503 (1979). — Yeh, F. C. and El-Kassaby, Y. A.: Enzyme variation in natural populations of Sitka spruce *(Picea sitchensis).* Genetic variation patterns among trees from 10 IUFRO provenances. Can. J. For. Res. **10***:* 415—422 (1980). — Yeh, F. C. and O'Malley, D.: Enzyme variations in natural populations of Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, from British Columbia. 1. Genetic Variation Patterns in Coastal populations. Silvae Genet. **29***:* 83—92 (1980). — Yeh, F. C.: Analyses of gene diversity in some species of conifers. In: Proc. of Symp. on Isozymes of North American Forest Trees and Forest Insects. July 27, 1979. Berkely, California. pp. 48—52 (1981). — Zackrisson, O.: Influence of forest fires on the North Swedish boreal forest. OIKOS **29***:* 22—32 (1977).

Geographical variation in the relative proportion of monoterpenes in cortical oleoresin of Pinus sylvestris in Sweden

By R. Yazdani[1], J. E. Nilsson[1] and T. Ericsson[2]

(Received 21st December 1984)

Summary

Quantities of several monoterpenes were determined by gas-chromatographic method in individuals belonging to 26 populations of *Pinus sylvestris* planted in Sävar, Sweden, latitude 63° 54', longitude 20°33', altitude 10 m. Pines originating from various parts of Sweden appear to have different monoterpene compositions. Southern populations contain more individuals with low limonene, low β-pinene and high Δ 3-carene than northern ones. The number of individuals with a high quantity of limonene gradually increases with latitude. The means of the six coefficients of variation for six major monoterpene variables demontrate that southern populations, when transferred from south to north, contain lower genetic variation than those from the central and northern Sweden.

Since there is a cline for relative proportion of limonene in Swedish *Pinus sylvestris* populations, it is proposed that such a pattern has evolutionary significance and is caused by the process of long term natural selection. Therefore, population descriptions based on monoterpene composition can be helpful in breeding work and gene resource conservation programs.

Key words: monoterpenes, *Pinus sylvestris,* population

Zusammenfassung

Nach gaschromatographischer Untersuchung haben quantitative Analysen verschiedener Monoterpene an Einzelbäumen von 26 Populationen der Kiefer *(Pinus sylvestris* L.) in einem Herkunftsversuch in Nordschweden (Sävar 64° 54') folgende Ergebnisse gezeigt:

Kiefern verschiedener schwedischer Herkünfte haben verschiedene Monoterpenmuster. In südlichen Populationen (56—60°) treten häufiger Individuen mit niedrigem Limonen- und β-Pinen- sowie hohem 3-Caren-Gehalt auf als in nördlichen Populationen (64—68°).

Mit zunehmender nördlicher Breite der Herkunft steigt die Anzahl der Individuen mit hohem Limonen-Gehalt.

Mittelwerte der 6 Variationskoeffizienten für 6 Hauptmonoterpen-Variable zeigen, daß südliche Herkünfte, die im Norden angebaut wurden, eine durchschnittlich geringere genetische Variation aufweisen als mittelschwedische (60—64°) und nordschwedische Herkünfte. Dieser Verlust genetischer Variation könnte die Folge einer Selektion am Anbauort sein.

Der gefundene Süd-Nord-Klin für Limonen in schwedischen Kiefern-Populationen hat als Folge langanhaltender natürlicher Selektion evolutionäre Signifikanz. Eine Herkunftsbeschreibung mit Hilfe von Monoterpenmustern kann daher wertvolle Hinweise für die Züchtung und Erhaltung von Genressourcen geben.

Introduction

Conifers produce the greatest amount of terpenes among all plant families. In *P. sylvestris* the heart wood contains 8% resin consisting of 25% monoterpenes (Parham 1976).

Monoterpene composition has been shown to be very informative in investigations of infrastructure and population variation for a wide variety of species. For the most part, monoterpene variation is found to be genetically determined and little influenced by environmental factors, and each component is identifiable with high accuracy (Squillace 1976). Studies of monoterpene composition are used for characterization of populations and provenances and determination of seed sources of *Pinus elliotti* (Gansel and Squillace 1976; Squillace 1977; Squillace *et al.* 1980), *Pinus contorta* (Forrest 1977) and *Pinus maritima* (Baradat *et al.* 1978). In *Picea abies* monoterpenes have been used as markers in clone identification (Esteban *et al.* 1976). Monoterpenes of the needles and oleoresin of *Pinus sylvestris* are under strong genetic control (Hiltunen 1975; Yazdani *et al.* 1982), and little influenced by the environment or the physiological condition of the trees (Yazdani unpublished data). Some monoterpene fractions show clinal variation among populations of *Pinus sylvestris* in Finland (Hiltunen *et al.* 1975).

Monoterpene composition seems to play an important role in the defense mechanism against pathogenic fungi (Rishbeth 1972; Schuck 1980), and has been found to be related to resistance against insect attacks (Hanover 1975).

Since monoterpene quantity in *P. sylvestris* is under strong genetic control, it can be used as a multigene marker for studying population structure. This study deals with a comparison of the geographical pattern of monoterpene composition among 26 populations of *P. sylvestris* in Sweden.

Materials and Methods

The reference material consisted of 26 representative and well-documented natural populations of *P. sylvestris* from different latitudes. Further details of the origins are presented in *Table 1* and *Fig. 1.* Seed was sown in 1971 and planted during autumn 1972 at Sävar outside, Umeå, Sweden. (Latitude 63° 54' N, longitude 20° 33 E and altitude 10 m) by the Institute for Forest Improvement.

---

[1] Department of Forest Genetics and Plant Physiology. The Swedish University of Agricultural Sciences, S-901 83 Umeå, Sweden

[2] Institute für Forest Improvement, Northern District, Box 3, S-910 36 Sävar, Sweden

Sample analysis

Oleoresin samples were obtained from tissue during late spring 1979 by cutting side branch buds. Exuded oleoresin was collected in capillary tubes, which were sealed and stored at -20^{0} C for several months until analysed. Samples were analysed by gas-chromatography using a capillary column (after Yazdani *et al.* 1982). Monoterpenes were quantified as the percentage contribution of each peak to the total monoterpenes present. Mean percentage value of needle dry matter content and relative annual growth for each population presented in this study was reported earlier by Ericsson and Remröd (1975). The number of trees sampled per population varied from 16 to 51.

