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SHORT COMMUNICATION

GEOGRAPHICAL VARIATION IN THE MONOTERPENE COMPOSITION OF RED SPRUCE*

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Abstract—Monoterpene composition of cortical oleoresin samples was determined for 14 populations of red spruce growing in a replicated plantation in Quebec, Canada. None of the 9 measured monoterpenes differed significantly among geographic seed sources. Individual tree variation in terpene concentration was slight despite the postulated occurrence of natural hybrids with black spruce in many of the seed sources sampled. The seed sources most similar to black spruce in morphology were no more similar in terpene composition to black spruce from Michigan than were sources of pure red spruce.

INTRODUCTION

GEOGRAPHIC seed sources of red spruce (*Picea rubens* Sarg., Pinaceae) planted in a common environment show considerable variation in growth characteristics and morphological traits. This is apparently due, at least partially, to hybridization with black spruce (*Picea mariana* (Mill.) B.S.P.) in areas where these two species are sympatric.[1–3] Although the 2 species and their natural hybrids have been studied in some detail, no systematic examination of chemical variation in relation to the taxonomic problem has been attempted. Monoterpene compounds in the oleoresin of conifers have been shown to be useful in defining genetic differentiation of populations of white spruce (*Picea glauca* (Moench) Voss) as well as of other tree species.[4] Therefore, analyses of these compounds in red spruce and also in black spruce may help to clarify the natural variation and evolutionary relationships among these and other North American spruces.

Previous analyses of the terpene composition of red spruce have been limited to the study of leaf oils in two geographic sources[5] and a genus-wide survey of essential oil composition, utilizing a limited number of red spruce grown in an arboretum in Europe.[6] Neither of these studies was designed to assess the range of variability in terpene composition associated with geographic origin, which was the objective of the work described here.

RESULTS AND DISCUSSION

Nine monoterpenes were detected in the cortical oleoresin of red spruce; 3-carene was consistently present in large amounts. Alpha- and β-pinene, terpinolene, and myrcene were

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the other terpenes present in measurable concentrations (Table 1). Camphene, limonene, β-phellandrene, and γ-terpinene each accounted for less than 2 per cent of the oleoresin.

TABLE 1. RANGES IN CONCENTRATIONS OF MAJOR CORTICAL MONOTERPENES IN 14 GEOGRAPHIC ORIGINS OF RED SPRUCE

Geographic origin	Monoterpene				
	α-Pinene	β-Pinene	Myrcene	3-Carene	Terpinolene
			(% of oleoresin)		
2019-North Carolina	2–7	5–11	1–2	19–30	2–3
2020-West Virginia	3–6	4–11	1–2	21–32	2–3
2021-Pennsylvania	3–10	2–15	1–3	11–36	1–3
2022-Massachusetts	2–13	4–12	0–1	17–32	1–3
2024-New York	1–5	4–9	1–2	17–32	1–3
2030-Maine	2–8	3–11	1–2	19–32	2–3
2031-New Hampshire	2–5	4–9	1–2	9–30	2–3
2032-Quebec	2–7	6–9	1–2	17–27	1–3
2033-Quebec	1–7	2–13	1–2	17–27	1–2
2100-Nova Scotia	2–10	3–10	1–2	15–29	1–2
2101-Nova Scotia	1–7	3–14	0–1	21–41	2–3
2102-New Brunswick	2–8	4–12	1–2	22–39	2–3
2103-New Brunswick	2–8	2–19	0–2	21–30	2–3
2505-New Brunswick	1–7	2–12	0–1	19–31	1–2

There were no significant differences in monoterpene concentrations between geographic sources of red spruce. The *F*-values for each monoterpene are as follows:

Monoterpene	F-Value	Monoterpene	F-Value
α-Pinene	1·12	Limonene	0·15
Camphene	1·43	β-Phellandrene	1·38
β-Pinene	0·27	γ-Terpinene	1·16
Myrcene	0·88	Terpinolene	1·35
3-Carne	0·96		

Moreover, the individual tree variation in monoterpene concentrations was much less than that encountered in white spruce.[4] The lack of variability in monoterpene concentrations is in contrast to the great deal of variability in morphology and growth characteristics[1–3] shown by these same sources of red spruce.

