GENETICS OF TERPENES

II. GENETIC VARIANCES AND INTERRELATIONSHIPS OF MONOTERPENE CONCENTRATIONS IN *PINUS MONTICOLA*

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1. INTRODUCTION

THE concentrations of monoterpene compounds in the cortical oleoresin of western white pine (*Pinus monticola* Dougl.) are gene regulated and are influenced relatively little by such non-genetic factors as season of year, site conditions, tree age, vigour, and position in the tree (Hanover, 1966a, b). Evidence has recently been presented that the terpene 3-carene is quantitatively and qualitatively controlled primarily by single dominant and recessive alleles at one locus (Hanover, 1966c). With the exception of 3-carene, information about the degree and mode of genetic control of monoterpene synthesis in species of the Pinaceae family is meagre despite the extensive use of terpenes for chemosystematic purposes.

The present study was designed to further clarify the inheritance patterns and interrelationships among the major monoterpenes of western white pine. In my earlier study (Hanover, 1966a), the progenies used to derive inheritance data resulted from a non-systematic biparental mating scheme and were based on parent-offspring regression analyses. Here I present the results of monoterpene analyses of parents and progenies from a factorial mating design.

2. MATERIALS AND METHODS

Western white pine seedlings representing 28 full-sib progenies were the sources of oleoresin for terpene analyses. The progenies were obtained by controlled pollinations among four pollen parents and seven seed parents using the factorial mating scheme (table 1). The pollen parents represent a random mating population growing in one locality near Fernwood, Idaho, while the seed parents were from several other local populations also in northern Idaho. Thus, it is assumed that the parental population is a sample from a large, randomly mating population of western white pine. Seeds from the crosses were sown in 3 × 3 × 8-inch tar paper plant bands in the nursery at Moscow, Idaho. Oleoresin samples were taken from eight to ten seedlings from each cross after 4 years of growth in the nursery.

The procedures for sampling oleoresin and obtaining quantitative analyses of the monoterpenes have been described in detail previously (Hanover, 1966b). Analyses were done by gas-liquid chromatography

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using a flame ionisation detector and a $\frac{1}{4}$ " $\times$ 6' stainless steel column packed with 10 per cent. polypropylene glycol on 60-80 mesh Diatoport W-AW. The column temperature was 95° C., injection port and detector temperatures were 196° C. and helium flow rate was 160 ml./min.

Quantitative estimates of the monoterpenes were derived from area integrator values and standard curves were prepared for five of the predominant monoterpenes in order to express the results on the basis of percentage of oleoresin. Qualitative determinations were done by comparing

TABLE 1

Model for analysis of variance of monoterpenes in progenies from factorial crossing experiment

Source	Degrees of freedom	Mean square	Expectations of mean squares	
			Components	Covariances
Between male parents	3	MS_M	$\sigma_W^2 + 8\sigma_{M \times F}^2 + 56\sigma_M^2$	$(\sigma_T^2 - \text{cov}_{FS}) + 8(\text{cov}_{FS} - \text{cov}_{FHS} - \text{cov}_M + 56 \text{cov}_{MHS})$
Between female parents	6	MS_F	$\sigma_W^2 + 8\sigma_{M \times F}^2 + 32\sigma_F^2$	$(\sigma_T^2 - \text{cov}_{FS}) + 8(\text{cov}_{FS} - \text{cov}_{FHS} - \text{cov}_M + 32 \text{cov}_{FHS})$
Male $\times$ female	18	$MS_{M \times F}$	$\sigma_W^2 + 8\sigma_{M \times F}^2$	$(\sigma_T^2 - \text{cov}_{FS}) + 8(\text{cov}_{FS} - \text{cov}_{FHS} - \text{cov}_M)$
Within crosses	196	MS_W	σ_W^2	$(\sigma_T^2 - \text{cov}_{FS})$

σ_W^2 = variance among individuals from the same mating.

$\sigma_{M \times F}^2$ = progeny variance due to male parent and female parent interaction.

σ_F^2 = progeny variance due to differences between female parents = cov_{FHS} .

σ_M^2 = progeny variance due to differences between male parents = cov_{MHS} .

σ_T^2 = total variance.

cov_{FS} = covariance between full sibs.

cov_{FHS} = covariance between maternal half-sibs.

cov_{MHS} = covariance between paternal half-sibs.

relative retention times of the oleoresin terpenes with those of known compounds. In order to substantiate the identifications, known monoterpenes were added to the white pine samples for peak enhancement. This procedure was repeated using a non-polar column packing (Apiezon L) in contrast to polypropylene glycol which is polar.

