that „the mode of heredity of the same monoterpene can vary not only between different species but also between different geographic origins of the same species: one concludes that simple genetic determinism occurs in cases in which the number of major genes segregating is reduced”. HANOVER (1966a) noted that though segregation ratios for 3-carene in *P. monticola* crosses fitted those expected for control by a single locus with two alleles and complete dominance, the amount of variation within dominant genotypes suggested that other types of gene action were involved. He states that a wider range of parental genotypes and number of progeny would be required to clarify 3-carene's mode of inheritance. HILTUNEN *et al.* (1975) selected crosses of Scots pine parents with extremes of 3-carene levels for segregation testing, and also noted wide ranges in their progeny, suggesting a strong effect on the major genes by modifying genes. The definition of the characters segregating in these tests depends on the population sampled, because the exact concentrations that differentiate “high” and “low” modes vary with the origin of the trees analysed (VON RUDLOFF and REHFELDT 1980). Classification of modes is especially ambiguous when relative data are used, as the cut-off point between modes for one terpene is affected by modes in others (GANSEL and SQUILLACE 1976, SQUILLACE *et al.* 1980). Though similar parts of the genome may effect terpene production in different trees, they may or may not be expressed as characters showing simple Mendelian segregation depending on the population sampled (i.e. what other genes are present in the parent trees), and how the character is described (i.e. whether relative or absolute data is used, what cut-off point between “high” and “low” modes is chosen).

Heritability estimates provide little information about the mode of inheritance of terpenes but are useful in establishing in particular populations that the level of a terpene is under sufficient genetic control to be useful as a taxonomic marker, for breeding or other purposes. Estimates of variances in clones and open-pollinated families for cortical monoterpenes in virginia pine (*P. virginiana*) indicated they were under rather strong genetic control, sufficient to allow changes in concentration through selec-

tion breeding (MEIER and GOGGANS 1977). BARADAT *et al.* (1972, 1975), and MARPEAU *et al.* (1975) used clonal variance and parent/progeny regression estimates of heritability to determine the age at which trees showed mature terpene patterns. The major cortical monoterpenes except camphene in *P. monticola* were shown to be highly gene regulated by clonal and parent/progeny heritability estimates (HANOVER 1966b, 1971). SQUILLACE (1971) calculated broad and narrow sense heritabilities for major cortical monoterpenes of slash pine, and cautioned that constraint due to the use of relative data can effect terpene heritabilities differentially.

Study of the inheritance of terpene composition in *P. contorta* has been limited to *contorta* \times *banksiana* hybrids, which were investigated partly to determine whether control of terpene levels by a few genes restricted the use of terpene analysis in studies of introgression (ZAVARIN *et al.* 1969). The authors were limited by the material available for analysis, and it was impossible to formulate an inheritance model, though major gene effects were suggested. Failure to recover levels of β -phellandrene equal to those in lodgepole in the progeny was suggested to be due to the effect of using relative data, in which the parental percentage of a terpene would not be expected against a hybrid background.

In an earlier study, (WHITE, 1983), the foliar terpenes of lodgepole pine were shown to fall into three biosynthetically correlated groups. The structural similarity of the terpenes which had high correlations, and the consistency of these correlations in trees of different parentage with different terpene patterns, indicated they were due to biosynthetic rather than genetic linkage, though genetic linkage could not be tested as controlled crosses were not available. They were obscured if results were expressed as relative rather than absolute amounts. The present study was undertaken to determine genetic variances for terpene production in a lodgepole pine seed orchard and clonal archive, to determine the effect on the variances of expressing results as percentages, and to examine terpene frequency distributions.

3. Methods and Materials

3.1 Plant Material and Chemical Analysis

The trees sampled were from a clonal archive and seedling seed orchard at Sör Amsberg, near Borlänge, Sweden, latitude 60°30', longitude 15°24', established after plus tree selection in British Columbia, Canada (SZIKLAI 1972). Scions for grafts and wild open-pollinated seed for establishing families had been collected in B. C. and grafted or sown in Sweden. Trees were ten-years-old from time of grafting or sowing when sampled. Samples were collected from ten clones and the corresponding seedling progeny, open-pollinated in the native stand. The origins of the ortets (mothers of open-pollinated families) are listed in Table 1.

Grafts were planted in ten tree rows, one row/clone, and seedlings were planted in single tree plots in sixty-five blocks, each family represented once/block. The soil is a well-drained fluvial sandy loam, with a slight slope and little variation between blocks. The scions had been subjected to sterilization procedures in connection with importation to Sweden and the grafts were weak and had high mortality. Selection of the ten clones was made largely on the basis of survival of sufficient ramets to allow collection of three second degree lateral twigs (1981 growth, collected in February 1982) from five ramets/clone. Sur-

Table 1. — Origin of ortets.