Red spruce and black spruce are considered to be closely related species.[7] Examination of the leaf oils of these 2 species by von Rudloff[5] indicated that only minor components occurring in amounts too small for positive identification vary between them. Von Schantz and Juvonen[6] also point out the similarity in terpene composition of the needle oils of red and of black spruce. The lack of variability in the monoterpenes of the cortical oleoresin between geographic sources of red spruce, despite the probable hybrid origin of some of the sources,[1] supports the theory for the close phylogenetic relationship between red spruce and black spruce. To further test this relationship, a total of 30 black spruce from 1 plantation in southern Michigan and 2 widely separated stands in Upper Michigan were sampled for monoterpene content in the same manner described for red spruce.

The same monoterpenes were found in the cortical oleoresin of both species (Table 2).

[7] J. W. WRIGHT, *Forest Sci.* **1**, 319 (1955).

The concentrations of β-pinene, 3-carene, limonene, β-phellandrene, and terpinolene differed significantly between red spruce and black spruce. Sources 2033-Quebec and 2505-New Brunswick resembled black spruce the most in morphology[1] and foliage phenolic content.[8] However, they showed no greater resemblance to Michigan black spruce in terpenes than did other sources of red spruce.

TABLE 2. MEANS AND RANGES OF MONOTERPENE CONCENTRATIONS IN RED SPRUCE AND IN BLACK SPRUCE

	Species			
	Red spruce		Black spruce	
Monoterpene	Mean	Range	Mean	Range
		(% of oleoresin)		
α-Pinene	4·0	1–13	3·6	1–11
β-Pinene*	6·6	2–19	2·7	1–5
Myrcene	1·2	0–3	1·2	1–2
3-Carene*	24·6	9–41	13·5	7–24
Limonene*	0·3	0–2	0·2	0–1
β-Phellandrene*	0·8	0–2	0·4	0–1
Terpinolene*	2·0	1–3	0·8	0–2

* Denotes that species are significantly different at the 5% level.

Red spruce and black spruce are much more similar to each other in terpene composition than they are to white spruce.[4] The amounts of α- and β-pinene, myrcene, limonene, and β-phellandrene were generally greater in white spruce than in black or red spruce. Conversely, the amounts of 3-carene and terpinolene were much greater in the latter 2 species.

It seems that the monoterpene composition of red and black spruce may have been established as a result of common ancestry and has undergone relatively little differentiation despite differences in climatic adaptation of the two species. Black spruce is strictly a boreal species, and red spruce is adapted to cool-temperate climates. Monoterpenes may be more suitable for detecting the phylogenetic relationships between species of spruce than for detecting natural hybrids between closely related species. The monoterpenes may have a low adaptive value and, therefore, would remain unchanged over long periods of time.

On the basis of monoterpene composition, sources of red spruce containing hybrids could not be distinguished from sources of pure red spruce. This could be due to a similarity of terpene composition between black spruce from the eastern portion of its range and red spruce. When rangewide provenance tests of black spruce become available, sources of black spruce from the east and from the west can be examined for their monoterpene content; and the hybrid situation in red spruce may be clarified.

Although the monoterpenes were not suitable for detecting hybridization between red spruce and black spruce, they may still be of value in studying hybridization of other spruce species.[9–11]

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EXPERIMENTAL

Cortical oleoresin samples were collected from 14 geographic origins of red spruce planted in a replicated growth test at Valcartier, Quebec, Canada. This plantation is maintained by the Forest Research Laboratory, Department of Forestry and Rural Development, Ste. Foy, Quebec. The plantation was established in 1959 with 2–2 stock and was 14-yr-old at the time of sampling. Growth characteristics and origin data for this material have been reported by Roche.[3]

The smallest and the largest healthy trees from each source were sampled from each of 5 replicates, making a total of 10 trees sampled from each source. Samples of oleoresin were taken over a 2-day period in late June, 1969. Small incisions were made into the phloem of the 1-yr-old internode of the main stem. 10 μl samples of the exuded oleoresin were immediately drawn into calibrated 20 μl capillary tubes. The capillary tubes containing the samples were placed in centrifuge tubes, were sealed, and were then refrigerated until analysis.

All samples were analyzed quantitatively soon after collection by GLC as described previously.[4] Monoterpene composition was expressed as a percentage of the oleoresin, and the total terpene content was obtained by summing all values for the individual terpene concentrations, except those of camphene and γ-terpinene, which occurred only in very low concentrations. An analysis of variance was performed on each set of data. Degrees of freedom were 13, 4, and 52 for origin, replicate, and error, respectively.

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Key Word Index—Picea rubens; Pinaceae; chemotaxonomy; monoterpenes; geographical uniformity.