Statistical treatment

Analysis of variance, discriminant analysis and t-test analysis have been used in the statistical treatment of monoterpene data. For the discriminant analysis, we have used a statistical program package of SPSS available at the computer centre of UMDAC, Umeå. For analysis of variance and t-test a local computer program was developed in our department. The possible nonnormality of the variables studied is not considered critical, since the analyses of variance and discriminant analyses are used only as numerical methods for calculating variance components and discriminant functions. No significance test are made for these methods. Only for the univariate t-tests of differences between populations are significance levels based on normality used. However, t-tests are known for the small influence of nonnormality on the significance levels. Therefore we have considered it relevant to ignore the possible deviations from the normal distribution in this report.

Results and Discussion

The six monoterpenes α-pinene, β-pinene, △ 3-carene, myrcene, limonene and β-phellandrene were found to be the major variable components and the three monoterpenes terpinolene, sabinene and α-terpinene to be the minor components. None of the three minor components represented more than 5 percent of the total monoterpene fraction of cortical oleoresin. The mean proportion of each monoterpene and a variation coefficient characteristic for each population are presented in *Table 2.*

Table 1. — Details of population origin, planted by the Institute for Forest Improvement at Sävar, Sweden. Data on relative growth increment and dry matter content are reported earlier by Ericsson and Remröd 1975.

1	2	3	4	5	6	7	8
1	Eb 61	Glimåkra	56.33	90	48.5	24.9	34.15
4	Eb 64	Svinhult	57,77	260	54.6	27.3	34.32
5	Eb 60	Skänksätter	58,53	25	57.0	29.5	34.26
6	Bg 423	Floda	59.03	60	55.8	26.8	35.52
7	Bg 424	Ockelbo	60.92	80	58.8	33.1	36.46
8	Bg 425	Söderbärke	60.17	170	57.7	29.4	37.57
12	Bg 429	Zon 61° 61°59'	61.50	310	69.4	39.2	37.43
13	Z 435	-"-	61.97	400	77.6	39.6	38.18
17	Bg 433	-"-	61.75	625	76.7	39.3	38.52
18	Bg 434	-"-	61.88	665	77.3	39.6	37.11
19	Y 852	Zon 62° - 62°59'	62.90	80	75.1	40.8	38.29
20	Y 825	Nordanende	62.58	225	66.4	34.7	37.45
25	Z 438	Zon 62° 62°59'	62.43	510	72.7	40.6	38.29
27	Z 440	-"-	62.17	625	81.2	44.3	37.45
28	Z 441	-"-	62.50	775	80.6	43.8	38.61
29	AC 511	Zon 63° - 63°59'	63.97	50	75.1	41.5	38.42
30	Y 854	-"-	63.52	150	68.0	35.1	37.85
34	Z 444	-"-	63.67	410	78.8	44.4	38.66
40	AC 515	Zon 64° - 64°59'	64.55	380	82.1	41.7	38.81
43	AC 518	Zon 65° - 65°59'	65.08	130	75.5	39.4	38.72
45	AC 520	-"-	65.00	320	75.7	41.5	38.81
48	Ac 521	Zon 65° - 65°59'	65.50	460	86.3	55.6	38.18
50	BD 418	Zon 66° - 66°59'	66.00	70	82.0	44.5	39.01
56	BD 410	Övertorneå	67.00	150	83.4	44.2	39.73
57	BD 411	Vijateievat	67.80	300	82.8	46.2	38.47
58	BD 412	Aijänrova	67.63	370	78.1	41.8	37.52

1 = Population no.
2 = Population letter
3 = Name
4 = Latitude
5 = Altitude m
6 = Relative growth increment — 74%
7 = Relative growth increment — 75%
8 = Dry matter content — 74%

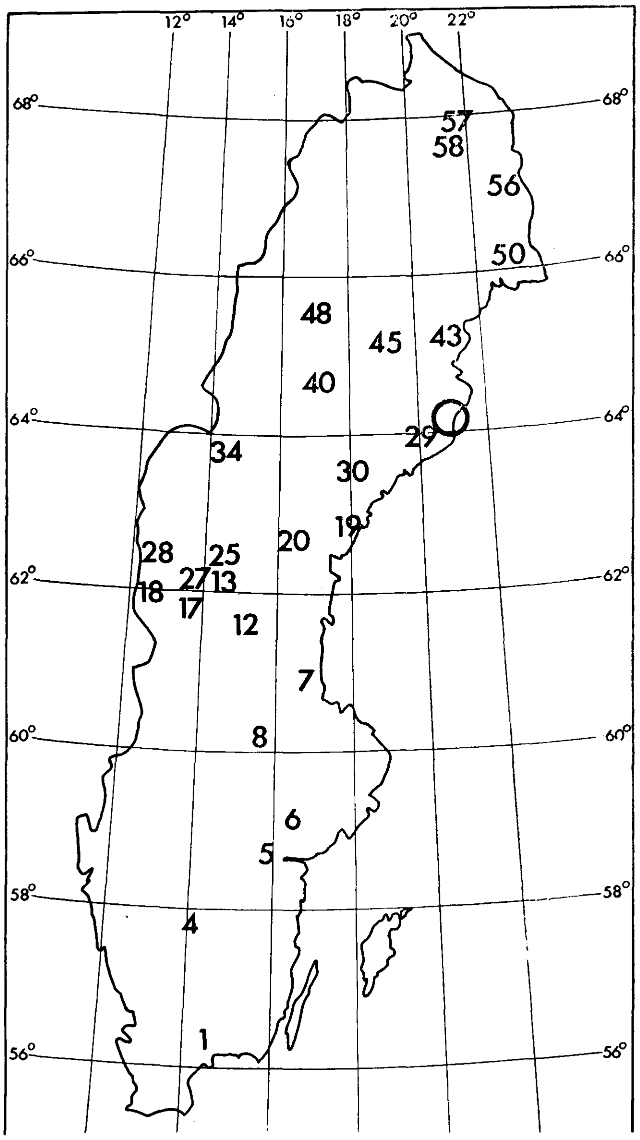

Figure 1. — Map of population origins. The location of planting site in Sävar outside Umeå is indicated by circle.

