PHYSIOLOGICAL, MORPHOLOGICAL, AND BIOCHEMICAL VARIATION IN WESTERN WHITE PINE (PINUS MONTICOLA DOUGL.) SEEDLINGS FROM DIFFERENT ALTITUDINAL SEED SOURCES IN IDAHO

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PHYSIOLOGICAL, MORPHOLOGICAL, AND BIOCHEMICAL VARIATION IN WESTERN WHITE PINE (PINUS MONTICOLA DOUGL.) SEEDLINGS FROM DIFFERENT ALTITUDINAL SEED SOURCES IN IDAHO

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Alden Miller Townsend

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ABSTRACT

PHYSIOLOGICAL, MORPHOLOGICAL, AND BIOCHEMICAL VARIATION IN WESTERN WHITE PINE (*PINUS MONTICOLA* DOUGL.) SEEDLINGS FROM DIFFERENT ALTITUDINAL SEED SOURCES IN IDAHO

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The objective was to determine the physiological and genetic variation among altitudinal seed sources of western white pine in Idaho. Progeny representing elevations ranging from 1500' to 5200' in each of four geographic areas were grown from seed in a common environment at the Michigan State University nursery.

Oleoresin monoterpene concentrations in 293 trees were measured by gas-liquid chromatogrphy and provided an estimate of the degree of non-adaptive genetic differentiation. Measurements of various photosynthetic parameters during dormancy and growth were used to determine the degree of physiological adaptation to the low temperatures, high light intensities, and low CO_2 partial pressures characteristic of high altitudes. Analyses of total height and weekly growth increments of three-year-old seedlings provided a measure of heritable morphological variation.

An overall lack of variation among seed sources associated with environmental gradients characterized the

traits which had been chosen for analysis. The monoterpenes analyzed were α-pinene, β-pinene, 3-carene, limonene, and β-phellandrene. The concentrations of α-pinene and limonene were significantly influenced by seed source. Terpene differences occurred between widely separated areas, and were unrelated to the elevation or latitude of the parent trees. Four pairs of terpenes showed significant correlations: α-pinene and limonene; β-pinene and limonene; β-pinene and 3-carene; β-phellandrene and 3-carene. Histograms of the individual terpenes indicated polygenic inheritance mechanisms for α-pinene, β-pinene, and limonene; and probably one- or two-gene control for 3-carene and myrcene. The distribution of β-phellandrene suggested control by several genes.

Photosynthesis was measured with an infrared-gas analyzer and four controlled-environment chambers in a closed system. Seedlings which were brought inside and immediately subjected to conditions of 22°C and 6600 ft-c did not show positive rates of net photosynthesis during February and March. Relatively small smounts of CO_2 absorption were observed when growth began in April. At only one (1400 ft-c) of the three (1400, 4600, and 6600 ft-c) light intensities was the seed source important in determining photosynthetic efficiency. At this light intensity and at both 22°C and 28°C, seedlings representing two high elevation (3500' and 5200') sources were superior to low elevation

(2100') seedlings in net photosynthesis. It is suggested that the high elevation sources have evolved a more efficient photosynthetic system in order to compensate for low CO_2 partial pressures. This hypothesis could not explain the lack of source differences at 4600 and 6600 ft-c, however. No progeny differences were observed in the CO_2 compensation point, at three light intensities and two temperatures (22° and 28°C). Within limits, the compensation point decreased with an increase in light intensity or with a decrease in temperature. Values of photorespiration, as estimated by the extrapolation of photosynthetic response lines to zero CO_2 concentration, did not help explain the inefficiency of low elevation sources at low light intensities.

Seed source was not significant in determining total height, beginning and ending dates of growth, frequency of lammas growth, and number of days to complete 75 percent of growth. Significant source differences were found in the number of days from January 1 required to complete 25 and 50 percent of seasonal growth but these differences were not related to latitude or elevation of the seed source. Average dates on the growth curve for the species were as follows: five percent of total growth, April 29; 25 percent, May 10; 50 percent, May 18; 75 percent, May 30; and 95 percent, August 4.

It was postulated that effective cross-pollination over long distances, genetic homeostasis, developmental plasticity, and flexibility of individual trees have been important in preventing selection pressures from creating large genetic differences among sources over this portion of the species' range.

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INTRODUCTION

Study of the genetic diversity that exists within most plant species has received increasing attention during the last 50 years. The pioneering efforts occurred in Europe during the last half of the nineteenth century, when several European forest botanists grew trees under the same environment directly from seed of different geographic origins. In this way, they were able to discriminate between genetic and environmental factors and assess the contributions of each to the phenotypic variability of the natural populations.

Later, Turesson (1922) studied genetic variation in a large number of herbaceous perennials by transplanting individuals into gardens and comparing them under relatively uniform conditions. His research resulted in the introduction of the ecotype concept, and formed the basis for many presently accepted genecological principles. In North America, the concerted research efforts of Clausen, Keck, and Hiesey (1947, 1958) were of significance in the substantiation and clarification of Turesson's work. Evidence of genetic differentiation within many tree species is now commonly accepted. However, little information is available about the inheritance of morphological and physiological responses of species to environmental gradients, such as those found in mountainous topography. The objective of this investigation was to determine the pattern of genetic variation among elevational seed sources of western white pine (*Pinus monticola* Dougl.) in northern Idaho (see Figure 1). This area is characterized by steep and broken topography, with wide climatic and altitudinal ranges and associated habitat variations. In this area, western white pine occurs over a broad elevational belt from 1000 to 6000 feet, in irregular, often attenuated bodies following the more moist creek bottoms, lower benches and flats, and northerly slopes (Wellner, 1962). In order to meet the general objective of describing genetic variation, the following characteristics were considered:

(1) Oleoresin monoterpene concentrations of 293 trees were measured in 1968 by gas chromatography and gave an estimate of non-adaptive genetic differentiation;

(2) From February to July, 1969, measurements of photosysthesis and respiration were made on three-year-old potted seedlings. The objective of these measurements was to determine the possibility of physiological adaptation to the low temperatures, high light intensities, and low carbon dioxide partial pressures characteristic of high altitudes.

