# VARIATION IN MONOTERPENE CONTENT AMONG GEOGRAPHIC[1] SOURCES OF EASTERN WHITE PINE

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ABSTRACT.--Variations of monoterpenes in cortical oleoresins and foliar samples were determined for seed from 16 provenances of eastern white pine (*Pinus strobus* L.). The experiment was analyzed using the "raw" and the arcsine "transformed" data. Alpha-pinene, camphene, and β-pinene varied between seed sources when "raw" data were analyzed and α-pinine, β-pinene, and myrcene varied between seed sources when "transformed" data were analyzed. No trend was detected for latitude or longitude of seed origin for any monoterpene. Foliar monoterpenes did not vary among seed sources and no geographic pattern or trend was indicated for any monoterpene.

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Previous studies have shown regional variation in the monoterpenes of certain coniferous species. For example, Gilmore (1971) found that α-pinene in loblolly pine increased almost linearly from the southernmost to northernmost seed source; Smith (1977) reported evidence to support the establishment of at least five regions and four transition-zone types of ponderosa pine; Hanover (1966) showed most of the variations in monoterpene content in western white pine to be genetically controlled; and Hilton (1968) found that the concentration of five monoterpenes in 23 geographic sources of eastern white pine varies, but he could not show distinct geographic patterns for these monoterpenes.

A range-wide provenance test of 16 sources of eastern white pine (*Pinus strobus* L.) planted in west central Illinois in 1959 provided an opportunity to study the relation between monoterpenes and

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geographic origin of white pine growing on a uniform site outside its natural range. The most southerly natural occurrence of white pine in the Mississippi River Valley is in northern Illinois, which is about 100 miles north of the plantation used in this study.

## METHODS

Seed from 16 provenances of eastern white pine were collected by the U.S. Forest Service (table 1) and sown during the 1957 and 1958 growing seasons in the State Tree Nursery at Morgantown, North Carolina.

Table 1.--Origin of eastern white pine provenances

| Location | North latitude | West longitude | Elevation in feet |
|---|---|---|---|
| Union County, Georgia (GA) | 34°46' | 84°03' | 2450 |
| Greene County, Tennessee (TN) | 36°00' | 82°48' | 2250 |
| Pulaski County, Virginia (VA) | 37°05' | 80°50' | 2400 |
| Monroe County, Pennsylvania (PA) | 41°05' | 75°25' | 1800 |
| Franklin County, New York (NY) | 44°25' | 74°15' | 1600 |
| Penobscot County, Maine (ME) | 44°51' | 68°38' | 150 |
| Ashland County, Ohio (OH) | 40°45' | 82°15' | 1000 |
| Allamakee County, Iowa (IA) | 43°28' | 91°30' | 1000 |
| Cass County, Minnesota (MN) | 47°23' | 94°25' | 1300 |
| Forest County, Wisconsin (WI) | 45°51' | 88°54' | 1500 |
| Newaygo County, Michigan (MI) | 43°30' | 85°40' | 600 |
| Algoma District, Ontario (ON) | 46°10' | 82°37' | 650 |
| Pontiac County, Quebec (PQ) | 47°30' | 77°00' | 1000 |
| Lunenburg County, Nova Scotia (NS) | 44°25' | 64°35' | 150 |
| Transylvania County, North Carolina (NC) | 35°14' | 82°38' | 2120 |
| Greenbrier County, West Virginia (WV) | 38°02' | 80°30' | 2600 |

In 1959, 2-0 seedlings of all provenances except Michigan were planted in 4-tree row plots, replicated 12 times. Seedlings from the Michigan source were planted in 1961 as 2-1 stock. The experimental trees were spaced 7 feet apart, in rows 14 feet apart. White pine seedlings planted in intervening "filler" rows were removed after the 1970 growing season. The plantation is located in the "sand hill" area of west central Illinois in Cass County. The soil type is a Plainfield fine sand (Typic Udipsamments) and is fairly uniform over the planted area. Growth and survival data have been obtained periodically; the last measurements were taken in the fall of 1973. Survival at the end of the first growing season ranged from 52 to 90 percent. Fail spots were replanted in 1961 with surplus stock (2-2) that had been grown an additional 2 years in the Mason State Tree Nursery in Illinois. Survival of provenances after the 1973 growing season ranged from 90 to 100 percent and averaged 97 percent.

The plantation was thinned to two trees per plot in December, 1974, when it was 16 years old. The uncut trees represented the best of the largest trees. Before the plantation was thinned, a cut was made through the bark at breast height on 8 to 10 sample trees per provenance. The oleoresin exuding from each cut was collected in a small vial, which was placed on ice for transport to the laboratory where the sample was frozen until it was analyzed.

Foliar samples were taken from the upper part of the crown of each sample tree when felled. The needles were placed in plastic bags in an ice cooler for transport to the laboratory where they were frozen until analyzed.

Cortical oleoresin samples were dissolved in hexane containing 670 ppm cumene and this solution was analyzed for monoterpenes. Each monoterpene was computed as percentage of weight of the oleoresin sample, using cumene as the internal standard.

