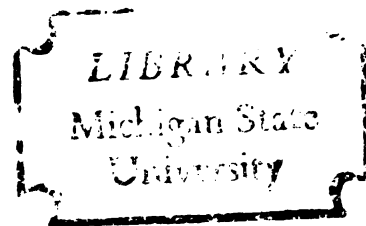


GENETIC VARIATION AND
INTERRELATIONSHIPS OF THE CORTICAL
MONOTERPENES, FOLIAR MINERAL
ELEMENTS, AND GROWTH
CHARACTERISTICS OF EASTERN
WHITE PINE

THESIS FOR DEGREE OF Ph.D.
MICHIGAN STATE UNIVERSITY

ROBERT L. HILTON

1968



This is to certify that the
thesis entitled
Genetic Variation and Interrelationships of the Cortical
Monoterpenes, Foliar Mineral Elements, and Growth
Characteristics of Eastern White Pine

presented by

Robert Louis Hilton

has been accepted towards fulfillment
of the requirements for

R. L. Hilton degree in Forestry

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Date Dec 3, 1968

ABSTRACT

GENETIC VARIATION AND INTERRELATIONSHIPS OF THE CORTICAL MONOTERPENES, FOLIAR MINERAL ELEMENTS, AND GROWTH CHARACTERISTICS OF EASTERN WHITE PINE

By

Robert Louis Hilton

Analysis of the cortical monoterpenes in 23 geographic sources - part of a range-wide provenance study - at four test sites in Lower Michigan revealed that sources differed significantly in their concentrations of alpha-pinene, limonene, myrcene, 3-carene and beta-phellandrene. Absence of distinct geographic patterns suggests that genetic drift is responsible for these differences.

The large percentage of variation in terpene levels due to site and source x site interaction demonstrates that monoterpene composition is highly dependent upon environmental influences. Only limonene and myrcene appear to be under strong genetic control. However, it is hypothesized that the large influence of environment on terpene composition was due to sampling widely diverse genotypes growing under drastically different conditions.

Simple correlations were calculated between each of several morphological traits and the concentrations of the individual monoterpenes for one southern plantation. There was a positive relationship between 3-carene and height growth. However, this relationship may be of no biological significance.

The cortical oleoresin was also analyzed in 37 half-sib families representing six geographic areas in Michigan. There were significant differences among families in the concentrations of eight out of nine monoterpenes. Seven of these terpenes varied significantly among families within stands.

Among the six geographic areas there were significant differences in the concentrations of alpha-pinene, 3-carene, myrcene and limonene. It is apparent that separation of Michigan's Lower and Upper Peninsulas by five miles of water has allowed differentiation with regard to the levels of three of these terpenes. There was very little variation among stands within the Lower and Upper Peninsulas. This probably reflects the continuity of eastern white pine within these areas.

Frequency distributions for the major terpenes in 131 trees strongly indicate multigenic inheritance patterns for alpha- and beta-pinenes. On the other hand, the concentrations of myrcene, 3-carene, limonene, and beta-phellandrene appear to be controlled by relatively few genes.

These data for 131 trees were also used to compute simple correlations between monoterpenes. There was a positive correlation ($r = .74$; significant at the 1% level) between 3-carene and terpinolene. Four negative correlations were significant at the 5 or 1% levels, i.e. alpha-pinene and 3-carene, beta-pinene and 3-carene, beta-pinene and

Robert Louis Hilton

terpinolene, alpha-pinene and myrcene.

It is pointed out that the exact relationships between these terpenes in biosynthetic pathways is difficult to determine from correlation analyses alone. Biochemical studies are needed to further define the exact modes of biosynthesis.

At two test sites of the range-wide provenance study referred to above cortical monoterpene concentrations were investigated at two different times of the year. It was determined that, with the exceptions of gamma-terpinene and terpinolene, reasonably accurate estimates of cortical monoterpene concentrations can be obtained by sampling only once during the dormant period.

Likewise, monoterpene levels in 1- and 2-year-old cortex were studied at these test sites. From the results it is hypothesized that there are differences in enzymatic activity between 1- and 2-year-old cortical tissues.

Here, also, 12 mineral elements were analyzed in the foliage of 15 geographic sources. There were significant differences among 14 seedlots growing at both locations in the concentrations of N, Cu and Zn. However, there were no geographic patterns. Likewise, there were no relationships between the concentrations of these three elements and any growth or morphological traits.

In view of the author's main objectives two general conclusions can be stated. First, there is genetic variation in both the cortical monoterpene and foliar mineral

Robert Louis Hilton

element compositions of eastern white pine. Second, in spite of these genetic differences there appear to be no correlated responses of biological significance with regard to the growth characteristics studied.

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Robert Louis Hilton

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Forestry

1968

40-811
6-6-67

ACKNOWLEDGMENTS

The author sincerely thanks the members of the Guidance Committee -- Drs. A.A. DeHertogh, J.E. Grafius, J.W. Hanover, C.J. Pollard, and J.W. Wright (Chairman) -- for their assistance.

Thanks are also due to W. Lemmien, J. Tobolski and J. Bright for their assistance in collecting oleoresin samples at the W.K. Kellogg and Fred Russ Forests. In addition, I am grateful to the Glidden Company of Jacksonville, Florida and the Hercules Powder Company of Wilmington, Delaware for supplying pure samples of monoterpenes.

My wife, Carol, provided much inspirational encouragement during the course of this study and actively participated in the preparation of the manuscript. To her I owe a special debt of gratitude.

This study was financed by the Cooperative State Research Service of the U.S. Department of Agriculture as part of the regional project NC-51 entitled, "Tree Improvement Through Selection and Breeding", and by the McIntire-Stennis Cooperative Forestry Research Program.

TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENTS.....	11
LIST OF TABLES.....	v
LIST OF FIGURES.....	vi11
 CHAPTER	
I. INTRODUCTION.....	1
Geographic Variation in Eastern White Pine...	1
Physiological traits.....	5
Monoterpenes.....	7
Mineral Elements.....	8
Objectives of Study.....	8
 II. GEOGRAPHIC VARIATION IN THE MONOTERPENE COMPOSITION OF EASTERN WHITE PINE.....	 10
Methods.....	13
Variation Among Geographic Sources.....	16
Results.....	21
Plantation Differences.....	24
Source x Plantation Interaction.....	27
Relationships Between Terpene Concentrations and Other Traits at Kellogg.....	39
Comparison of Cortical and Xylem Oleoresin...	41
 III. VARIATION, INHERITANCE AND RELATIONSHIPS OF THE CORTICAL MONOTERPENES IN HALF-SIB FAMILIES OF EASTERN WHITE PINE FROM SIX AREAS IN MICHIGAN...	 43
Methods.....	46
Sources of Variation in Terpene Concentrations	49
Genetic Control of Terpene Levels.....	54
Growth Rate and Terpene Concentrations.....	55
Half-sib Families at Allegan.....	56
Inheritance Patterns.....	60

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	PAGE
CHAPTER	
III. (cont'd.)	
Simple Correlations Between Monoterpenes.....	64
Methods.....	65
Results.....	67
Discussion.....	69
IV. EFFECTS OF SAMPLING TIME AND AGE OF TISSUE ON CORTICAL MONOTERPENE LEVELS IN EASTERN WHITE PINE.....	71
Time of Sampling.....	71
Methods.....	74
Results and discussion.....	75
Age of Tissue.....	77
Methods.....	79
Results and discussion.....	80
Conclusions.....	84
V. GEOGRAPHIC VARIATION IN THE FOLIAR MINERAL COM- POSITION OF EASTERN WHITE PINE.....	87
Methods.....	89
Results and Discussion.....	90
Plantation differences.....	93
Relative importance of geographic origin, planting location and interaction of these factors.....	95
LITERATURE CITED.....	99
VITA.....	104
APPENDIX.....	106

LIST OF TABLES

TABLE	PAGE
1.--Concentrations of five monoterpenes in 23 seedlots growing at four locations in southern Michigan.	22
2.--Comparison of F values derived from analyses of variance of the terpene concentrations among seedlots growing in either two or four plantations.	23
3.--Comparison of monoterpene compositions at four plantations in southern Michigan.	25
4.--Percent of variation in the terpene composition of geographic origins due to seedlot, plantation, seedlot x plantation interaction, and error (calculated from the data for eight seedlots in all four plantations).	32
5.--Percent of variation in the terpene composition of geographic origins due to seedlot, plantation, seedlot x plantation interaction and error (calculated from the data for 12 seedlots at the Kellogg and Russ Forests).	34
6.--Geographic variation in growth, female flower production and foliar characteristics among 14 seedlots at the W.K. Kellogg Forest (from both personal measurement and Wright, 1968).	40
7.--Monoterpene composition of half-sib families growing at the W.K. Kellogg Forest in southwestern Michigan.	50
8.--Monoterpene composition of progeny originating from six areas in Michigan and growing at the W.K. Kellogg Forest.	52
9.--Sources of variation in the terpene composition of half-sib families growing at the W.K. Kellogg Forest.	53

TABLE

PAGE

10.--Levels of three monoterpenes in half-sib families growing at the Allegan State Recreation Area in west-central Michigan.	57
11.--Monoterpene composition of progeny originating from five areas in Michigan and growing at the Allegan State Recreation Area.	58
12.--Sources of variation in the terpene composition of half-sib families growing at the Allegan State Recreation Area.	59
13.--Simple correlations between monoterpenes analyzed in 84 trees from half-sib families at the W.K. Kellogg Forest.	66
14.--Percent of variation in terpene composition due to seedlot, time of sampling, and error, and the reliability of sampling at only one time of the year.	76
15.--Average monoterpene levels in 1- and 2-year-old cortex of geographic origins at two locations in southwestern Michigan.	81
16.--Percent of variation in the terpene composition of geographic origins due to seedlot, age of tissue, seedlot x age of tissue interaction, and error.	85
17.--Foliar nitrogen, copper, and zinc concentrations of 15 seedlots growing at two places in southwestern Michigan.	91
18.--Comparison of foliar mineral element concentrations at two plantations in southwestern Michigan.	94
19.--Percent of variation in mineral element concentration due to seedlot, plantation, seedlot x plantation interaction, and error, and the reliability of analyzing foliage from 40 trees.	96
20.--Comparison of foliar mineral element concentrations in eastern white pine and in four other pine species (From Wright, personal communication).	97

TABLE	PAGE
21.--Monoterpene composition of 47 trees from five half-sib families at Allegan State Recreation Area.	106
22.--Monoterpene composition of 84 trees from five half-sib families at the W.K. Kellogg Forest.	109

LIST OF FIGURES

FIGURE	PAGE
1.--Natural distribution of eastern white pine in the United States and Canada (shaded) and origin of 23 geographic sources sampled in this study.	12
2.--Locations of provenance test plantations in Michigan - Fred Russ Forest (R), W.K. Kellogg Forest (K), Newaygo Research Forest (N), Pine River Research Forest (P).	15
3.--The Kellogg plantation pictured here is eight years old from seed.	29
4.--The Newaygo plantation pictured here is nine years old from seed. Note the poor growth in comparison with that at the Kellogg plantation in Figure 3.	31
5.--Locations of 48 parents in Michigan. Grand Traverse County (G), Iron County (I), Lake County (L), Newaygo County (N), and Schoolcraft County (S).	45
6.--Frequency distributions of the six major cortical monoterpenes in eastern white pine. Basis, 131 trees.	62
7.--Hypothesized pathways for the biosynthesis of eight monoterpenes (after Juvonen, 1966).	83

CHAPTER I

INTRODUCTION

Provenance studies have demonstrated that eastern white pine (Pinus strobus) is genetically variable with regard to growth rate and a number of other morphological as well as physiological traits. The best evidence comes from a U.S. Forest Service study initiated in 1955. Interim results have been published for plantations in the southern Appalachians by Sluder (1963), in the Northeast by Santamour (1960), and in the Midwest by Funk (1964). The 10-year results of this investigation as well as other studies are thoroughly reviewed by Wright (1968). A synopsis of this and other papers is given below.

GEOGRAPHIC VARIATION IN EASTERN WHITE PINE

The earliest geographic origin test, established in Massachusetts in 1937, was described by Pauley et al. (1955). Eighty of the 159 seed sources were local collections from Massachusetts. In addition there were 27 New Hampshire, 17 New York, 21 Ontario, 3 Michigan, 9 Minnesota and 2 Virginia sources. The Massachusetts origins displayed the best growth rate.

In 16 plantations of the U.S. Forest Service study located from Georgia to Pennsylvania and in central Michigan

and southern Ontario, seedlots 1-GA, 2-NC and 3-TENN grew 25 percent faster than the average for all seedlots. Sources 6-PENN, 16-OHIO and 24-ONT grew at an intermediate rate. Eight other seedlots grew at a rate below the plantation averages. Thus, with few exceptions, seedlots from the southern Appalachians grew the fastest.

In contrast to the above, seedlots 31-WISC. and 32-MICH grew well while seedlots 1-GA, 3-TENN, 14-ME and 23-QUE grew poorly in four plantations located in the northern Lake States and in northern Ontario. In general, the fastest growing sources originated south of the test sites.

Genys (1968) reported on another provenance test in Maryland in which 99 geographic sources were studied. Two-year nursery data revealed the same general results reported above for 16 south-central test plantations in the U.S. Forest Service study. Growth was fastest in seedlots from the southern Appalachians, Pennsylvania, Ohio, and southern Ontario and slowest in seedlots from the northern Lake States and portions of Canada.

In all three studies Virginia sources grew the poorest. Hence, one should not generalize concerning the performance of southern Appalachian white pine. Similarly, the superior growth rate of southern Ontario sources does not allow one to generalize concerning growth rates among northern sources.

Variation among individual trees and stands was investigated in a half-sib progeny test started by Michigan State University in 1962 (Wright, 1968). Seed was collected from

123 parents over 14 counties in both the Lower and Upper Peninsulas of Michigan. Height measurements in four southern Michigan plantations at age 6 revealed that there were no significant differences in growth rate among progenies from the same stand. However, there were significant differences among progenies due to region of origin. Seedlots from the west-central Lower Peninsula grew fastest. Growth rate was significantly less among progenies from the northeastern Lower Peninsula and particularly among those from the Upper Peninsula.

Morphological characteristics other than growth rate have also been studied. Wright et al. (1963) reported significant differences in lammas growth (1 percent level) among 15 origins of the U.S. Forest Service study in a test plantation in southern Michigan. However, there was no pattern to these differences. Likewise, Santamour (1960) discovered no correlation between latitude of seed source and percent of seedlings with lammas growth at a plantation site in New Jersey.

Wright (1968) summarized the data for female flower production at five plantation sites of the U.S. Forest Service study. Slow growing seedlots 20-NS, 21-NB and 28-MINN produced the greatest quantities of female flowers. Other slow growing and all five southern Appalachian origins displayed very little flowering.

Numerous foliar traits were investigated among 15 geographic origins of the U.S. Forest Service study in southern

Michigan. Seedlots differed significantly but to a very small degree in most traits (Wright et al., 1963; Wright, 1968).

Although there were significant differences among seedlots in the width and length of the needles, number of endodermal cells, number of resin canals, number of stomata, and period of needle retention, no patterns were observed. However, southern origins generally possessed the greatest number of serrations per millimeter of needle length. Likewise, eastern origins could be classified as having blue foliage and northwest origins plus seedlot 14-ME as having yellow-green foliage.

In contrast to the above, a study, conducted in Connecticut, of eight seed sources at age three by Mergen (1963) revealed that northern trees had the greatest number of serrations. Also, there was a strong relationship between latitude of seed source and number of stomata, number of resin canals and average needle length. Southern origins possessed the largest number of stomata and the longest needles, but the fewest resin ducts.

In addition, Genys (1965) studied cotyledon numbers among 16 geographic sources of a 99-origin Maryland provenance test. Although none of the progenies had uniform numbers of cotyledons, seedlings from the most northern and southern locations possessed the greatest numbers and differed significantly (1 percent level) from sources representing the more central parts of the species' range.

PHYSIOLOGICAL TRAITS

Mergen (1963) reported the responses of several geographic sources, grown in Connecticut, to varying photoperiods and temperatures. In one investigation he brought three-year-old seedlings of eight seed sources into the greenhouse each month from September to January and exposed them to light periods of 8- and 16-hours. For the 8-hour light treatment there were significant differences among seedlots in the initiation of growth. Northern sources were the first to break dormancy but grew less than southern sources. For the 16-hour light treatment there were no significant differences among seed sources in time of bud break. However, southern Appalachian seedlots grew, on the average, 50 percent more than northern seedlots.