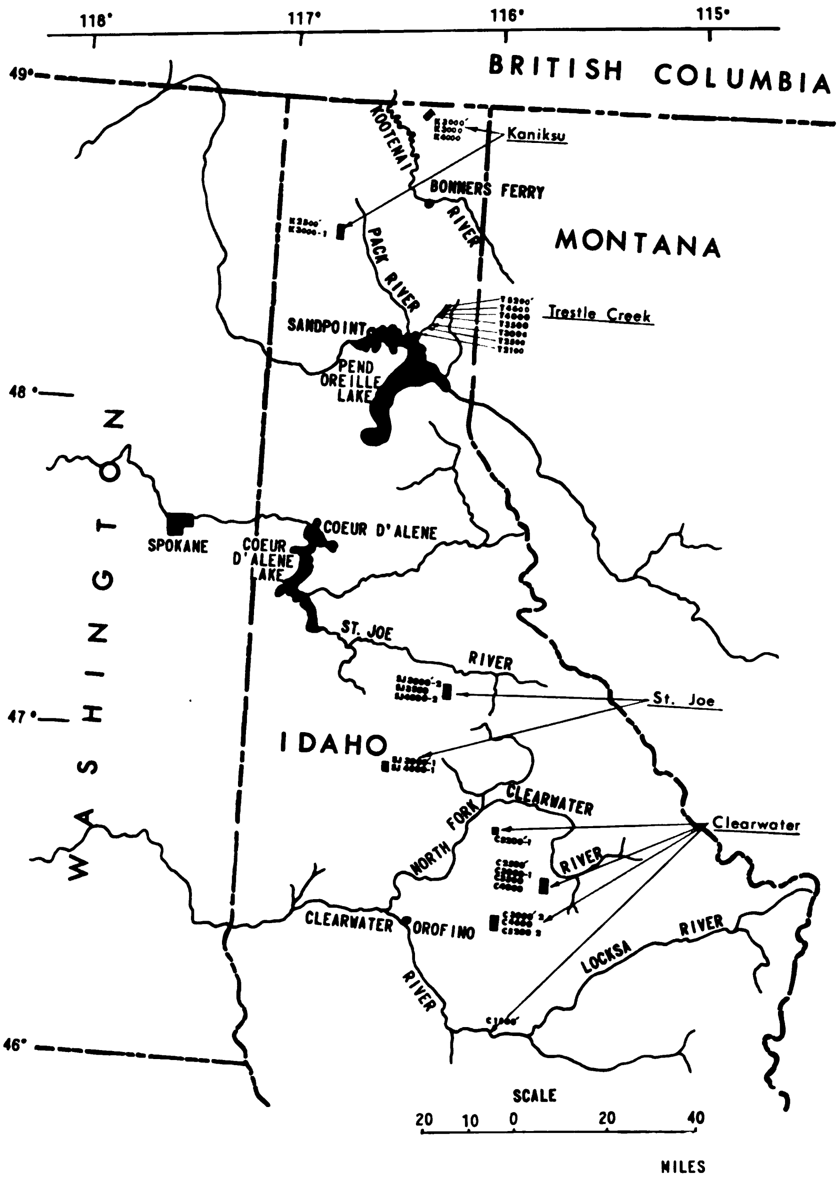

Figure 1. Location of western white pine seed sources used in this study.

(3) Analyses of total height and weekly growth increments of the three-year-old seedlings in 1969 provided a measure of the heritable morphological variation among elevational populations.

The seedlings studied were grown from bulked seed representing eight trees in each of 41 elevational plots. The seed was sown in November, 1965, in a randomized block progeny test at the Tree Research Center, Michigan State University. In the spring of 1968, the two-year-old seedlings were transplanted to give wider spacing in the nursery bed.

CHAPTER I

VARIATION IN MONOTERPENES

Introduction

Recent evidence suggests that monoterpenes in some plants are subject to metabolic turnover (Burbott and Loomis, 1969). Large differences in terpenoid levels both within and between plant species has stimulated their extensive use in biochemical systematic studies (Alston and Turner, 1963; Mirov, 1958; Hanover and Furniss, 1966; Zavarin *et al.*, 1966; Hilton, 1968; and Tobolski, 1968). Hanover (1966a) demonstrated strong genetic control of monoterpene concentrations, and suggested their use as markers for population genetic studies involving diverse environmental gredients.

In many species of the genus *Pinus*, the cortex is characterized by the presence of numerous large resin canals. These canals provide a source of monoterpenes very close to their site of synthesis, which is presumed to be in the thin-walled epithelial cells which surround the resin ducts. In their study of cortical oleoresin from 12 geographic sources of slash pine (*Pinus elliottii*) Squillace and Fisher (1966) found significant differences in myrcene, β-pinene, and β-phellandrene. Both β-pinene and β-phellandrene showed geographic variability patterns very similar to those expressed for height, needle length, and stomata number.

Tobolski (1968) reported significant differences in 11 cortical monoterpenes collected from 108 European and Asian seed sources of Scotch pine (Pinus sylvestris). The terpene 3-carene was absent in most southern populations but increased northward to a high of 63 percent of the monoterpene fraction. α-pinene also exhibited wide variability, but in the opposite direction; Spanish sources showed 69 percent α-pinene but some Scandinavian sources were as low as five percent. Evolution of the species as related to Pleistocene glaciation was postulated to be the primary causative factor for the observed variation.

Hilton (1968) studied cortical monoterpenes in 23 geographic sources of eastern white pine (Pinus strobus). Sources differed significantly in their concentrations of α-pinene, limonene, myrcene, 3-carene, and β-phellandrene. Hilton suggested genetic drift as the cause of the differences, all of which displayed no distinct geographic patterns.

Most of the previous biosystematic investigations of monoterpene levels have involved intraspecific variation associated with widely separated portions of the total range of the species. This type of variation is particularly large where the species involved has a wide distribution.

Variation occurring within relatively small portions of the total range of a species has received very little attention.

Here I report on the variation in monoterpene composition occurring within a relatively small, continuous portion of the range of western white pine. The seedlings used in this study were grown from seed collected in several elevational locations in Idaho, and are being used in an intensive study of local populations in four general geographic areas.

Materials and Methods

Western white pine seed was collected in 1962 through 1964 from elevational plots containing eight parent trees chosen at random from an area of about one acre. The seed sources used for monoterpene analyses are listed in Table 1 and mapped in Figure 1. Seed from each plot was bulked into one seed lot and planted in four replicates at the Michigan State nursery at East Lansing. All collections and analyses of oleoresin were done between May and September, 1968, when the seedlings were two years old. Because slight variation can exist in monoterpene composition among different aged tissues (Hanover, 1966b; Tobolski, 1968), sampling was limited to the one-year-old main leader. The cortex was sliced with a razor blade and the resulting oleoresin exudate was quickly drawn into a 50 µl glass capillary tube, which was then placed in a centrifuge tube, sealed, and refrigerated until analyses were made, usually within several hours.

The analysis of monoterpenes was done by gas-liquid chromatography using a hydrogen flame detector according to the following procedure: Before injection, each sample of oleoresin was diluted with 30 µl of acetone and a 2 µl aliquot of this acetone-resin mixture was injected. Separations were obtained with a 1/4" x 8' stainless steel column packed with 10 percent polypropylene glycol on Chromosorb G-AW. Temperature of the injection port was 195°C, hydrogen flame detector, 200°C, and helium flow rate was 130 ml/min. The identification of individual terpenes was determined by comparing retention times of unknowns with those of known compounds, and by peak enhancement of unknowns using standards. The concentrations of the monoterpenes were expressed as a proportion of the oleoresin.

Results and Discussion

Elevational and Geographic Variation

The terpenes which were analyzed are α-pinene, β-pinene, 3-carene, limonene, and β-phellandrene (Table 1). Other monoterpenes detected but not included in the analysis are camphene, γ-terpinene, myrcene, and terpinolene. Standard concentration curves were not prepared for these compounds because they represent only a few percent of the

Table 1. Monoterpene concentrations in two-year-old western pine cortex in relation to geographic origin.