β-pinene: The mean proportion of β-pinene for populations from south of latitude 62^{0} N is lower than for more northern ones. However, population 56 in the north shows a very low mean proportion of this monoterpene. The variation may be a result of ecotypic differentiation for this population.

△ 3-Carene: In every population, △ 3-carene shows the highest mean proportion of all the monoterpenes examined, and also contributes most to the variation among populations. Individuals with high proportions of △ 3-carene are more prevalent in southern populations than in northern ones, although southern population 7 has a low mean proportion. In Finland the variation of △ 3-carene leaf extract of *P. sylvestris* has been found to be clinal (Hiltunen *et al.* 1976).

α-pinene, myrcene and β-phellandrene: No specific pattern of variation were detected for α-pinene, β-phellandrene or myrcene in the populations from different latitudes. The mean proportion of α-pinene shows relatively

Table 2. — Population origin, mean proportion ($\bar{X}$) and variation coefficient ($\frac{S}{\bar{X}}$) among trees within populations for six major monoterpenes (S^2 = estimated variance among trees within a population).

Pop No	No of trees	α-Pinene		β-Pinene		Δ3-Carene		Myrcene		Limonene		β-Phellandrene		Mean variation coefficients for six monoterpene variables
		$\bar{X}$ (%)	$\frac{S}{\bar{X}}$	$\bar{X}$ (%)	$\frac{S}{\bar{X}}$	$\bar{X}$ (%)	$\frac{S}{\bar{X}}$	$\bar{X}$ (%)	$\frac{S}{\bar{X}}$	$\bar{X}$ (%)	$\frac{S}{\bar{X}}$	$\bar{X}$ (%)	$\frac{S}{\bar{X}}$	$\frac{\sum_{i=1}^{6} \frac{S_i}{\bar{X}_i}}{6}$
1	31	7.19	0.34	6.74	1.33	53.1	0.31	5.34	0.78	3.44	1.84	14.1	0.71	0.88
4	25	7.55	1.00	5.30	1.00	54.18	1.00	5.30	1.00	4.07	1.00	12.53	1.00	1.00
5	20	9.96	0.61	7.54	1.22	41.12	0.48	13.18	0.58	3.48	1.47	18.41	0.52	0.81
6	26	10.86	0.88	7.36	1.14	51.14	0.47	8.11	1.02	4.27	1.79	10.08	0.98	1.04
7	16	11.98	0.34	9.96	0.74	24.88	0.86	20.65	0.67	3.88	1.34	23.83	0.33	0.71
8	38	12.69	0.77	7.66	0.86	39.63	0.60	11.35	0.97	5.18	1.51	15.05	0.67	0.90
12	18	15.05	0.54	9.66	0.97	47.37	0.36	2.87	0.33	10.19	1.35	8.18	1.08	0.77
13	20	12.32	1.16	11.88	0.98	47.94	0.54	4.58	1.15	10.77	2.03	7.97	0.74	1.10
17	38	8.16	0.34	12.87	1.00	48.97	0.40	4.10	0.85	5.03	1.44	12.94	0.86	0.82
18	40	12.05	0.63	19.12	0.80	33.61	0.73	6.74	0.80	10.54	1.05	9.90	1.16	0.86
19	48	9.93	0.60	11.33	0.98	43.07	0.49	5.19	1.08	10.53	1.20	12.95	0.67	0.84
20	51	11.29	0.53	18.03	0.85	40.65	0.59	5.31	0.97	9.76	1.11	8.41	1.05	0.85
25	51	11.26	0.48	20.03	0.82	34.20	0.68	6.91	0.90	9.08	1.15	12.63	0.80	0.81
27	20	13.36	0.53	20.95	0.64	36.76	0.66	4.83	0.93	7.60	1.03	10.28	0.89	0.78
28	30	8.93	0.49	12.40	1.07	52.37	0.39	4.75	0.79	6.59	1.67	7.00	1.09	0.92
29	40	9.97	0.48	13.05	1.02	40.28	0.56	4.73	1.11	11.69	1.06	13.51	0.80	0.84
30	39	11.26	0.43	24.27	0.58	31.38	0.67	12.06	0.79	9.20	1.00	6.31	1.02	0.75
34	39	10.69	0.42	16.63	0.94	43.61	0.56	6.19	1.15	8.38	1.32	6.93	1.20	0.93
40	18	10.36	0.40	13.44	1.18	42.17	0.58	5.98	1.22	12.57	1.59	8.59	1.04	1.00
43	34	11.98	0.44	17.21	1.04	25.87	0.95	10.42	1.04	14.78	1.20	14.88	0.89	0.93
45	19	10.70	0.28	13.76	0.90	23.72	0.81	13.29	0.97	14.65	1.35	17.88	0.51	0.80
48	31	9.29	0.44	11.40	0.95	39.73	0.55	7.81	1.25	9.50	1.00	14.10	0.74	0.82
50	49	10.30	0.49	14.91	0.99	34.60	0.70	6.93	1.43	13.76	1.07	13.92	0.84	0.92
55	29	9.98	0.53	7.17	1.03	33.70	0.57	8.58	1.16	11.96	1.18	20.86	0.57	0.84
57	17	11.94	0.35	19.62	0.82	33.13	0.83	11.47	0.85	6.82	1.48	12.04	0.60	0.82
58	40	8.59	0.32	14.20	0.83	40.16	0.48	7.25	0.83	6.99	1.29	14.44	0.79	0.76

small differences. The lowest β-phellandrene and myrcene proportions were found among individuals of populations around latitude 62^0—63^0.

Limonene: Populations from the southern part of Sweden show very low proportions of limonene, which increase with higher latitude. Populations of similar geographical origin show greater similarity in limonene content than do those of distant origin. The changes in mean limonene content are small between latitude 56^0 to 61^0, but increase from 61^0 northward. Two populations, 57 and 58 above latitude 67^0, show a decline in the content of this monoterpene.