Using the same materials specified above Mergen placed 3-year-old seedlings in growth chambers during January and grew them at a day temperature of 80° F. and night temperatures of 39°, 60° and 80° F. All origins grew best at a night temperature of 60° F. At this temperature southern origins grew the fastest. Growth was noticeably poor in northern origins grown at a night temperature of 80° F. and in southern origins grown at 39° F. These data may explain why southern Appalachian seedlots have not performed well in provenance test plantations established in the northern Lake States.

Mergen also reported that the foliage of southern Appalachian seedlots was severely discolored when subjected to a

temperature of -75° F. On the other hand, northern seedlots suffered no damage.

Furthermore, Mergen (1963) studied the stratification requirements of seedlots from North Carolina, New Brunswick and New Hampshire. Seed of the latter origin needed very little stratification while that of the New Brunswick and North Carolina origins germinated only when stratified 28 or more days.

A more intensive study of 11 seedlots by Fowler and Dwight (1964) demonstrated that the percent germination of all seedlots was positively correlated with length of the stratification period. However, seed of four northern origins germinated fairly well even with no treatment while the seed of most southern origins germinated poorly unless stratified at least 60 days.

Apparently geographic origins of eastern white pine are adapted to local climatic conditions. The seed of southern origins possesses a greater degree of dormancy to prevent germination and subsequent mortality during the relatively warm but often harsh winter weather characteristic of southern areas.

Thus, provenance testing has established the presence, and in some instances, the patterns of genetic variation in numerous morphological and physiological traits of eastern white pine. However, there is a noticeable absence of data pertaining to chemical constituents of this species.

MONOTERPENES

Monoterpenes are 10-carbon compounds classified among the isoprenoids because they may be considered derivatives of isoprene, C_5H_8 . The simplest monoterpenes, the aliphatic compounds myrcene and ocimene, are thought to be formed from geraniol pyrophosphate by phosphatase activity and dehydration. Most of the monoterpenes are cyclic and are presumed to be derived by cyclization of geraniol or geraniol pyrophosphate (Bonner and Varner, 1965).

The importance of monoterpenes in taxonomic investigations has been stressed by Mirov (1961; 1963). Studies which have shown that the terpenes are under strong genetic control have justified their use in a phylogenetic approach to plant classification. Already there have been several successful attempts to use the qualitative and quantitative monoterpene composition of xylem oleoresin as an aid to taxonomic study in pine species (Forde and Blight, 1964; Iconomou et al., 1964; Mirov, 1961; Mirov et al., 1965; 1966a; 1966b; Pelouquin, 1964; Squillace and Fisher, 1966; Tobolski, 1968; Williams and Bannister, 1962; and Zavarin et al., 1966).

More recently the monoterpene composition of cortical oleoresin has been studied (Hanover, 1966a; 1966b; 1966c; Squillace and Fisher, 1966; and Tobolski, 1968). As stated by Hanover (1966b) the close proximity of the cortical terpenes to the site of their synthesis, the epithelial cells, and to the photosynthetic tissue renders them most reliable for genetic studies.

The function(s) of terpenes in the plant has not been resolved. Recently they have been implicated as factors influencing insect resistance. Although some investigators regard terpenes only as waste products of normal metabolism, others reject this hypothesis because of the large amounts of energy expended by the plant in producing considerable quantities of terpenes.

MINERAL ELEMENTS

Mineral elements analyses of whole plants or parts thereof have been previously used to investigate the role of nutrients in plant metabolism. More recently foliar analyses in replicated provenance tests have been used to detect genetic differences among geographic sources (Gerhold, 1959; Lee, 1966; Mergen and Worrall, 1965; and Steinbeck, 1966).

OBJECTIVES OF STUDY

Following, in Chapters 2 to 5, are the results from investigations of the cortical monoterpenes and foliar mineral elements found in eastern white pine. These studies were conducted in lower Michigan in four provenance test plantations which are part of the 1955 U.S. Forest Service study, and in two other half-sib progeny test plantations established by Michigan State University in 1962.

The purpose of these investigations was fourfold: (1) To provide further knowledge concerning the patterns of genetic differentiation among geographic origins. (2) To

uncover some correlated responses which would aid in the genetic improvement of eastern white pine by selection and breeding. (3) To determine possible modes of inheritance and biosynthesis of the cortical monoterpenes through analyses of their concentrations in individual trees. This information is important to understanding the progress and patterns of differentiation among geographic sources in regard to terpene levels. (4) To determine the direction and magnitude of the effects of sampling time and age of tissue sampled on terpene concentrations. These factors must be investigated if one is to make valid comparisons among different studies of the cortical monoterpenes.

CHAPTER II

GEOGRAPHIC VARIATION IN THE MONOTERPENE COMPOSITION OF EASTERN WHITE PINE

A total of 23 geographic sources of eastern white pine (Pinus strobus) were planted in four experimental plantations in Lower Michigan in 1960 and 1962. These sources are a portion of 33 original seedlots assembled for a range-wide provenance test by the U.S. Forest Service in 1955. The origins of these seedlots are shown in Figure 1. Fifteen seedlots are represented at the W.K. Kellogg Forest in Kalamazoo County, 14 at the Fred Russ Forest in Cass County, 16 at the Newaygo Research Forest in Newaygo County, and 18 at the Pine River Research Forest in Wexford County.

Each of the four plantations follows a randomized complete block design. The Kellogg and Russ plantations contain ten replications of 4-tree plots. Both were established with 2-1 stock in the spring of 1960 and were ten years old from seed when this study was conducted. The Newaygo Plantation consists of four replications with 81-tree plots; that at Pine River contains 25 replications with 1-tree plots. These latter two plantations were established with 2-2 stock in the spring of 1962 and were nine years old from seed when sampled. The location of these four plantations is shown in

Figure 1.--Natural distribution of eastern white pine in the United States and Canada (shaded) and origin of 23 geographic sources sampled in this study.

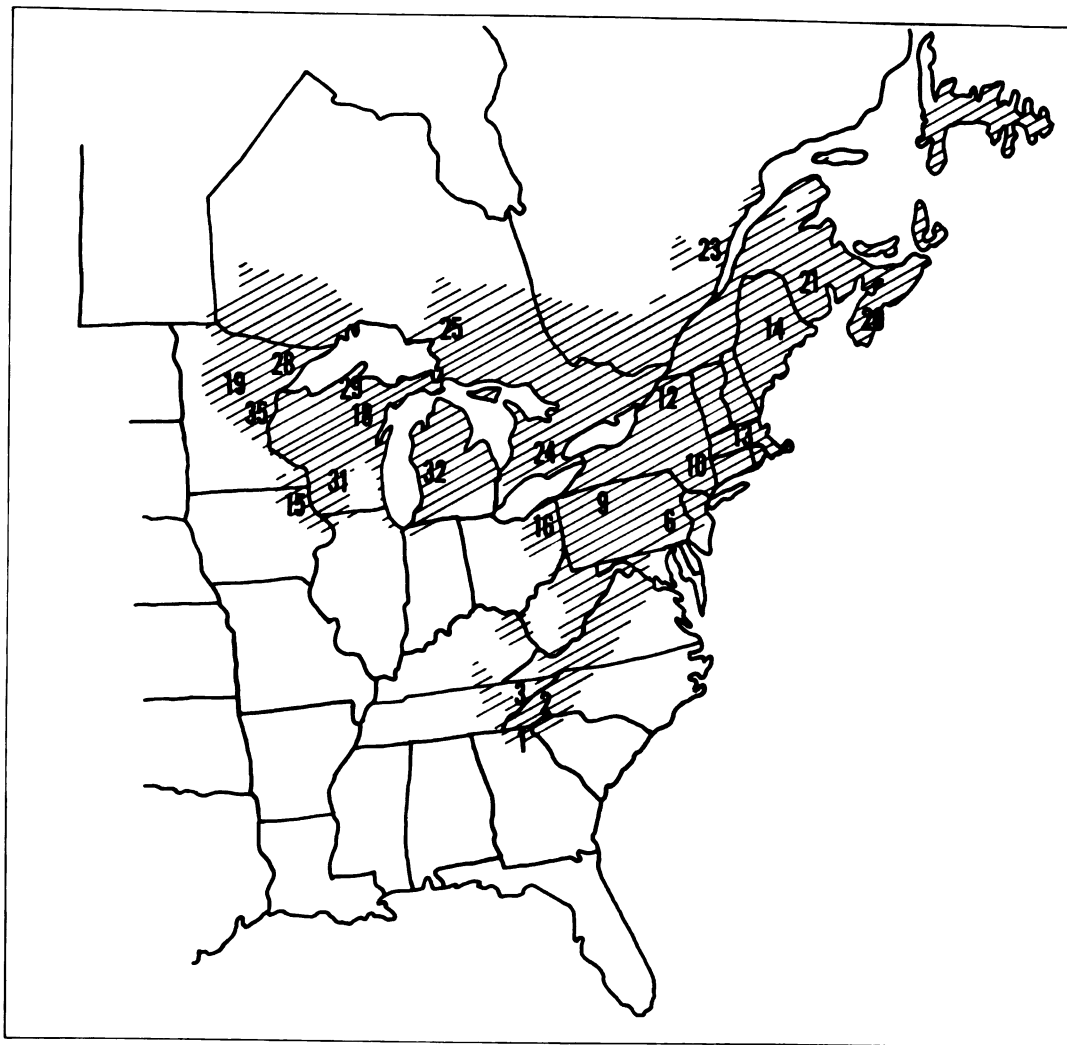


Figure 1.--Natural distribution of eastern white pine in the United States and Canada (shaded) and origin of 23 geographic sources sampled in this study.

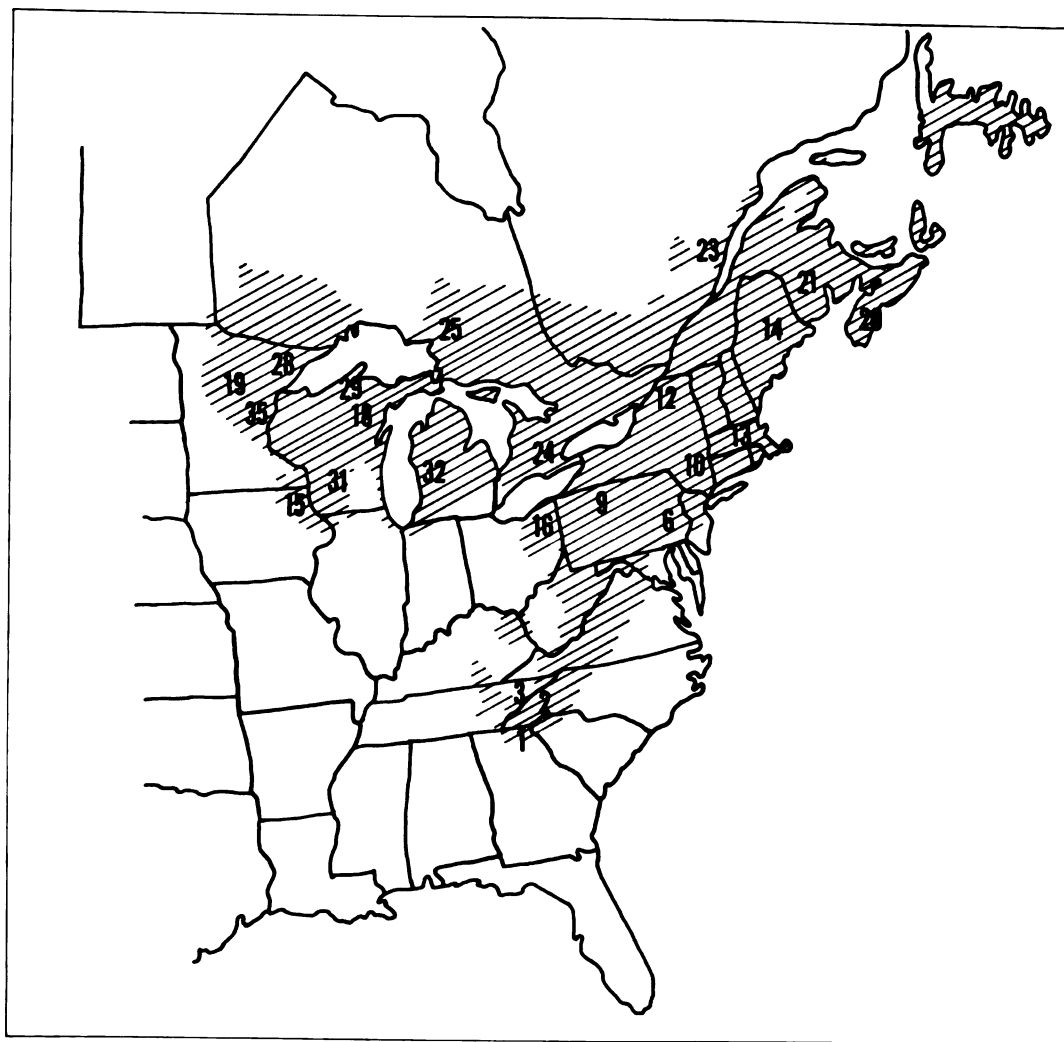


Figure 2.

Methods

Cortical oleoresin was collected at the four plantations during the spring of 1967. For each seedlot oleoresin samples from individual trees were bulked over each half of a plantation. This provided two replicate determinations for each seedlot. The values obtained therefrom were used in subsequent analyses of variance to compute error mean squares for testing seedlot x plantation interaction.

At the Kellogg and Russ plantations the tallest tree of each plot was sampled at the 3-year-old node. The cortex of the previous year's (1965) lateral growth was cut with a razor blade and 20 microliters of the exuding resin was drawn into capillary tubes one mm. in diameter. The oleoresin from five trees comprised each bulk sample. The Kellogg samples were collected March 24, 1967, stored at 40° F. and analyzed March 29, 1967. The Russ samples were collected March 31, 1967, similarly stored, and analyzed April 26, 1967.

At the Newaygo plantation the tallest three trees per plot were similarly sampled at the 2-, 3- and 4-year-old branch whorls. A bulk sample consisted of 120 microliters of oleoresin from a total of six trees. Bulk collections at the Pine River plantation contained a total of 100 microliters of oleoresin from the 2-, 3-, and 4-year-old branch whorls of five trees per source selected over 11 replicates

Figure 2.--Locations of provenance test plantations in Michigan - Fred Russ Forest (R), W.K. Kellogg Forest (K), Newaygo Research Forest (N), Pine River Research Forest (P).

for good thrift. The Newaygo samples were collected April 19, 1967, stored at 40° F., and analyzed April 26, 1967. The Pine River samples were collected April 23, 1967, similarly stored and analyzed April 27, 1967.

Each sample was diluted 1:1 with pentane and analyzed by gas-liquid chromatography. Three microliters of the diluted oleoresin were injected into an F & M gas chromatograph, Model 700, having a thermal conductivity detector. A stainless steel column measuring 6 feet x $\frac{1}{8}$ inch and containing 15% β, β -oxydipropionitrile on chromosorb G support was used. The analytical conditions were as follows: column temperature, 61° to 65° C.; injection port temperature, 150° to 151° C.; detector temperature, 154° to 160° C.; and helium flow rate, 100 to 110 ml. per minute.

All of the individual monoterpenes, except beta-phellandrene, were identified by comparing relative retention times with those of known compounds at the same operating conditions and by comparison with values published by Klouwen and ter Heide (1962). Beta-phellandrene was identified only by the latter procedure.

VARIATION AMONG GEOGRAPHIC SOURCES

Several investigators have demonstrated intraspecific variation in the monoterpene composition of pine species. Samples of xylem oleoresin were collected from Monterey pine (Pinus radiata) trees growing in three native California stands. The stand average varied from 23 to 34 percent for

alpha-pinene; from 64 to 76 percent for beta-pinene. Other terpenes were present in very minute quantities (Bannister et al., 1962).