Tree number	Location	Latitude	Longitude
9	North of Fort St. John	54°44'10"	124°09'15"
16	-"	54°57'45"	124°15'
19	-"	55°05'10"	124°12'40"
30	-"	55°27'40"	124°08'50"
39	-"	55°40'	124°25'30"
50	Near Wonowon	56°40'30"	121°40'30"
59	Near Chetwynd	55°46'	121°34'50"
73	Parsnip Reach	55°21'45"	123°09'50"
76	-"	55°26'40"	123°12'20"
89	-"	55°06'40"	122°56'40"

Table 2. — Analysis of variance.

Source	df	Mean squares	Expectation of mean squares
Total	n-1		
Between groups	df _B	MS _B	$\sigma^2_E + k\sigma^2_B$
Within group	df _E	MS _E	σ^2_E

where:

- n = total number of trees (= 50 for clones, 203 for families)
df_B = degrees of freedom, between groups (= 9)
df_E = degrees of freedom, within groups (= 40 for clones, 193 for families)
k = number of individuals/group (= 5 for clones, for families 19–21 adjusted to a constant according to BECKER 1975).
 σ^2_B = additive plus dominance and all interaction variances for clones, additive plus maternal variances only for families.
 σ^2_E = environmental variance.

vival of seedlings was good, 88–98 % for all families except 89 and 9 which had 78 and 82 % survival respectively. From seedlings, ten second degree lateral twigs were collected in February 1982 (1981 growth) around the tree from the upper second and third branch whorls of 19–21 trees/family. Details of terpene extraction and analysis were reported previously (WHITE, 1983). Analytical er-

ror was ± 0.1 mg/10 g fresh weight. Average foliar dry weight was 41.3 ± 1.8 % and did not vary significantly between families.

3.2 Statistical analysis

Individual terpenes and total terpenes were studied by one-way analysis of variance, by partitioning the total

Table 3. — Heritability estimates and confidence intervals based on absolute amounts of terpenes and % of monoterpenes.

Terpene	Intraclass correlations, clones		4 × Intraclass correlations, families		Parent-offspring regression		
	Absolute amount	% of monoterpenes	Absolute amount	% of monoterpenes	Absolute amount	% of monoterpenes	
Monocyclic monoterpenes + sabinene	β -Phellandrene	0.85 (1) (0.68 - 0.95) (2)	0.75 (0.53 - 0.92)	0.69 (0.26 - 1.80)	1.35 (0.70 - 2.59)	0.39	0.74**
	Limonene	0.77 (0.56 - 0.93)	0.57 (0.30 - 0.84)	0.55 (0.17 - 1.58)	0.61 (0.22 - 1.69)	0.47	0.90**
	cis-Ocimene	0.83 (0.65 - 0.95)	0.71 (0.47 - 0.90)	0.15 (-0.03 - 0.75)	0.11 (-0.06 - 0.70)	0.19	0.68**
	α -Phellandrene	0.73 (0.50 - 0.91)	0.19 (-0.02 - 0.57)	0.63 (0.23 - 1.71)	0.47 (0.14 - 1.45)	0.43	0.47
	Myrcene	0.65 (0.39 - 0.88)	0.08 (-0.09 - 0.45)	0.13 (-0.04 - 0.75)	0.03 (-0.10 - 0.50)	0.20	0
	3-Carene	0.47 (0.19 - 0.78)	0.61 (0.34 - 0.86)	0.47 (0.13 - 1.46)	0.06 (0.08 - 0.59)	0.06	0
	Sabinene	0.29 (0.05 - 0.66)	-0.03 (-0.14 - 0.29)	0.27 (0.02 - 1.05)	0.07 (0.08 - 0.61)	0.05	0.19
Bicyclic monoterpenes	Bornyl acetate	0.81 (0.61 - 0.94)	0.91 (0.80 - 0.97)	0.79 (0.32 - 1.95)	1.65 (0.92 - 2.85)	0.97**	0.98**
	Tricyclene	0.73 (0.49 - 0.91)	0.91 (0.81 - 0.98)	1.04 (0.48 - 2.26)	1.55 (0.84 - 2.77)	0.98**	0.98**
	Camphene	0.66 (0.41 - 0.88)	0.92 (0.82 - 0.98)	1.10 (.52 - 2.33)	1.77 (1.02 - 2.95)	0.98**	0.98**
	α -Pinene	0.61 (0.34 - 0.86)	0.92 (0.82 - 0.98)	0.66 (0.24 - 1.75)	1.78 (1.03 - 2.95)	0.66*	0.97**
	β -Pinene	0.79 (0.59 - 0.93)	0.74 (0.51 - 0.91)	0.17 (0.02 - 0.86)	0.35 (0.07 - 1.22)	0.26	0.66*
Sesquiterpenes	Cadinenes 1	0.08 (-0.09 - 0.44)	—	0.06 (-0.08 - 0.58)	—	0	—
	Cadinenes 2	0.07 (-0.09 - 0.44)	—	0.05 (-0.08 - 0.54)	—	0	—
	Cadinols	0.47 (0.21 - 0.79)	—	0.07 (-0.07 - 0.63)	—	0	—
Total monoterps.	0.68 (0.43 - 0.87)	—	0.25 (0.02 - 1.03)	—	0.07	—	
Total terpenes	0.62 (0.38 - 0.87)	—	0.14 (-0.04 - 0.77)	—	0.03	—	