Seed Source (North to South)	No. of Trees	Monoterpene				
		α-pinene	β-pinene	3-carene	Limonene	β-phellandrene
		---------Mean percent of oleoresin---------				
Kaniksu						
2500'	25	11.6	31.2	3.4	2.2	1.8
4000'	25	10.9	34.0	1.5	2.1	2.6
Mean	50	11.2	32.6	2.5	2.2	2.2
Trestle Creek						
2100'	53	13.6	38.3	2.8	3.0	2.5
3500'	49	11.5	35.5	3.9	2.4	2.5
5200'	41	12.5	33.7	4.1	2.8	2.5
Mean	143	12.6	36.0	3.6	2.7	2.5
St. Joe						
3000'-2	25	15.0	35.8	2.5	3.4	3.6
4000'-2	25	12.0	36.1	3.0	2.5	3.2
Mean	50	13.5	35.9	2.7	2.9	3.4
Clearwater						
2500'	25	11.1	34.1	4.1	3.0	2.4
5200'-2	25	14.3	35.6	3.8	3.4	3.0
Mean	50	12.7	34.8	3.9	3.2	2.7
Mean, all sources	293	12.5	35.2	3.3	3.2	2.7

total monoterpene fraction. Ranges for each terpene were: α-pinene, 2.7 to 34.4; β-pinene, 4.4 to 73.4; 3-carene, 0 to 23.9; limonene, 0.4 to 10.1; and β-phellandrene, 0.3 to 10.8 percent of oleoresin.

Simple correlation analyses between individual terpenes and elevation of the seed source showed no significant relationships:

Monoterpene	Terpene--Elevation Correlation Coefficients
α-pinene	-0.0046 N.S.
β-pinene	-0.0639 N.S.
3-carene	0.0493 N.S.
Limonene	0.0068 N.S.
β-phellandrene	0.0673 N.S.

In order to examine the variation pattern more closely, an analysis of variance was done using each individual stand of origin as a source. Significant differences between sources were found in the concentrations of α-pinene (significance level $P = 0.0005$) and limonene ($P = 0.004$), but the variation pattern appeared to be random and unrelated to elevational or latitudinal differences (Table 1). α-pinene and limonene have varied with location in several other species. For example, significant variation in α-pinene among three populations of *Pinus radiata* in California was reported by Bannister *et al*. (1962).

Hanover and Furniss (1966) distinguished three populations of Douglas-fir on the basis of α-pinene and limonene.

If high or low terpene concentrations are adaptively advantageous, then one might expect natural selection to effect significant correlations between elevation and latitude of seed source and monoterpene levels. As we have seen, however, neither elevation nor latitude and their associated environmental gradients appear to be related to the pattern observed. There is a possibility, therefore, that terpene synthesizing ability is non-adaptive, contributing little to energy production or to biochemical adaptation. Other recent work (Hilton, 1968; Tobolski, 1968) also supports this hypothesis.

King and Jukes (1969) present evidence that considerable opportunity exists at the molecular level for random genetic changes that have no effect upon the fitness of the organism. They estimated that five to 10 percent of all mutations are selectively neutral. Without additional evidence for linkage and natural selection variation in monoterpene concentrations is probably best explained by genetic drift. Random fluctuation and stabilization of gene frequencies could occur in the smaller population subunits of the species' range. Changes in a non-adaptive trait such as terpene concentration could accumulate in each of these subunits. The results of this study, therefore, may be a measure of the manifestations of genetic drift on intraspecific differentiation over varying geographic distances.

The effects of seed source on monoterpene concentrations were also analyzed by using Tukey's multiple range test. The results showed that significant differences in both α-pinene and limonene exist among, but not within, the four geographic areas (Table 2). Significant differences in α-pinene concentration were found between the St. Joe 3000' plot and three other plots in each of the other geographic areas. Limonene also differed between populations. The Kaniksu 4000' plot, for example, was significantly different from two distant populations. The greatest genetic differences were found between Kaniksu 4000' and St. Joe 3000', both from near the northern and southern limits, respectively, of the range sampled. These two sources differed in both limonene and α-pinene levels (Table 2).

Table 2.--Seed sources found significantly different by Tukey's test of α-pinene and limonene concentrations.

Seed Sources Compared				Difference Between Sources
α-pinene:				
St. Joe	3000'	Kaniksu	4000'	4.1**
St. Joe	3000'	Clearwater	2500'	3.9*
St. Joe	3000'	Trestle Creek	3500'	3.5*
Limonene:				
St. Joe	3000'	Kaniksu	4000'	1.3*
Clearwater	5200'	Kaniksu	4000'	1.3*

**Significant at the 1% level.
*Significant at the 5% level.

These results suggest that populations must be widely separated to diverge significantly in concentrations of the monoterpenes. It appears that few cross-fertilization barriers exist which isolate contiguous populations from each other to increase the effectiveness of random fixation and loss of genes.

Of the nine populations, Kaniksu 4000' was lowest in limonene, α-pinene, and 3-carene; and its neighbor, Kaniksu 2500', produced the lowest quantities of β-pinene and β-phellandrene. If values for these two populations are averaged, the most northerly forest, Kaniksu, displays the lowest synthesis of each of the five monoterpenes measured (Table 1). Thus, the northernmost seed sources centered in the Kaniksu National Forest appear to be genetically distinguishable from the western white pine to the south. Because of the low level of significance ($P = 0.10$), variation in the monoterpene fraction of the oleoresin is probably insufficient to differentiate between each of the four geographic areas.

Simple Correlations between Monoterpenes

Correlations between monoterpenes may provide indirect evidence regarding their biosynthetic or genetic relationships. The material in this study offered substantial individual tree variation to test for correlations between monoterpenes without the complicating effects of large intraspecific geographic differences. Four pairs of terpenes showed statistically significant correlations: α-pinene and limonene; β-pinene and limonene; β-pinene and 3-carene; and β-phellandrene and 3-carene. Listed below are the significant correlations found in western white pine along with those of three other species for comparison.

	Western white pine (293 trees)	D.-fir xylem[1] (94 trees)	Scotch pine[2] (54 trees)	Eastern white pine[3] (84 trees)
α-pinene/ limonene	0.246**	-0.31**	N.S.	N.S.
β-pinene/ limonene	0.204**	0.66*	N.S.	N.S.
β-pinene/ 3-carene	-0.214**	N.S.	-0.74**	-0.43**
β-phell./ 3-carene	-0.170*	-	-0.42**	N.S.

*0.05 level
**0.01 level

[1] Hanover and Furniss, 1966.

[2] Tobolski, 1968.

[3] Hilton, 1968.

Hanover (1966a) reported significant correlations for western white pine of 0.256 between limonene and α-pinene, and -0.279 between β-pinene and 3-carene. The negative relationship between β-pinene and 3-carene is reported also in several other species, including Scotch pine (Tobolski, 1968), eastern white pine (Hilton, 1968), and ponderosa pine (Smith, 1964). There are several possible explanations for negative correlations. Alternative pathways from a common precursor, such as the carbonium ion proposed by Juvonen (1966), or from a "pool" of geranyl pyrophosphate as suggested by Tobolski (1968), could account for this negative relationship. Because several possible interpretations can be given to significant correlations among the monoterpenes, however, conclusive evidence will be derived only from the use of tracers and the isolation and characterization of specific substrates and enzymes.

Inheritance of Monoterpenes

Because environmental modification of terpene concentrations is small (Hanover, 1966b), inheritance patterns may be directly discerned from frequency distributions of the terpenes. Figure 2 presents the histograms of six monoterpenes from western white pine.

Normal distribution of α-pinene suggests multiple gene inheritance. Polygenic control for this terpene has also been confirmed in *Pinus elliottii* (Squillace and Fisher, 1966), *Pinus sylvestris* (Tobolski, 1968), and in *Pinus strobus* (Hilton, 1968).