Mirov et al. (1965) analyzed a composite sample of xylem oleoresin from 400 slash pine (Pinus elliotii var. elliotii) trees from northern Florida. Beta-phellandrene accounted for only 3 percent of the total monoterpene content in contrast to 19 percent found earlier by Mirov in a composite sample of oleoresin from Pinus elliotii var. densa from southern Florida. Squillace and Fisher (1966) studied the xylem oleoresin from five geographic sources of slash pine. There were significant differences among sources in the concentrations of beta-pinene and myrcene. No geographical patterns were observed.

Squillace and Fisher (1966) also investigated the cortical oleoresin of slash pine. They sampled 12 geographic sources distributed from central Florida to central Georgia. There were significant differences in myrcene content. However, no geographic pattern was evident. There were significant differences in the levels of beta-pinene and beta-phellandrene as well. The latter two terpenes followed north-south patterns that can be compared to similar variation patterns for several traits as shown below. These authors did not imply any cause and effect relationships.

Region	Height (Age 1)	Needle Length	Stomata per mm.	Beta- pinene	Beta-phel- landrene
	<u>cm.</u>	<u>cm.</u>	<u>number</u>	<u>percent</u>	<u>percent</u>
S. Florida	9-19	16-20	8.6-8.8	--	--
Central Florida	10-28	16-19	8.2-8.8	10-35	22-40
N. Florida and S.C. to La.	24-30	14-15	8.8-9.2	36-55	9-18

Forde and Blight (1964) analyzed 58 samples of xylem oleoresin from Bishop pine (Pinus muricata) collected in eight native stands. Variation in terpene concentrations was strongly related to morphological differences. These authors recognized three chemical races, i.e. a northern (96 to 99 percent alpha-pinene), central (73 to 89 percent 3-carene), and a southern race (50 to 75 percent sabinene and 18 to 43 percent terpinolene). Mirov et al. (1966b) further defined the geographic limits of these three chemical races after sampling 127 trees at 16 additional locations. These investigators regard alpha-pinene as the "ancestral" terpene of pines belonging to the Subsection Oocarpae. On this basis they deduced that Bishop pine originated in the northern portion of its range and differentiated into chemical races as it spread southward.

Smith (1967b) studied the monoterpene composition of the xylem oleoresin from 20 Coulter (Pinus coulteri), 46

Washoe (Pinus washoensis), and 128 Jeffrey (Pinus jeffreyi) trees which represented a large portion of the range of each species. The terpene fraction of Jeffrey pine oleoresin varied little over the locations sampled. It consisted of 88 to 99 percent heptane. On the other hand, there was considerable variation in Coulter pine with regard to the levels of alpha-pinene (25 to 51 percent), beta-phellandrene (21 to 37 percent), and myrcene (6 to 42 percent). Samples of Washoe pine displayed considerable variation in the amounts of beta-pinene (0 to 33 percent), 3-carene (45 to 78 percent), and myrcene (5 to 20 percent). However, for both Coulter and Washoe pines there were no distinct geographical patterns in terpene composition.

Xylem oleoresin was collected from 94 Douglas-fir (Pseudotsuga menziesii var. glauca) trees growing at three locations in Idaho and Montana. One Idaho population contained the highest levels of alpha-pinene and the lowest levels of beta-pinene, 3-carene, and limonene (Hanover and Furniss, 1966).

Zavarin et al. (1966) examined the monoterpene fraction of xylem oleoresin from Pinus khasya in Southeast Asia. Four samples were obtained from Assam and Burma and one from Viet Nam. Each sample contained the oleoresin from several trees. As shown in the following tabulation some of the stands from Assam and Burma differed considerably from the Viet Nam stand.

Terpene	Assam and Burma	Viet Nam
	<u>percent of monoterpenes</u>	
Beta-pinene	2-52	2
Longifolene	1-5	trace
Beta-phellandrene	1-3	17

Smith (1964b) studied the xylem oleoresin in 64 ponderosa pine (Pinus ponderosa) trees growing at eight places in the mountains of California. There was a wide range in monoterpene composition among the individual trees, and no geographic patterns were observed.

Terpene analysis of ponderosa pine trees from 40 locations was reported by Peloquin (1964). Trees from southern California and Arizona were different from trees over the rest of the range.

Tobolski (1968) reported the cortical monoterpene composition among 108 seed sources of Scotch pine (Pinus sylvestris) from Europe and Asia. There were significant differences among sources in all 11 monoterpenes. Alpha-pinene and 3-carene varied the most. The latter compound was absent in most southern populations and increased northward, reaching a high of 63 percent. Alpha-pinene showed a different trend. It varied from 5 percent in Scandinavia to 69 percent in Spain. These two terpenes were the most useful in distinguishing previously named varieties of Scotch pine.

RESULTS

In this investigation the concentrations of five monoterpenes differed among seedlots (Table 1). Eight seedlots were represented in all four plantations. Most of the other 15 seedlots were represented only at the Kellogg and Russ or only at the Newaygo and Pine River plantations. Values for sources not present at all four locations were adjusted to account for plantation differences.

Analyses of variance were calculated using only data obtained from the eight seedlots growing at all four test sites. There were significant differences among seedlots in levels of alpha-pinene, limonene, and myrcene. However, further analyses for 12 seedlots growing in the Kellogg and Russ plantations revealed significant differences in concentrations of two additional terpenes, i.e. 3-carene and beta-phellandrene (Table 2).

The significant variation among sources in limonene content can largely be attributed to the high concentrations of this chemical in seedlots 18-WISC and 20-NS.

Fast-growing southern seed sources contained relatively high amounts of 3-carene. Seedlots 24-ONT and 32-MICH, both fast-growing, contained the highest levels of 3-carene among northern seed sources. However, seedlots 13-MASS, 15-IOWA and 21-NB, characterized by slow to moderate growth rates, also had relatively high concentrations of 3-carene.

There were no other terpenes in which it was possible to recognize geographic trends. Seedlots differed, but

Table 1.--Concentrations of five monoterpenes in 23 seedlots growing at four locations in southern Michigan.

Seedlot	Planted At ^(a)	Monoterpene				
		Alpha- pinene	3- carene	Myr- cene	Limo- nene	Beta-phel- landrene
		-----percent of total monoterpenes-----				
1 GEO	K R N P	22	14	11	6	7
2 NC		17	25	14	5	4
3 TENN	K R N P	24	16	9	7	8
6 PENN	K R N P	33	15	4	3	8
9 PENN	K R	21	25	22	3	3
10 NY	K R	36	23	5	4	3
12 NY	K R N P	29	5	15	4	8
13 MASS	K R	41	10	4	3	3
14 MAINE	K	35	6	10	4	5
15 IOWA		26	11	9	6	8
16 OHIO		27	2	12	2	6
18 WISC		27	4	12	12	8
19 MINN	K	29	2	14	3	6
20 NS	K R N P	24	6	7	16	8
21 NB	K R	28	11	9	9	4
23 QUE		26	6	36	3	5
24 ONT	K R	28	16	4	5	4
25 ONT	K R N P	26	5	15	3	6
28 MINN	K R N P	31	7	12	3	6
29 MICH	K R N P	26	4	19	4	7
31 WISC		35	5	14	3	9
32 MICH		21	14	14	6	5
35 MINN		30	2	12	4	10
LSD (.05) ^(b)		10.7	17.3	12.4	5.3	5.3
LSD (.01)		15.8	25.6	14.3	7.9	7.9

(a) Letters denote Kellogg, Russ, Newaygo and Pine River plantations.

(b) These LSD values are conservative insofar as it was assumed that each seedlot was represented at only two locations. To compute the smaller values applicable to seedlots at four locations it is necessary only to multiply by .7.

Table 2.--Comparison of F values derived from analyses of variance of the terpene concentrations among seedlots growing in either two or four plantations.^(a)

Monoterpene	F value	
	Kellogg and Russ	All plantations
Alpha-pinene	2.58	2.81*
Beta-pinene	2.01	.66
Camphene	1.86	1.74
3-carene	6.93**	1.76
Myrcene	13.91**	5.56**
Limonene	17.72**	16.22**
Beta-phellandrene	3.99*	.57
Gamma-terpinene	.94	.63
Terpinolene	1.94	.77

(a) F values for Kellogg and Russ are based on 12 seedlots represented at both test sites; those for all four plantations are based on eight seedlots represented at each test site.

*, ** - Significant at the 5 and 1 percent levels, respectively.

distant sources were often as similar to each other as were neighboring sources. In other words, differentiation into distinct varieties has not occurred.

The extensive range of eastern white pine from Newfoundland to Georgia has provided enough geographic isolation so that lack of gene exchange could permit seed sources to differentiate with regard to cortical monoterpene composition. The absence of geographical patterns suggests that genetic drift has been responsible for the differentiation observed in this study.

PLANTATION DIFFERENCES

There were significant differences among the four plantations in the concentrations of alpha-pinene, beta-phellandrene, gamma-terpinene, and terpinolene (Table 3). Apparently environmental factors influenced either the formation or the stability of these four terpenes.

Major differences occurred between the two southern and two central Michigan plantations. Alpha-pinene was lower and beta-phellandrene, gamma-terpinene and terpinolene were higher at Kellogg and Russ than at the Nawaygo and Pine River plantations. Thus, it is inviting to conclude that environmental factors which vary with latitude, especially light and temperature, influenced monoterpene levels.

Reported variability in leaf oil composition of peppermint clones (Mentha piperita var. Black Mitcham) due to location, year, and cultural treatment prompted Burbott and Loomis (1967) to investigate the effects of light and

Table 3.--Comparison of monoterpene compositions at four plantations in southern Michigan.

Terpene	Monoterpene composition at			
	W.K. Kellogg Forest	Fred Russ Forest	Newaygo Research Forest	Pine River Research Forest
	<u>-----percent of total monoterpenes-----</u>			
Alpha-pinene**	24.0	24.5	27.3	31.9
Beta-pinene	32.9	36.3	37.0	32.6
Camphene	3.2	3.4	3.3	3.6
3-carene	9.7	7.2	9.7	9.0
Myrcene	12.2	11.9	10.9	11.4
Limonene	5.6	5.5	6.6	5.5
Beta-phellandrene**	9.4	9.3	4.5	4.8
Gamma-terpinene**	1.2	.7	.1	.4
Terpinolene*	1.8	1.2	.6	.8
Total	100.0	100.0	100.0	100.0

*, ** - Indicate significant differences among plantations at the 5 and 1 percent levels, respectively.

temperature on monoterpene concentrations. Using plants of a single clone of the Black Mitcham variety these investigators employed several combinations of temperature and photoperiod in a growth chamber. A minimum of six analyses were made for each set of conditions. It was discovered that short or cool nights increased the levels of methone and decreased the levels of menthofuran and pulegone. These effects were thought to be indirect and mediated through the concentrations of respiratory substrates in the cells producing terpenes.

Based on analyses of four trees Juvonen (1966) reported that levels of alpha-pinene in Scotch pine foliage are positively correlated with temperature.

The data for peppermint and Scotch pine support the hypothesis that light and/or temperature affect the cortical monoterpene composition of eastern white pine. However, as shown in the following tabulation there are only slight differences in temperature and length of growing season between southern and central Michigan. Although I have no data,

Plantations	Av. Temperature		Growing Season
	Jan.	July	
	<u>°F.</u>	<u>°F.</u>	<u>days</u>
Kellogg and Russ	24.2	73.3	158
Newaygo and Pine River	20.6	70.6	142

differences in photoperiod are probably slight also. Other environmental factors may influence monoterpene levels.

The Kellogg and Russ plantations have grown four to five times faster than the Newaygo and Pine River plantations. Height growth differences between the Kellogg and Newaygo plantations are shown in Figures 3 and 4. Possibly differences in vigor are somehow responsible for the quantitative differences in terpene composition. Evidence for this viewpoint can be obtained from a study of Scotch pine by Tobolski (1968). He demonstrated that defoliation by the European sawfly (Neodiprion sertifer) significantly affected the levels of alpha-pinene, beta-pinene, 3-carene and total monoterpenes in the cortical tissues. Within trees terpene concentrations were higher in moderately attacked branches (35 to 65 percent) than in those only lightly attacked (5 to 25 percent).

Positive identification of the environmental factors influencing terpene levels in this study and the mechanism of such action must await further experimentation.

SOURCE X PLANTATION INTERACTION

The importance of geographic origin (genotype), plantation (environment), and the interaction of these two factors (genotype x environment interaction) upon monoterpene composition is shown in Table 4. Most striking is the amount of interaction for six of the nine monoterpenes. This, combined with a significant effect of location on the levels of four

Figure 3.--The Kellogg plantation pictured here is eight years old from seed.

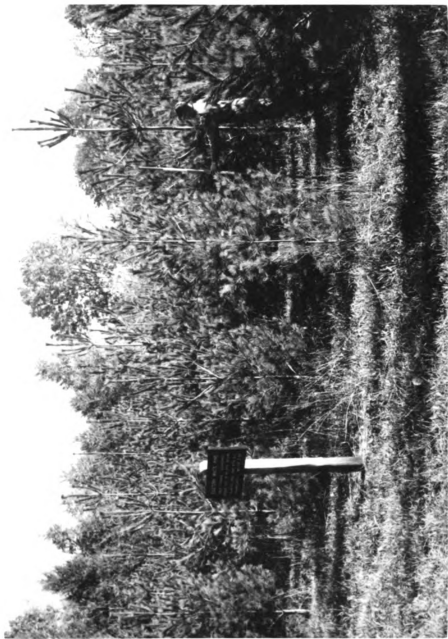


Figure 4.--The Newaygo plantation pictured here is nine years old from seed. Note the poor growth in comparison with that at the Kellogg plantation in Figure 3.



Table 4.--Percent of variation in the terpene composition of geographic origins due to seedlot, plantation, seedlot x plantation interaction, and error (calculated from the data for eight seedlots in all four plantations).

Monoterpene	Percent of Variance Due to:			
	Seedlot	Plantation	Seedlot x Plantation	Error
Alpha-pinene	20*	22**	43**	15
Beta-pinene	0	0	87**	13
Camphene	13	0	68**	19
3-carene	14	0	73**	13
Myrcene	46**	0	40**	14
Limonene	71**	0	19*	10
Beta-phellandrene	0	67**	26**	7
Gamma-terpinene	0	68**	25**	7
Terpinolene	0	44*	46**	10

*, ** - Significant at the 5 and 1 percent levels, respectively.

terpenes illustrates that monoterpene composition is highly dependent upon environmental influences. Only the variability in concentrations of limonene and myrcene appear to be largely due to genetic differences.

In comparison with the combined analyses for all four plantations, data for the Kellogg and Russ Forest plantations only revealed that environmental factors had comparatively small effect on terpene levels (Table 5). Hence, much of the environmental influence on terpene composition reported above is the result of differences in terpene values between the southern and central plantations. The striking differences in growth between the Kellogg and Newaygo plantations exemplifies these widely different environments.

Several authors have reported that the monoterpene composition of Pinus wood oleoresin is determined predominantly by the genotype of the organism. In his investigation of the turpentine composition in Pinus attenuata x radiata hybrids Forde (1964) found that when two individuals of a clone were sampled, the monoterpene composition agreed quite closely.

Forde and Blight (1964) collected 58 oleoresin samples from Bishop pine growing in eight locations. They discovered that variation among samples from the same area was insignificant in comparison with differences among areas. For example, differences of approximately 90 percent in alpha-pinene content were evident among some areas while differences within areas were generally on the order of 5 percent or less.

12. The following are the names of the persons who have been
appointed to the various committees of the Board of Directors.

Table 5.--Percent of variation in the terpene composition of geographic origins due to seedlot, plantation, seedlot x plantation interaction and error (calculated from the data for 12 seedlots at the Kellogg and Russ Forests).

Monoterpene	Percent of Variance Due to:			
	Seedlot	Plantation	Seedlot x Plantation	Error
Alpha-pinene	34	10	43**	13
Beta-pinene	29	0	59**	12
Camphene	16	33**	37*	14
3-carene	64**	0	21	15
Myrcene	75**	0	12	13
Limonene	77**	0	9	14
Beta-phellandrene	49*	0	32	19
Gamma-terpinene	0	32*	53**	15
Terpinolene	21	15*	46*	18

*, ** - Significant at the 5 and 1 percent levels, respectively.

Also, the turpentine from both wild and planted trees of the same geographic origin agreed quite closely.