Area integrator values were used directly as a measure of concentration for all statistical analyses which were done with the aid of an IBM 709 computer. Analyses of variance and covariance were used to estimate variance components for each terpene. The model for these analyses is given in table 1.

Phenotypic correlations were calculated for all combinations of the terpenes to provide information about the interrelationships among them. Although genotypic correlations were calculated they are not given because of insufficient observations to provide valid estimates. Correlations were estimated using the following formula:

$$r_p = \frac{\text{COV}_{W(xy)} + \text{COV}_{M(xy)} + \text{COV}_{F(xy)}}{\sqrt{(\sigma_{W(x)}^2 + \sigma_{M(x)}^2 + \sigma_{F(x)}^2)(\sigma_{W(y)}^2 + \sigma_{M(y)}^2 + \sigma_{F(y)}^2)}}$$

where x and y represent the individual monoterpenes.

3. RESULTS AND DISCUSSION

A total of eight measurable monoterpene peaks were identified and used in the analyses. In subsequent work, using other columns, we have also detected β -phellandrene in western white pine. Therefore, the possibility exists that small amounts of this compound may be obscured by limonene both of which have very similar retention times. Emphasis here is given to α -pinene, β -pinene, myrcene, 3-carene, and limonene which are the major monoterpenes of western white pine cortex oleoresin. Camphene, γ -terpene, and terpinolene were also detected and included in the statistical analyses although these compounds represent only a few percent of the total monoterpene fraction. The individual tree data for one terpene, 3-carene, have already been reported upon elsewhere (Hanover, 1966c). Because chi-square analyses indicated single gene control for this compound, 3-carene is included in the present quantitative genetic analyses for comparative purposes.

When inheritance studies are limited to analyses of progenies derived from a small number of parents it is imperative that the parental variation be considered in the interpretation of progeny data. Hence, the monoterpene compositions of the 11 parents used are shown in table 2, along with the

TABLE 2

Concentrations of the five major monoterpenes in cortex oleoresin from western white pine parents and progenies. Parental concentrations are given in parentheses after each parent. Progeny values are means of 8 to 10 individuals*

α -PINENE								
Male parent	Female parent							Mean
	103 (18.6%)	118 (6.8%)	140 (13.9%)	150 (9.6%)	181 (14.1%)	195 (7.9%)	214 (10.4%)	
	Percentage of oleoresin							
17 (4.0%)	10.5	6.6	6.1	7.7	6.1	6.7	5.8	7.1
19 (4.7%)	7.8	7.6	6.7	8.2	7.2	5.7	6.8	7.2
22 (2.9%)	11.0	8.4	6.6	6.6	7.3	7.0	5.4	7.5
58 (5.2%)	12.5	6.5	6.8	6.7	6.9	6.2	6.6	7.5
Mean (4.2%)	10.5	7.3	6.6	7.3	6.9	6.4	6.2	7.3
β -PINENE								
Male parent	Female parent							Mean
	103 (8.2%)	118 (7.8%)	140 (7.3%)	150 (7.6%)	181 (5.4%)	195 (9.5%)	214 (10.8%)	
	Percentage of oleoresin							
17 (4.2%)	10.7	19.5	18.2	23.2	20.4	19.9	21.8	19.1
19 (2.1%)	14.6	19.7	18.4	22.7	19.0	18.2	20.9	19.1
22 (4.9%)	15.5	22.4	18.9	20.7	20.8	18.6	17.4	19.2
58 (12.8%)	14.5	19.5	18.6	22.7	20.0	20.0	21.9	19.6
Mean (6.0%)	13.8	20.3	18.5	22.3	20.0	19.2	20.5	19.2

TABLE 2 (*continued*)