Multigenic control is also suggested for β-pinene. Although this agrees with Hilton's (1968) results, simple inheritance of β-pinene has been inferred from Tobolski's (1968) work with cortical oleoresin of Scotch pine, which displayed a tri-modal β-pinene pattern. Geographic variation may have biased the results in Scotch pine.

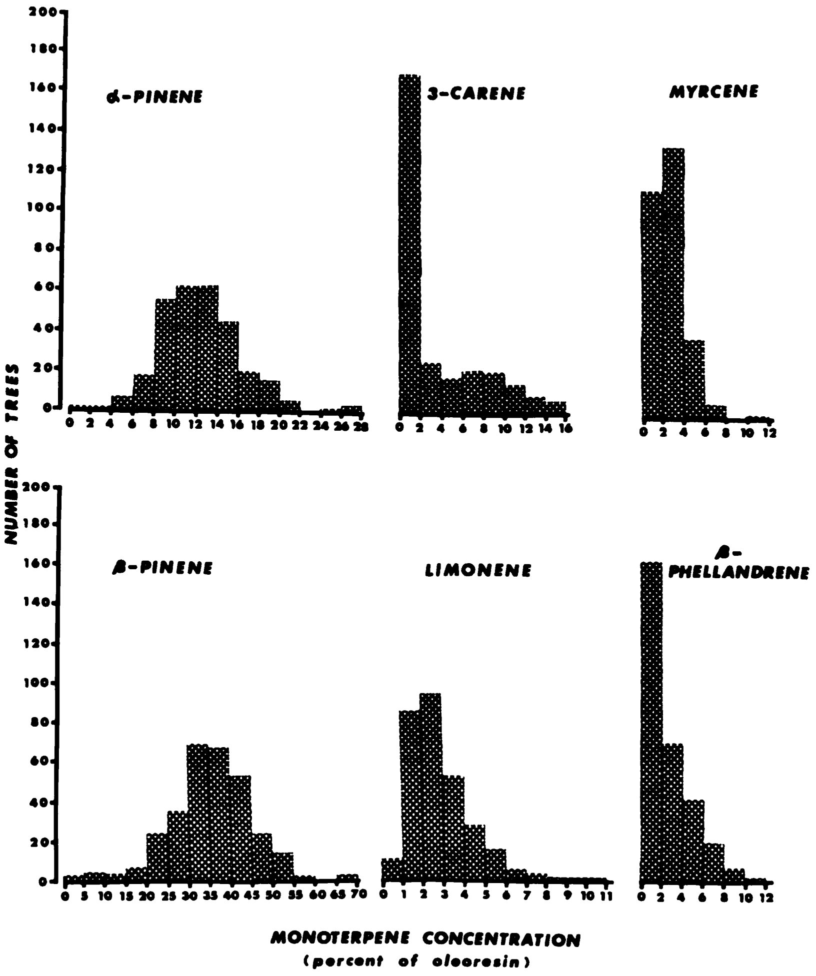

Figure 2. Histograms of monoterpenes from 293 western white pine seedlings.

The histogram of limonene contains many classes, most of which follow a continuous distribution, suggesting polygenic inheritance. This inference is in contrast to that made by two other workers (Hilton, 1968; Tobolski, 1968) who suggested control by one or two genes.

The presence of modifying genes has been postulated for the control of 3-carene in western white pine by Hanover (1966c). He hypothesized that 3-carene level is principally determined by a single dominant and recessive gene pair designated C/c. The trimodal inheritance pattern of 3-carene in this study suggests an incomplete dominance; the intermediate class represents either heterozygous genotypes or modifier genes acting with the major genes. The distribution of 3-carene into three classes has also been found in Scotch pine (Tobolski, 1968), and in eastern white pine (Hilton, 1968).

One or two gene control of myrcene is indicated from its histogram. Simple gene control for β-phellandrene is not clear as for myrcene, but nevertheless the distribution suggests control by few genes. Relatively simple control of these two terpenes has also been shown in Scotch pine (Tobolski, 1968), slash pine (Squillace and Fisher, 1966), and eastern white pine (Hilton, 1968).

CHAPTER II

VARIATION IN PHOTOSYNTHESIS AND RESPIRATION

Introduction

One primary requirement for the evolutionary success of a population of forest trees is that it be capable of making reasonably efficient use of the energy available in its natural habitat. Photosynthesis provides the original source of chemical energy and the primary substrates to drive all other biological activity. In each particular environment photosynthetic efficiency is a vital factor in determining the success of a given population. The question may be asked: Does the photosynthetic efficiency differ within species among populations from contrasting habitats as a result of genetic adaptation?

Genetic differences in photosynthesis have been demonstrated in several plant species. Milner and Hiesey (1964) showed that saturation light intensities of *Mimulus cardinalis* from California increase with elevations of the native habitats. Billings, Clebsch, and Mooney (1961) found that *Oxyria digyna* plants from an alpine race were clearly more effective in fixing CO_2 and in utilizing high light intensities than a sea-level race. Bjorkman and Holmgren (1963) reported that races of Solidago virgaurea from shaded habitats were able to utilize weak light more efficiently than races from exposed habitats. They were also able to relate the temperature optimum for net photosynthesis to the climatic origin of the race. Bourdeau (1963) demonstrated that Pinus strobus seedlings from southern origins were less efficient at low temperatures than more northerly seedlings. Krueger and Ferrell (1965) found a higher light compensation point at 20°C for Douglas-fir seedlings from Montana than for seedlings of the same species from Vancouver Island.

The purpose of the following photosynthetic experiments was to compare physiological responses of different elevational seed source progenies of western white pine grown in an uniform environment. These experiments comprise a major portion of a study designed to determine whether elevational races or ecotypes of western white pine exist in one portion of the species' range.

Materials and Methods

Western white pine seed was collected from plots located at elevations varying from 1500' to 5200' in each of four national forests in Idaho. Seed collected from eight trees in each plot was bulked into single seedlots and sown in November, 1965, in a completely randomized four-replicate nursery progeny test at Michigan State

University. In April, 1968, approximately 150 of the three-year-old seedlings representing 11 plots were potted and kept outdoors prior to use.

Photosynthetic parameters were measured in four separate controlled-environment chambers constructed of acrylite. Each chamber is approximately 50 liters in volume and is surrounded by a water jacket necessary to stabilize temperature. Four 300-watt reflector spot bulbs were immersed in a water jacket above each chamber (Figure 3). Additional temperature and light control was provided by a large Sherer-Gillette controlled environment room, into which all four of the acrylite chambers were placed. A Beckman infrared gas analyzer Model 215 and a Servoriter recorder were used to detect and record CO_2 concentration changes. Small Rotron fans circulated air inside the chambers, and a Cole-Parmer variable speed pump maintained a flow rate of 900 ml/min in a closed system. A "Drierite" ($CaSO_4$) desiccant column removed water vapor from the air flowing into the gas analyzer.

