components which contribute to its external aroma. It might provide a means for aroma classification of dessert plums.

EXPERIMENTAL

Extraction and analysis of epicuticular wax and wax volatiles. Intact plums 'Golden Egg' (1 kg) were individually immersed for 30 sec in 1 l. of CH_2Cl_2. The extract was coned to dryness using a rotary evaporator, yielding 280 mg of dried extract. The qualitative and quantitative composition of the CH_2Cl_2 extract was determined using published TLC, PLC and GC–MS methods [9, 10].

Examination of odorous wax fraction. The fraction (R_f in C_6H_6 = aldehyde) was taken up in 1 ml of hexane and examined by GLC and GC–MS.

GLC. Carried out using an FID instrument, with a N_2 flow of 40 ml/min. Injection port and detector temps of 250°. Temp. programming was used from 65–210° at 8°/min, then isothermal. Two columns were used: (i) 3.6 m × 3.2 mm glass column packed with 10% Carbowax 20M. (ii) 3.6 m × 3.2 mm glass column packed with 10% SE30 + 0.5% Carbowax 20M.

MS. Recorded on an LKB 9000 coupled GC–MS operating at 70 eV and a separator temp. of 250°, using same columns as above. He flow was 30 ml/min and the GLC was programmed at 6°/min, otherwise conditions were similar to those for GLC analysis.

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RELATION OF CORTICAL MONOTERPENOID COMPOSITION OF *ABIES* TO TREE AGE AND SIZE

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Abstract—Correlations between the monoterpenoid compositions of cortical oleoresin on the one hand and tree age and diameter at breast height on the other, were determined for seven fir species of North America. In most cases neither age nor diameter related significantly to the composition of monoterpenoids, suggesting that it was unnecessary to standardize oleoresin sampling for chemosystematic purposes.

INTRODUCTION

In an earlier paper [1] we reported on changes in composition of cortical monoterpenoids of several species of *Abies* as a function of the age of the cortex, i.e., with the height of individual trees. The data indicated that large changes take place only in the uppermost portions of tree stems, and that the variability within older portions of the stems was minor compared with differences between individual trees. These older portions of the stems included as a rule the areas of bark blistering—the areas where cortical extrusions filled with oleoresin are formed as the result of local enlargement of resin canals. The absence, or near absence, of the mentioned nongenetic changes along the blistered portion of the stem made it possible to use the composition of cortical oleoresins from blisters directly in our chemosystematic studies without any additional corrections.

The terpenoid variability with age of cortex within a plant must be distinguished, however, from the variability with age or size of individual plants. The latter can be studied as a correlation between the percentages of individual terpenoids and age or diameter at breast height (DBH) of trees. In principle, besides nongenetic factors such correlations (i.e. chemophenotypically different compositions of a taxon within different age groups) could also be genetically caused, and could include a dynamic situation with a composition of a gene pool changing in time, or a stationary situation with differences in selection for a particular genotype within specific age groups.

In the present study we examined the relationships between chemical composition of cortical monoterpenoids on the one hand and tree age and DBH on the other using 420 *Abies* trees belonging to seven different species and collected in 51 different geographic areas.

RESULTS AND DISCUSSION

The taxa investigated included *A. magnifica* A. Murr., *A. procera* Rehd., *A. lasiocarpa* (Hook.) Nutt. var. *lasiocarpa* and var. *arizonica* (Merriam) Lemm., *A. balsamea* (L.) Mill., *A. fraseri* (Pursh) Poir., *A. grandis* (Dougl.) Lindl., and *A. concolor* (Gord. & Glend.) Lindl. var. *concolor* and var. *lowiana* (Gord.) Lemm. The geographic locations of the trees sampled are given in Table 1, and are more exactly delineated in our other publications [2–5]. In each geographic location mostly eight, in a few cases 10, trees were sampled for oleoresin, and concurrently age at breast height (about 1.4 m above the ground) was estimated from an increment core and the diameter at breast height (DBH) was measured. The percent composition and content of monoterpenoid hydrocarbons of each sample was determined by GLC and coefficients of correlation, r, were calculated between age and DBH on one side and chemical percentage data on the other. To increase the significance of statistics, the data for several, mostly four, populations belonging to the same species or variety and located geographically close to each other, were combined and to eliminate the influence of the variability between populations the correlation coefficients were calculated using within-population components of variances and covariances.

The correlations obtained were generally low and explained on the average only 3.7% of the total variability with age and only 4.9% of that with DBH. The cumulative distribution of the correlation coefficients obtained agreed roughly, particularly in the case of r_{age}, with theoretical distribution of sample correlation coefficients calculated assuming absence of any relationships, i.e. for $\rho = 0$ (Fig. 1). Only a few correlation coefficients proved to be significant on the 5% or 1% level. Since, on other occasions, the terpenoid values have been demonstrated to be nonparametrically distributed, either skew or polymodal, the significance of these correlations was checked by calculation of the mean nonparametric rank correlation ($\overline{Rs}$) for each set of populations. Two 1% relationships—age/β-pinene in *A. grandis* (southern Oregon, $r = +0.487$) and DBH/limonene in *A. magnifica* ($r = +0.516$)—and three 5% relationships—DBH/α-pinene in *A. concolor* var. *lowiana* ($r = +0.315$), age/limonene in *A. magnifica* ($r = +0.478$), and age/terpene content of oleoresin in *A. grandis* (southern Oregon, $r = +0.429$)—withstood this test. The fact that these correlations represent only a small fraction out of nearly 200 determined correlation coefficients argues for their being normal stray values and could comprise one explanation. At the same time it cannot be excluded that the relatively weak but real relationships between age/size and terpenoid composition of cortex within a single tree found by us earlier [1] have to do with these correlations. Specifically in the case of *A. magnifica* a positive correlation between percent limonene and age of cortex was reported for a younger (1–25 years) portion of one tree, a portion which borders on the average age (25.8 years) of *A. magnifica* trees used in this investigation.