Variation within and between populations

A simple measure of variation coefficient $\frac{S}{\bar{X}}$ is calculated for six major monoterpenes *(Table 2)*. $\bar{X}$ = mean proportion of monoterpene for trees in the population. The variation coefficient shows large differences among populations with respect to within population variation of monoterpene proportions. The mean variation coefficient for six monoterpenes demonstrates small differences among populations.

Within and between populations, variance components for nine different monoterpenes variables were estimated by analysis of variance *(Table 3)*. The within population variance components are much larger than the between population components. The magnitude of the variance for each of the nine variables is almost the same. On average about 88 percent of the variation exists within and 12 percent between populations.

Discriminant analysis

The variation between populations was studied by discriminant analysis *(Table 4)*. By this method the original monoterpene variables can be decreased to a smaller num-

Table 3. — Estimated variance components within (σ^2_B) and between (σ^2_W) 26 populations of *Pinus sylvestris* expressed as proportions of (σ^2_W + σ^2_B) for different monoterpene variables. The analyses of variance were based on monoterpene proportions of single trees.

Monoterpene variables	Within pop variance, % $100\,\sigma^2_W/(\sigma^2_W+\sigma^2_B)$	Between pop variance, % $100\,\sigma^2_B/(\sigma^2_W+\sigma^2_B)$
α-Pinene	92.8	7.2
β-Pinene	87.2	12.8
Sabinene	88.3	11.7
Δ3-Carene	88.8	11.2
Myrcene	83.6	16.4
Terpinolene	85.5	14.5
Limonene	92.7	7.3
β-Phellandrene	89.7	10.3
Mean	88.4	11.6

Table 4. — Actual and predicted population membership according to a discriminant analysis based on the proportion of nine monoterpenes of 827 trees in 26 populations.

Population No	No of trees	Altitude	PREDICTED PROVENANCE MEMBERSHIP 1	4	5	6	7	8	12	13	17	18	19	20	25	27	28	29	30	34	40	43	45	48	50	56	57	58
1	31	90	32.3	25.8	3.2	0	0	0	3.2	0	9.7	0	3.2	0	3.2	0	3.2	3.2	0	0	0	0	0	0	0	3.2	0	9.7
4	25	260	16.0	36.0	0	0	0	0	8.0	0	16.0	4.0	0	0	0	4.0	0	8.0	0	0	4.0	0	0	0	0	0	0	4.0
5	20	25	0	0	60.0	5.0	10.0	5.0	0	0	10.0	0	0	0	0	5.0	0	0	0	0	0	0	0	0	0	0	5.0	0
6	26	60	0	11.5	15.4	15.4	0	0	3.8	0	34.6	0	3.8	0	0	3.8	0	0	3.8	0	0	0	0	0	0	0	3.8	3.8
7	16	80	0	6.3	0	0	87.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.3	0
8	38	170	10.5	21.1	10.5	0	0	15.8	7.9	0	7.9	0	2.6	0	0	0	0	5.3	0	0	5.3	0	5.3	0	0	0	0	7.9
12	18	310	5.6	0	0	0	0	0	72.2	0	0	0	5.6	0	0	0	0	0	0	0	11.1	0	0	0	0	0	5.6	0
13	20	400	10.0	5.0	0	5.0	0	0	10.0	15.0	25.0	0	5.0	0	0	5.0	0	5.0	5.0	0	0	0	0	5.0	0	0	5.0	0
17	38	625	2.6	5.3	0	0	0	0	0	0	68.4	0	5.3	0	0	5.3	0	2.6	0	0	5.3	0	0	0	2.6	0	0	2.6
18	40	665	2.5	12.5	2.5	2.5	2.5	5.0	2.5	0	10.0	10.0	0	2.5	2.5	2.5	0	0	2.5	2.5	2.5	5.0	5.0	0	2.5	2.5	0	20.0
19	48	80	0	4.2	8.3	0	8.3	2.1	0	2.1	39.6	0	18.8	0	0	4.2	0	4.2	2.1	0	0	0	2.1	0	4.2	0	0	0
20	51	225	9.8	2.0	5.9	0	0	2.0	2.0	3.9	21.6	0	5.9	3.9	2.0	2.0	2.0	5.9	3.9	3.9	13.7	0	0	2.0	0	0	2.0	5.9
25	51	510	7.8	3.9	5.9	3.9	3.9	0	2.0	3.9	17.3	0	2.0	5.9	2.0	3.9	0	3.9	5.9	5.9	5.9	2.0	2.0	0	2.0	2.0	7.8	0
27	20	625	0	5.0	0	0	0	0	10.0	0	30.0	0	10.0	0	0	35.0	0	0	5.0	5.0	0	0	0	0	0	0	0	0
28	30	775	3.3	3.3	0	0	0	0	16.7	0	30.0	0	3.3	3.3	3.3	3.3	16.7	0	3.3	3.3	0	0	0	0	3.3	0	3.3	3.3
29	40	50	0	2.5	2.5	0	2.5	0	0	2.5	40.0	0	12.5	0	0	0	0	20.0	2.5	2.5	5.0	2.5	0	0	2.5	2.5	0	0
30	39	150	0	2.6	5.1	5.1	2.6	2.6	0	2.6	7.7	2.6	7.7	5.1	0	5.1	2.6	2.6	25.6	2.6	0	5.1	0	0	0	0	12.8	0
34	39	410	0	7.7	2.6	5.1	0	5.1	12.8	0	12.8	0	7.7	2.6	0	5.1	0	2.6	7.7	12.8	7.7	5.1	0	0	2.6	0	0	0
40	18	380	0	5.6	0	0	5.6	0	5.6	0	11.1	0	5.6	0	0	16.7	0	0	5.6	5.6	16.7	0	0	0	0	16.7	5.6	0
43	34	130	0	2.9	0	0	17.6	0	0	2.9	26.5	0	0	0	0	0	2.9	2.9	0	0	2.9	17.6	2.9	0	5.9	5.9	8.8	0
45	19	320	21.1	10.5	0	0	5.3	5.3	0	0	0	5.3	0	0	0	0	0	0	10.5	0	0	0	26.3	0	0	10.5	5.3	0
48	31	460	3.2	3.2	6.5	0	0	0	6.5	0	35.5	0	9.7	0	0	0	6.5	6.5	3.2	0	3.2	0	6.5	6.5	3.2	0	0	0
50	49	70	6.1	0	2.0	2.0	4.1	0	10.2	0	14.3	0	14.3	0	0	4.1	2.0	0	6.1	4.1	4.1	4.1	2.0	0	6.1	0	14.3	0
56	29	150	6.9	20.7	3.4	0	3.4	0	0	0	6.9	0	3.4	0	0	0	0	0	0	0	6.9	0	6.9	3.4	0	24.1	3.4	10.3
57	17	300	0	0	0	0	0	0	17.6	0	0	0	5.9	0	0	0	0	0	11.8	0	0	0	0	0	5.9	0	58.8	0
58	40	370	22.5	17.5	2.5	0	0	0	0	0	12.5	0	0	0	0	0	0	5.0	0	0	0	0	7.5	0	2.5	2.5	0	27.5
Total No	827																											

