Phytochemistry, 1972, Vol. 11, pp. 3255 to 3257. Pergamon Press. Printed in England.

IDENTIFICATION OF BLUE SPRUCE CULTIVARS BY ANALYSIS OF CORTICAL OLEORESIN MONOTERPENES*

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(*Revised Received* 10 *July* 1972. *Accepted* 24 *July* 1972)

Key Word Index—*Picea pungens*; Pinaceae; oleoresin; monoterpenes; cultivar identification.

Abstract—The monoterpenes of ten blue spruce cultivars were analyzed to determine the feasibility of chemical identification. There were highly significant differences between cultivars in six of the seven major monoterpenes. Of the 45 possible pairs of cultivars, 37 can be distinguished from each other on the basis of monoterpene composition.

INTRODUCTION

BLUE SPRUCE (*Picea pungens* Engelm.) is a widely used ornamental conifer. Individuals with exceptional foliage color and/or form have been discovered and propagated by grafting. The resulting clones have been designated as cultivars. Large numbers of ramets whose clonal identity is uncertain are sometimes produced because of error during grafting and handling. It is usually desirable to re-establish the identity of such individuals. The gross morphology of recently grafted individuals often does not provide an adequate basis for their clonal identification.

The monoterpene composition of the cortical oleoresin in conifers is under strong genetic control and is little influenced by the environment.[1] Variations in monoterpene composition which may occur between internodes formed during different growing seasons[2] or are associated with season of the year[3] do not diminish the usefulness of the monoterpenes if sampling is done on tissue of uniform age over a short period of time. Here we report the results of a test on the use of monoterpenes for identifying important cultivars of blue spruce.

RESULTS AND DISCUSSION

The major monoterpenes detected were α-pinene, camphene, β-pinene, myrcene, 3-carene, limonene and terpinolene. β-Phellandrene and γ-terpinene occurred occasionally, but only in trace amounts. The mean monoterpene composition of each cultivar is presented in Table 1. The *F*-values from the analysis of variance, and the number of pairs of cultivars that can be distinguished from each other with reference to an individual monoterpene appear in Table 2.

* Michigan Agricultural Experiment Station Journal Article No. 5852.

[1] J. W. HANOVER, *Phytochem.* **5**, 713 (1966).
[2] R. ROBERTS, *Phytochem.* **9**, 809 (1970).
[3] P. ADAMS, *Phytochem.* **9**, 397 (1970).

In preliminary studies on this material, considerable variation occurred in the monoterpene composition of the cortical oleoresin between internodes which had elongated during the current growing season, and those which had elongated during the previous growing season. Therefore, it is imperative in comparative work of this type that similar tissues be sampled in each case.

TABLE 1. MEAN COMPOSITION OF OLEORESIN MONOTERPENES AND STATISTICAL DIFFERENCES BETWEEN 10 CULTIVARS OF BLUE SPRUCE*

Cultivar	No. of trees sampled	Monoterpenes							
		α-Pinene	Camphene	β-Pinene	Myrcene	3-Carene	Limonene	Terpinolene	Total monoterpenes
		% of oleoresin							
Argentea	6	12·7ab	0·4a	5·7a	0·8bc	2·5cd	2·7cd	0·4bc	25·2a
Compacta									
Thume	4	11·3bc	0·3a	5·4ab	0·7c	1·7d	1·3d	0·6abc†	21·4a
Globosa	4	13·6ab	0·4a	5·2ab	0·9abc	2·0cd	1·6d	0·5bc	24·1a
Hoopsii	5	8·8c	0·3a	3·8ab	0·9abc	7·3a	4·7ab	1·0a	26·8a
Hunnewelliana	8	12·7ab	0·4a	5·1ab	0·8bc	2·4cd	1·6d	0·6bc†	23·5a
Koster	4	9·6bc	0·4a	4·4ab	1·0abc	2·7cd	5·8a	0·8ab	24·6a
Mission Blue	4	10·0bc	0·3a	4·9ab	0·8bc	2·6cd	3·8bc	0·4bc	22·8a
Moerheimi	5	15·9a	0·5a	3·9ab	1·2a	0·7d	3·9bc	0·3c	26·4a
Pendula	4	10·4bc	0·2a	3·9ab	0·7c	6·3ab	2·5cd	0·8ab	24·8a
Thomsen	5	10·8bc	0·3a	3·4b	1·1ab	4·4bc	5·7a	0·7abc	26·4a

* For a given monoterpene, cultivarietal means not followed by a common letter are statistically different from one another at the 5% level as determined by Tukey's honestly significant difference test (ω-procedure).
† Different sample sizes result in 0·6 being statistically different than 1·0 in one case and not in another.

The results show that 37 of 45 possible pairs of cultivars can be distinguished from each other on the basis of their monoterpene composition. Thus, in most cases, an analysis of the monoterpene composition of the cortical oleoresin can be a valuable tool in identifying blue spruce cultivars.

TABLE 2. ANALYSIS OF VARIANCE OF MONOTERPENE CONCENTRATIONS IN 10 BLUE SPRUCE CULTIVARS AND NUMBER OF PAIRS OF CULTIVARS SEPARATED BY EACH MONOTERPENE

Monoterpene	F-value	Pairs separated	Monoterpene	F-value	Pairs separated
Limonene	23·32†	25	Terpinolene	4·82†	7
3-carene	16·80†	17	β-Pinene	2·96*	1
α-Pinene	5·90†	9	Camphene	1·44 n.s.	0
Myrcene	4·98†	7	Total Monoterpenes	1·86 n.s.	0

* Significant at 1% level. † Significant at 0·1% level. n.s. Not significant at 5% level.

One precaution suggested in employing this technique is that absolute concentrations given in this paper need not be matched exactly. A comparison of relative monoterpene compositions may be sufficient because of slight variations that may occur due to technique or other non-genetic causes.

EXPERIMENTAL

4–8 specimens of each of the 10 following cultivars of blue spruce were acquired from a commercial nursery: 'Argentea', 'Compacta Thume', 'Globosa', 'Hoopsii', 'Hunnewelliana', 'Koster', 'Mission Blue', 'Moerheimi', 'Pendula' and 'Thomsen'. Samples of oleoresin were collected near the end of the growing season by making an incision in the cortex of an internode formed the previous year. 20 min after incision, a known volume of exuded oleoresin was collected in a 20-μl capillary tube. The sample was then dissolved in acetone and analyzed by GLC using an F & M Model 700 instrument with a hydrogen flame ionization detector and a digital integrator. The chromatographic column was 6 mm $\times$ 240 cm stainless steel packed with Chromosorb W-AW coated with 10% polypropylene glycol. All peaks and shoulders were readily distinguishable except in a few instances when very low amounts of β-phellandrene occurred with very large amounts of limonene.

Identity of the various compounds was determined by comparing relative retention times, and co-chromatography, with known pure monoterpenes. The quantity of each monoterpene was then computed from standard curves as a percent of oleoresin. For each monoterpene and the total of major monoterpenes, an analysis of variance was performed, and statistically significant differences between cultivars were determined by Tukey's honestly significant difference test (ω-procedure).