- 1) heritability estimate
2) approximate confidence interval

- ** h² significant at p = 0.01
*) h² significant at p = 0.05

variation in clones and families into components of within — and between — group variation (Table 2).

Environmental variance was probably minimal in the trees sampled, as the clones were planted in rows and there was little soil or topographic difference between seedling blocks.

Broad sense heritabilities were estimated from the clonal data as intraclass correlations:

$$H^2 = \frac{\sigma_B^2}{\sigma_B^2 + \sigma_E^2}$$

Narrow sense heritabilities were estimated from the family data as four times the intraclass correlations:

$$h^2 = 4 \frac{\sigma_B^2}{\sigma_B^2 + \sigma_E^2} \quad (\text{FALCONER 1981}).$$

Approximate confidence limits were calculated according to BECKER 1975. Limits are approximations as the data deviated from normal distributions. Narrow sense heritabilities were also estimated from the regression of progeny on maternal parent,

$$h^2 = 2 K_1 \times r_{OP}$$

(FRANKLIN 1974), where r_{OP} is the correlation coefficient between clone and family means, and K_1 is a correction factor.

Significance levels are those of the corresponding regression coefficients. Results were calculated on the basis of:

- absolute amount
- % of total terpenes
- % of total monoterpenes
- arc/sine transformed proportions of monoterpenes (clones only).

Frequency distributions were calculated for terpenes in each family and in all seedlings combined after dividing the range over all samples for each terpene into ten classes. Results were expressed as absolute values and % of total terpenes.

4. Results

Heritability estimates and confidence intervals for absolute and relative amounts of terpenes are given in Table 3. Estimates based on percentage of total terpenes or arc/sine transformed percentages did not differ greatly from those based on percentage of monoterpenes and are not given.

Trends in heritability estimates differed for three groups of terpenes: the monocyclic monoterpenes (α - and β -phellandrene, limonene, cis-ocimene, myrcene and 3-carene) plus sabinene, the bicyclic monoterpenes (bornyl acetate, tricyclene, camphene, α - and β -pinene); and the sesquiterpenes (cadinenes and cadinols). Heritability estimates for the sesquiterpenes which were monitored in this study were low with wide confidence intervals, whether based on absolute amount or percentage of total terpenes, and will not be discussed further.

For the bicyclic monoterpenes, heritability estimates were higher calculated on percentage than on absolute data for clonal and family intraclass correlations, and approximately the same based on family/clonal regressions. For the monocyclic monoterpenes, clonal intraclass correlations were higher based on absolute than percentage data. Estimates based on percentage data tended to be higher than those based on absolute data for heritabilities derived from intra-family correlations. Estimates were higher for percentage data with family/clonal regressions