ber of discriminant function: $f_1, f_2 \ldots\ldots, f_k$, where f_k explains as much as possible of the remaining variation after $f_1 \ldots\ldots f_{k-1}$ have been formed. Discriminant analysis was performed on the arc sine transformed monoterpene proportions of 827 trees from 26 populations.

Table 5. — Eigenvalues and coefficients of the first four discriminant functions for nine monoterpenes in 26 populations of *P. sylvestris.*

Monoterpene	Discriminant function f_1	f_2	f_3	f_4	
	Discriminant coefficients				
α-Pinene	-.0215	-.0769	.3309	.8830	
β-Pinene	.3582	-.2787	-.4160	-.4047	
Sabinene	-.3671	.0323	-1.0074	1.5571	
Δ3-Carene	1.6874	.1458	2.3029	-.0922	
Myrcene	.1345	.6043	-.0599	.0713	
α-Terpinene	-1.048	-.0988	-.0248	-.0173	
Limonene	-.0054	-.2046	.0494	.0010	
β-Phellandrene	-.0316	.4498	.7447	-.6728	
Terpinolene	-.6922	-.5502	-.6690	-1.1089	
Eigenvalue e_i	.3445	.2291	.1704	.1131	$\sum_{i=1}^{8} e_i = 1.108$
% of variance	30.37	20.20	15.02	9.97	
% cumulative	30.37	50.57	65.59	75.56	

In *Table 5* eigen-values (expressing the proportion of the total variation explained by its corresponding discriminant function) and the coefficients of the first four discriminant functions are given (example discr function 1 = $-0.0215 \times \alpha$-pinene + $0.4582 \cdot \beta$-pinene + .. $-0.6922 \cdot$ Terpinolene). The first discriminant function explains 40% of the total variation among populations. The highest loading (coefficient) 1.69 in *Table 5* is given to $\triangle$ 3-carene, which indicates that this is the variable that best discriminates between the 26 populations.

Each tree in the analysis was allocated to the population to which it had the highest probability of belonging according to discriminant values of the first six discriminant functions. These predicted population memberships are summarized in *Table 4.* The larger the proportion of trees allocated to the actual population, the better that population is assumed to be discriminated from the others. From *Table 4* it is obvious that southern populations are often better discriminated than northern ones. Especially well discriminated are the southern populations 5, 7, 12, 17 and the northern population 57. No correlation was observed between degree of population discrimination and altitudinal differences.

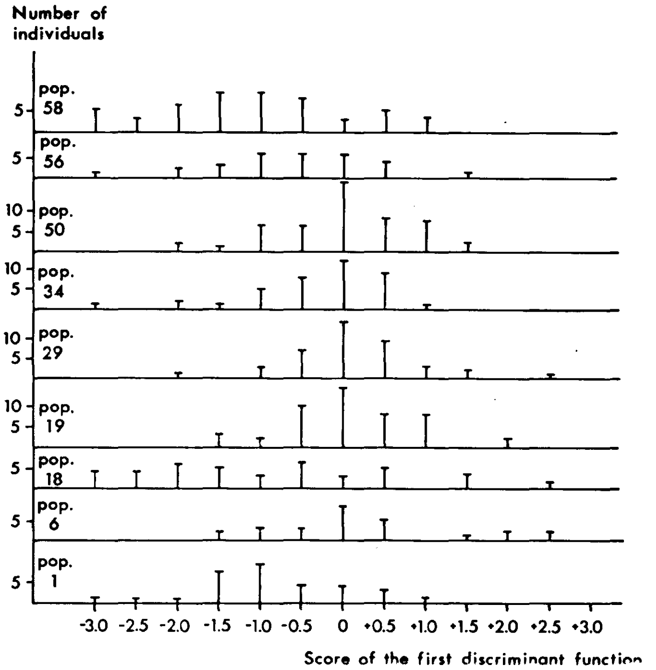

Figure 2. — Histograms showing the frequency distribution upon the first discriminant function of individuals of nine selected populations (class interval 0.5 units).

Frequency distributions of the individuals from nine randomly selected populations with at least 25 individuals each upon the first discriminant function are presented in *Figure 2*. There are visible differences in distribution patterns among several populations, which are most likely due to ecotypic differentiation among these populations.

In southern Sweden, populations with good discrimination tend to have low variation coefficients *(Tables 2, 4)*. This could be a result of transfer in a northward direction. Many individuals from southern populations are dying because of the severity of climate in the north. If terpene content is related to frost hardiness, the decrease in genetic variation should effect both characters in the same direction. The removal of non-adapted individuals most likely decreases the level of genetic variation within these populations. If terpene content is related to frost hardiness, the decrease in genetic variation should effect both characters in the same direction. The low discrimination of many populations might also be a result of high variability in environmental conditions which cause broad genetic variation within the population. The most heterogenous populations with the highest coefficient of variation and lowest discrimination are numbers 6, 13 and 40. These populations may be more exposed to inter-breeding with others and pollen and seed dispersal may be promoted over relatively longer distances by wind. In central Sweden and around latitude 62°—63°, several populations show high frequencies of trees allocated to population 17. This could be the result of the existence of some similarity in the gene pool among populations at these latitudes.

