MONOTERPENE VARIABILITY ON BALSAM FIR (Abies balsamea (L.) MILLER) PROVENANCES

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ABSTRACT

Morphology and phenology of balsam firs growing in Maritime and northeastern U. S. areas differ from trees growing at lower altitudes and in western U. S. locations. Gas liquid chromatography was used to study monoterpene content of trees from mountain, Maritime, and low-altitude provenances. Significant differences were found in the relative percentage of three of the seven monoterpenes present. Two groups, Maritime-mountain and low-altitude, can be separated by the ratio of beta-alpha-pinene.

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INTRODUCTION

Balsam firs (Abies balsamea (L,) Milli growing in Maritime and high altitude areas differ in morphology and phenology from those growing at lower altitudes or more western locations. Corresponding chemical differences, if present, might be used to distinguish between populations of seedlings. Geographic variability in the relative amounts of the various monoterpenes present in balsam oleoresin (Zavarin and Snajberk, 1972) and balsam foliage extracts (Lester, 1974) has been reported. The purpose of this study was to establish the typical monoterpene profiles for populations over the range of balsam fir and to use this information to infer origin or to select potentially superior trees in transplant beds.

Two groups of trees were tested, provenance study outplantings and trees growing on natural sites. Plant material for provenance testing was collected from nine-year-old trees at the University of Vermont Research Forest at Wolcott, Vermont. [2] In situ collections were made from high and low altitude sites in Vermont. These study sites are illustrated in Figure 1. Provenances are summarized in Table 1.

MATERIALS AND METHODS

Six to eight twigs of current year's growth were collected from each of ten trees for each site or provenance. These were sealed in separate plastic bags and refrigerated until analyzed.

Duplicate samples from each tree were placed in vacuum flasks equipped with corks and three-way stopcocks. After a fifteen minute incubation at 30°C, a five ml. sample of headspace air was withdrawn and injected directly on to the chromatographic column. All analyses were performed on a Nuclear Chicago 5000 gas-liquid chromatograph machine with a flame ionization detector. Columns were 12 foot x 1/8 inch O.D. stainless steel packed with 15% 1, 2, 3, TRIS (2 cyaneothoxy) propane on Chromosorb P, AW DMCS treated, 80/100 mesh. Column temperature was 75o C, injection temperature 170°C, and detector temperature, 300°C. Nitrogen flow rate was 72 ml./minute. Peak quantification was by Disc integrator, Peak identification was by peak enhancement with known compounds and by comparison of relative retention times with those in the literature (Klouwen and ter Heide, 1962).

RESULTS AND DISCUSSION

Balsam fir foliage emitted the same seven monoterpenes which have been reported in blister oleoresin (Zavarin and Snajberk, 1972) and in leaf and twig extracts (Lester, 1974). Monoterpene ranges and mean percentages for two typical provenances are given in Table 2. Alpha-pinene, beta-pinene and 3-carene are the most important components comprising about eighty percent of the total, and also the most interesting because they vary significantly with provenance. Alpha-pinene and beta-pinene correlate with longitude and with altitude, the highest alpha- and lowest beta-pinene levels being in mountain or maritime provenances.

[2]Much of this seed was supplied by Dr. Donald T. Lester, Department of Forestry, University of Wisconsin.