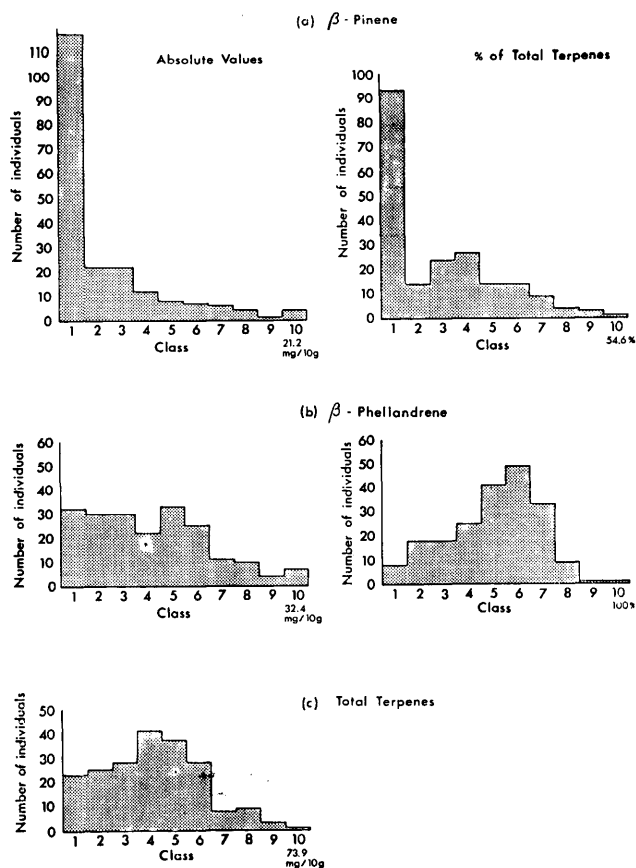


Figure 1. — Frequency distributions for (a) pinenes, (b) monocyclic monoterpenes and (c) total terpenes in seedlings from all families combined.

— left: based on absolute values, right: based on % of total terpenes.

— each class 1/10th of range over all samples, lower figures show upper limits of ranges.

for those monocyclic terpenes for which the regression was significant.

For the monocyclic monoterpenes, “broad sense heritabilities” estimated by clonal intraclass correlations were generally higher than “narrow sense heritabilities” based on family intraclass correlations, but somewhat less than those based on regression of families on clones. For the bicyclic monoterpenes, “broad sense heritability” was lower than the intra family estimate of “narrow sense heritability” particularly for percentage data. “Broad sense heritability” was also generally lower than the estimate based on family/clonal regressions.

Frequency distributions in seedlings of all families combined, based on absolute and percentage data, for terpenes representative of the pinane and monocyclic monoterpene groups (β -pinene and β -phellandrene) and for total monoterpenes are given in Figure 1. Frequency distributions in three individual families and all families combined for a terpene representative of the camphane group (bornyl acetate) are given in Figure 2.

Frequency distributions for α -pinene and most of the monocyclic monoterpenes were similar to that for β -pinene (Figure 1a). Absolute data had a single mode in the lowest class with a pronounced skew to the right or a poorly separated higher mode. The effect of expressing data as percentage of total terpenes was to decrease the size of the low mode and introduce or increase a higher mode. The

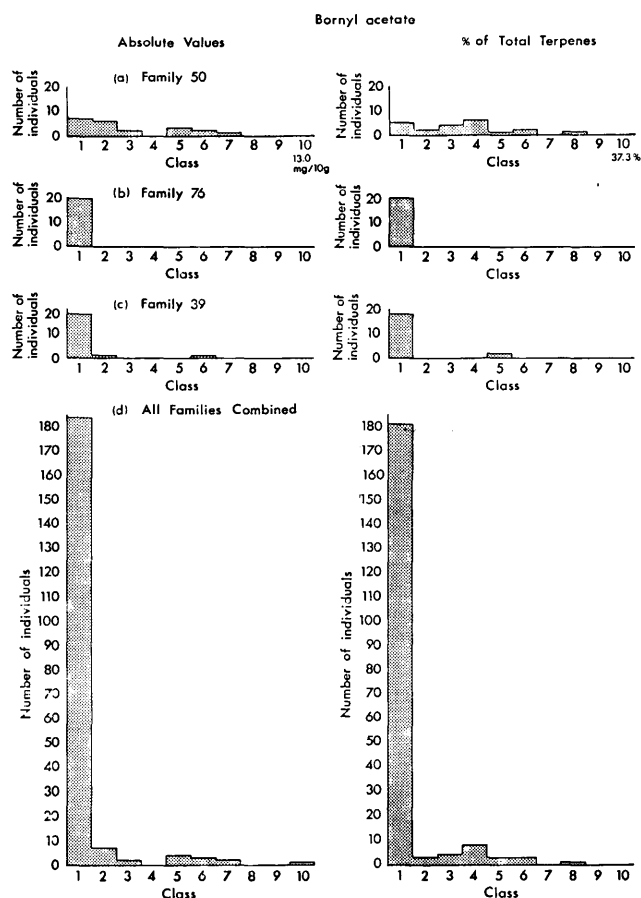


Figure 2. — Frequency distribution in (a-c) 3 separate families and (d) all families combined for a representative camphane skeleton bicyclic monoterpene.

— left: based on absolute values, right: based on % of total terpenes.