There is no indication that the central part of Sweden has the most variable populations, as was assumed by Sylven (1916) and Kiellander (1974). On the contrary, variable populations are spread out in different parts of Sweden. Based on studies of some physiological characters like dry matter content and catalase activity in needles, *P. sylvestris* shows clinal variation from south to north with no intermediate zone with highest variability (Langlet 1936). In populations of *P. contorta* in northwestern America the most variable populations occurred in central British Columbia, which is the centre of the natural range of this species. The most distinct and homogenous populations were found on the periphery of the range (Forrest 1977).

T-test analysis

Populations of *P. sylvestris* from different parts of their natural range can be compared with respect to homogeneity or heterogeneity in monoterpene patterns. The populations have been grouped into three different regions: south (latitude 56—61° N), central (61—64° N) and north (64—68° N) Sweden. Pairs of populations within each of these three regions are compared by t-test analysis with respect to six major monoterpenes. The results of these comparisons are presented in *Table 6*. Pairwise comparisons among the southern populations show that most population differentiations are due to differences in level of △ 3-carene

Table 6. — Comparison between different geographical groups of *P. sylvestris* populations by t-test analysis. Only significant differences in monoterpene variables are shown.

Population No.	1	4	5	6	7	8
1		NS	3-carene* Myr***	NS	α-pin*** 3-carene*** Myr*** β-phe***	α-pin*** 3-carene*** Myr**
4			3-carene* Myr***	NS	α-pin*** 3-carene*** Myr*** β-phe***	α-pin** 3-carene** Myr**
5				Myr * β-phe **	3-carene*	NS
6					3-carene*** Myr*** β-phe ***	β-phe*
7						3-carene * Myr* β-phe ***
8						

Table 6. — Continued

Population no.	12	13	17	18	19	20	25	27	28	29	30	34
12		NS	α-pin*** Myr*	β-pinene** 3-carene* Myr***	α-pin * Myr ** β-phe *	β-pin** Myr**	β-pin** 3-carene* Myr ***	β-pin**	α-pin** Myr**	α-pin* Myr*	β-pinene*** 3-carene** Myr***	α-pin* β-pin* Myr*
13			β-phe *	β-pin * 3-car *	β-phe **	NS	β-pin * β -car * β -phe *	β-pin *	NS	β-phe *	β-pin*** 3-car ** Myr ***	NS
17				α-pin** 3-car** Myr** Lim*	Lim **	α-pin** Lim * β-phe*	α-pin*** β-pin** 3-car** Myr** Lim*	α-pin** β-pin*	β-phe**	α-pin* Lim**	α-pin* β-pin*** 3-car*** Myr*** Lim* β-phe**	α-pin** β-ph**
18					β-pin**	NS	NS	NS	α-pin* 3-car***	NS	Myr **	NS
19						β-pin* β-phe**	β-pin** 3-car*	β-pin**	β-phe**	NS	β-pin*** 3-car* Myr*** β-phe***	β-phe***
20							β-phe*	NS	α-pin* 3-car*	β-phe*	β-pin* Myr***	NS
25								NS	α-pin * β-pin * 3-car *** β-phe **	β-pin *	Myr ** β-ph ***	β-phe **
27									α-pin* β-pin* 3-car*	β-pin*	Myr***	NS
28										β-car* β-phe**	α-pin* β-pin*** 3-car*** Myr***	NS
29											β-pin*** Myr*** β-phe**	β-phe**
30												β-pin* β-car* Myr**
34												

* Significant at 5 per cent level
** Significant at 1 per cent level
*** Significant at 0.1 per cent level

Population no.	40	43	45	48	50	56	57	58
40		3-carene * β-phe *	3-carene * Myr * β-phe **	NS	β-phe *	β-phe ***	NS	β-phe *
43			NS	α-pin * 3-carene *	NS	β-pin **	Lim *	α-pin *** β-carene ** Lim *
45				3-carene *	NS	β-pin *	β-phe *	α-pin **
48					NS	β-phe *	α-pinene *	NS
50						β-pin ** β-phe *	Lim *	α-pin * Lim **
56							β-pin ** β-phe **	β-pin ** β-phe *
57								α-pin **
58								

and myrcene. Limonene and β-pinene do not show significant differences in any comparison. In central Sweden the major monoterpene which contributes most to the variation appears to be β-pinene and the minor one limonene. In northern Sweden, myrcene contributes least to the differences among populations. It seems that populations in the north differ to a lesser extent with respect to single monoterpene patterns than populations in central and southern Sweden. Two populations 57 and 58 in the north close to latitude 68°, show rather low quantities of limonene. These two populations might to some extent be influenced by continental climate conditions (according to Odin *et al.*

1983) and show some deviation in the pattern of monoterpene distribution. Our recent study on several natural populations around latitude 68^0 N-indicate that individuals with a high proportion of limonene are more frequent among populations of this region than in any other part of Sweden (Yazdani unpublished data). This finding is important and may help us in future to determine the genetic constitution of northern populations in harsh climates.

Variations among native *P. sylvestris* populations in Scotland based on monoterpene analysis were also divisible into several areas of biochemical similarity (Forrest 1980). He suggested that monoterpenes should be used as a guide for choosing sources of genetic material in reforestation programmes for *P. sylvestris.* Investigation of several groups of young natural regenerations of *P. sylvestris* in Scotland shows that these young populations are very similar to the parental stands, but reveals small consistent differences and a surprisingly high degree of genotypic variation (Forrest 1980). Our recent study on several adjacent populations of *P. sylvestris* from different geographical regions demonstrates that adjacent populations show more similarity to each other than distant populations (Yazdani unpublished data). Genotypic variations in six continental sources of *P. sylvestris* were analysed by Forrest (1982), and compared with the chemotypic characteristic of the native Scottish population. Forrest used the data to provide an explanation for the sequential post-glacial migration of *P. sylvestris* from the continent into Scotland. This information confirms that certain compositions of monoterpenes are more site specific than randomly varied. Since we have also found clines for monoterpene pattern in *P. sylvestris* in Sweden, we assume that the pattern of monoterpene variation in these populations is influenced by the process of natural selection. Therefore site classification based on monoterpene data can be helpful in planning for gene resource conservation, design of breeding strategies, and how the transfer of breeding material within a certain geographical range should be done.