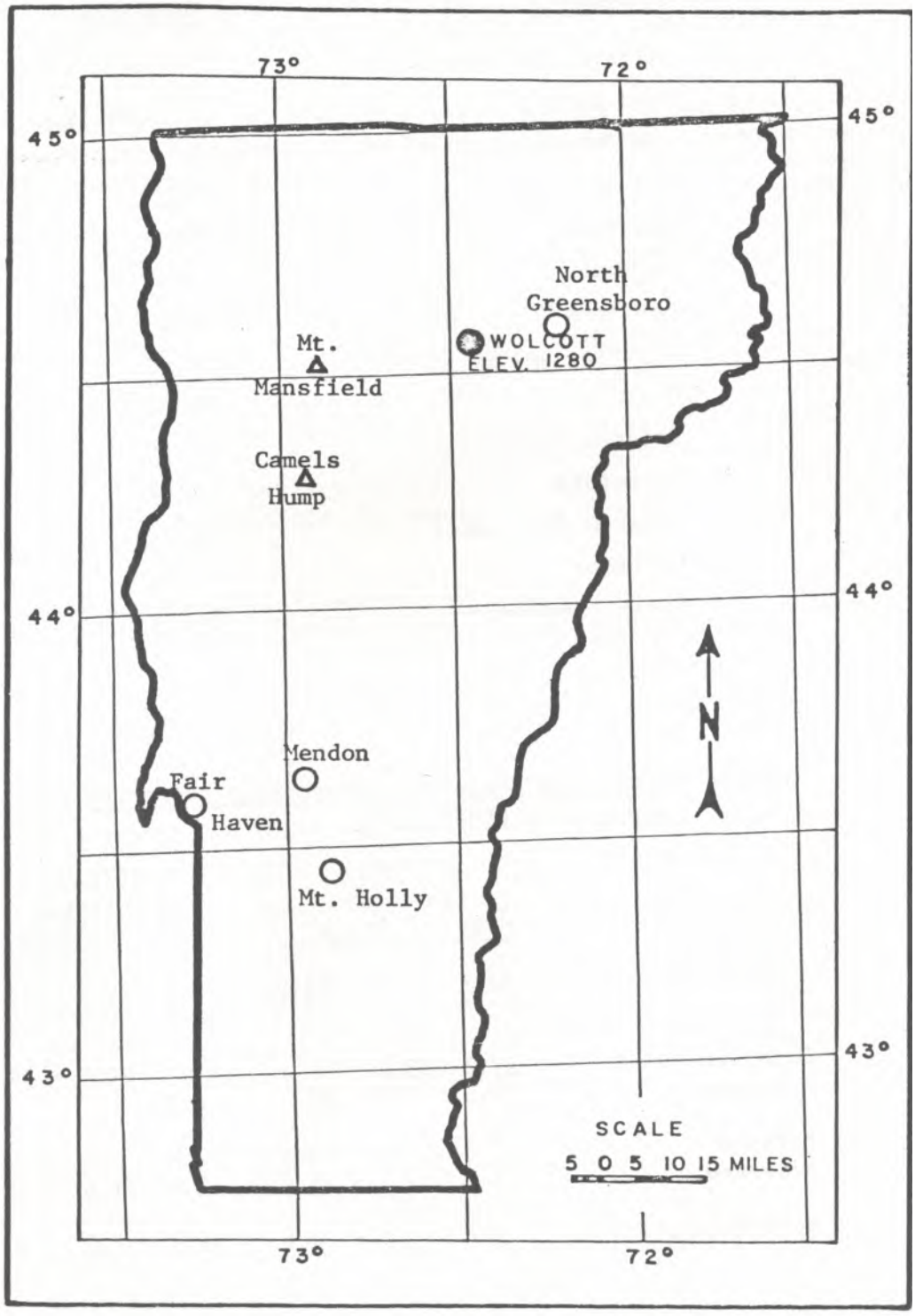

Figure 1. A map of Vermont showing study sites and the location of provenance outplantings.

Table 1.--Provenances sampled.

Provenance	Longitude
Vermont (Mt. Mansfield)	72°45'
Nova Scotia	62°, 64°15'
New Brunswick	66°00'
Maine	68°37'
Quebec	69°01'
Vermont	72°59'
New York	74°07'
Ontario (Balsam Creek)	79°10'
Michigan	84°37'
Ontario (Hearst)	85°55'
Minnesota	91°47'
Ontario (Red Lake)	93°55'
Manitoba	101°00'
North Carolina (Fraser fir)	82°15'

Table 2.--Means and ranges of monoterpene concentrations in two balsam fir provenances.

	EASTERN (Vermont)		WESTERN (Manitoba)	
Monoterpene	Mean	Range	Mean	Range
Alpha-pinene	32.5	23.9 - 49.9	29.4	25.9 - 35.5
Camphene	11.0	6.4 - 15.5	13.4	5.3 - 19.9
Beta-pinene	37.3	23.7 - 49.1	45.3	43.4 - 58.4
3-carene	10.9	0.0 - 20.4	0.0	
Myrcene	1.3	.5 - 3.5	.9	.4 - 1.6
Limonene	3.8	.5 - 6.4	3.4	1.5 - 6.8
Beta-phellandrene	3.2	1.0 - 4.8	6.5	.6 - 9.7

Three-carene was absent in all western trees sampled, only occasionally absent in eastern trees. Because these three are related in a negative correlation pattern with beta-pinene replacing 3-carene at least in part, the ratio beta-alpha-pinene may be more meaningful in some cases than either value alone.