— each class 1/10th of range over all samples (including clones, not shown): lower figures in 1 (a) show upper limit of range.

frequency distribution for β -phellandrene (Figure 1b) which was frequently the major monoterpene, had a middle mode poorly differentiated from a broad low mode when data were plotted as absolute amounts. The effect of expressing results as percentages was to remove the low mode, leaving a single mode skewed to the left. The frequency distribution for total monoterpenes (Figure 1a) was rather similar to that for β -phellandrene. Frequency distributions for monocyclic monoterpenes were similar in individual families to those in all families combined.

Frequency distributions for the camphane skeleton bicyclic monoterpenes differed greatly in family 50 from those in other families (Figure 1a). In family 50, the camphanes were frequently the major monoterpenes, and their frequency distributions in this family showed a middle mode and a broad low mode based on absolute data. The effect of expressing results as percentages was to decrease the low mode and increase the size of the middle mode, while displacing it to the left. Families 76, 73, 89, 16 and 9 contained exclusively trees with small amounts of camphanes (cf. Figure 2b), while families 59, 39, 30, and 19 contained a few trees with large amounts of camphanes (cf. Figure 2c). The effect of changing from absolute to percentage data was again to increase the size of the middle mode while displacing it to the left. The same trend occurred in frequency distributions for bornyl acetate in seedlings of all families combined (Figure 2d).

There was a considerable range in total terpene production in different ramets of the same clone, though individual terpenes were generally produced in the same proportions in each ramet. However, for the two clones with the lowest total terpene production, 73 and 9, proportions varied. Clone 73 contained 3 ramets with $> 45\%$ β -pinene and $< 22\%$ β -phellandrene, and 2 ramets with $< 3\%$ β -pinene and $> 75\%$ β -phellandrene. In clone 9 β -phellandrene was the major terpene in all ramets, but in two ramets total terpene production was so low that only β -phellandrene and cadinol could be quantified and all other terpenes had 0% values. As a consequence, while variances for clonal data tended to be reduced when results were expressed as percentages they increased for clones 9 and 73, and these two clones were major contributors to total clonal variance based on percentage data. If these clones were omitted from the calculations, heritability estimates from clonal data were higher based on percentage than absolute data.

5. Discussion

5.1 Within clone variation

The unexpectedly high variation in proportions of terpenes within the two clones with very low total terpenes appears to be related to within tree variation in resin canals of the original ortet, which is propagated and possibly amplified as stable variation between grafts in a clone. The relationship between resin canal variability and terpene production is presently being investigated. The assumption that grafts express exact reproductions of a genotype is not valid for vegetative characters effected by apical determinism (WARING 1978). The variance due to epistasis is not restricted to between clone variance in such cases. Grafts had lower total terpene production than seedlings, possibly another manifestation of apical determinism, i. e. of a more "mature" physiological state in grafts. This was reflected in high, positive regression constants for family/clonal regressions based on absolute amounts of terpenes, which were 90.5 and 30.9 for the major constituents β -phellandrene and β -pinene and > 3.0 for most of the compounds studied. In contrast, regression constants based on percentage data were < 0.1 except for that for β -phellandrene, which was 0.3. The possibility of incorrect labelling of ramets in the two low terpene clones cannot be eliminated but seems unlikely. Incorrect substitution of ramets from other clones would have resulted in grafts with much higher total amounts of terpenes than the rest of the grafts in the clone.

5.2 Use of absolute versus relative data

In general, heritability estimates were higher based on relative than absolute data. Comparisons of estimates based on variances for non-normally distributed data, such as the skewed distributions of absolute amounts of terpenes in families (Figure 1) can be expected to be affected by scale transformation (FALCONER 1981). In this case the effect appears to have a biological explanation, namely that major genetic control is exercised on the proportions of terpenes produced while absolute amounts are strongly affected by physiological factors such as tree age. Small amounts of the camphane skeleton terpenes were produced in all families. For example all but two trees of family 76 illustrated in Figure 2b contained measurable amounts of bornyl acetate, up to 0.9 mg/10 g foliage, though all fell into the lowest class of the range for bornyl acetate. Major genetic control of terpene synthesis

appears not to involve the presence or absence of specific synthetases but rather to depend on factors which control the steady-state levels of their products. These factors include enzyme concentration, compartmentalization, and activity, which are governed by genes controlling protein transcription and translocation, and tissue and organelle structure, as well as those controlling the synthetase's structure.