MYRCENE								
	Female parent							
Male parent	103 (1.2%)	118 †	140 (0.9%)	150 †	181 (4.2%)	195 (1.1%)	214 (1.1%)	Mean (1.7%)
Percentage of oleoresin								
17 (5.3%)	1.8	1.7	1.8	2.3	2.3	2.5	2.1	2.1
19 (3.3%)	1.7	1.3	1.5	1.5	1.9	1.6	1.5	1.5
22 (4.9%)	2.5	2.2	2.1	1.5	2.3	2.0	1.5	2.0
58 (9.0%)	1.7	1.3	1.5	1.6	2.2	1.8	1.6	1.7
Mean (5.6%)	1.9	1.6	1.7	1.7	2.1	2.0	1.7	1.8
3-CARENE								
	Female parent							
Male parent	103 (0.7%)	118 (6.2%)	140 (1.2%)	150 (8.5%)	181 (2.9%)	195 (0.6%)	214 (0.5%)	Mean (2.9%)
Percentage of oleoresin								
17 (0.0%)	1.1	7.4	2.5	5.2	3.9	1.6	3.6	3.6
19 (23.7%)	22.1	15.7	15.8	15.5	16.4	19.4	19.8	17.8
22 (13.6%)	10.1	15.1	8.3	20.3	12.8	8.9	16.6	13.2
58 (6.8%)	7.6	13.3	5.5	13.0	8.0	5.6	8.0	8.7
Mean (11.0)	10.2	12.9	8.0	13.5	10.3	8.9	12.0	10.8
LIMONENE								
	Female parent							
Male parent	103 (10.2%)	118 (9.7%)	140 (13.7%)	150 (8.1%)	181 (8.6%)	195 (26.8%)	214 (11.3%)	Mean (12.6%)
Percentage of oleoresin								
17 (8.4%)	4.2	3.1	4.7	2.8	3.0	8.2	3.6	4.2
19 (3.0%)	2.2	2.2	2.6	2.2	2.2	5.3	2.8	2.8
22 (0.9%)	2.3	3.0	3.9	2.0	2.3	6.5	2.4	3.2
58 (2.4%)	2.0	2.5	4.1	2.2	1.7	6.6	2.6	3.1
Mean (3.7%)	2.7	2.7	3.8	2.3	2.3	6.6	2.8	3.3
TOTAL MONOTERPENES								
	Female parent							
Male parent	103 (44.8%)	118 (40.1%)	140 (41.0%)	150 (42.4%)	181 (41.8%)	195 (49.1%)	214 (39.4%)	Mean (42.7%)
Percentage of oleoresin								
17 (21.5%)	28.3	38.1	33.3	41.1	35.8	39.0	36.9	36.1
19 (35.1%)	48.4	46.5	45.0	50.2	46.6	50.2	51.8	48.4
22 (28.6%)	41.5	51.0	39.9	51.0	45.6	43.0	43.4	45.0
58 (33.7%)	38.2	43.2	36.5	46.2	39.8	40.2	40.8	40.5
Mean (29.7%)	39.1	44.8	38.7	47.1	41.7	43.1	43.2	42.5

* Other monoterpenes detected but not included in table are camphene, γ -terpinene, and terpinolene. Standard concentration curves were not prepared for these compounds which represent only a few percent of the total monoterpene fraction.

† No measurement obtained.

progeny data. The range and variation in parental values for several of the terpenes differs considerably between sets of male and female parents. For example, α -pinene concentrations are substantially greater and more variable in the female parents than in the male parents. The parental data will be referred to again later in relation to the progeny data.

(a) *Progeny analyses*

One of the striking results which is most obvious upon examination of the parent-progeny compositions is an age effect in the case of β -pinene (table 2). The concentration of β -pinene in each biparental progeny group far exceeded that of either parent and the mean of all progenies was 19.2 per cent. compared with means of 6.0 per cent. and 7.9 per cent. for the male and female parent sets, respectively. Recent observations in our laboratory on other species have verified this age relationship with β -pinene concentrations in cortical oleoresin. The physiological significance of this finding must await further testing but conceivably it may be related to the juvenile-mature transition in pine. There was also a tendency towards a higher level of limonene in the parents than in progeny, although this pattern, which was opposite to that of β -pinene, was not as clear-cut. The other terpenes and total monoterpenes showed no such age effects.

TABLE 3
*F-values from variance analyses of concentrations of monoterpenes
in western white pine progenies*

Monoterpene	Source of variation		
	Male parent	Female parent	Male $\times$ Female
α -Pinene	0.42	20.11**	1.93*
Camphene	1.64	1.41	1.28
β -Pinene	0.38	14.12**	1.10
Myrcene	9.45**	3.22**	1.36
3-Carene	55.32**	4.29**	1.73*
Limonene	14.82**	51.98**	1.21
γ -Terpinene	4.74**	4.40**	2.42**
Terpinolene	10.60**	3.24**	1.99**
Total monoterpenes	8.15**	6.15**	1.26

* Significant at the 5 per cent. level. ** Significant at the 1 per cent. level.