Based upon data from previous investigations involving several Pinus species which illustrated the gross similarity of turpentine composition of trees growing within and outside their native ranges, Mirov (1961) concluded that the terpene composition of xylem oleoresin is a genetically fixed character. Likewise, based upon samples obtained from several locations in Canada, Michigan and Minnesota von Rudloff (1967) reported that ecological differences did not affect leaf oil composition of white (Picea glauca) and black spruce (Picea mariana). Von Rudloff and Hefendehl (1966) found almost no variation in the terpene composition of the oil of Mentha arvensis var. glabrata collected from different localities. Baker and Smith (in Mirov, 1948) also have reported the constancy of the oil of Eucalyptus globulus from several localities.

Squillace and Fisher (1966) obtained intraclass correlations for both progeny and clonal data from which they concluded that most of the cortical terpenes in slash pine are strongly inherited. Particularly convincing are the data for two representative clones; as an example, differences among these clones in alpha-pinene content were five times greater than differences among ramets within clones. In the case of each clone some of the ramets were rooted cuttings from one location while others were grafted trees from another location.

Hanover (1966a; 1966b) has given similar evidence for 5 of 6 cortical monoterpenes studied in western white pine (Pinus monticola). Hanover's conclusions are primarily based on analyses of two grafted trees from each of four clones growing in three widely different environments in northern Idaho in addition to heritability estimates derived from breeding data. For each clone percentages of any given terpene component generally varied by only 2 or 3 percent among the three sites. On the other hand, differences among clones were considerably greater except in the case of alpha-pinene. Using data from the progeny of 17 crosses among nine parents at one location in Idaho heritability estimates were derived from regression analyses of offspring on mid-parent values. For all terpenes, except camphene, narrow sense heritability estimates approximating unity were obtained. However, large standard errors averaging 0.5 were also reported.

Tobolski (1968) investigated ten seed sources of Scotch pine growing at two locations in Michigan. He determined that location and seed source x location interaction accounted for an insignificant amount of the variation in 10 of 11 cortical terpene components in comparison with variation attributable to differences among geographic sources.

In view of this evidence for strong regulation of both wood and cortical terpenes by gene action it might be assumed that collection time, collection technique, storage, non-random distribution of genotypes within seedlots over locations, sampling error or all five factors have influenced the results

presented in Tables 4 and 5. But all available evidence implicates these factors only to a small degree.

Seedlings of each seedlot were randomly assigned to a plantation at time of outplanting. Thus, there is no reason to suspect that genotypes within seedlots were stratified by location.

Sampling error is expected due to the presence of different genotypes from one plantation to the next. This is particularly expected for terpenes controlled by relatively few genes having low frequencies such as 3-carene, myrcene, limonene and beta-phellandrene (Chapter 3). However, source x replication interaction variance within plantations should also increase as a result of sampling error. Since this variance tests source x plantation interaction and contributes to the total variance, there is little reason to suspect that sampling error is the cause of the large and statistically significant interaction variances found in this study.

The fact that oleoresin was collected at the 2-, 3-, and 4-year-old branch whorls at the Newaygo and Pine River plantations and only at the 3-year-old branch whorl at the Russ and Kellogg Forest plantations must be considered. However, preliminary investigations on two trees of each of five different geographic origins at the Kellogg Forest revealed no significant variation in the monoterpene composition of 2-year-old cortex of lateral branches located at the 3- and 5-year-old nodes.

Reasonable care was exercised to collect equal amounts of oleoresin from each tree, and all samples, especially those from the same plantation, were stored for approximately equal periods under the same conditions.

Finally the influence of sampling time was probably negligible since samples were collected during a 30-day period in March and April. A study of the effects of sampling time (Chapter 4) demonstrated that monoterpene concentrations did not differ appreciably when samples were collected in July and March or in October and March.

It should be noted that some of the environmental influences on terpene concentration may be more apparent than real. Inasmuch as the terpene concentrations were measured as percentage of total monoterpene content, it is highly probable that significant changes in the levels of one or more terpenes caused others to change also. However, it is most likely that there were some real environmental influences on terpene composition. The differences in vigor and growth between the southern and central Michigan plantations further reflect these environmental influences.

In reconciling the results of this investigation with those reported by other authors, it should be noted that clonal data provide the most convincing evidence for lack of environmental influences on terpene composition. However, clonal and seedling data may not be comparable. Ideally clones are better suited to investigations of environmental effects. But unless one employs several clones genetically

very dissimilar, seedlings with their wide genetic base may better depict the influence of environment under natural conditions. Hence, it is possible that environmental influences on terpene composition occurred in this study largely as a result of sampling widely diverse genotypes growing under drastically different conditions.

RELATIONSHIPS BETWEEN TERPENE CONCENTRATIONS AND OTHER TRAITS
AT KELLOGG

Growth and morphological data for the Kellogg Forest plantation are presented in Table 6. Using seedlot means as items, simple correlations were calculated between those data and the concentrations of the individual monoterpenes. Seedlots which were high in terpinolene contained the fewest resin canals. This negative correlation ($r = -.62$) is significant at the 5% level. However, it may not be biologically meaningful because one expects some significant correlations on the basis of chance alone. Other correlations were non-significant.

These correlations based on Kellogg data alone may not have revealed all the growth-terpene relationships. Wright (1968) has summarized growth data for all origins tested in numerous southern plantations. Seedlots 1-GA, 2-NC, 3-TENN, 6-PENN and 9-PENN grew rapidly. All had high quantities of 3-carene (Table 1). Similarly, fast-growing northern seedlots 10-NY, 24-ONT, and 32-MICH were high in 3-carene.

Tobolski (1968) found that high amounts of 3-carene

Table 6.--Geographic variation in growth, female flower production and foliar characteristics among 14 seedlots at the W.K. Kellogg Forest (from both personal measurement and Wright, 1968).

Seedlot (Age 10)	Trees With		Needle Retention (b)	Needle		Stomata tions per mm.	Serra-		Endo-
	Mean Height (Age 10)	Female Shoots Flowers Color (a)		Length	Width		tions	Resin	
	feet	%		mm.	mm.			Canals	dermal Cells
1 GEO	10.7	25	11	64	.77	61	33	2.4	13
3 TENN	11.9	25	10	67	.79	65	32	2.2	12
6 PENN	11.4	55	11	63	.73	60	25	2.1	11
9 PENN	11.3	40	8	70	.74	60	20	2.1	11
10 NY	11.4	40	10	63	.72	58	45	2.2	12
12 NY	10.4	70	10	69	.74	54	23	2.3	12
13 MASS	10.9	40	11	64	.71	59	26	2.2	13
14 MAINE	9.8	60	10	68	.73	58	18	2.5	12
20 NS	9.4	35	13	66	.76	61	23	2.5	13
21 NB	9.7	60	10	68	.75	66	22	2.3	13
24 ONT	11.5	40	8	66	.72	60	21	2.1	13
25 ONT	11.0	80	10	66	.72	58	21	2.3	10
28 MINN	9.6	80	7	64	.72	56	18	2.3	13
29 MICH	9.4	55	9	60	.76	56	16	2.2	15
Mean	10.6	51	15	66	.74	59	24	2.3	12
F Value	8.2**	4.3**	8.8**	12.0**	5.4**	3.9**	5.6**	3.0**	1.9**

(a) 1 = green; 10 = blue-green

(b) 0 = complete loss of previous year's needles; 16 = maximum retention and needles green

** - Significant at the 1 percent level

occurred in slow-growing Scotch pine from northern Eurasia and fast-growing ones from central Europe. Trees with a moderate growth rate from southern Europe were low in 3-carene. This trend differs from that in eastern white pine.

Although I did not find any other terpene-growth relationships, some have been reported in the literature. Hanover (1966a) obtained a significant negative correlation between growth and both alpha-pinene ($r = -.32$) and total monoterpene content ($r = -.38$) of cortical tissue from western white pine. Squillace and Fisher (1966) found a strong positive relationship between height growth and beta-pinene levels in the cortex of slash pine.

There are no consistent trends among species. If there is a cause and effect relationship between the monoterpenes and growth - the evidence is at best scanty - the mechanism might differ from species to species.

COMPARISON OF CORTICAL AND XYLEM OLEORESIN

Mirov (1961) found that the turpentine of xylem oleoresin collected from eastern white pine in northern Minnesota contained 75 percent alpha-pinene, 15 percent beta-pinene, 4 percent oxygenated compounds, and less than 1 percent of a tricyclic sesquiterpene. Specific tests for 3-carene were negative. The latter finding is not surprising. Analysis of 131 individual trees (Chapter 3) has shown that 3-carene is relatively rare in cortical tissue.

On the other hand, this study showed that approximately 60 percent of the monoterpene fraction of the cortical

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oleoresin is composed of alpha and beta-pinenes. The remainder is largely composed of 3-carene, myrcene, limonene and beta-phellandrene with smaller amounts of camphene, gamma-terpinene and terpinolene. Analysis of half-sib progenies by slightly different analytical methods has also shown the presence of small quantities of alpha-terpinene and para-cymene (Chapter 3).

CHAPTER III

VARIATION, INHERITANCE AND RELATIONSHIPS OF THE CORTICAL MONOTERPENES IN HALF-SIB FAMILIES OF EASTERN WHITE PINE FROM SIX AREAS IN MICHIGAN

Previous studies in the genus Pinus have only partly answered two basic questions which are important in evolving efficient experimental approaches in investigations of the cortical monoterpenes. First, how much variation in terpene concentrations exists within and between stands? Second, what modes of inheritance and biosynthetic pathways are suggested by the distributions of terpene levels in individual trees? To discover solutions to these questions this investigation was performed on half-sib progenies which are part of a program to provide genetic improvement in eastern white pine (Pinus strobus) for planting in Michigan.

In 1960, open-pollinated seed was collected from 123 average white pines located in 26 stands in 14 counties in the upper half of Michigan's Lower Peninsula and Upper Peninsula. The locations of 48 parents whose progenies were studied here are shown in Figure 5. Eight permanent test plantations were established in various parts of the state. I studied one at W.K. Kellogg Forest in Kalamazoo County and one at Allegan State Recreation Area in Allegan County.

Both plantations are located in the southern portion of

Figure 5.--Locations of 48 parents in Michigan. Grand Traverse County (G), Iron County (I), Lake County (L), Newaygo County (N), and Schoolcraft County (S).

Michigan's Lower Peninsula and were established during the spring of 1965 with 1-2 stock planted at an 8 x 8-foot spacing. Furthermore, each plantation follows a randomized complete block design with 4-tree plots. There are seven replications at Kellogg and ten at Allegan.

The Kellogg plantation occupies a loam soil which was previously covered with a dense blue-grass sod. Just prior to planting the sod was treated with amino-triazole. Follow-up weed control has been necessary each year. Only 6 percent of the trees were dead three years after outplanting. The average height of this plantation at age six from seed was 60 cm. The standard deviation of a seedlot mean was 9 cm.

The Allegan plantation was established on a Plainfield sand having a sparse cover of grass, cactus, lichens and dewberry. No weed control has been necessary. Nine percent of the trees had died three years after outplanting. Average height of this plantation at age six from seed was 50 cm. The standard deviation of a seedlot mean was 7 cm.

Methods

Samples of oleoresin were collected at the Allegan State Recreation Area July 26, 1966. A branch was removed from the 3-year-old node of each tree and freshly exuding oleoresin from the 2-year-old cortex was collected in half-dram vials. For each family the oleoresin of 20 trees in replications 1 to 5 was bulked. Likewise, a 20-tree sample was collected from replications 6 to 10 for each family. This sampling scheme was chosen because it provided maximum efficiency for

detecting differences in terpene composition between families. To study inheritance patterns and possible biosynthetic pathways for terpene synthesis, additional collections were made for 8 to 10 individual trees in each of five families. All samples were stored in half-dram vials at 40° F. for ten months prior to chromatographic analysis.

Sampling was carried out at the Kellogg Forest May 16 to 20, 1967. The 1965 (2-year-old) cortical tissues of several lateral branches located at the 2-year-old node were cut with a razor blade. From each tree ten microliters of oleoresin were drawn into a capillary tube one mm. in diameter. The oleoresin of 12 trees per family was bulked over replications 1 to 3. Similarly a 12-tree sample of each family was obtained from replications 4 to 6. Separate 20-microliter samples were collected from 13 to 20 individual trees in each of five families. All samples were stored in half-dram vials at 40° F. and analyzed within four weeks.

Terpene analyses were performed on an F & M, Model 700, gas chromatograph equipped with a thermal conductivity detector. All Allegan and the Kellogg bulk samples were analyzed on an 8-foot x $\frac{1}{4}$ inch stainless steel column packed with 10 percent β, β -oxydipropionitrile on a chromosorb G support. Raw oleoresin was diluted 1:1 with pentane. A 3-microliter sample was analyzed. The Kellogg single tree collections were analyzed on a 6-foot x $\frac{1}{4}$ inch stainless steel column containing 10 percent polypropylene glycol on chromosorb W-AW. The 20 microliter samples were diluted

with 50 microliters of acetone and 3 microliters of this mixture were analyzed. The analytical conditions for both columns are shown in the following tabulation:

Samples	Helium flow rate (ml./ minute)	Temperature ($^{\circ}$ C.) of		
		Injection port	Detector	Column
Allegan, Kellogg bulk	120-130	153-155	153-160	70-72
Kellogg single tree	100	200-201	188-189	98-99

The use of acetone as a solvent, rather than the more volatile pentane, permitted more exact control of the sample volume analyzed. Thus, with acetone each terpene was expressed as a percentage of the oleoresin as well as a percentage of the total monoterpene content. In addition, alpha-terpinene and para-cymene were detected for the first time in the cortex of eastern white pine using these analytical procedures.

Identification of all monoterpenes except beta-phellandrene was accomplished by comparing relative retention times with those of known compounds analyzed under the same conditions. Further identification was obtained by comparing relative retention times with those published by Klouwen and

ter Heide (1962) who used the same liquid phase. Beta-phellandrene was identified only by the latter procedure.

SOURCES OF VARIATION IN TERPENE CONCENTRATIONS

There were significant differences among the 37 families sampled at the Kellogg Forest in the levels of all monoterpenes except gamma-terpinene (Table 7).

There was appreciable variation within stands in concentrations of nearly all terpenes. Only for alpha-pinene were the within-stand differences non-significant. Variation was particularly evident for the most heavily sampled stands from Lake and Grand Traverse Counties. Schoolcraft and Iron Counties of the geographically isolated Upper Peninsula were the only stands within which limonene varied significantly.

Within stands there were large differences in the levels of beta-pinene and 3-carene. In the case of camphene and terpinolene the differences were only one-fourth as large. Except for myrcene in Iron County, there were moderate differences in the concentrations of myrcene, limonene and beta-phellandrene.

Among the six geographic areas sampled there were significant differences in the amounts of alpha-pinene, 3-carene, myrcene and limonene (Table 8). Many of these differences are reflected in the low amounts of alpha-pinene and high amounts of myrcene and limonene in Schoolcraft and Iron Counties of the Upper Peninsula. Statistical evidence for this is shown in Table 9. Approximately 50 percent of

Table 7.--Monoterpene composition of half-sib families growing at the W.K. Kellogg Forest in southwestern Michigan.

Monoterpene								
Family	Pinene		Cam-	3-ca-	Myr-	Limo-	Beta-phel-	Terpin-
No.	Alpha	Beta	phene	rene	cene	nene	landrene	olene
MSFG-								
-----percent of monoterpenes-----								
Lake County, Area One								
5009	35	37	5	2	5	4	9*	2
5017	34	44	4	2	7	4	4	1
5022	29	47	4	4	5	3	7*	1
5023	34	44	5	3	6	4	3	1
5034	33	42	4	7	4	5	4	1
Lake County, Area Two								
5005	28	40*	3	6	8	3	10*	1
5010	24	38*	3	7	18*	4	3	1
5012	35	45*	4	2	5	4	4	1
5013	29	24	3	25*	5	5	4	4*
5014	25	36*	4	16*	5	6	4	2
5015	29	32*	4	19*	5	4	3	2
5019	37	29	6*	11*	6	4	4	2
5021	30	20	4	27*	6	5	4	4*
5029	34	39*	4	9	5	4	4	1
5035	27	53*	3	6	5	3	3	1
Grand Traverse County, Stand One								
5038	32	27	4	14*	13	4	4	2
5039	28	36	3	3	17*	5	5	1
5040	30	40*	6*	3	9	4	6*	1
5041	30	42*	4	4	12	4	3	1
5042	22	40*	4	3	16*	5	9*	1
5043	37	28	5*	6	6	6	8*	1
5044	39	38*	4	3	5	5	5	1
5046	22	31	3	6	19*	4	10*	2
5048	34	32	4	6	7	5	10*	2
5049	37	39*	5*	2	6	4	6*	1

Table 7 (cont'd.)