Of the exceptions to the generalization that heritability estimates were higher based on relative data, one, the case of monocyclic terpenes in grafts, has been discussed. The two clones with low, variable terpenes did not contain large amounts of bicyclic monoterpenes, consequently heritabilities for these terpenes were higher based on relative data in clones as well as families. The other major exception is that for bicyclic terpene heritability estimates based on family/clonal regressions. Except for β -pinene, bicyclic terpene heritability estimates based on family/clonal regressions are intermediate to those based on either family or clonal intraclass correlations using relative data, while using absolute data the family/clonal regressions give values often higher than other estimates. It appears that the tendency for seedlings to produce more terpenes than grafts is especially pronounced for trees producing larger amounts of these four bicyclic terpenes. The rather high heritability estimates for family/clonal regressions of bicyclic terpenes based on absolute amounts may be the result of comparing parents and progeny at different developmental stages characterized by different levels of total terpene production.

5.3 "Broad-" versus "narrow-sense" heritability

Heritability estimates for relative amounts of terpenes were high and did not differ greatly for "broad sense heritability" and either estimate of "narrow sense heritability", indicating little dominance or gene interaction effects. In the case of the bicyclic monoterpenes there was a tendency for "narrow sense heritability" based on percentages to be somewhat greater than "broad sense heritability". In estimating "narrow sense heritability" as four times intrafamily correlations or twice the family/clonal regression coefficient the assumption is made that open-pollinated families represent half-sib families derived from the same pollen population, and the multiplicands should be reduced if some of the progeny are actually full-sibs or if the pollen populations differ. This is the case, especially for family 50 which contained most of the trees with large amounts of bicyclic terpenes. The mother tree was the only one sampled which came from east of the Rocky Mountains, where the ranges of *P. contorta* and *P. banksiana* overlap. Compatibility barriers may have decreased the number of pollen parents, and the terpene patterns of the father population may have been very different for this family. Thus deviation from random mating likely contributed to the high heritability estimates for the bicyclic terpenes.

The data illustrate the sampling dependence of heritability estimates based on intraclass correlations, none of which would have been as large if clone and family 50 had not been sampled.

5.4 Frequency distributions

Frequency distributions for monoterpenes did not suggest simple models for genetic control. They indicated clearly the skewed tendency for data relating to compounds non-essential to intermediary metabolism. Many

individuals did not contain appreciable amounts of the compounds studied, and the lowest class was enlarged, partly as a consequence of instrumental detection limits. By expressing results as percentages based on the sum of the compounds studied some observations were moved into higher classes, decreasing the size of the lowest class and introducing a second higher mode continuous with the low mode. The distributions were consistent with control by additive genes. In the case of bornyl acetate a smaller number of major genes may have contributed to the disjunct distribution for absolute amounts observed in family 50. However, in the absence of controlled crosses, segregation for major genes could not be tested.

5.5 Concluding remarks

These data indicate that monoterpene content in lodgepole pine is under strong genetic control, mainly by additive genes. Control appears to be exercised not on the presence or absence of specific synthetases, but on factors which control the metabolic steady state of the products of these enzymes. Such factors include enzyme concentration, compartmentalization, and activity and can be expected to be governed by a complex of genes. Major gene effects on terpene levels may occur following crosses between trees with very different terpene composition, as would occur following introgression between *P. contorta* and *P. banksiana*.

6. Acknowledgements

The authors are indebted to Professor DAG LINDGREN, Swedish University of Agricultural Sciences, Umeå, Sweden, and Dr. OUTI MUONA, University of Oulu, Finland, for helpful discussions, to Stora Kopparberg-Bergvik Forestry Division for the use of their seed orchard at Sör Amsberg, and to IRÈNE KLING for field assistance.