The progeny data were subjected to analyses of variance and the calculated *F*-values are summarised in table 3. All terpenes except camphene showed highly significant variation of either paternal or maternal progeny groups. The existence of comparatively little variation in α -pinene and β -pinene in the paternal parents (table 2) probably contributed to non-significance in the male component compared with high significance in the female component for these terpenes. Some significant male $\times$ female interaction was evident although the magnitude was relatively small.

A more meaningful analysis of the phenotypic variances is provided in table 4, which lists the variance components as percentages of total variance calculated according to methods previously described (Hanover and Barnes, 1963). Varying degrees of genetic control over terpene concentrations are suggested by these results. However, as mentioned earlier, these results should be interpreted in the light of the limited number of parents used and

parent variability for a specific terpene. Limonene shows a high maternal parent effect and a low paternal parent effect which probably reflects the greater range of variation in maternal than paternal parent data for this compound. This is certainly true for 3-carene which is controlled primarily by genes at one loci (Hanover, 1966c). Inspection of the individual tree data for 3-carene revealed segregation into two major classes and the chi-square test was appropriate. When the methods of quantitative genetic analyses are applied to the 3-carene data as was done here the strong, simple gene control over synthesis of this compound is only slightly obscured. One reason for this may be that a greater proportion of the within-progeny

TABLE 4

Estimates of components of phenotypic variance for monoterpene concentrations in western white pine. Variance components are expressed as percentages of total variance

Monoterpene	Variance component as percentage of total variance				Total variance
	σ_M^2	σ_F^2	$\sigma_{M \times F}^2$	σ_W^2	
α -Pinene	nd*	34†	7†	59	15,250
Camphene	1	0	3	96	71
β -Pinene	nd	29†	1	70	56,100
Myrcene	11†	5†	4	80	525
3-Carene	45†	4†	4†	47	35,890
Limonene	9	55†	1	35	10,369
γ -Terpinene	3	5	14†	78	58
Terpinolene	12†	3	9†	76	370
Total monoterpenes	9†	12†	2	77	96,320

* "nd" denotes an undefined statistic arising from a negative variance component.

† Indicates that the male, female, or male $\times$ female component estimate is equal to or greater than its standard error.

component of variance (σ_W^2) for 3-carene compared with the other terpenes is due to gene segregation. Segregation occurred within progeny groups for the other terpenes but classes were not as clearly defined as in the case of 3-carene. Myrcene and terpinolene tended to vary in a pattern more suggestive of a simple inheritance mechanism but involving more than a single gene pair. α -pinene, β -pinene and limonene appear to be inherited as typical quantitative characters judging from the individual tree data.

The male $\times$ female component of variance gives an estimate of $\frac{1}{4}$ of the dominance variance (Becker, 1967). Although this component was a significant portion of the total variances for α -pinene, 3-carene, γ -terpinene and terpinolene (table 4) it is not large relative to the male or female components for the major monoterpenes. In the cases of terpinolene and γ -terpinene dominance may be important.

(b) Heritability estimates

Despite limitations in the number and variability of parents a further estimate of the strength of genetic control over monoterpene concentrations may be gained by calculating heritability values. Two common methods were used on the terpene data: covariances between the half-sib progenies and regression of the mean of the progeny on the mid-parent value. The computational procedures for estimates based on covariance analyses were

given in an earlier paper (Hanover and Barnes, 1963). The mid-parent-offspring regression coefficient is a direct estimate of narrow sense heritability assuming negligible age effects on terpene composition (Falconer, 1960).

The two heritability estimates for each terpene are listed in table 5.

TABLE 5

Estimates of heritability of monoterpene concentrations in western white pine

Monoterpene	Heritability	
	cov*	op†
α -Pinene	65 $\pm$ 39	51
Camphene	2 $\pm$ 9	7
β -Pinene	56 $\pm$ 32	> 100
Myrcene	33 $\pm$ 25	62
3-Carene	97 $\pm$ 65	64
Limonene	> 100 $\pm$ 92	50
γ -Terpinene	16 $\pm$ 20	13
Terpinolene	29 $\pm$ 26	24
Total monoterpenes	42 $\pm$ 29	28

* Estimated from covariances between half-sib progenies. Standard errors (S.E.) were calculated according to Becker (1967).

† Estimated from regression of mean of offspring on mid-parent value (27 d.f.).