Monoterpene								
Family	Pinene		Cam-	3-ca-	Myr-	Limo-	Beta-phel-	Terpin-
No.	Alpha	Beta	phene	rene	cene	nene	landrene	olene
MSFG-								
-----percent of monoterpenes-----								
Grand Traverse County, Stand Two								
5064	34	26	5	18	6	5	3	3
5066	21	45*	4	3	19	4	3	1
5072	42	33	5	6	4	3	6	1
Schoolcraft County								
5101	14	33	2	22*	17	5	4	3*
5102	24	31	4	4	19	12*	4	1
5103	26	28	4	3	16	14*	6	1
5104	20	29	4	11	19	5	8*	3*
Iron County								
5119	28	34*	3	5	20	4	4	1
5121	19	22	5*	2	40*	5	5	1
5122	28	38*	4	2	18	4	5	1
5123	25	35*	4	2	10	16*	6	1
5124	24	43*	4	6	11	7	3	1

* - Significantly different at the 5 percent level from the lowest concentration in the same forest or county.

Table 8.--Monoterpene composition of progeny originating from six areas in Michigan and growing at the W.K. Kellogg Forest.

Monoterpene	Manistee National Forest	Lake County	Grand Traverse Stand One	County Stand Two	Schoolcraft County	Iron County
-----percent of total monoterpenes-----						
Alpha-pinene**	33	30	31	32	21	25
Beta-pinene	43	35	35	35	30	34
Camphene	4	4	4	5	3	4
3-carene*	4	13	5	9	10	4
Myrcene**	6	7	11	9	18	20
Limonene**	4	4	5	4	9	7
Beta-phellandrene	5	4	7	4	6	5
Gamma-terpinene	.4	1	1	1	1	.4
Terpinolene	1	2	1	1	2	1

*, ** - Indicates significant differences among areas at the 5 and 1 percent levels, respectively.

Table 9.--Sources of variation in the terpene composition of half-sib families growing at the W.K. Kellogg Forest.

Monoterpene	Percent of Variance Due to:			
	Families Within Stands	Stands Within Regions	Regions of Origin	Error
Alpha-pinene	14	0	44**	42
Beta-pinene	66**	4	7	23
Camphene	43**	6	0	51
3-carene	48**	29*	0	23
Myrcene	28**	0	58**	14
Limonene	26**	0	50**	24
Beta-phellandrene	61**	16	0	23
Gamma-terpinene	10	16*	0	74
Terpinolene	48**	14	0	38

*, ** - Significant at the 5 and 1 percent levels, respectively.

the total variation in these terpenes was due to Regions of Origin. Separation of the Lower and Upper Peninsulas by five miles of water has apparently caused differentiation with regard to the levels of these terpenes.

A large portion of the variability in the concentrations of beta-pinene, camphene, 3-carene, beta-phellandrene and terpinolene can be attributed to differences among families within stands (Table 9). Very little was due to differences among stands within the Lower and Upper Peninsulas. This pattern of differentiation is not surprising in view of the continuity of eastern white pine within each Peninsula.

GENETIC CONTROL OF TERPENE LEVELS

The large percentages of variation attributable to family differences suggest that the monoterpenes are highly regulated by gene activity (Table 9). Gamma-terpinene is the only terpene for which this conclusion does not hold.

On the basis of data collected from eight geographic sources growing at four test sites in southern Michigan (Chapter 1) it was previously demonstrated that plantation and source x plantation interaction accounted for much of the variation in 7 of 9 terpenes. However, these results were largely attributed to the severity of the growing conditions at two sites.

Based upon the results of these two experiments and those reported in the literature (reviewed in Chapter 1) it is probable that monoterpene composition is highly influenced

by gene activity which can be obscured under very severe growing conditions.

GROWTH RATE AND TERPENE CONCENTRATIONS

Growth data at age six for four southern Michigan plantations, including Kellogg and Allegan, have been reported by Wright (1968). The results for all plantations showed that there were no significant differences among families from the same stand. However, there were significant differences due to Region of Origin. The following tabulation is taken from Wright's paper:

Region of Origin	Average Height as Percent of Plantation Mean	
Upper Peninsula	82	(78 to 86) ^a
Northeastern Lower Peninsula	92	(88 to 95)
West-central Lower Peninsula	106	(100 to 111)

(a) Range in means for three to eight stands sampled within each region.

Growth data support the conclusion reached above that the majority of differentiation has occurred between the geographically isolated Lower and Upper Peninsulas.

There were no strong relationships between the levels of six terpenes and growth. This is illustrated by the fact that within the Lower Peninsula stands there were no significant differences in height growth while there were

differences in terpene concentrations. There is an obvious relationship between the concentrations of three terpenes (alpha-pinene, myrcene, and limonene) and growth rate. Such was not the case in the range-wide provenance test reported in Chapter 1. However, in that study there was a positive relationship between 3-carene and growth rate. Hence, in this study it is likely that there are no cause and effect relationships between growth and the concentrations of alpha-pinene, myrcene, and limonene.

HALF-SIB FAMILIES AT ALLEGAN

The data for 21 families growing at the Allegan State Recreation Area are presented in Tables 10, 11 and 12. In general, differences among families and stands were much less pronounced than at the Kellogg Forest. This can be attributed to sampling only 21 families from the Lower peninsula. Also, the data are subject to more experimental error because of the less precise sampling techniques employed.

Concentrations of some terpenes were seemingly higher at Kellogg than at Allegan. This is evident from the average concentrations of myrcene and beta-phellandrene in ten families (5009, 5015, 5017, 5023, 5029, 5035, 5038, 5040, 5046, and 5072) represented at both locations. On the other hand, the levels of beta-pinene were higher at Allegan than at Kellogg. Deterioration of the Allegan samples probably occurred during the 10-month storage period preceding chromatographic analysis. The fact that the terpenes were

Table 10.--Levels of three monoterpenes in half-sib families growing at the Allegan State Recreation Area in west-central Michigan.

Family No. MSFG-	Monoterpene		
	Beta-pinene	Myrcene	Beta-phellandrene
-----percent of total monoterpenes-----			
Lake County, Area One			
5009	40	8	1.8*
5017	43	5	.6
5023	47	4	.7
Newaygo County			
5002	43	3	2.2*
5031	30	13	.9
5037	32	6	1.2
Lake County, Area Two			
5015	46	2	.7
5020	40	15*	.6
5029	46	2	1.4*
5032	36	3	1.0
5035	42	2	.8
Grand Traverse County, Stand One			
5038	18	8	1.2
5040	42*	3	.7
5045	40*	6	1.4
5046	40*	13*	2.1*
5051	48*	1	.8
5057	37*	2	1.0
Grand Traverse County, Stand Two			
5068	33	4	.7
5069	33	14*	.8
5071	36	15*	1.4
5072	40	4	.8

* - Significantly different at the 5 percent level from the lowest concentration in the same forest or county.

Table 11.--Monoterpene composition of progeny originating from five areas in Michigan and growing at the Allegan State Recreation Area.

Monoterpene (a)	<u>Manistee National Forest</u>		Lake County	<u>Grand Traverse County</u>	
	Stand One	Stand Two		Stand One	Stand Two
	-----percent of total monoterpenes-----				
Alpha-pinene	42	47	40	46	43
Beta-pinene	44	35	42	38	35
Camphene	4	4	3	3	4
3-carene	1	3	8	4	4
Myrcene	6	7	5	6	9
Limonene	2	2	1	1	4
Beta-phellandrene	1	1	1	1	1
Terpinolene	.3	.4	.3	.4	.2

(a) There were no significant differences among areas for any of the monoterpenes.

Table 12.--Sources of variation in the terpene composition
of half-sib families growing at the Allegan State
Recreation Area.

Monoterpene	Percent of Variance Due to:		
	Families Within Stands	Stand of Origin	Error
Alpha-pinene	26	3	71
Beta-pinene	46*	2	52
Camphene	4	16	80
3-carene	22	16	62
Myrcene	64**	0	36
Limonene	33	0	67
Beta-phellandrene	65**	0	35
Terpinolene	0	1	99

*, ** - Significant at the 5 and 1 percent levels,
respectively.

measured as percentages of total monoterpene content rather than as percentages of the oleoresin probably accounts for the higher concentrations of beta-pinene at Allegan. Thus, the differences in terpene levels between the two plantations may be due to sample age rather than to site.

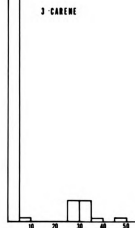
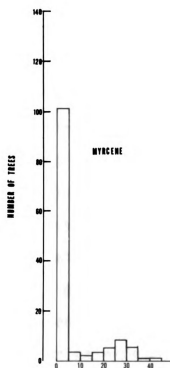
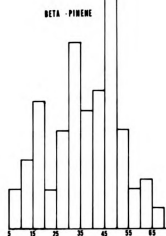
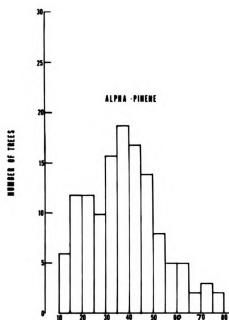
INHERITANCE PATTERNS

As previously described, oleoresin was collected from 8 to 10 individual trees in each of five families at Allegan. Similarly 13 to 20 trees were sampled in each of five other families at Kellogg. Frequency distributions were constructed for the six major monoterpenes using the combined data for 131 trees (Figure 6).

The levels of alpha- and beta-pinenes were more or less normally distributed and varied over wide limits. Although some non-genetic variation may have been present, multigenic inheritance is indicated for these two terpenes. Squillace and Fisher (1966) came to the same conclusion for alpha-pinene in both cortex and xylem oleoresin of slash pine (Pinus elliotii). Although multigenic inheritance was postulated for beta-pinene in the xylem, control by only a few genes was suggested for this terpene in the cortex. Tobolski (1968) reported a trimodal distribution pattern for beta-pinene in the cortex of Scotch pine (Pinus sylvestris), also indicating control by a few genes.

Evidence given above demonstrated that environmental factors had little influence on the levels of beta-pinene in

Figure 6.--Frequency distributions of the six major cortical monoterpenes in eastern white pine. Basis, 131 trees.



PERCENT OF TOTAL MONOTERPENES

this study. Thus, the normal distribution for this terpene in eastern white pine definitely points to a multigenic inheritance pattern. Therefore, it appears that beta-pinene is controlled by more genes in eastern white pine than is the case in slash and Scotch pines.

The distribution pattern for myrcene was unusual. It was largely bimodal (less than or greater than 5 percent of the total monoterpenes), but more sampling is required to define this pattern. Squillace and Fisher (1966) reported a similar distribution for myrcene in the cortical tissue of slash pine. They interpreted their results as indicative of control by a few genes. This hypothesis seems well founded for eastern white pine also in view of the fact that Tobolski (1968) reported a trimodal distribution for myrcene in the cortex of Scotch pine.

The concentrations of 3-carene, limonene and beta-phellandrene followed bi- or trimodal distribution patterns, suggesting control by a few genes. Tobolski (1968) arrived at a similar conclusion for these three terpenes in Scotch pine. Based upon data from a 2-parent progeny test Hanover (1966c) postulated that 3-carene in the cortex of western white pine (Pinus monticola) is primarily determined by a single dominant-recessive gene pair designated C/c. Epistatic gene action or male/female interaction were also suggested as factors that could influence the concentration of 3-carene.

SIMPLE CORRELATIONS BETWEEN MONOTERPENES

Several investigators have reported statistical correlations and apparent relationships between monoterpenes in individual trees (Hanover, 1966a; Smith, 1964b; 1967b; Pelouquin, 1964; Tobolski, 1968). If carefully interpreted, this information can be utilized to guide future research on terpene biosynthesis. However, final confirmation of all biochemical pathways must depend on isolation of specific enzymes and characterization of their behavior in vitro.

The major difficulty in using simple correlations arises from their many possible interpretations. A positive correlation may indicate that two compounds are synthesized in the same chemical pathway. For example, if compound B is derived from compound A, one expects the production of B to be related to the amount of A present. But if A and B are synthesized at the same rate from a common precursor, a positive relationship will also result. Likewise, if the precursor is not present in limited amounts, A and B may be positively correlated since one is not produced at the expense of the other. In addition, a positive correlation may indicate the linkage of genes responsible for the synthesis of A and B.

A negative correlation could imply that two compounds are formed in different pathways. On the other hand, it might also imply that two compounds synthesized in the same pathway are produced at different times or are metabolized at different rates. Furthermore, a negative correlation

could signify genetic linkage.

Both positive and negative relationships could occur merely as a result of sampling geographic sources that are genetically distinct.

Based upon the above considerations, the task of elucidating biosynthetic pathways with simple correlation analyses appears practically impossible. However, if some relationships are later confirmed in several species, their interpretation will be made somewhat easier.

METHODS

Simple correlations between monoterpenes were calculated using the data obtained to study inheritance patterns. The results for 84 individual trees at Kellogg are given in Table 13. Integrator values representing actual levels of the terpenes in the oleoresin were used as items.

Alpha-terpinene, gamma-terpinene and para-cymene were present in such small quantities that their measurements may have been subject to considerable error. Hence, these terpenes are not considered.

The origins of the trees at Kellogg are shown below:

Family (MSFG) No.	No. Trees Sampled	County
5036	19	Lake
5051	16	Grand Traverse, stand one
5059	20	Grand Traverse, stand one
5063	16	Grand Traverse, stand two
5100	13	Schoolcraft

Table 13.--Simple correlations between monoterpenes analyzed in 84 trees from half-sib families at the W.K. Kellogg Forest (integrator values used as items).

Monoterpenes								
	Alpha-pinene	Beta-pinene	Camphene	3-carene	Myrcene	Limonene	Beta-phellandrene	Terpinolene
Beta-pinene	NS							
Camphene	.67**	NS						
3-carene	-.26*	-.43**	NS					
Myrcene	-.48**	NS	NS	NS				
Limonene	NS	NS	NS	NS	NS			
Beta-phellandrene	NS	NS	NS	NS	NS	NS		
Terpinolene	NS	-.39**	NS	.74**	NS	NS	NS	
Total monoterpenes	.55**	.58**	.34**	NS	NS	.24*	NS	NS

*, ** - Significant at the 5 and 1 percent levels, respectively.

Allegan data for 47 trees from five other families (5038, 5042, 5045, 5048, and 5056) were expressed as percentages of total monoterpene content. The Kellogg data were transformed to percentages of total monoterpenes as well and additional simple correlations were calculated for both sets of data. The results did not differ from those presented in Table 13.

RESULTS

The high positive correlation between alpha-pinene and camphene ($r = .67$) may be an artifact. Camphene was usually present in very small amounts and was eluted from the column immediately after alpha-pinene. High levels of the latter caused some "tailing" and inflation of the measured camphene contents.

The concentrations of alpha-pinene, beta-pinene, camphene, and limonene were all positively correlated with total monoterpene content. A similar finding for 5 of 6 monoterpenes in western white pine was reported by Hanover (1966a). He pointed out the obvious nature of these relationships since total monoterpene content is a function of the quantities of the individual terpenes comprising this mixture.

There was also a significant positive correlation between 3-carene and terpinolene ($r = .74$). This relationship appears to be well founded. In his study of the cortical monoterpenes in 54 Scotch pine trees from southern Sweden, western Germany, and Yugoslavia, Tobolski (1968) obtained a significant correlation ($r = .98$) between these two compounds.

This relationship also occurred in a study of the xylem oleoresin collected from four other pine species, i.e. Pinus jeffreyi, P. washoensis, P. contorta and P. coulteri (Smith, 1967b). Furthermore, Townsend and Wilkinson (personal communication) obtained significant correlations between 3-carene and terpinolene in western white pine and white spruce (Picea glauca), respectively.