Mean percents of the major monoterpenes and beta-alpha-pinene ratios for each provenance are summarized in Table 3, for Vermont study sites in Table 4. The provenances tested fall into two major groups, those west and those east of 80°W. The western group is distinguished by the absence of 3-carene and by higher beta-pinene levels. The eastern block can be further divided into a mountain-maritime groug which as beta-alpha-pinene ratios less than one, and an intermediate (70 W to 80°W) low altitude group with ratios greater than one. The populations tested on natural sites fall into the same pattern, with low altitude trees having more beta- than alpha-pinene, but lower levels of beta-pinene than western trees. High altitude trees have higher alpha-pinene levels. Ratios for provenance out-plantings are lower than those for populations on natural sites. Possibly an unavoidable bias is introduced by selecting trees which produce seed. It may be that parent trees with high alpha- and low beta-pinene are better suited to the mountain environment and therefore produce more seed.

Table 3.--Summary of mean percents of the major monoterpenes and beta/alpha pinene ratios by provenance.

Provenance	Alpha-pinene	Beta-pinene	Three-carene	Beta/Alpha pinene
Mt. Mansfield	47.0	27.1	8.7	.58
	47.9	24.2	9.5	.51
Nova Scotia	35.0	32.1	13.5	.92
New Brunswick	37.7	31.8	13.2	.84
Maine	33.4	31.8	13.0	.95
Quebec	40.9	29.3	12.0	.72
Vermont	32.5	37.5	15.0	1.14
New York	30.4	34.2	14.7	1.13
Ontario	31.8	32.8	15.0	1.06
Michigan	30.9	48.7	none	1.60
Ontario	31.7	44.8	none	1.41
Minnesota	30.1	48.0	none	1.60
Ontario	26.5	49.9	none	1.88
Manitoba	29.4	45.3	none	1.58

Table 4.--Summary of mean percents of the major provenances and beta/alpha pinene ratios of Vermont study sites by elevation.

Elevation (ft. above msl)	Site	Alpha-pinene	Beta-pinene	Three-carene	Beta/Alpha pinene
3800+	Mt. Mansfield	36.1	33.8	12.3	.93
3800+	Camels Hump	38.7	28.0	9.8	.76
1600	No. Breensboro	28.7	34.8	17.7	1.23
1400	Mendon	28.3	39.4	13.9	1.39
1400	Mt. Holly	25.1	43.3	16.1	1.72
1200	Wolcott	31.2	38.4	13.1	1.22
350	Fair Haven	23.9	41.8	14.2	1.74

A cluster analysis can be done by plotting provenance mean levels of beta- against alpha-pinene. This separates wester, eastern, and maritime-mountain provenances (Figures 3 and 4). Table 5 compares these three groups with Fraser fir (Abies fraseri (Pursh.) Poir.). All of the Fraser first sampled produced 3-carene. Provenance beta-alpha-pinene ratios were less than 0.5, all trees tested had more alpha than beta pinene.

Group and provenance monoterpene percentages are a reflection of the monoterpene levels of individual trees, The differences shown are a function of higher or lower levels of the alpha- or beta-pinene in particular trees and also of the number of trees showing these differences. Thus, only four percent of the western trees have more alpha- than beta-pinene and only two percent of these trees have more than 40 percent alpha-pinene. In contrast, 95 percent of the mountain trees have more alpha- than beta-pinene and 70 percent of the mountain trees have more than 40 percent alpha-pinene. All of the Fraser firs tested had alpha-pinene levels greater than 40 percent. Tree to tree differences within a provenance are quite large (Table 2) so that when the ratios of individual trees are plotted there is some overlap between groups, particularly in the eastern intermediate group.

Results can be summarized:

1. Differences in monoterpene profiles exist between populations.

2. Monoterpene levels are under strong genetic control and are therefore heritable.

3. It is possible to make probability statements about the origin of groups of trees and, in some cases, with caution, of individuals.