Measurements--During Dormancy

During February and March, 1969, comparisons of photosynthetic response to 22°C and 6600 ft-c were made between high (5200') and low (2100') Trestle Creek seed sources, and between two widely separated seed sources, Clearwater 3500' and Kaniksu 3500'. Prior to each analysis,

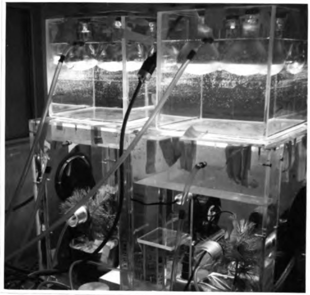

Figure 3. Two of the four controlled environment chambers used in this study.

four seedlings--two from each of the two seed sources to be compared--were picked from the nursery at random, brought into the laboratory, and placed in the four acrylic chambers at conditions of 22°C and 6600 ft-c. Polyethylene bags were wrapped completely around the seedling pots to decrease the effect of evaporation and soil respiration. Net respiration was measured under continuous light for four hours. Dark respiration was measured for two hours immediately following the light period.

Measurements--During Growth

From April to July, daily measurements were made to compare seedlings from three sources, Trestle Creek 2100', 3500', and 5200', in a completely randomized design. Two seedlings representing the same source were placed together inside one of the four chambers. By using all four chambers, measurements could therefore be made on eight trees simultaneously. A minimum of 16 trees from each Trestle Creek source were measured. Light intensities were adjusted to 1400 ft-c, 4600 ft-c, or 6600 ft-c, and temperatures were maintained at either 22°C or 28°C. The rate of depletion of CO_2 from 550 ppm to the compensation point was used as the measure of net photosynthesis. Rates were computed from slopes of lines drawn tangent to the recorder tracings at every 50 ppm interval starting from 500 ppm.

Results and Discussion

Photosynthesis of Dormant Seedlings

During February and March, 1969, 16 trees from each of two sources, Testle Creek 2100' and Trestle Creek 5200', were compared with each other in a paired-t design. No differences were found between the high and the low elevation sources in their response to constant conditions of 6600 ft-c and 22°C. Comparison of eight seedlings from the most northerly source, Kaniksu 3500', with eight seedlings from the southernmost seed source, Clearwater 3500', also failed to show significant differences in net respiration.

Because no significant genetic differences were found, all seedlings were considered together to provide information on the average physiological condition of dormant western white pine trees. Seedlings brought inside and immediately measured during February and March displayed no net photosynthesis (Figure 4).

Several trees were exposed to varying lengths of pre-conditioning at 22°C which showed that at least 72 hours were required for most seedlings to achieve net photosynthesis at 22°C and 6600 ft-c.

These results corroborate the general observation that dormant pine trees are reluctant to show net photosynthesis when brought directly inside during the winter. Several days preconditioning in warm temperatures may be required before seedlings display a capacity for net photosynthesis in the winter. Bourdeau (1959) brought four-year-old Scotch pine seedlings indoors in March and found that three out of nine reached positive rates of net photosynthesis in less than 24 hours, and the balance after less than 48 hours. This response is comparable to that shown by western white pine during March.

It should be emphasized that the response of quiescent seedlings during winter to the unusually high temperature of 22°C gives no sure indication of the net photosynthetic capacity at colder, more natural temperatures. For example, Freeland (1945) observed net photosynthesis in Pinus sylvestris, Picea mariana, and Pinus austriaca nigra down to -6°C at various times from January to late March. Parker (1953) reported net photosynthesis in Picea abies at

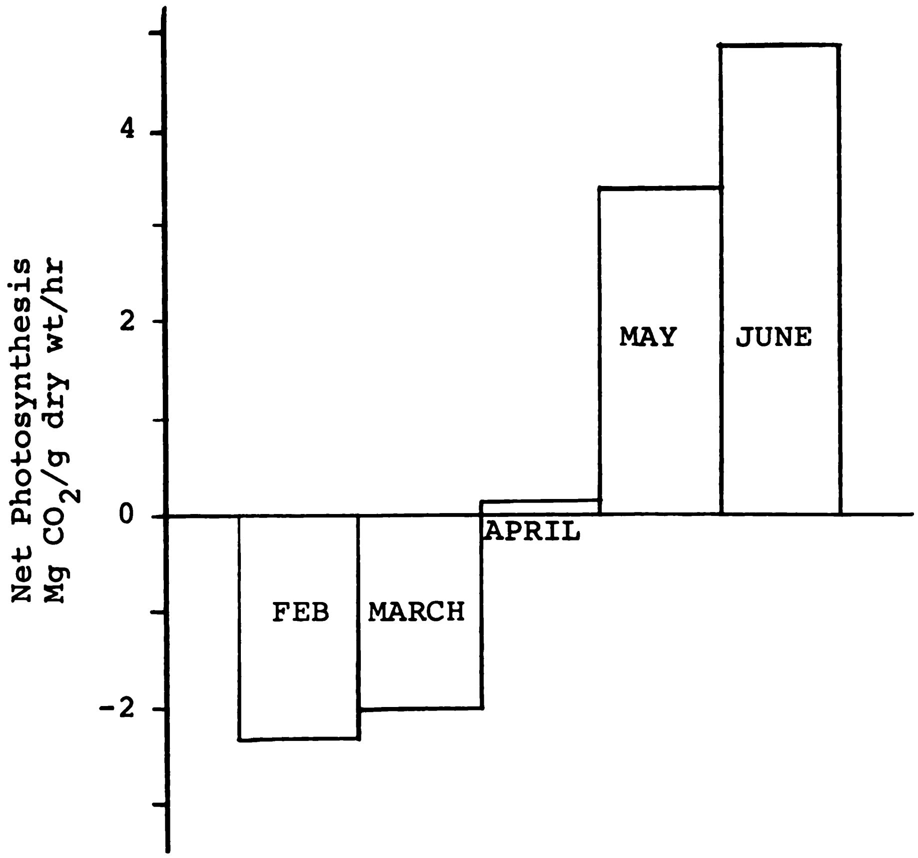

Figure 4.--Seasonal changes in net photosynthesis at 6600 ft-c, 22°C, and 300 ppm CO_2, based on an average of at least 16 seedlings measured each month.

temperatures below freezing. The capacity for net photosynthesis at even the optimum temperature during winter is small, however, when compared to summer CO_2 uptake (Kramer, 1958). Among the causes suggested for this curtailment of net photosynthesis are an end-product accumulation of sugars; a disruption of the chloroplasts (Perry and Baldwin, 1966); stomatal closure; and biochemical factors related to cold hardiness (Parker, 1963).

The first signs of a positive net photosynthesis coincided with the initiation of spring growth. Because the amount of CO_2 uptake and new carbohydrate synthesis is very small, however, large amounts of stored carbohydrates must be utilized early in shoot expansion. Translocated carbohydrates are especially important at this time, because the expanding shoot is a center of rapid metabolism and intense respiration. Kozlowski and Gentile (1958) found a marked and sudden increase in respiration of _Pinus strobus_ buds at the time of bud break. Bourdeau (1959) found high respiration rates in the elongating shoots of _Picea abies_ and _Picea pungens_ during May, followed by a decline and leveling off at the end of the first flush of growth in June. Net photosynthesis in these species was negative until after this initial growth period.

The increase in CO_2 uptake in early May can be partially attributed to the rapid expansion and increased photosynthetic efficiency of the new needles. It is unlikely that the new foliage contributed to net photosynthesis in early and mid-April, when the needles just beginning to appear were tightly enclosed in their fascicle sheaths.