Four correlations were negative (significant at the 5 or 1 percent levels) - alpha-pinene and 3-carene; beta-pinene and 3-carene; beta-pinene and terpinolene; alpha-pinene and myrcene. All except the latter were also reported by Tobolski (1968) in his study of Scotch pine cortical oleoresin. He obtained the values shown in the following tabulation:

Correlation Between	r
Alpha-pinene and 3-carene	-.71
Beta-pinene and 3-carene	-.74(a)
Beta-pinene and terpinolene	-.28

(a) Statistically significant only when 28 trees containing large amounts of 3-carene and terpinolene were examined.

On the other hand, Tobolski (1968) found a positive correlation ($r = .64$) between alpha-pinene and myrcene. Likewise, this correlation was positive ($r = .22$) in the

wood oleoresin of 94 Douglas-fir (Pseudotsuga menziesii) trees sampled at three locations in Idaho and Montana (Hanover and Furniss, 1966).

Smith (1964b) also reported a strong inverse relationship between the amounts of beta-pinene and 3-carene in the xylem oleoresin of 64 ponderosa pines (Pinus ponderosa) sampled at eight places in California. Peloquin (1964) confirmed Smith's finding except in samples collected from southern California and Arizona.

DISCUSSION

Among the correlations obtained in this study only the relationship between alpha-pinene and myrcene appears to be highly questionable on the basis of results obtained from investigations of other species.

The positive correlation between 3-carene and terpinolene has been confirmed in six pines and in one species of spruce. Whether or not these two compounds are synthesized in the same biosynthetic pathway is still a matter of conjecture.

Three negative relationships are worthy of more study, i.e. correlations between alpha-pinene and 3-carene; beta-pinene and 3-carene; beta-pinene and terpinolene. One can hypothesize a common precursor (a carbonium ion) for each using the scheme for monoterpene biosynthesis proposed by Juvonen (1966). However, Tobolski (1968) has pointed out that the common precursor could simply be a "pool" of

garanylpyrophosphate. This compound is known to be a precursor of all the monoterpenes.

As described above other interpretations of these relationships are possible. Further experiments may provide solutions to the actual significance of these findings.

CHAPTER IV

EFFECTS OF SAMPLING TIME AND AGE OF TISSUE ON CORTICAL MONOTERPENE LEVELS IN EASTERN WHITE PINE

In order to compare the results from different studies of the monoterpene composition of oleoresin one must know the importance of several potentially important variables such as the time of sampling and age of the tissue sampled. The range-wide provenance study of eastern white pine (Pinus strobus) growing in southern Michigan at the W.K. Kellogg and Fred Russ Forests, described in Chapter 2, furnished the material for the following investigations which were designed to provide information on the relative importance of these factors as compared to that of provenance.

TIME OF SAMPLING

Previous studies indicate that in comparison with individual tree differences there is insignificant seasonal variation in the monoterpene composition of xylem oleoresin from Monterey pine (Pinus radiata) (Bannister et al., 1959; 1962). In a more comprehensive study which entailed sampling eight trees each month for one year Blight and McDonald (1964) obtained a mean standard deviation of only 1.5 percent.

In his study of the monoterpene composition of the wood oleoresin of ponderosa pine (Pinus ponderosa) Smith (1964b)

sampled one tree 14 times from June to September, 11 trees during August and March, and one tree during July of three consecutive years and in March of a fourth year. The concentrations of each of seven terpenes, expressed as percentages of total monoterpene content, varied by only 1 or 2 percent between the dormant and growing seasons.

On the basis of his own work and that of several other investigations Mirov (1961) concluded that the terpene composition of xylem oleoresin varies little during the growing season in several species of pines. His statement applies particularly to bulk samples in which unpredictable seasonal changes in the optical rotation of the turpentine of individual trees are likely to compensate for one another.

Likewise, Emboden and Lewis (1967) reported very small seasonal differences in the monoterpene composition of essential oil collected from one Salvia leucophylla plant during February, April and June of the same year.

In contrast to the above findings, a study of the monoterpenes of the xylem oleoresin in five Scotch pine (Pinus sylvestris) trees by Juvonen (1966) demonstrated significant quantitative variation during the course of the growing season while variability during the dormant period was less. Sampling was performed several times each month from July to October. No seasonal patterns were observed.

Another study by Tobolski (1968) which involved an analysis of the seasonal variation of the monoterpenes in eight Scotch pine trees sampled nine times over a 13-month period,

disclosed that time of sampling significantly affected the levels of 7 of 11 monoterpenes. However, time of sampling accounted for less than 4 percent of the total variation in five of these terpenes. Only in the case of para-cymene and camphene was there any appreciable contribution to the overall monoterpene variation due to time of sampling.

Seasonal variation was also reported for the monoterpenes in the xylem oleoresin of Douglas-fir (Pseudotsuga menziesii var. glauca) by Hanover and Furniss (1966). Fifteen trees were sampled in one area of Montana during June and October. Although there were sizable increases in the levels of alpha- and beta-pinenes during this period, seasonal variation was significantly less than the variation among individual trees. The alpha-pinene content of the 15 trees averaged 22 percent in June and 29 percent in October. However, the level of this compound in individual trees varied as much as 23 percent between seasons. Likewise, beta-pinene content averaged 5 percent in June and 6 percent in October, but varied up to 3 percent in individual trees. The concentrations of myrcene, 3-carene and limonene remained relatively constant.

Squillace and Fisher (1966) reported on seasonal variation in the monoterpene levels of both cortex and xylem oleoresin from slash pine (Pinus elliotii). Over a 3-year period, cortical and stem oleoresin samples were collected twice from 18 and 9 trees, respectively. Although there were no seasonal patterns standard deviations ranging from

2 to 9 percent were obtained. The greatest variation was found in the levels of beta-pinene and beta-phellandrene in the cortex.

Methods

Ten geographic origins of eastern white pine (1-GA, 3-TENN, 6-PA, 10-NY, 12-NY, 14-ME, 20-NS, 21-NB, 28-MINN, and 29-MICH) growing at the W.K. Kellogg Forest were sampled October 21, 1966 and March 20, 1967. Eleven origins (1-GA, 3-TENN, 9-PA, 10-NY, 12-NY, 13-MASS, 21-NB, 24-ONT, 25-ONT, 28-MINN and 29-MICH) were sampled at Fred Russ Forest on July 25, 1966 and March 31, 1967. Both forests are located in southwestern Michigan.

All the 1966 samples were collected by removing a single branch from the 3-year-old node of each tree. The bark was then cut and a drop of freshly exuding cortical oleoresin collected. In each replication, samples were taken from all four trees of a given seedlot. The oleoresin from the 20 trees in replications 1 to 5 was bulked for each seedlot. Likewise, oleoresin from the 20 trees in replications 6 to 10 comprised a second bulk sample.

Whereas all four trees of a plot were sampled in 1966, only the tallest tree per plot was sampled for each seedlot in a replication in 1967. An incision was made into the cortical tissue of an intact branch at the 3-year-old node and 20 microliters of exuding oleoresin were drawn into a 1-mm. capillary tube. Again two bulk samples were collected

for each seedlot, each sample representing the oleoresin from five trees. All samples were stored in half-dram vials at 40° F. until analyzed.

All samples were analyzed on an F & M, Model 700, gas chromatograph equipped with a thermal conductivity detector. A stainless steel column, measuring 6 feet x $\frac{1}{8}$ inch, packed with 15% β, β -oxydipropionitrile on a chromosorb G support was used. For each sample a 3-microliter injection of oleoresin diluted approximately 1:1 with pentane was analyzed under the following conditions: injection port temperature 150° C.; detector temperature 153-160° C.; column temperature 61-65° C.; and helium flow rate of 110 ml. per minute. The 1966 samples were analyzed six to eight months after collection; the 1967 samples one to four weeks after collection.

Monoterpene identification was accomplished by comparison with retention time of single known compounds and known mixtures analyzed at the same time, and with the data of Klouwen and ter Heide (1962) who used the same liquid phase. However, beta-phellandrene was identified only on the basis of published data.

Results and Discussion

The relative importance of provenance, time of sampling and error are shown in Table 14. The influence of time was negligible for most monoterpenes. There were changes in the levels of gamma-terpinene and terpinolene with time at the Russ Forest, but this may have been due to prolonged storage.

Table 14.--Percent of variation in terpene composition due to seedlot, time of sampling, and error, and the reliability of sampling at only one time of the year.

	Percent of Variance Due to:			Coefficient of Variation ^(a) (%)
Monoterpene	Seedlot	Time	Error	
W.K. Kellogg Forest				
Alpha-pinene	82**	0	18	12
Beta-pinene	75**	0	25	11
Camphene	31*	54**	15	14
3-carene	88**	0	12	28
Myrcene	89**	0	11	18
Limonene	60*	0	40	44
Beta-phellandrene	44	0	56	24
Gamma-terpinene	0	0	100	58
Terpinolene	21	0	79	55
Fred Russ Forest				
Alpha-pinene	86**	0	14	10
Beta-pinene	82**	4	14	10
Camphene	83**	0	17	12
3-carene	75**	0	25	31
Myrcene	91**	0	9	17
Limonene	82**	0	18	23
Beta-phellandrene	84**	0	16	22
Gamma-terpinene	18	26*	56	46
Terpinolene	34*	46**	20	34

(a) Coefficient of variation is the standard deviation of a single time of sampling divided by the mean of the experiment expressed as a percentage.

*, ** - Indicates significance at the 5 and 1 percent levels, respectively.

Forde and Blight (1964) reported that samples of xylem oleoresin, especially those containing terpinolene, deteriorated when stored at 4° C. in the dark. Likewise, Ikeda et al. (1961) demonstrated the spontaneous oxidation of gamma-terpinene to para-cymene in stored samples of lemon oil. I did not detect para-cymene in this investigation possibly due to the procedures employed. Also, gamma-terpinene and terpinolene were present in such small quantities that their measurement may be subject to considerable error.

At the Kellogg Forest the concentration of camphene decreased from October to March, averaging 4.5 and 3.3 percents, respectively. This probably reflected changes within the trees rather than deterioration during storage.

The coefficients of variation in Table 14 illustrate the magnitude of variability in terpene composition to be expected when sampling is carried out only once. For all terpenes, except gamma-terpinene and terpinolene, reasonably accurate estimates of quantitative values can be obtained by sampling only once during the dormant period.

AGE OF TISSUE

Past investigations have generally demonstrated that the age of xylem tissue has little effect on the composition of the oleoresin from several Pinus species. Smith (1964b) discovered differences in terpene levels at heights of 3 and 60 feet in one ponderosa pine stem as shown below.

Height (feet)	Monoterpene	
	beta-pinene plus myrcene	limonene plus beta-phellandrene
3	48	5
60	46	7

Samples from five other individuals revealed that myrcene averaged 8 and 9 percents at 3 and 15 feet respectively.

Smith (1964a; 1967a) also investigated the inter-ring constancy of monoterpene composition in one tree of the following species: Pinus coulteri, Pinus echinata x Pinus taeda, Pinus contorta var. murrayana x Pinus contorta var. contorta and Pinus ponderosa (two trees). He found no appreciable difference in the monoterpene composition of oleoresin samples collected from annual rings of different age and from the whole cross section of the stem.

Samples of oleoresin were collected by Blight and McDonald (1964) from the stems of three Monterey pine trees at heights of $\frac{1}{2}$, 4, 29, and 50 feet. These investigators obtained an average standard deviation of only 0.7 percent of the total monoterpene content for all the monoterpenes.

Hanover (1966b) investigated the cortical oleoresin of three western white pine (Pinus monticola) trees. He found that the current year's tissue sampled in July had less alpha-pinene, beta-pinene and limonene but higher

levels of myrcene and 3-carene than did 1-, 2-, or 3-year-old tissues. Also, oleoresin collected from the main stem contained more alpha- and beta-pinene and less myrcene and 3-carene. There was also a net decrease in the total amount of monoterpenes in the oleoresin of the main stem.

Tobolski (1968) reported on variation in the levels of five monoterpenes sampled from 1-, 2-, and 3-year-old cortex in four trees of different geographic origins of Scotch pine. The older tissues contained more alpha-pinene and beta-pinene and less myrcene, 3-carene and limonene as shown in the following tabulation:

Age of tissue (years)	Monoterpene				
	alpha-pinene	beta-pinene	myrcene	3-carene	limonene
	<u>-----percent of total monoterpenes-----</u>				
1	16	21	21	30	4
2	18	30	19	25	3
3	24	30	15	23	2

Methods

At the time of the 1966 sampling, oleoresin was collected from the 1965 (2-year-old) and 1966 (1-year-old) cortex of the same branch at the 3-year-old node of each tree. For all seedlots a 20-tree bulk sample was obtained from each

half of the plantation. Ten seedlots were sampled at the W.K. Kellogg Forest and eight at the Russ Forest. Chemical analyses were performed in the same manner as described above.

Results and Discussion

Tissue age affected the concentration of several monoterpenes. With the exception of one seedlot at each of the two plantations the levels of beta-pinene were lower in the 1965 (2-year-old) cortex than in the 1966 (1-year-old) cortex. However, with few exceptions camphene, beta-phellandrene and terpinolene occurred in higher amounts in the 1965 cortex. Likewise, limonene at the Russ Forest and gamma-terpinene at the Kellogg Forest were present in consistently higher amounts in the 1965 cortex. The direction and magnitude of these differences for all seedlots combined are shown in Table 15. In all other instances there were no significant effects of cortical age on monoterpene levels.

These results differ from those cited above for the cortical monoterpenes of three western white pine and four Scotch pine trees. Although the results in this study were obtained from approximately 700 trees representing ten geographic origins the use of bulk samples precludes any exact determination of how monoterpene levels vary between 1- and 2-year-old cortex of individual trees. Examination of the values for pairs of bulk samples for each seedlot indicates that there may be considerable differences in the behavior of individual trees with regard to the levels of beta-pinene,

Table 15.--Average monoterpene levels in 1- and 2-year-old cortex of geographic origins at two locations in southwestern Michigan.

Monoterpene*	Composition of oleoresin at:			
	W.K. Kellogg Forest 1-year-old	2-year-old	Fred Russ Forest 1-year-old	2-year-old
	-----Percent of total monoterpenes-----			
Beta-pinene	36.9	30.9	43.3	33.8
Camphene	2.5	4.5	2.0	4.2
Beta-phellandrene	5.2	8.2	2.0	4.2
Gamma-terpinene	.4	.8	---	---
Limonene	---	---	2.6	4.2
Terpinolene	.9	1.7	.6	.8

* - The only monoterpenes shown are those whose levels varied significantly between 1- and 2-year-old cortical tissue.

gamma-terpinene and terpinolene at the Kellogg Forest, and terpinolene at the Russ Forest in different aged cortex. However, environmental factors could also be responsible for these differences.

Variations in terpene levels between cortical tissues of different ages may reflect differences in rates of synthesis or remetabolism. Beta-phellandrene, gamma-terpinene and terpinolene in wood oleoresin often diminish spontaneously with time. Thus, it is possible that the higher amounts of these chemicals in the older cortex is a reflection of more rapid rates of synthesis, while beta-pinene decreased percentage-wise solely as a result of these increases.

If, in fact, there is a decreased rate of synthesis of beta-pinene in the older cortex this would further substantiate the presence of different enzymatic activities between 1- and 2-year-old cortex. As shown in Figure 7 camphene, limonene, gamma-terpinene, terpinolene and beta-pinene may all be derived, at least ultimately, from the carbonium ion II in the biosynthetic scheme for monoterpenes proposed by Juvonen (1966). The simplest hypothesis suggests less enzymatic activity for the conversion of carbonium ion II to carbonium ion V in the older cortex. Furthermore, the presence of higher amounts of beta-phellandrene in the older cortex suggests preferential conversion of carbonium ion VIII to beta-phellandrene rather than beta-pinene.