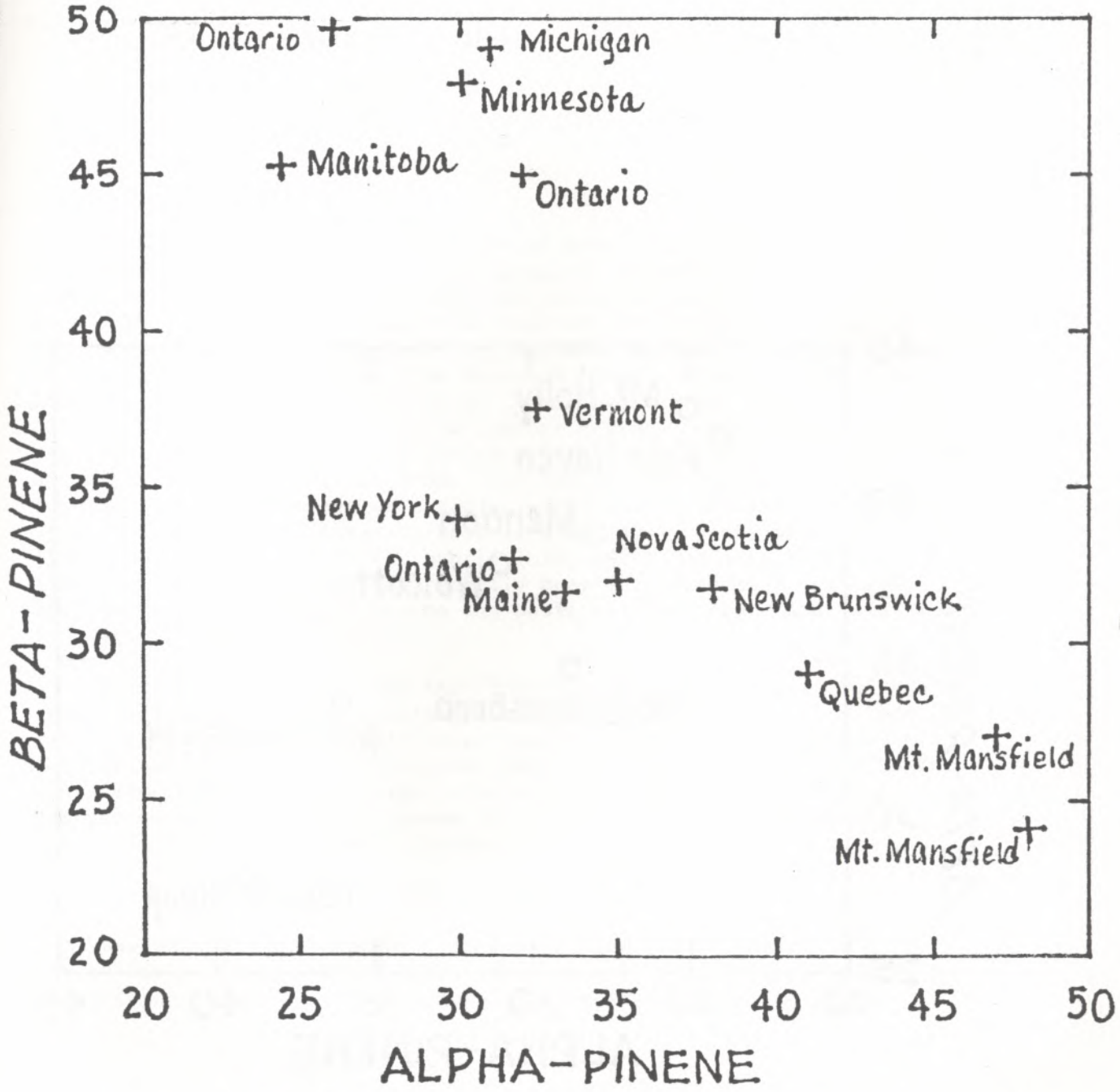

Figure 2. A plot of beta- on alpha-pinene separates western, eastern intermediate and maritime-mountain populations.