Monoterpenes in the foliar samples were determined by injecting a solid segment of needle without cumene into the gas chromatograph, as described by Roberts (1968). The content of each foliar monoterpene was expressed as a percentage of the total monoterpene concentration in the sample. Otherwise, analytical procedures were the same as those for the cortical oleoresin samples.

Samples were analyzed with a Hewlett-Packard Model 5750 Gas Chromatograph equipped with dual hydrogen-air ionization detectors using stainless steel columns with 20 percent carbowax 20M liquid phase on 60-80 mesh, acid-washed chromosorb W solid support. Operating conditions were: injection port, 200°C; detector 225°C; column 110°C; and He flow 17 $cm^3$/min.

The raw data were statistically analyzed as a complete randomized design, and differences between provenances for each significant variable were determined by the least-significant-difference method. In addition, the raw data of the monoterpenes in the cortical oleoresin samples were transformed using an arcsine function and analyzed as were the raw data.

## RESULTS AND DISCUSSION

The average height and diameter (d.b.h.) of sample trees according to seed source are shown in Table 2. The average height of all sample trees was equal to the plantation average (26 feet), whereas the diameter of cut trees was slightly smaller than the plantation average - 5.0 vs. 5.3 inches. Differences in average height or average diameter between sample and all trees in a seed source was small, never exceeding 2 feet in height or 0.6 inches in diameter. Therefore, the sample trees were considered to be reasonably representative of the plantation.

Table 2.--Average heights and diameters (d.b.h.) of sample trees at end of 1973 growing season by seed source

| Seed source | Height[1/] | Seed source | Diameter[1/] |
|---|---|---|---|
| | feet | | inches |
| GA | 31 | TN | 6.4 |
| TN | 29 | GA | 6.2 |
| NC | 29 | PA | 5.8 |
| PA | 29 | OH | 5.7 |
| WI | 27 | NC | 5.6 |
| MI | 27 | MI | 5.5 |
| OH | 27 | WI | 5.2 |
| NS | 26 | WV | 5.0 |
| WV | 26 | NS | 4.9 |
| MN | 25 | MN | 4.9 |
| ON | 25 | VA | 4.5 |
| NY | 25 | PQ | 4.5 |
| VA | 24 | ON | 4.5 |
| PQ | 23 | NY | 4.3 |
| IA | 20 | ME | 3.6 |
| ME | 19 | IA | 3.6 |
| Average | 26 | Average | 5.0 |

1/ Any two averages not included within the same line appearing to the right of each ranking of source are significantly different at 5-percent level.

Six monoterpenes were found in the cortical oleoresin of all seed sources. The order in which they eluted from the gas chromatographic column were α-pinene, camphene, β-pinene, myrcene, limonene, and β-phelledrene. Hilton (1968) reported two additional monoterpenes in eastern white pine than were reported in this study: 3-carene and terpinolene. This discrepancy can be explained by the fact that 3-carene was eluting from the gas chromatographic column at the same time as our marker cumene and could not be detected. This resulted in the amount of cumene appearing greater under the curve on the chromatogram chart than its actual amount. Therefore, an error might have been introduced in computing the quantities of monoterpenes in a sample. But when we determined the monoterpenes in a number of our cortical oleoresin samples without using cumene, we found that the concentrations of 3-carene was low when compared to the other monoterpenes in the sample and in some samples it was not detected. So, we feel confident that our conclusions from this study are valid and would not change, even if some other marker was used that would not interfere with the monoterpenes eluting from the gas chromatograph. We did not report terpinolene in the study as it was detected in only a few samples and the incidence was too small and varied among seed source to be considered.

The composition of those monoterpenes in cortical oleoresins that varied significantly among seed sources and the ranking of these sources is shown in Table 3. Although significant differences were found between provenances for α-pinene, camphene, and β-pinene when the raw data were analyzed, no distinct geographic concentration pattern was established because no significant correlation was found between latitude or longitude of the seed source and any of the monoterpenes in the oleoresin. These results agree with Hilton (1968) who did not establish a monoterpene pattern for eastern white pine. But it is interesting to note that the New York seed source had the highest concentration of camphene and β-pinene and the next to highest concentration of α-pinene. On the other hand, the Ohio seed source had the lowest concentration of camphene and β-pinene but was average in its concentration of α-pinene. The raw data averages of the three monoterpenes that did not differ among seed source were: myrcene, 2.5; limonene, 0.1; and β-phelledrene, 0.8.

When the raw data of cortical oleoresin was converted using arcsine transformations, the analyses showed that α-pinene, β-pinene, and myrcene varied significantly among seed sources at the 5-percent level, whereas camphene differences only approached significance at this level (table 4). But the relative rank of seed sources for each monoterpene was almost the same regardless of the method of anlaysis used (raw data or transormation).