The relative growth increment and dry matter content in the populations mentioned above have been studied by Ericsson and Remröd (1975). Both characters show clinal variation in populations from south to north *(Table 1).* Comparison between populations based on these three characters-limonene, dry matter content and relative growth increment shows that populations originating from north and south of latitude 61^0, respectively, are quite different with respect to these characters, and do not overlap with each other.

Correlation coefficients were calculated for dry matter content, relative growth increment, and limonene with latitude. Dry matter content has a correlation coefficient $r = 0.76$ and relative growth increment for the years 1974 and 1975 have correlations $r = 0.86$ and $r = 0.79$, respectively. The corresponding correlation coefficient for limonene with latitude is $r = 0.42$. The correlation coefficient based on population mean values for dry matter content and limonene content is $r = 0.82$. Whether there exists any interrelationship between the two characters within populations has not yet been investigated. Since correlations were found on the population level between frost damage and dry matter content of the apical or lateral shoots in seedlings of *P. sylvestris,* and *P. contorta* (Dormling *et al.* 1977; Jonsson *et al.* 1981), it would be of interest to investigate whether the high quantity of limonene has any connection with frost tolerance within populations.

Acknowledgements

This work was supported by the Swedish Council for Forestry and Agricultural Research. We thank Professor Lennart Bondesson for help in statistical treatment of data. Professor Gösta Ericsson, Professor Dag Lindgren and Dr. Dag Rudin for advice during the early stages of this research and Dr. Ladislav Paule for valuable comments on the manuscript. Lars-Göran Lejdebro and Gun Lindqvist are thanked for collecting material for this study. We also thank Barbro Harbom, Maj-Lene Aman, Karin Ljung and Karin Lundström for technical assistance, and Helene Risberg and Gertrud Lestander for typing the manuscript.

Literature Cited

Baradat, Ph., Bernard-Dagan, C. and Marpeau, A.: Variation of terpenes within and between populations of maritime pine. Proceedings of the conference on biochemical genetics of forest trees. November 7—8, 151—169. (1978). Umeå, Sweden. — Bergman, F.: The allelic distribution at an acid phosphatase locus in Norway spruce *(Picea abies)* along similar climatic gradients. Theoret. and Appl. Genet. **52**, 57—64 (1978). — Conkle, M. T.: Isozyme variation and linkage in six conifer species. Proceedings of the symposium on isozymes of American Forest Trees and Forest Insects, July 27, 11—17. 1979, Berkely, California (1979). — Curie, R. P. and Ledig, F. T.: Analysis of population structure from allozyme frequencies. Proceedings of the 14th Southern Forest Tree Improvement Conference, Gainseville, FL. 14—16 June, 1977, 246—253 (1977). — Ericsson, T. and Remröd, J.: Rutiner och resultat från härdighetstester i Sävar. Föreningen Skogsträdsförädling och Institutet för Skogsförbättring, Arsbok 1975, 152—170 (1975). — Esteban, I., Bergmann, F., Gregorius, H. R. and Hiltunen, O.: Composition and genetics of monoterpene from cortical oleoresin of Norway spruce and their significance for clone identification. Silvae Genetica **25***:* 59—66 (1976). — Forrest, G. I.: Geographical variation in the monoterpenes of the resin of *Pinus contorta.* E. E. C. symposium on forest tree biochemistry. Eur. 5885. 55—71 (1977). — Forrest, G. I.: Genotypic variation among native Scots pine populations in Scotland based on monoterpene analysis. Forestry **53** (2), 101—128 (1980). — Forrest, G. I.: Relationship of some European Scots pine populations to native Scottish Woodlands based on monoterpene analysis. Forestry **55** (1), 55—65 (1982). — Gansel, C. R. and Squillace, A. E.: Geographic variation of monoterpene in cortical oleoresin of Slash pine. Silvae Genetica **25** (5—6), 150—154 (1976). — Grant, M. C. and Mitton, J. B.: Genetic differentiation among growth forms of Engelmann spruce and subalpine fir at tree line. Arct. Alp. Res. **9***:* 259—263 (1977). — Hannover, J. W.: Comparative physiology of eastern and western white pines: oleoresin composition and viscosity. Forest Sci **21***:* 214—221 (1975). — Hiltunen, R., Juvonen, S. and Tigerstedt, P. M. A.: Geographical variation in some monoterpenes in Scots pine *(Pinus sylvestres* L.) in Finland. Farm. Natisblad **84***:* 73—82 (1976). — Kiellander, C.-L.: Genekologi i naturen. Kompendium i skogsgenetik. Skogshögskolans kompendieförmedling, 12 pages (1974). — Langlet, O.: Studier över tallens fysiologiska variabilitet och dess samband med klimatet. Ett bidrag till kännedom om tallens ekotyper. Medd. Statens Skogsförsöksanst. **29** (4), 219—406 (1936). — Lundkvist, K.: Allozyme frequency distribution in four Swedish populations of Norway spruce *(Picea abies* K.). Hereditas **90***:* 127—143 (1979). — Mitton, J. B., Linhart, Y. B., Hamrick, J. L. and Beckman, J. S.: Observations on the genetic structure and mating system of Ponderosa pine in the Colorado front range. Theor. Appl. Genetic **51***:* 5—13 (1977). — Mitton, J. B., Linhart, Y. B., Sturgeon, K. B. and Hamrick, J. L.: Allozyme polymorphisms detected in mature needle tissue of Ponderosa pine. Journal of Heredity **70***:* 86—89 (1979). — Mitton, J. B., Sturgeon, K. B. and Davis, M. L.: Genetic differentiation in Ponderosa pine along a steep elevational transect. Silvae Genetica **29***:* 76—81 (1980). — Odin, H., Eriksson, B. and Kurth, Perttu: Temperature climate maps for Swedish forestry. Reports in Forest Ecology and Forest Soils. Department of Forest Soils, Swedish University of Agricultural Sciences. (1983). — Parham, M. R.: Stimulation of oleoresin yield in conifers. Outlook on Agriculture **9***:* 76—81 (1976). — Rishbeth, J.: Resistance to fungal pathogens of tree roots. Proceedings of the Royal Society, London B 181: 333—351 (1972). — Schuck, H. J.: Monoterpenes and resistance of conifers to fungi. Proceedings of the 3rd International Workshop on the Genetics of Host-Parasite Interactions in Forestry, Wageningen (Netherlands) 14—21, Sept 1980. PUDOC, Wageningen (Netherlands ISBN 169—175. (1982). — Squillace, A. E.: Analyses of monoterpenes of conifers by gas-liquid chromatography. In: J. P. Miksche (ed.), Modern Methods in Forest Genetics, p. 120—157. Springer-Verlag, N. Y. 288