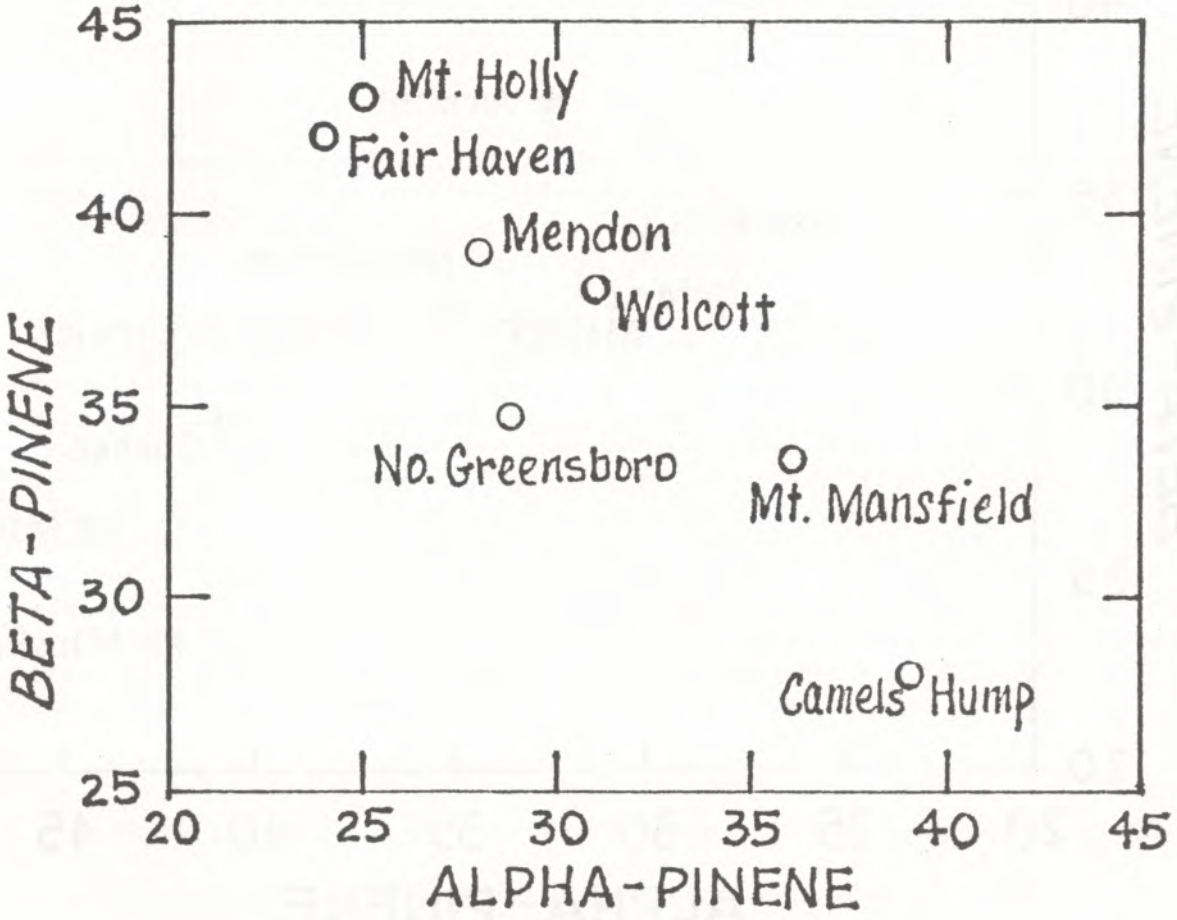

Figure 3. A plot of beta- on alpha-pinene shows the correlation of both variables with altitude.

Table 5.--Major monoterpene percents and ratios for Fraser fir and three groups of balsam fir.

	Alpha-pinene	Beta-pinene	Beta/Alpha pinene
West	29.6	47.6	1.61
East (low altitude)	31.6	34.9	1.10
Mountain-maritime	40.3	29.0	.72
Fraser fir	52.2	17.0	.32

What these results mean in terms of practical application can best be demonstrated by illustration. A Wolcott, Vermont, Christmas tree grower, Mr. John A. Young, allowed testing of three firs growing in his yard. Their origin was known to be southern and it had been supposed that they were Fraser firs. In fact they had been used as the Fraser parent in Fraser balsam fir crosses at the Research Forest. Their monoterpene profiles are summarized in Table 6. Tree number 1 has more beta- than alpha-pinene, tree number 2 has a typical Fraser fir beta-alpha-pinene ratio but no 3-carene, a very un-Fraser trait. Tree number 3 looks like a typical mountain-maritime tree. Further discussion with Mr. Young revelaed that these trees were dug as seedlings in the intermediate range in Virginia which has been characterized variously as balsam fir (Fernald, 1950), as balsam x Fraser fir hybrid (Liu, 1971), or as a separate species (Fulling, 1936) and which has recently been described as A. balsamea (L). Mill. var. phanerolepis Fern. on the basis of monoterpene analysis (Thor and Barnett, 1974),

There is some indication that 3-carene may be related to foliage characteristics in eastern balsam firs. A random group of trees from one seed source had significantly lower levels (6.9%) than a group selected for foliage color (18.1%) from the same source. Progeny and grafts from a superior parent all had high 3-carene percentages. Trees from a plantation in New Hampshire known for superior Christmas trees have generally higher levels of 3-carene.

CONCLUSIONS

Analysis of monoterpenes from fir foliage has potential as a tool for selecting trees best suited to grow in a particular environment, for identifying hybrids and, for selecting potentially superior trees.

Table 6.--Monoterpene profiles of "John Young Fraser Firs."

	Tree Number 1	2	3	Mean
Alpha-pinene	32.4	50,3	30,4	37.0
Camphene	2.5	13.9	6.2	7.5
Beta-pinene	37.7	19.6	25.0	27.4
Three-carene	12.0	0.0	15.1	9.0
Myrcene	1.1	2.3	2.6	2.0
Limonene	14.3	11.8	20.1	15.4
Beta-phellandrene	.6	2.7	1.6	1.6

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