The fact that alpha-pinene concentrations did not differ between the 1- and 2-year-old cortex agrees with Juvonen's

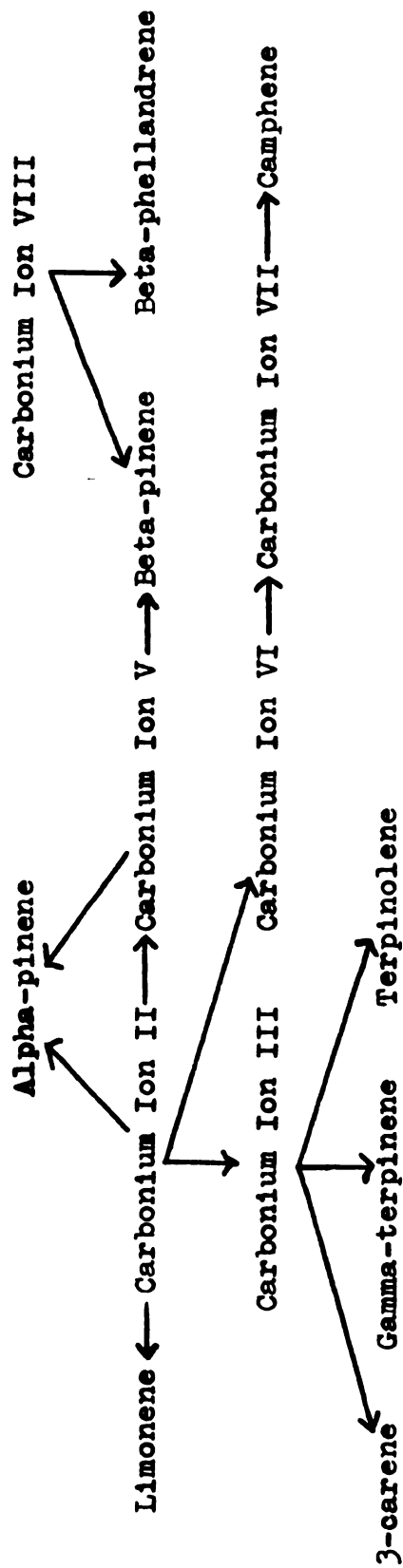


Figure 7.--Hypothesized pathways for the biosynthesis of eight monoterpenes (after Juvonen, 1966).

hypothesis that the primary precursor of this terpene is carbonium ion II rather than carbonium ion V. On the other hand, one might expect more 3-carene in the 2-year-old cortex due to less conversion of carbonium ion II to carbonium ion V. This was not the case. Several factors could be operating to maintain relatively constant levels of 3-carene in different age cortical tissues. These include the affinity of an enzyme for its substrate, rate of metabolism, feedback inhibition, etc.

The contribution of tissue age to the variability of a particular monoterpene appears to vary with planting location as the data for beta-phellandrene and terpinolene in both plantations, of limonene and gamma-terpinene in one or the other of the plantations indicate (Tables 15 and 16).

CONCLUSIONS

Although the monoterpene composition of any given seedlot is expected to vary with age of the tissue sampled, the lack of seedlot x tissue age interaction clearly illustrates that the relative seedlot values for all monoterpenes will be the same as long as oleoresin is collected from cortex of identical age. Thus, consistent sampling of either 1- or 2-year-old cortex should be used to determine cortical monoterpene composition. As previously determined, sampling need be carried out only once during the dormant period for determination of the levels of the major monoterpenes. However, the choice of age of tissue to sample and time of sampling must necessarily depend on the objective of the

Table 16.--Percent of variation in the terpene composition of geographic origins due to seedlot, age of tissue, seedlot x age of tissue interaction, and error.

Monoterpene	Percent of Variance Due to:			
	Seedlot	Age of Tissue	Seedlot x Tissue Age	Error
W.K. Kellogg Forest				
Alpha-pinene	49**	1	0	50
Beta-pinene	28*	16**	0	56
Camphene	18*	53**	0	29
3-carene	61**	0	0	39
Myrcene	72**	0	0	28
Limonene	46**	0	0	54
Beta-phellandrene	31**	33**	0	36
Gamma-terpinene	8	14*	0	78
Terpinolene	39*	12*	3	46
Fred Russ Forest				
Alpha-pinene	56*	0	6	38
Beta-pinene	42**	21**	0	37
Camphene	17*	61**	1	21
3-carene	62**	1	0	37
Myrcene	64**	2	0	34
Limonene	47**	15**	0	38
Beta-phellandrene	11	54**	0	35
Gamma-terpinene	6	5	0	89
Terpinolene	49**	3*	0	48

*, ** - Indicates significance at the 5 and 1 percent levels, respectively.

investigator. It is only logical that many studies of physiological significance of the monoterpenes should be carried out during the growing season on actively growing tissue.

CHAPTER V

GEOGRAPHIC VARIATION IN THE FOLIAR MINERAL COMPOSITION OF EASTERN WHITE PINE

Provenance studies have established that eastern white pine (Pinus strobus) is genetically variable with regard to numerous morphological and physiological traits (Bourdeau, 1963; Fowler and Dwight, 1964; Funk, 1964; Genys, 1965; 1968; Mergen, 1963; Pauley et al., 1955; Santamour, 1960; Sluder, 1963; Wright et al., 1963; and Wright, 1968). A detailed review of specific traits was given in Chapter 1. To further define the variation patterns within this species and to discover why certain geographic sources exhibit superior growth rates, this study of the foliar mineral elements was performed on 15 seedlots of the 1955 U.S. Forest Service provenance test at two southern Michigan locations.

Investigations of a similar nature have been carried out on several other tree species. Gerhold (1959) reported that six geographic origins of Scotch pine (Pinus sylvestris) sampled at 19 years of age differed significantly in the foliar contents of N, Ca, Mg and Fe.

Steinbeck (1966) determined the foliar mineral element levels in 45 origins of 4-year-old Scotch pine growing at three locations in southern Michigan. All 12 elements measured varied significantly among the three test sites.

However, there were significant differences among seed sources only in the concentrations of N, P, Na, Mg and B. Of these elements nitrogen and magnesium were related to the current year's internode growth. In the case of nitrogen a positive relationship was found at one site and a negative relationship at another. On the other hand, fast-growing origins contained high levels of magnesium at all sites. A cause and effect relationship was suggested.

Corsican seedlots of Austrian pine (Pinus nigra) were found to differ considerably from other seedlots in several foliar mineral elements. However, there was no significant correlation between height growth and the levels of these elements (Lee, 1966).

Wright (personal communication) found differences between two varieties of ponderosa pine (Pinus ponderosa) in the levels of N, K, P, Ca, B, Zn, Na and Mg. In comparison with Rocky Mountain seedlots, those from the Pacific Coast contained higher concentrations of all these elements. There were significant intra-varietal differences for N, K, P, Mg and B as well, especially with regard to the Pacific Coast variety. Wright also discovered that various geographic sources of Virginia pine (Pinus virginiana) differed significantly in the levels of N, P, Mg, B, Zn, Al and Cu.

Mergen and Worrall (1965) studied the foliar mineral element contents of jack pine (Pinus banksiana) seedlots from the eastern, central and western portions of the species' range. Seedlings were grown under three photoperiods and

three combinations of day/night temperatures and harvested after 90 days. There were significant differences among seed sources in the N, P, K and ash contents. However, these genetic differences were both enhanced and obscured by a particular set of environmental conditions.

Methods

During the last week of March 1966 foliage samples were collected from 14 geographic sources of the range-wide provenance study located at the Fred Russ Experimental Forest in Kalamazoo County, Michigan. The study consists of ten replications of 4-tree plots. Needle fascioles were removed from an area extending two inches below the terminal bud of a branch located at the 3-year-old node. The foliage from replications 1-5 and 6-10 were bulked for seven origins. The foliage of the remaining seven origins was bulked over all ten replications. A minimum of 100 fascioles was obtained for each bulk sample and each tree and 4-tree plot was equally represented.

The foliage samples were stored at 40° F. for four days and then dried at 70° C. for 48 hours. Later the samples were ground in an intermediate Wiley mill fitted with a 20-mesh sieve. Mineral element analysis was performed by Michigan State University's Plant Analysis Laboratory. Nitrogen concentrations were determined with a micro-Kjeldahl apparatus while the concentrations of 11 other elements were made using a direct-reading spectrograph.

Similar procedures were employed at the W.K. Kellogg Forest in Kalamazoo County, Michigan where 15 geographic origins were sampled on December 8, 1965. The season, position on the tree, and drying procedures specified above are in accordance with the recommendations set forth by White (1954) for foliar analyses of pine species.

The locations of the Russ and Kellogg Forests, and the origins of the geographic sources sampled in this study are shown in Figures 1 and 2 (Chapter 1).

Results and Discussion

The 14 seedlots growing at both locations differed significantly only in the concentrations of N, Cu and Zn (Table 17). There are no consistent north-south or east-west trends in the levels of these elements. However, seedlots 6-PENN and 19-MINN contained relatively low concentrations of all three elements. Any one or a combination of three factors could be responsible for the differences among seedlots. As pointed out by Steinbeck (1966), variation in foliar mineral element concentrations may reflect differences among trees in their ability to extract, translocate or metabolize nutrients.

Although N, Cu and Zn varied among seedlots there are no clear-cut relationships between the concentrations of these elements and any growth or morphological traits. In contrast, Mitchell (1939) found strong positive relationships between the internal concentrations of N, P, K, and

Table 17.--Foliar nitrogen, copper, and zinc concentrations
of 15 seedlots growing at two places in south-
western Michigan.

Seedlot	Element		
	Nitrogen	Copper	Zinc
	<u>-----percent of mean-----</u>		
1 GEO	91	123	103
3 TENN	91	150	105
6 PENN	98	96	92
9 PENN	102	118	101
10 NY	102	107	101
12 NY	102	96	101
13 MASS	102	86	110
14 MAINE	105	54	79
19 MINN	98	86	94
20 NS	101	96	97
21 NB	102	80	88
24 ONT	102	93	101
25 ONT	103	96	108
28 MINN	102	94	110
29 MICH	99	123	110
Mean	1.87%	6.2ppm	46ppm

Ca and the dry weight yield of 4-month-old eastern white pine seedlings grown in sand cultures. More than 200 seedlings were analyzed for each element. Corrections were made for differences in food reserves of the seed. Dry weight yield reached a maximum but varied little over the ranges in concentration shown below.

Element	Range in percent of dry weight
Nitrogen	2.50 to 3.26
Phosphorus	.56 to .67
Potassium	1.50 to 1.72
Calcium	.28 to .33

As pointed out by Mitchell, these values for 1-year-old seedlings are probably too low for optimum yields in older trees. Yet the average foliar levels of these four elements in 10-year-old trees at the Kellogg and Russ Forests were considerably below those given above as shown in the following tabulation:

Element	Plantation	
	Kellogg	Russ
	---percent---	
Nitrogen	1.90	1.85
Phosphorus	.24	.18
Potassium	.56	.47
Calcium	.40	.47

Thus, one cannot explain a lack of correlation between nutrient concentrations and growth in this study on the basis that nutrient levels were optimum. Apparently these relationships became obscured after the first year and particularly after outplanting as competition for light, moisture and growing space became more acute. There is an inverse relationship between the concentrations of N and Cu ($r = -.80$; significant at the 1% level). However, this may be of no physiological importance.

Plantation Differences

The concentrations of all the mineral elements, except aluminum, differed significantly at the 1% level between the two plantations (Table 18). Geographic origins growing at the Fred Russ Forest contained lower concentrations of all the macro elements measured except calcium. On the other hand, with the exception of copper, the concentrations of the micro elements were higher at the Russ Forest. Aluminum, a non-essential element, was found in equal concentrations in both plantations.

Growth at the Fred Russ plantation lags approximately two years behind that at the W.K. Kellogg plantation. One possible explanation lies in the higher concentrations of the macro elements at Kellogg. However, it is likely that both growth rate and uptake of the macro elements were enhanced in the Kellogg plantation as a result of superior weed control. While chemicals were employed at the W.K. Kellogg Forest plantation to eliminate weeds completely,

Table 18.--Comparison of foliar mineral element concentrations at two plantations in southwestern Michigan.

Mineral Element ^(a)	Unit	Foliar concentration at:	
		W.K. Kellogg Forest	Fred Russ Forest
Nitrogen	%	1.90	1.85
Potassium	%	.56	.46
Phosphorus	%	.24	.18
Magnesium	%	.16	.13
Calcium	%	.40	.47
Sodium	ppm	339	518
Manganese	ppm	301	405
Iron	ppm	141	196
Copper	ppm	7.0	5.7
Boron	ppm	24	31
Zinc	ppm	44	48
Aluminum	ppm	252	252

(a) Between plantation differences were significant at the one percent level for all elements except aluminum which was nonsignificant.

only mowing was used to control competition at the Fred Russ plantation.

Relative Importance of Geographic Origin, Planting Location and Interaction of These Factors

With the exceptions of N, Cu and Zn, which vary significantly among geographic origins, the variability in concentrations of the mineral elements in the foliage of eastern white pine is largely due to plantation differences (Table 19). Seedlot x plantation interaction, although significant for 8 of the 12 elements analyzed, accounted for very little of the variability encountered with the exception of manganese and particularly aluminum. Thus, the geographic origins utilized in this study accumulated vastly different but relatively the same amounts of the majority of the mineral elements at the two locations.

The coefficients of variation in Table 19 illustrate that reliable estimates of the foliar mineral element levels can be obtained using 40-tree samples. These values were computed by dividing the standard deviation of a 40-tree sample by the mean of the experiment. This result was expressed as a percentage. With the exception of two elements these values ranged from one to ten percent. The coefficients of variation for copper and manganese were 16 and 18 percents, respectively. Hence, if these elements are of special interest, sampling of more than 40 trees is warranted.

Coefficients of variation have been calculated for 12

Table 19.--Percent of variation in mineral element concentration due to seedlot, plantation, seedlot x plantation interaction, and error, and the reliability of analyzing foliage from 40 trees.

Mineral Element	Percent of Variance Due to:				Coefficient of Variation ^(a) (%)
	Seedlot	Plantation	Seedlot x Plantation	Error	
Nitrogen	74**	16**	1	9	1.4
Potassium	3	90**	5*	2	3.5
Phosphorus	0	92**	7**	1	5.7
Magnesium	7	78**	8	7	7.2
Calcium	7	65**	22**	6	7.6
Sodium	3	85**	10**	2	11.1
Manganese	0	57**	37**	6	17.7
Iron	9	76**	6	9	10.2
Copper	46**	23**	16	15	16.1
Boron	12	71**	13*	4	8.8
Zinc	37*	29**	23*	11	6.8
Aluminum	2	0	74*	24	8.7

(a) Coefficient of variation is the standard deviation of a 40-tree sample divided by the mean of the experiment and expressed as a percentage.

*, ** - Indicates significance at the 5 and 1 percent levels, respectively.

Table 20.--Comparison of foliar mineral element concentrations in eastern white pine and in four other pine species (from Wright, personal communication).

Mineral Element	Unit	Foliar Concentration in:		
		Eastern White Pine	<u>Four Other Pinus Species</u> (a)	
Nitrogen	%	1.87	1.38 to	1.88
Potassium	%	.51	.46 to	.64
Phosphorus	%	.21	.17 to	.22
Magnesium	%	.15	.08 to	.14
Calcium	%	.43	.22 to	.41
Sodium	ppm	433	67 to	514
Manganese	ppm	353	121 to	728
Iron	ppm	170	53 to	176
Copper	ppm	6.1	6.9 to	15.2
Boron	ppm	28	18 to	32
Zinc	ppm	46	29 to	63
Aluminum	ppm	252	158 to	1164

(a) Pinus virginiana, P. ponderosa, P. sylvestris, P. nigra, and P. strobus.

foliar elements analyzed in geographic origin studies of Virginia, ponderosa, Scotch, Austrian and eastern white pines growing at the W.K. Kellogg Forest (unpublished data at Mich. State Univ.). The collection and analytical methods were the same as described above. Although the coefficients of variation for a particular element varied among the five species, there were no significant differences. These results indicate that one can use an average experimental error for all species to conserve time and expense in future foliar analysis studies.

The results obtained in this study generally indicate that eastern white pine is less variable genetically with regard to foliar mineral element levels than are Scotch, European black, ponderosa, and Virginia pines.

Compared to these other four species eastern white pine is relatively high in N, Ca, Mg and P and low in Cu. It ranks with all the others in the concentrations of seven additional elements studied (Table 20). If foliar concentrations of the mineral elements reflect to a high degree the ability of each species to extract nutrients from the soil, one may conclude that eastern white pine is at least slightly more site demanding with regard to soil nutrients.

As is the case with Scotch pine site has a pronounced effect on the foliar mineral element composition of eastern white pine.

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Genetic variation and interrelationships of the
cortical monoterpenes, foliar mineral elements,
and growth characteristics of eastern white pine.