The five major monoterpenes gave fairly high values for one or both methods of derivation. The minor terpenes gave lower values, which was probably due in part to the measurement error associated with compounds occurring as 1-2 per cent. or less of the oleoresin. Also, the somewhat small parental sample size may of course affect the precision of these estimates.

(c) *Correlation analyses*

Several workers and I have done simple correlation analyses among the monoterpenes in efforts to determine whether or not they vary independently of one another. Depending upon the particular populations involved, significant correlations have been found for many combinations of monoterpenes (Hanover, 1966a; Hanover and Furniss, 1966; Hilton, 1968; Tobolski, 1968). However, many of these correlations are not consistent and are dependent upon the background terpene composition of the populations as Tobolski (1968) has demonstrated in *Pinus sylvestris*.

Phenotypic correlation coefficients were calculated for the monoterpenes as described under methods and the results are shown in table 6. Only one phenotypic correlation coefficient is relatively high: 0.61 for 3-carene and terpinolene. Others are statistically significant but of dubious biological significance. Correlations between the major individual terpenes and total terpenes should be high because the latter is merely a summation of the former.

From the results of correlation analyses among monoterpenes of western white pine and other species we have examined we may conclude that 3-carene and terpinolene are quite surely biologically related, probably through biochemical or genetic linkage. More direct experimental methods are now needed to clarify this relationship. There are only two other

consistent interrelations among the terpenes to suggest that other than relatively independent inheritance mechanisms exist for each terpene considered here. Exceptions to this conclusion may be those for α -pinene and β -pinene and myrcene and 3-carene. Hanover and Furniss (1966) in a study of *Pseudotsuga menziesii* terpenes and Tobolski (1968) studying *Pinus*

TABLE 6

Phenotypic correlations between monoterpenes in western white pine progenies (196 d.f.)

	Camphene	β -Pinene	Myrcene	3-Carene	Limonene	γ -Terpinene	Terpinolene	Total monoterpenes
α -Pinene	0.19	-0.22	0.25	-0.02	-0.08	-0.08	0.29	0.43
Camphene	—	0.00	0.38	-0.13	0.23	0.09	0.27	0.19
β -Pinene	—	—	0.10	-0.14	-0.07	-0.13	-0.08	0.66
Myrcene	—	—	—	-0.30	0.21	-0.03	0.05	0.15
3-Carene	—	—	—	—	-0.26	0.19	0.61	0.54
Limonene	—	—	—	—	—	0.17	0.18	0.31
γ -Terpinene	—	—	—	—	—	—	0.21	0.07
Terpinolene	—	—	—	—	—	—	—	0.47

$$r(196 \text{ d.f.}, P = 0.01) = 0.183.$$

sylvestris terpenes have also reported strong negative correlations for these pairs of compounds.

The relationship, if any, between monoterpene synthesis and growth rate of trees is unknown and must be answered by research using several types of approaches. Simple correlation analyses between mean progeny height growth or breeding value and individual terpenes could provide

TABLE 7

Simple correlations between monoterpenes and mean height growth of 2-year-old progenies

Monoterpene	r^*
α -Pinene	-0.17
Camphene	-0.08
β -Pinene	-0.08
Myrcene	0.35
3-Carene	-0.56**
Limonene	0.10
γ -Terpinene	-0.51**
Terpinolene	-0.63**
Total monoterpenes	-0.57**

* 26 degrees of freedom.

** Significant at the 1 per cent. level.

indirect evidence for or against a relationship (table 7). As reported in my earlier study (Hanover, 1966a), negative correlations between the terpenes and growth rate predominate and several are highly significant. However, only the correlation between total monoterpenes and growth is consistently significant between the two independent studies. We are currently investigating the possibility of a biological relationship between a high monoterpene-oriented metabolism and slow growth potential in conifers.

4. SUMMARY

1. Analyses of the monoterpene compounds in western white pine parents and progeny from a factorial mating design show that these chemicals are under strong, predictable genetic control probably involving one to several loci.

2. One compound, β -pinene, consistently occurs in substantially larger concentrations in progeny than in parents, which suggests an age effect of possible physiological significance.

3. A strong positive relationship exists between the concentrations of 3-carene and terpinolene. Two other pairs of monoterpenes, α -pinene and β -pinene and myrcene and 3-carene are negatively correlated. Except for these interrelationships the compounds appear to be independently inherited.

4. Negative correlations predominate between monoterpene concentrations and mean progeny height growth rate. Further work is needed to determine the biological basis, if any, for this relationship.

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