There were some tree-to-tree variations in the composition of the monoterpene fractions in the cortical oleoresin, not only for all trees but also for trees within a seed source. Similar results have been shown by numerous investigators for other tree species.

The average concentrations and range of monoterpenes in the foliage are shown in table 5. Foliar monoterpenes varied widely among trees and there was no significant difference between seed sources and no geographic concentration pattern or trend was indicated. It would have been interesting from an academic viewpoint to determine the chemical composition of the four unknown monoterpenes. But because of the findings in this phase of the study, the time and expense could not be justified at this time.

## CONCLUSION

Although we found distinct differences in some cortical monoterpenes between provenances, white pine does not seem to exhibit a distinct geographic concentration pattern for any of the detected monoterpenes. As shown in this and other studies, cortical and foliar monoterpenes vary widely between trees within a seed source.

Table 3.--Ranking of white pine sources by average percentages of raw data of α-pinene, camphene, and β-pinene in cortical oleoresin samples[1/]

| α-pinene | | Camphene | | β-pinene | |
|---|---|---|---|---|---|
| Source | Oleoresin | Source | Oleoresin | Source | Oleoresin |
| | Percent | | Percent | | Percent |
| PA | 8.11 | NY | 1.28 | NY | 8.41 |
| NY | 7.09 | ME | 1.20 | WV | 6.96 |
| WV | 6.36 | WV | 1.11 | MN | 6.70 |
| ME | 6.25 | PA | 1.09 | ON | 6.68 |
| PQ | 5,83 | GA | 1.03 | NC | 6.12 |
| MN | 5.70 | IA | 0.94 | IA | 6.09 |
| OH | 5.64 | PQ | 0.93 | VA | 5.95 |
| TN | 5.60 | MN | 0.92 | PQ | 5.93 |
| GA | 5.38 | NC | 0.91 | TN | 5.88 |
| ON | 5.29 | TN | 0.87 | GA | 5.59 |
| NC | 4.80 | ON | 0.85 | WI | 5.37 |
| NS | 4.61 | MI | 0.85 | MI | 4.97 |
| WI | 4.55 | WI | 0.73 | ME | 4.92 |
| IA | 4.47 | NS | 0.66 | PA | 4.69 |
| VA | 4.22 | VA | 0.60 | NS | 4.57 |
| MI | 3.98 | OH | 0.60 | OH | 1.88 |
| Average | 5.49 | Average | 0.91 | Average | 5.66 |

1/ Any two averages not included within the same line appearing to the right of each ranking of source are significantly different at 5-percent level.

Table 4.--Ranking of white pine sources by average percentages based on transformed data of α-pinene, β-pinene, and myrcene in cortical oleoresin samples [1/]

| α-pinene | | β-pinene | | Myrcene | |
|---|---|---|---|---|---|
| Source | Oleoresin | Source | Oleoresin | Source | Oleoresin |
| | Percent | | Percent | | Percent |
| PA | 7.7 | NY | 7.8 | OH | 7.6 |
| NY | 7.0 | MN | 6.5 | PA | 6.2 |
| ME | 6.0 | WV | 6.3 | WV | 5.8 |
| WV | 5.9 | IA | 5.9 | MI | 2.8 |
| PQ | 5.7 | NC | 5.8 | NS | 2.9 |
| MN | 5.7 | TN | 5.7 | NC | 2.5 |
| OH | 5.6 | PQ | 5.7 | TN | 2.0 |
| TN | 5.5 | VA | 5.6 | VA | 1.7 |
| GA | 5.1 | GA | 5.3 | WI | 1.8 |
| ON | 5.1 | ON | 5.2 | IA | 1.6 |
| NC | 4.6 | WI | 4.9 | ME | 1.6 |
| WI | 4.4 | MI | 4.8 | PQ | 1.2 |
| NS | 4.4 | ME | 4.7 | NY | 1.2 |
| IA | 4.4 | PA | 4.3 | GA | 0.6 |
| VA | 4.1 | NS | 4.1 | MN | 0.6 |
| MI | 3.9 | OH | 1.7 | ON | 0.4 |
| Average | 5.3 | Average | 5.3 | Average | 5.7 |

1/ Any two averages not included within the same line appearing to the right of each ranking of source are significantly different at 5-percent level.

Table 5.--Monoterpene content of foliar oleoresin for 16 geographic sources of eastern white pine (raw data)

(In percent)

| Terpene | Range of source means | Average of source means |
|---|---|---|
| α-pinene | 4.7 - 10.3 | 6.8 |
| Camphene | 53.6 - 65.6 | 59.0 |
| β-pinene | 5.0 - 18.0 | 12.4 |
| Myrcene | 16.6 - 23.3 | 19.4 |
| Limonene | 5.2 - 13.3 | 8.7 |
| Unknown 1 | 1.0 - 10.7 | 5.3 |
| Unknown 2 | 1.0 - 3.5 | 1.8 |
| Unknown 3 | 1.0 - 7.0 | 2.1 |
| Unknown 4 | 3.4 - 8.7 | 5.5 |

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