Photosynthesis During Active Growth

Photosynthetic Efficiency.--A factorial analysis of Trestle Creek 2100', 3500', and 5200' sources at three light intensities showed that the overall effect of seed source on photosynthetic efficiency was non-significant (Table 3). Genetic differences were found, however, at the lowest light intensity, 1400 ft-c, at both 22°C and 28°C (Tables 4 and 5). Under these conditions, seedlings from Trestle Creek 2100' showed less photosynthetic efficiency when compared to the progeny of Trestle Creek 3500' and 5200' (Figure 5). Decker (1947) has pointed out that, although carbon dioxide concentration may be the same at high altitudes and low altitudes, the partial pressure decreases with decreasing atmospheric pressure, and this decreases the actual amount of CO_2 diffusing into the leaf. Billings *et al*. (1961) demonstrated that alpine plants of *Oxyria digyna* were more effective in fixing carbon dioxide than sea-level plants of the same species. In a similar way, perhaps the Trestle Creek 3500' and 5200' sources have evolved a more efficient photosynthetic system in order to compensate for low CO_2 partial pressures. This adaptation seems unlikely, however, because of the overall lack

Table 3.--Analysis of net photosynthesis of Trestle Creek sources at different light and CO_2 levels.

Source of Variation	Percent of Total Variance
Source	0
Light	62**
CO_2	14**
Source x light	0
Source x CO_2	0
Light x CO_2	0
Source x light x CO_2	0
Error	24

**Significant at the 1% level.

Table 4.--Analysis of variance of net photosynthesis by three Trestle Creek sources at 1400 ft-c; 500, 450, 400, and 350 ppm CO_2; 22° and 28°C; based on 24 pairs of trees.

Source of Variation	Percent of Total Variance
Source	5**
Temperature	22**
CO_2	18**
Source x temperature	2
Source x CO_2	0
Temperature x CO_2	0
Source x temperature x CO_2	0
Error	53

**Significant at the 1% level.

Table 5.--Analysis of variance of net photosynthesis of juvenile foliage by Trestle Creek sources at 2100', 3500', and 5200', at 500, 450, 400, 350 ppm CO_2, 22°C, and 1400 ft-c.

Source of Variation	Percent of Total Variance
Source	9**
CO_2	26**
Source x CO_2	0
Error	65

**Significant at the 1% level.

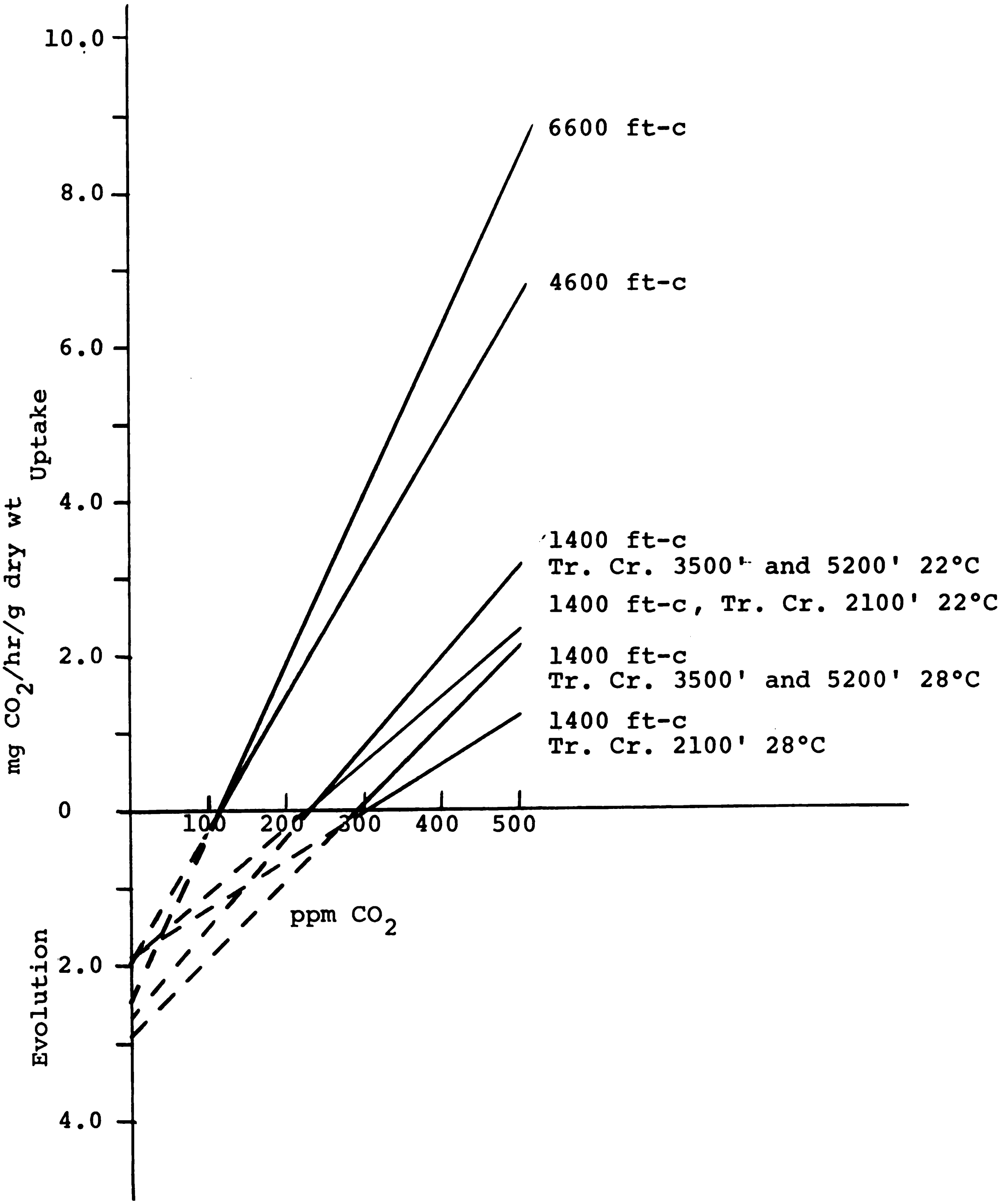

Figure 5.--The rate of net photosynthesis and respiration of western white pine seedlings as a function of CO_2 concentrations at different light intensities and temperatures.

of source differences at 4600 and 6600 ft-c. Decker (1959) compared a clone of Mimulus spp. which grows in the lowlands with one growing at high altitudes. He measured photosynthetic rates as a function of CO_2 concentration at three temperatures and found that both types of Mimulus spp. yielded the same response to the treatments. This evidence did not support the concept of an improved CO_2 absorption system for the alpine plant. The validity of this concept remains unresolved. Perhaps differences in the photosynthetic mechanism develop only when plants are actually grown at high altitudes.

To determine the contribution of mature foliage to photosynthesis, all foliage except the new needles was stripped from the seedlings, and apparent photosynthesis of these seedlings was measured at 1400 ft-c and 22°C. At 1400 ft-c, the same source differences were expressed by the juvenile foliage as by the intact seedlings. When the rate was expressed on a per unit weight of foliage basis, photosynthesis of intact seedlings was markedly less than for seedlings with older foliage removed. The photosynthetic superiority of current-year foliage has been demonstrated in other conifers (Clark, 1961; Freeland, 1952). However, the data presented here should be interpreted with caution. It is very possible that the lower rates of the intact plants are caused by mutual shading of the needles, resulting in less actual light interception by each individual needle. Less interception of light would account for the lower apparent CO_2 uptake by mature foliage in an intact plant. Bormann (1956) has demonstrated the importance of mutual shading in loblolly pine (*Pinus taeda*) seedlings.