Outline of studies:

Major subject: Forestry, Minor subjects: Forest
genetics, Plant physiology.

University of Massachusetts, B.S., 1963

University of Massachusetts, M.S., 1965

Michigan State University, Ph.D., 1968

Biographical items:

Born May 22, 1941, in Boston, Massachusetts.

Married Carol A. Keirstead on August 25, 1962.

Two children: Heather Joan, born Nov. 16, 1963
and Scott Andrew, born July 14, 1966.

Experience:

Research assistant at the University of Massachu-
setts from September 1963 to June 1965, and

research associate at Michigan State University from June 1966 to September 1967. Timber stand improvement work for the U.S. Forest Service on the Stanislaus National Forest, California from June to August 1961. Cortical oleoresin analyses at Michigan State University from June to August 1966. Assistant Professor of Biology, State College at Boston, September 1967 to present.

Memberships:

Alpha Zeta

Xi Sigma Pi

Phi Kappa Phi

Sigma Xi

Society of American Foresters

APPENDIX

Table 21.--Monoterpene composition of 47 trees from five half-sib families at Allegan State Recreation Area.

Monoterpenes									
Tree No.	Alpha-pinene	Beta-pinene	Camphene	3-carene	Myrcene	Limonene	Beta-phellandrene	Gamma-terpinene	Terpinolene
-----percent of monoterpenes-----									
MSFG 5038					Grand Traverse County				
1	22.6	17.6	3.1	26.5	23.6	2.7	1.2	.8	1.8
2	22.2	15.4	3.0	32.1	22.4	.8	.8	1.0	2.4
3	50.1	10.3	3.6	29.7	2.3	1.0	.8	.2	1.8
4	34.7	10.0	3.9	24.2	22.4	1.8	.9	.3	1.8
5	70.6	17.6	6.8	.9	1.4	1.4	1.1	.0	.2
6	71.9	15.0	6.3	1.0	1.8	1.8	1.8	.0	.3
7	26.9	10.0	4.1	25.4	27.2	2.5	1.6	.2	2.1
8	37.4	25.2	5.3	.5	28.0	1.7	1.2	.2	.5
9	77.3	11.6	7.5	.3	1.4	1.1	.6	.0	.2
10	74.9	14.8	6.7	.2	1.1	1.0	.8	.2	.4
MSFG 5042					Grand Traverse County				
11	34.3	54.2	3.2	.8	4.6	1.0	1.4	.0	.4
12	34.0	16.8	4.3	.4	40.5	2.2	1.7	.0	.0
13	23.3	62.9	1.2	8.8	1.9	1.9	.0	.0	.0
14	35.7	32.3	3.8	.3	24.0	2.7	.7	.0	.3
15	47.4	5.6	7.3	.6	32.7	4.3	1.2	.3	.6
16	33.3	61.4	1.7	1.0	1.0	.7	.7	.0	.0

Table 21 (cont'd.)

Monoterpenes									
Tree No.	Alpha-pinene	Beta-pinene	Camphene	3-carene	Myrcene	Limonene	Beta-phellandrene	Gamma-terpinene	Terpinolene
-----percent of monoterpenes-----									
MSFG 5042									
17	69.2	18.8	6.4	.2	1.6	2.6	.9	.0	.2
18	28.6	60.3	2.3	.8	2.0	2.3	3.4	.3	.0
MSFG 5045									
19	45.4	50.7	1.9	.2	.7	.5	.5	.0	.0
20	57.7	38.2	2.3	.2	.8	.5	.2	.0	.0
21	36.5	55.1	3.0	1.0	1.5	1.3	1.3	.0	.2
22	50.4	38.7	3.6	.6	1.8	1.6	3.2	.0	.2
23	57.4	33.8	4.7	.7	1.6	1.1	.4	.0	.2
24	43.4	50.8	2.1	1.0	1.0	.9	.7	.0	.0
25	62.3	25.1	5.2	.9	1.8	1.8	2.7	.0	.2
26	54.5	40.2	1.2	.6	1.4	1.2	.6	.0	.2
27	40.0	51.5	4.6	.8	1.5	1.5	.0	.0	.0
28	58.7	30.8	4.1	.4	1.7	2.1	2.1	.0	.0
MSFG 5048									
29	44.8	48.1	2.1	.4	1.0	.6	2.9	.0	.2
30	44.3	45.7	2.6	.8	1.2	1.0	4.1	.0	.3
31	60.2	27.9	5.4	.8	1.9	2.2	1.1	.1	.3

Table 21 (cont'd.)

Monoterpenes									
Tree No.	Alpha-pinene	Beta-pinene	Camphene	3-carene	Myrcene	Limonene	Beta-phellandrene	Gamma-terpinene	Terpinolene
-----percent of monoterpenes-----									
MSFG 5048					Grand Traverse County				
32	41.7	47.4	2.6	1.0	1.3	1.4	4.3	.0	.3
33	41.9	49.9	3.4	.9	1.5	.9	1.3	.0	.2
34	63.8	19.5	4.1	.4	1.7	1.0	9.2	.0	.3
35	45.1	47.4	2.5	.9	1.6	1.6	.7	.0	.2
36	57.8	34.0	1.9	.6	1.3	1.0	3.2	.0	.3
37	59.5	32.8	3.8	.6	1.5	1.3	.4	.0	.2
MSFG 5056					Grand Traverse County				
38	38.9	56.9	.9	.5	.9	.9	.9	.0	.0
39	49.7	40.5	3.5	.8	1.4	2.2	1.9	.0	.0
40	43.7	45.7	3.1	1.3	2.0	2.0	2.0	.0	.2
41	44.1	45.9	4.9	1.1	1.3	2.0	.5	.0	.2
42	52.0	33.8	5.4	1.4	2.2	2.2	2.7	.0	.3
43	46.4	44.4	4.0	.7	1.3	2.6	.7	.0	.0
44	52.5	38.3	4.7	.3	1.1	2.3	.6	.0	.0
45	44.2	39.9	2.7	1.0	8.6	1.9	1.2	.2	.3
46	41.0	51.9	4.8	.7	.4	1.1	.0	.0	.0
47	52.0	35.5	4.5	1.6	2.3	1.9	1.9	.0	.2

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Table 22.--Monoterpene composition of 84 trees from five half-sib families at the W.K. Kellogg Forest.

Tree No.	Monoterpenes										Terpin-olene	
	Pinenes Alpha Beta	Cam- phene	3-ca- rene	Myr- cene	Terpinenes Alpha Gamma	Limo- nene	Beta-phel- landrene	Para- cymene				
-----percent of monoterpenes-----												
	MSFG 5036										Lake County	
1	32.8	47.3	4.7	1.2	3.9	0	3.4	4.4	7	1.5		
2	31.9	17.6	3.1	28.8	3.6	1.6	2.2	3.3	2.2	4.9		
3	35.9	40.7	5.0	1.8	4.8	1.8	3.5	3.1	1.8	1.8		
4	47.0	25.7	4.5	1.3	2.4	1.6	5.3	5.3	2.7	4.0		
5	39.0	44.7	2.0	1.2	2.9	1.2	2.5	3.4	2.0	1.1		
6	32.4	47.9	5.0	1.2	3.5	1.0	2.9	3.3	1.2	1.4		
7	42.5	37.1	6.1	1.1	3.4	.7	2.9	2.7	1.8	1.6		
8	35.5	36.0	5.1	1.1	2.7	.0	2.2	9.4	3.0	5.1		
9	41.0	40.2	4.6	1.0	3.6	1.0	2.4	3.2	.0	3.0		
10	39.0	44.9	4.6	.9	3.1	.4	2.4	2.4	.0	2.2		
11	31.3	49.8	5.4	1.6	4.0	.0	2.6	3.1	1.2	.9		
12	43.2	44.6	3.4	.2	2.0	.0	1.6	1.8	1.2	2.0		
13	29.1	18.6	3.9	33.7	2.8	1.1	1.8	2.8	.9	5.3		
14	74.1	8.5	5.2	.2	1.4	.3	3.4	3.2	1.2	2.5		
15	34.4	47.5	3.9	1.0	2.9	.8	3.7	3.3	1.2	1.0		
16	48.4	32.6	7.9	.8	2.9	.8	2.5	2.6	.5	1.1		
17	55.5	20.7	9.7	.7	3.2	.7	4.1	3.2	1.0	1.2		
18	44.2	27.8	6.8	1.6	3.3	.7	1.6	9.6	2.1	2.1		

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Table 22 (cont'd.)

Monoterpenes										
Tree No.	<u>Pinenes</u> Alpha Beta	Cam- phene	3-ca- rene	Myr- cene	<u>Terpinenes</u> Alpha Gamma	Limo- nene	Beta-phenel- landrene	Para- cymene	Terpin- olene	
-----percent of monoterpenes-----										
MSFG 5036										
19	46.2	28.1	8.1	2.0	4.1	.0	3.2	2.8	.0	5.3
MSFG 5051										
20	32.5	46.3	5.6	.5	3.2	.0	1.8	9.0	.0	1.0
21	23.2	58.8	1.7	1.8	3.3	.0	3.3	4.2	1.8	2.9
22	15.6	67.2	1.3	.8	3.6	.5	1.8	8.1	.3	.8
23	44.4	37.6	5.8	1.3	3.3	.8	3.3	2.5	.2	.8
24	40.1	34.8	7.0	1.8	3.0	1.2	3.2	3.8	1.8	2.5
25	18.8	38.9	2.2	28.9	3.1	.6	2.2	2.0	.2	3.1
26	46.0	32.2	5.6	1.4	2.7	1.0	4.1	3.9	1.2	1.8
27	18.0	32.0	2.8	1.2	3.4	.6	6.2	2.5	1.9	3.4
28	31.3	46.2	6.8	1.0	4.0	1.0	3.8	2.6	1.2	2.2
29	31.8	48.1	3.6	.6	2.8	.4	2.2	8.2	.2	2.0
30	21.5	61.1	2.2	1.1	2.6	.7	1.8	7.0	.0	1.8
31	45.4	43.7	2.1	.2	2.3	.2	2.5	1.7	.4	1.5
32	36.1	41.1	5.8	.2	3.0	.2	2.3	7.3	.2	3.8
33	24.4	46.3	3.2	1.0	3.4	1.2	3.8	10.5	1.8	3.0
34	26.4	48.8	3.0	1.0	3.9	1.0	2.0	12.8	.5	.7
35	39.1	44.5	7.7	.7	3.7	.3	1.7	1.7	.0	.7

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Table 22 (cont'd.)

Monoterpenes										
Tree No.	Pinenes Alpha Beta	Cam- phene	3-ca- rene	Myr- cene	Terpinenes Alpha Gamma	Limo- nene	Beta-phel- landrene	Para- cymene	Terpin- olene	
-----percent of monoterpenes-----										
MSFG 5059					Grand Traverse County					
36	37.6	43.2	4.0	1.2	3.5	.8	.0	3.1	2.9	1.5
37	25.5	26.4	3.8	.0	32.2	.0	.0	4.2	2.9	1.7
38	35.2	46.8	1.2	.8	2.9	.7	.5	4.4	3.0	1.5
39	46.9	31.6	4.5	1.0	3.5	.6	.6	4.5	3.5	1.2
40	17.6	30.7	1.4	1.9	32.8	1.7	.0	6.4	1.9	2.1
41	32.1	40.6	5.3	.8	4.0	.0	.0	3.2	7.6	2.8
42	42.9	40.5	2.2	.8	3.4	1.0	.0	3.4	3.2	1.0
43	25.0	57.9	2.8	1.0	3.8	.7	.0	3.1	2.9	.7
44	14.6	42.5	2.5	.6	24.7	.4	.0	2.7	9.5	.0
45	19.0	31.5	1.7	.8	2.6	.0	.0	2.6	2.4	.9
46	11.9	7.3	1.4	33.2	13.8	.0	.0	5.1	4.0	.4
47	43.8	34.6	2.5	1.5	2.7	1.5	.0	4.4	4.2	1.5
48	51.6	22.4	6.3	1.4	3.6	1.4	.0	5.6	3.4	1.6
49	34.6	44.6	3.0	1.4	4.1	1.0	.0	3.6	4.1	1.2
50	48.6	33.0	4.8	.6	2.8	.6	.0	3.7	3.0	.9
51	24.3	59.6	1.0	1.2	3.2	.4	.0	2.0	6.0	.8
52	38.2	46.2	3.7	.8	2.9	.7	.3	2.9	1.9	1.2
53	32.7	49.2	4.7	.8	3.1	.6	.0	3.3	3.0	1.0
54	39.0	31.9	6.0	1.1	3.2	.0	.6	1.5	15.1	.0
55	46.5	30.7	6.7	1.2	3.0	.0	.0	4.0	3.2	1.7

Table 22 (cont'd.)

Monoterpenes										
Tree No.	Pinenes Alpha Beta	Cam- phene	3-ca- rene	Myr- cene	Terpinenes Alpha Gamma	Limo- nene	Beta-phel- landrene	Para- cymene	Terpin- olene	
-----percent of monoterpenes-----										
	MSFG 5063					Grand Traverse County				
56	12.2	39.7	3.7	1.1	33.5	1.1	.0	3.4	2.3	1.6
57	21.6	26.3	5.4	1.5	30.4	1.3	.0	5.6	3.2	3.2
58	18.1	53.3	3.0	.7	3.1	.0	.0	14.9	3.0	2.6
59	17.3	46.9	2.1	.9	21.1	.0	.0	4.3	4.0	1.6
60	12.0	65.5	1.9	.0	14.4	.7	.0	1.9	2.1	.7
61	24.9	33.8	4.2	.7	28.2	.5	.0	2.8	2.6	1.6
62	18.1	30.9	4.1	.8	34.6	.8	1.0	3.0	3.0	2.6
63	18.2	8.5	4.5	.0	60.3	.6	.0	3.3	1.8	2.1
64	13.1	35.5	2.3	1.2	28.0	1.2	.6	2.1	11.8	2.7
65	25.4	31.0	3.3	24.8	1.7	1.1	.0	2.8	2.7	5.6
66	24.4	28.4	3.5	.0	29.4	1.4	.0	4.4	3.3	3.0
67	25.8	52.6	4.8	1.9	3.7	1.0	.0	2.7	4.5	2.1
68	17.7	43.0	2.0	.8	18.5	1.0	.0	2.0	11.4	2.4
69	60.9	18.0	7.4	.8	2.5	.8	.8	2.6	4.0	1.3
70	37.7	46.9	3.9	.6	3.1	.8	.0	2.1	3.1	1.2
71	48.6	33.2	6.7	.6	4.2	.7	.0	2.2	2.5	.4

Table 22 (cont'd.)

Monoterpenes										
Tree No.	<u>Pinenes</u> Alpha Beta	Cam- phene	3-oa- rene	Myr- cene	<u>Terpinenes</u> Alpha Gamma	Limo- nene	Beta-phen- landrene	Para- cymene	Terpin- olene	
-----percent of <u>monoterpenes</u> -----										
	MSFG 5100					Schoolcraft County				
72	18.9	47.8	3.2	1.3	3.4	.0	20.9	2.1	.9	1.5
73	28.3	13.9	4.9	32.2	2.7	.6	3.0	3.8	2.2	7.4
74	35.1	49.2	3.6	1.0	3.4	.6	2.4	2.2	.0	2.4
75	22.4	33.8	3.4	26.9	1.7	1.1	2.4	3.2	1.5	3.4
76	32.4	16.6	4.8	30.4	2.4	1.7	2.8	3.0	1.3	4.5
77	11.3	16.2	3.7	36.2	15.5	1.6	3.0	3.2	2.8	6.5
78	24.3	52.2	4.2	1.7	3.6	.6	1.9	10.1	.4	.8
79	19.8	28.6	6.5	2.0	29.0	.0	5.9	2.4	2.8	3.0
80	32.4	49.4	3.2	.8	3.4	.6	1.4	7.6	.8	3
81	68.1	14.9	7.7	.4	2.2	.2	3.2	1.2	.4	1.6
82	37.2	13.8	5.2	31.3	1.7	.7	3.0	2.2	1.0	3.9
83	34.3	10.5	4.6	32.7	2.4	1.1	1.6	7.6	1.6	3.5
84	13.1	21.6	3.2	28.0	12.7	1.7	3.0	3.4	6.9	6.2

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