7. Literature Cited

- BARADAT, P., BERNARD-DAGAN, C., FILLON, C., MARPEAU, A., and PAULY, G.: Les terpènes du pin maritime: aspects biologiques et génétiques. II. Hérité de la teneur en monoterpenes. Ann. Sci. forest. 29, 307-334 (1972). — BARADAT, P., BERNARD-DAGAN, C., PAULY, G., and ZIMMERMAN-FILLON, C.: Les terpènes du pin maritime: aspects biologiques et génétiques. III. Hérité de la teneur en myrcène. Ann. Sci. forest. 32, 29-54 (1975). — BECKER, W. A.: Manual of Quantitative Genetics. Washington State University Press. 3rd edition. 170 pp. (1975). — BERNARD-DAGAN, C., FILLON, C., PAULY, G., BARADAT, P., and ILLY, G.: Les terpènes du pin maritime: aspects biologiques et génétiques. I. Variabilité de la composition monoterpénique dans un individu, entre individus et entre provenances. Ann. Sci. forest. 28, 223-258 (1971). — FALCONER, D. S.: Introduction to Quantitative Genetics. Longman, London. 2nd edition. 340 pp. (1981). — FORDE, M. B.: Inheritance of turpentine composition in *Pinus attenuata* × *radiata* hybrids. New Zealand J. Bot. 2, 53-59 (1964). — FORREST, G. D.: Geographical variation in the monoterpenes of *Pinus contorta* oeloresin. Biochem. Syst. Ecol. 8, 343-359 (1980). — FORREST, G. D.: Geographical variation in oleoresin composition of *Pinus contorta* from natural stands and planted seed collections. Biochem. Syst. Ecol. 9, 97-103 (1981). — FRANKLIN, E. C.: Adjustment of heritabilities estimated by correlation coefficients for typical forest tree breeding experiments. Silvae Genetica 23, 176-180 (1974). — GANSEL, C. R., and SQUILLACE, A. E.: Geographical variation of monoterpene in cortical oleoresin of slash pine. Silvae Genetica 25, 150-154 (1976). — HANOVER, J. W.: Inheritance of 3-carene concentration in *Pinus monticola* DOUGL. Forest Sci. 12, 447-450 (1966a). — HANOVER, J. W.: Genetics of terpenes. I. Gene control of monoterpene levels in *Pinus monticola*. DOUGL. Heredity 21, 73-84 (1966b). — HANOVER, J. W.: Genetics of terpenes. II. Genetic variances and interrelationships of monoterpene concentrations in *Pinus monticola*. Heredity 27, 237-245 (1971). — HILTUNEN, R., TIGERSTEDT, P. M. A., JUVONEN, S., and POHJOLA, J.: Inheritance of 3-carene quantity in *Pinus sylvestris* L. Fram. Aink.-Farm. Notisblad 84, 69-72 (1975). — HUNT, R. S., and VON RUDLOFF, E.: Leaf-oil-terpene variation in western white pine populations of the Pacific northwest. For. Sci. 23, 507-515 (1977). — MARPEAU, A., BARADAT, P., and BERNARD-DAGAN, C.: Les terpènes du pin maritime: aspects biologiques et génétiques. IV. Hérité de la teneur en deux sesquiterpènes: le

longifolène et le caryophyllène. Ann. Sci. forest. 32, 185—203 (1975). — MEIR, R. J. and GOGGANS, J. F.: Heritability and correlations of the cortical monoterpenes of virginia pine (*Pinus virginiana* MILL.). Silvae Genetica 27, 79—84 (1978). — MIROV, N. T.: Composition of turpentine of lodgepole × jack pine hybrids. Can. J. Bot. 34, 443—457 (1956). — PAULY, G., and VON RUDLOFF, E.: Chemosystematic studies in the genus *Pinus*: the leaf oil of *Pinus contorta* var. *latifolia*. Can. J. Bot. 49, 1201—1210 (1971). — ROCKWOOD, D. L.: Variation in the monoterpene composition of two oleoresin systems of loblolly pine. For. Sci. 19, 147—153 (1973). — VON RUDLOFF, E., and NYLAND, E.: Chemosystematic studies in the genus *Pinus*. III. The leaf oil terpene composition of lodgepole pine from the Yukon Territory. Can. J. Bot. 57, 1367—1370 (1979). — VON RUDLOFF, E., and REHFELDT, G. E.: Chemosystematic studies in the genus *Pseudotsuga*. IV. Inheritance and geographical variation in the leaf oil terpenes of Douglas-fir from the Pacific Northwest. Can. J. Bot. 58, 546—556 (1980). — SQUILLACE, A. E.: Inheritance of monoterpene composition in cortical oleoresin of slash pine. For.

Sci. 17, 381—387 (1971). — SQUILLACE, A. E., WELLS, O. O., and ROCKWOOD, D. L.: Inheritance of monoterpene composition in cortical oleoresin of loblolly pine. Silvae Genetica 29, 141—151 (1980). — SZIKLAI, O.: Plus tree selection of *Pinus contorta* var. *latifolia* in central British Columbia. Stora Kopparberg report 67—7295. 67 pp. (1972). — WARING, P. F.: Determination in plant development. Bot. Mag. Tokyo Special Issue 1, 3—17 (1978). — WHITE, E. E.: Biosynthetic implications of terpene correlations in *Pinus contorta*. Phytochem. 22, 1399—1405 (1983). — WILKINSON, R. C., HANOVER, J. W., WRIGHT, J. W., and FLAKE, R. H.: Genetic variation in the monoterpene composition of white spruce. For. Sci. 17, 83—90 (1971). — ZAVARIN, E., CRITCHFIELD, W. B., and SNAJBERK, K.: Turpentine composition of *Pinus contorta* × *Pinus banksiana* hybrids and hybrid derivatives. Can. J. Bot. 47, 1443—1453 (1969). — ZAVARIN, E., SNAJBERK, K., REICHERT, T., and TSIEN, E.: On the geographic variability of the monoterpenes from the cortical blister oleoresin of *Abies lasiocarpa*. Phytochem. 9, 377—395 (1970).