In all sources, a shift in temperature from 22°C to 28°C resulted in a marked decrease in net photosynthesis (Tables 4 and 5; Figure 5). It should be apparent, therefore, that the optimum temperature at 1400 ft-c for rapidly growing western white pine seedlings must be lower than 28°C, and perhaps is even less than 22°C. The decreased CO_2 uptake at high temperatures can be attributed to increased respiration which is discussed later.

Effect of CO_2.--Figure 5 illustrates that photosynthetic uptake varied directly with carbon dioxide concentration from the compensation point to 500 ppm CO_2; an orthogonal analysis indicated that a linear line best describes this response. Figure 5 also demonstrates the importance of high light intensities in modifying the net CO_2 absorption; the slope of the photosynthetic-response lines to increased CO_2 levels is greater for high than for low light intensities. This effect has been described in several tree (Kramer and Kozloswki, 1960) and crop (Leopold, 1964) species. It is clear that small changes in atmospheric concentrations of CO_2 could cause large changes in rates of photosynthesis, especially at the high light intensities occurring in the field.

Effect of Light.--The shape of the light curves in Figure 6 indicates that, even at 6600 ft-c, the seedlings were far from light saturation. This was particularly evident at high CO_2 concentrations; the saturation light intensity appeared to be much lower at low CO_2 levels (Figure 6). The curves are similar to those derived from Bourdeau's (1963) experiments with three-year-old *Pinus strobus*, except that *Pinus monticola* appears less efficient under illumination of less than 2000 ft-c. There is good evidence that inefficiency at low intensities can be partially attributed to inherent differences in respiratory activity (Grime, 1966), and to shading of the needles by each other. Kramer and Clark (1947), for example, found that when clusters of loblolly pine needles were spread out so that each one was fully exposed to light, they appeared to be as efficient at low light intensity as broadleaf species.

Photorespiration.--There is now a clear distinction between the processes of light and dark respiration, based on their differential sensitivity to oxygen, temperature, inhibitors, and on the specific activity of substrates used for respiration after the supply of $^{14}CO_2$. It is therefore not valid to assume respiratory activity to be identical in light and in darkness. One major problem has involved the measurement of photorespiration. Because photosynthesis and photorespiration take place simultaneously

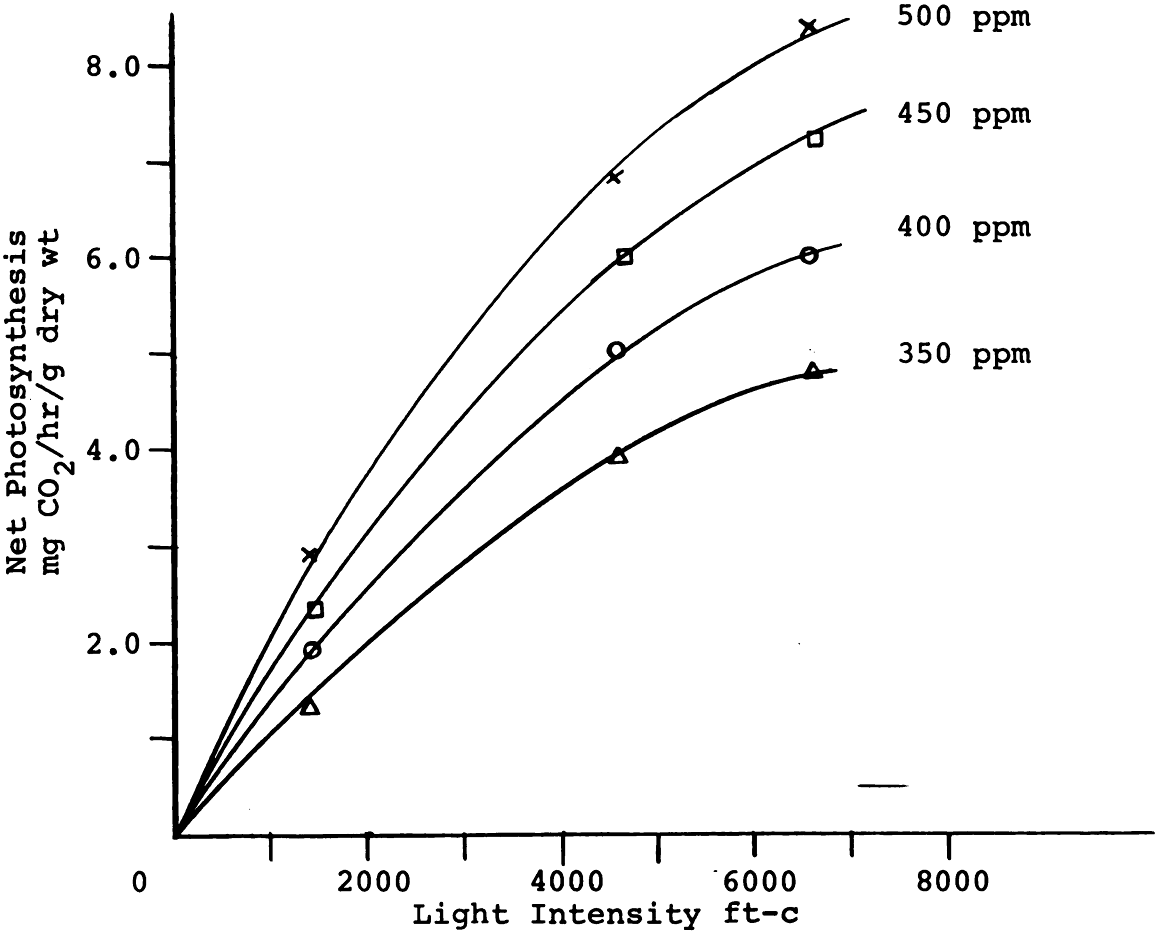

Figure 6.--Light intensity curves for net photosynthesis by western white pine seedlings at different CO_2 concentrations.

in green plants in light, the estimation of either process requires means of distinguishing one from the other. Zelitch (1966) concluded that all the techniques now used to estimate photorespiration are inaccurate because they cannot account for the internal cycling of carbon dioxide; that amount of CO_2 evolved in respiration may not be released to the outside atmosphere but refixed in photosynthesis.

In order to estimate photorespiration, the photosynthetic response curves were extrapolated to zero CO_2 concentration (Figure 5). Although it is simple and easy to apply to photosynthetic data, this method does have major limitations. One serious error may result from assuming that the straight line relationship which exists above the compensation point continues to be linear below this point. Brix (1968) compared the extrapolation method with a technique involving CO_2 evolution into a CO_2-free air-stream, and presented evidence that the slope of the line does in fact change below the compensation point. The accuracy of the method is also dependent on how large the stomatal and internal diffusive resistances are together with the "resistance" to refixation of evolved CO_2 (Bravdo, 1968).

The rates of photorespiration derived by the extrapolation method are shown in Table 6, and indicate the differences between sources in the slopes of the photosynthetic response lines. High rates of net photosynthesis are a result of a low ratio of photorespiration to total

Table 6.--Estimated photorespiration (mg $CO_2 \cdot hr^{-1} \cdot g^{-1}$dry wt) of 48 western white pine seedlings from three sources in relation to light intensity and temperature.