Generally the results point out that age/size of a tree could contribute only occasionally towards terpenoid variability within the cortex, but the effect appears to be of a second order if sampling is confined to blistered portions of the stem. As a method for reduction of non-genetic factors in chemosystematics the standardization

Table 1. Provenance of *Abies* samples and terpenoids considered in calculations

Population numbers	Taxon	Number of trees	Geographic area	Terpenoids considered in computation*
17, 22, 23, 28	*A. procera*	4 × 8	Southern Oregon and Northern California [10]	αP, Ca, βP, 3C, My, Li, βPh, TT
18, 20, 21, 27	*A. procera*	4 × 8	Central and Northern Oregon [10]	αP, Ca, βP, 3C, My, Li, βPh, TT
26, 32, 34, 36	*A. magnifica*	4 × 8	Sierra Nevada and Cascades of California [10]	αP, βP, 3C, My, Li, βPh, TT
39, 66, 72, 73, 74, 80	*A. concolor* var. *lowiana*	6 × 8	Sierra Nevada and Cascades of California [5]	αP, Ca, βP, 3C, My, Li, βPh, TT
70,71	*A. concolor* var. *concolor*	2 × 8	Utah and New Mexico [5]	αP, Ca, βP, 3C, My, Li, βPh, Te, TT
41, 42, 67, 77	*A. grandis*	4 × 8	Southern Oregon [10]	αP, Ca, βP, My, Li, βPh, TT
44, 45, 46, 68	*A. grandis*	4 × 8	Northwestern Oregon [10]	αP, Ca, βP, 3C, My, Li, βPh, TT
47, 48, 60, 69	*A. grandis*	4 × 8	Southern Washington and Northernmost Oregon [10]	Tr, αP, Ca, βP, My, Li, βPh, TT
81, 82, 83, 84, 85, 87	*A. lasiocarpa* var. *lasiocarpa*	6 × 8	West Central Alberta [2]	αP, βP, 3C, My, Li, βPh, TT
101, 105, 107, 109	*A. lasiocarpa* var. *arizonica*	4 × 8	New Mexico and Arizona [3]	αP, βP, My, Li, βPh, TT
25, 48, 49, 51, 52, 62	*A. balsamea*	6 × 10	Manitoba, Alberta and Saskatchewan [4]	αP, βP, Li, βPh, TT
79, 115, 116	*A. fraseri*	3 × 8	W. Virginia, N. Carolina and Virginia [4]	αP, Ca, βP, 3C, My, Li, βPh, TT

Abbreviations: Tr = tricyclene: αP = α-pinene: Ca = camphene: βP = β-pinene: 3C = 3-carene: My = myrcene: Li =limonene: βPh = β-phellandrene: Te = terpinolene: TT = monoterpenoid hydrocarbon content of oleoresins.

of sampling on age or DBH, while laborious, is likely to eliminate only negligible amounts of such extraneous factors and does not appear necessary. At the same time it should be emphasized that in the experiments performed tree age varied between 5 and 95 years, with a mean age and mean within-population range of 28.4 and 24.9 yr, respectively, and DBH between 2.8 and 42.7 cm, with a mean DBH and mean within-population range of 13.9 cm and 13.9 cm, respectively; it is possible that the effect would be higher if the ranges were larger, particularly if they included younger and metabolically more active trees.

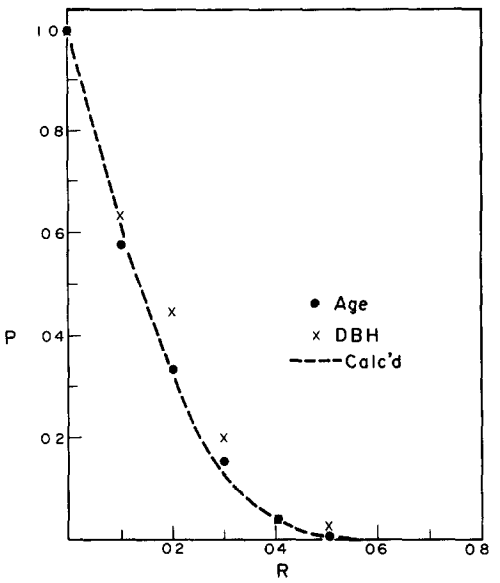

Fig. 1. Calculated probability P for $\rho = 0$ and df = 26 of finding an $|r| \geqslant R$, and found cumulative frequency of r_{age} and r_{DBH}. A slightly higher-than-expected frequency of r_{DBH} at R = 0.2 could indicate that growth rate might slightly influence the terpenoid composition.

EXPERIMENTAL

Collection and analysis of oleoresin samples has been described before [6]. Also given in the same sources is an accurate geographic location for most of the populations sampled; the latitudes and longitudes for the remaining populations will be published shortly in related papers. Tree age was measured at breast height using increment cores. Analysis of covariance, calculation of correlation coefficients and rank correlations and tests of significance were performed using standard procedures [7]. In Fig. 1 where $N > 32$ or $N < 32$ the found values of r were adjusted to r at N = 32 (df = 26) and the same P (probability of a larger value), using tables for r vs df and P. Theoretical distribution of values for sample correlation coefficients r, for $\rho = 0$ and df = 26 was computed from Fisher's [8] t–r relationship using calculated values for t [9].

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