p. (1976). — Squillace, A. E.: Monoterpene composition of cortical oleoresin in *Pinus elliotti:* and its utility in genetic research. E. E. C. symposium on forest tree biochemistry EUR 5885, 39—53 (1977). — Squillace, A. E., Schreuder, H. T. and Bhattacharyya, H. T.: Identification of seed origin of slash pine plantations. Silvae Genetica **29** (3—4): 152—154 (1980). — Sylven, N.: Den nordsvenska tallen. Medd. Statens Skogsförsöksanst. **13—14**: 9—110 (1916). — Yazdani, R., Rudin, D., Alden, T., Lindgren, D., Harbom, B. and Ljung, K.: Inheritance pattern of five monoterpenes in Scots pine *(Pinus sylvestris* L.). Hereditas **97**: 261—272 (1982). — Yeh, F. C. and El-Kassaby, Y.: Enzyme variation in natural populations of Sitka spruce *(Picea sitchensis).* 1. Genetic variation patterns among trees from 10 IUFRO provenances. Canadian Journal of Forest Research **10**: 415—422 (1980).

Genetic variation of Melampsora leaf rust resistance in progenies of crossings between and within Populus tremula and P. tremuloides clones

By L. A. Gallo1), B. R. Stephan and D. Krusche

Federal Research Centre for Forestry and Forest Products,
Institute of Forest Genetics and Forest Tree Breeding,
Sieker Landstr. 2, D-2070 Grosshansdorf
Federal Republic of Germany

(Received 18th January 1985)

Summary

A total of 49 families of controlled crosses between and within *Populus tremula* and *P. tremuloides* were investigated concerning their resistance to *Melampsora* leaf rust, presumably caused by *M. magnusiana.* The leaf rust attack was evaluated at the end of both the first and the second vegetation period and showed highly significant genetic variation between the interspecific crossings. The *P. tremuloides* families showed the highest resistance, *P. tremula* families were severely attacked. The interspecific hybrid families showed an intermediate behaviour. A quantitative genetic analysis of the data showed strong additive genetic variation. Therefore, *Melampsora* leaf rust resistance might be incorporated successfully into further breeding programs in aspen and hybrid aspen.

Key words: Populus tremula, P. tremuloides, inter- and intraspecific crossings, *Melampsora* leaf rust, resistance, additive genetic effects.

Zusammenfassung

Insgesamt 49 Familien aus kontrollierten Kreuzungen zwischen und innerhalb der *Leuce*-Pappelarten *Populus tremula* und *P. tremuloides* wurden nach der ersten und zweiten Vegetationsperiode auf Blattrostbefall bonitiert. Untersuchungen an verschiedenen Entwicklungsstadien des Parasiten ergaben, daß *Melampsora magnusiana* als Erreger dieser Rostkrankheit in Betracht kommt. Zwischen den Kreuzungsfamilien bestanden hoch signifikante Befallsunterschiede. *P. tremuloides*-Familien zeigten die größte Resistenz gegen den Pilz, *P. tremula*-Familien waren dagegen am stärksten befallen. Die interspezifischen Hybridfamilien verhielten sich intermediär. Eine quantitativ-genetische Analyse ergab, daß sich die Rostresistenz wie ein quantitatives Merkmal verhält, d.h. sie wird offenbar durch zahlreiche Gene mit additiver Wirkung vererbt. Die Selektion blattrostresistenter Elternbäume führt demnach zu resistenteren Nachkommen. Rostresistenz kann daher mit Erfolg in die weiteren Züchtungsprogramme bei Aspe integriert werden.

1. Introduction

The European aspen *Populus tremula* L. and the north American aspen *P. tremuloides* Michx. of the poplar section *Leuce* Duby, and their hybrids are fast growing hardwood species, which are used in the paper industry as well as for biomass and energy production. Aspens are valuable and tolerant forest tree species for marginal sites. Therefore, they are gaining more and more importance in silviculture. The interspecific hybrids between the two aspen species exhibit often a better growth performance than the parent species, as many hybridization programs have shown (Melchior and Seitz 1966, Hattemer and Seitz 1967, Melchior 1985, Weisgerber 1983).

In connection with the testing of the general performance also the behaviour against the more important fungal diseases should necessarily be known. Aspens can be attacked by a wide range of leaf, twig, branch, and stem diseases (Butin 1957, FAO 1979). One of the commonest, most serious, and widely distributed leaf disease of aspens is the *Melampsora* leaf rust, caused by several species (Gremmen 1954, Pinon 1973, FAO 1979, Butin 1983), which are difficult to distinguish morphologically. Heavy rust infection can result in an early leaf fall, a reduction of increment, an increase of susceptibility to winter frost, an easy entry to other parasites, and a higher mortality of young plants (Butin and Zycha 1973, FAO 1979, Schwerdtfeger 1981, Phillips and Burdekin 1982, Butin 1983). But poplar species show a high genetic variation of resistance to *Melampsora* rust species. These differences should be integrated into breeding programs of aspen.

In the following paper results are given on the genetic variation of *Melamsora* leaf rust infection of crossings between and within *Populus tremula* and *P. tremuloides.* The crossings were made originally with the purpose to study growth performance of families, combining ability and possible heterosis. Results on such traits will be published elsewhere (Gallo and Melchior, in preparation).

2. Materials and Methods

2.1. *Origin of the aspen clones*

For the crossing experiments five female and three male clones of *P. tremula,* and two female and four male clones of *P. tremuloides* were used. The origin of the parent trees is shown in *Table 1.* The clones were selected phenotypically on the basis of their growth performance, stem form

---

Dedictated to Dr. G. H. Melchior on his 60th birthday 1)
Dirección: Calle 34 No 309, 1900 La Plata, Argentina. Send Reprint requests to B. R. Stephan.