Results of a 70 years old Scots pine (*Pinus sylvestris* L.) provenance experiment in Pulawy, Poland

By J. OLEKSYN and M. GIERTYCH

Polish Academy of Sciences,
Institute of Dendrology,
62-035 Kórnik, Poland

(Received 25th March 1983)

Summary

The authors have found and described a provenance experiment on *Pinus sylvestris* L. established in 1912 by S. Z. KURDIANI in Pulawy, Poland. On the area provenances are represented from 15 provinces of pre-revolutionary Russia, from central Poland to eastern Siberia and from the White sea to the Caucasus. Most productive were provenances Ukraine (Volynia, Kiev), Latvia (Kurland) and from Poland (Lublin) — the local material. Least productive were pines from Siberia, Urals (Ufa, Jenisejsk, Tobolsk) and from Turkey (Kars). The factor affecting productivity most was the number of trees per ha, ie. survival. The earlier conclusions from a survey of the whole experimental series made by the authors are confirmed on this location, namely that the most adaptable provenances come from the region of mixed forests. It is suggested that the reason lies in the evolutionary adaptation to competition with broadleaf species.

A comparison of two progenies of trees differing in size of heartwood indicates that this trait is not heritable.

Key words: *Pinus sylvestris*, provenance, Genotype × environment interaction, heart-wood.

Zusammenfassung

Die Autoren beschreiben einen von ihnen wiederentdeckten Kiefernprovenienzversuch (*Pinus sylvestris* L.), angelegt im Jahre 1912 durch S. Z. KURDIANI bei Pulawy in Polen. Auf dieser Versuchsfläche befinden sich Kiefernherkünfte aus 15 Provinzen des ehemaligen kaiserlichen Russlands von Mittelpolen bis Ostsibirien und vom Weissen Meer bis zum Kaukasus. Den höchsten Massenzuwachs erreichten Herkünfte aus der Ukraine (Wolhynien, Kiew), aus Lettland (Kurland) und aus Polen (Lublin — die einheimische Provenienz). Am wenigsten leistungsfähig sind in dieser Hinsicht die Herkünfte aus Sibirien, aus dem Uralgebirge (Jenisejsk, Tobolsk) und aus der heutigen Türkei (Kars). Der Massenzuwachs wurde am meisten durch

die Anzahl der Bäume/ha (Anzahl der überlebenden Bäume) beeinflusst.

Auf diese Weise wurden früher gezogene Schlußfolgerungen aus einer Übersicht des ganzen Versuches bestätigt, daß nämlich die anpassungsfähigsten Herkünfte aus der Mischwaldzone stammen. Es wird angenommen, daß die Ursache dieses Verhaltens in der evolutionsbedingten Anpassung an die Konkurrenz der Laubbölzer liegt.

Ein Vergleich zweier Nachkommenschaften, die sich durch die Größe des Kernholzanteiles unterscheiden, läßt den Schluß zu, daß diese Eigenschaft nicht vererbt wird.

Introduction

In the years 1910—1916 in the European part of the Russian Empire several provenance experiments on Scots pine (*Pinus sylvestris* L.) have been established according to the methodology and partially under the supervision of V. D. OGIEVSKIJ. The two World Wars and various changes of frontiers have caused that out of the 21 places which received seeds for the experiment publications are known from only 7 locations (GIERTYCH and OLEKSYN 1981).

One of the experimental areas has been localized in the former experimental forest "Ruda" of the Novo-Aleksandria Institute of Rural Economy and Forestry in Pulawy, Poland. Information about this has been found in the annual report on forest research for 1910 (ANONYM. 1911) from prerevolutionary Russia. The scientific philosophy behind the experiment and its direct supervision were credited to S. Z. KURDIANI (ANONYM. 1914). He was a 1900 graduate from the Pulawy Institute and later, in 1914, became an assistant to the Chair of Forestry at the Institute lecturing dendrology (STRZEMSKI 1980).

Polish foresters have known about the experiment, however efforts made in the late forties to find it have failed because no plans nor archival documents on the experi-