(ft-c)	Trestle Creek 2100'		Trestle Creek 3500' and 5200'	
	22°C	28°C	22°C	28°C
Dark	2.5	2.8	2.5	2.8
1400	1.9	1.9	2.7	2.9
4600	1.9		1.9	
6600	2.4		2.4	

photosynthesis (Zelitch, 1966). Therefore, one would expect a higher rate of photorespiration at 1400 ft-c in the less efficient source, Trestle Creek 2100', compared to the average of the more efficient high elevation sources, Trestle Creek 3500' and 5200'. However, photorespiration as measured by the extrapolation method was lower in the less efficient Trestle Creek source, at both 22°C and 28°C (Table 6). Zelitch and Day (1968) state that little variation in gross photosynthesis has been found within plant species. It seems unlikely, then, that the high elevation sources are enough superior in total, or gross, photosynthesis to more than compensate for high photorespiration. It is more probable that the extrapolation method is much too inaccurate, and should not be relied upon to give precise measures of true photorespiration.

Only at 1400 ft-c and in the high-elevation progeny was the estimate of photorespiration greater than dark respiration (Table 6). All sources increased their rate of photorespiration when light intensity was changed from 4600 to 6600 ft-c. An inexplicable decrease in photorespiration, however, was observed between 1400 ft-c and 4600 ft-c. This data is in contrast with that presented by Brix (1968), who showed that with increases in light intensity above 1000 ft-c, the rate of CO_2 evolution in Douglas-fir rose to a steady rate above that in the dark at 6°, 20°, and 28°C. Holmgren and Jarvis (1967) observed a ratio of light to dark respiration in _Rumex_ _acetosa_ leaves of 1.6 in CO_2-free air at 21°C; Moss (1966) found a corresponding ratio of 1.5 in tobacco at 25°C.

In the dark and at 1400 ft-c, the rate of CO_2 evolution shown by the high elevation sources increased with an increase in temperature from 22°C to 28°C. No similar increase was observed in the Trestle Creek 2100' seed source. More extensive experiments on photorespiration are required to define temperature and seed source effects at all light intensities.

_CO_2 Compensation Point_.--Perhaps a better measure of the effect of light and temperature on respiration is the CO_2 compensation point, or the atmospheric concentration of CO_2 at which respiratory release of CO_2 from illuminated leaves is in balance with photosynthetic CO_2 fixation. Seed source proved non-significant in determining the compensation point at three light intensities and two temperatures

(Tables 7 and 8). At 22°C, a change in light intensity from 1400 ft-c to 4600 ft-c markedly lowered the compensation point, but a further increase from 4600 to 6600 ft-c caused no significant change (Table 9).

Raising the temperature from 22°C to 28°C increased the compensation point, at both high and low light intensities (Table 9). Because a decrease in total photosynthesis with the increase from 22°C to 28°C appears unlikely (Kramer, 1958), temperature must be raising respiration more than photosynthesis. This explanation has been given for similar temperature responses by Decker (1959) for *Mimulus* and by Brix (1968) for Douglas-fir.

It is evident that the temperature effect was more pronounced at low than at high light intensities. Brix (1968) observed a similar temperature effect on Douglas-fir seedlings. He reasoned that low light was more restrictive for photosynthesis than for respiration.

The increase in photosynthetic efficiency at low CO_2 concentrations as the growing season progressed was indicated by a corresponding decrease in compensation points (Table 9). The implications of high CO_2 compensation points in April are important; when growth is just beginning, the photosynthetic abailities of seedlings at normal CO_2 concentrations can be almost negligible. This would be especially true at low light intensities and high temperatures, conditions which promote compensation points above the 300 ppm average of atmospheric air.

Table 7.--Analysis of variance of CO_2 compensation points for three Trestle Creek sources at three light intensities and constant temperature of 22°C. Analysis was based on 48 trees.

Source of Variation	Variance Ratio (F)
Source	0.3
Light	120.7**
Source x Light	0.2

**Significant at the 1% level.

Table 8.--Analysis of variance of CO_2 compensation points for three Trestle Creek sources at 22°C and 28°C, and 1400 ft-c. Analysis was based on 24 pairs of trees, or 48 trees total.

Source of Variation	Variance Ratio (F)
Source	0.4
Temperature	24.8**
Source x temperature	0.7

**Significant at the 1% level.

Table 9.--Mean CO_2 compensation points of western white pine seedlings from Trestle Creek, Idaho seed source. Twelve seedlings were measured in April and 48 seedlings in late May and June.

Light Intensity	22°C		28°C	
	April	Late May and June	April	Late May and June
ft-c	ppm CO_2			
1400	332	251	553	305
4600	--(1)	138	--(1)	--(1)
6600	265	141	313	--(1)

1) Measurements not obtained.

CHAPTER III

VARIATION IN HEIGHT GROWTH

Introduction

Variation in height growth among western white pine populations growing naturally at different elevations in Idaho has been demonstrated by Barnes (1967). He found that periodic height growth was significantly less for trees at 4600 feet and higher than for trees at elevations ranging from 2500 to 4000 feet. Squillace and Bingham (1958) presented evidence that these differences are under genetic control. Growth characteristics of progeny were associated with the elevation of the seed source; progenies from high-elevation sites grew faster at high elevations than did those from low-elevation sites. They concluded that selection pressures that influence seed germination and growth rate restrict the permanent infiltration of genes from trees on adjacent but radically different sites.

Differences in height growth of elevational seed source progeny also have been reported for lodgepole pine (Critchfield, 1957), and for ponderosa and Jeffrey pines (Callaham and Liddicoet, 1961). Ponderosa and Jeffrey pine grown at three elevations for 20 years showed heritable differences in height and diameter growth associated with the elevation of the seed tree. Generally, local seed sources grew best at the three planting sites--960', 2730', and 5650' above sea level.

Materials and Methods

As previously described, western white pine seedlings from 41 seed sources and sown in October, 1965, were grown in four replicates at Michigan State's Tree Research Center. Growth curve patterns were determined by making weekly measurements from March to October, 1969, on 240 trees. To insure measurement from a constant point, insect pins were inserted at or below the 1967 node in each tree selected for observation. A total of 16 trees equally distributed among four blocks represented each of the 15 sources studied.

The study of source differences was based on analyses of variance of the number of days from January 1, 1969, to 5, 25, 50, 75, and 95 percent of the total seasonal growth. The number of days to 5 and 95 percent of growth was used to define the beginning and ending dates of growth. The number of days required to complete 25, 50, and 75 percent of growth was used as a relative measure of the rapidity of growth.

In late August, 1969, measurements were made of the total height of the first 10 trees representing each source and block. The means of the 10 trees measured in each replicate were used in an analysis of variance of seed source effects.

Results and Discussion

Total Height.--Analysis of variance of total height after four seasons of growth indicated that no differences in total height growth exist among progeny of the 27 seed sources examined. Variation could be attributed to blocks within the nursery beds but not to origin of seed sources (Table 10). It is possible, however, that the nursery environment masked genetic differences in height growth, as Hermann and Lavender (1968) found for Douglas-fir. Also, inherent differences in height growth may be more apparent if trees are grown at different elevations and under environmental conditions which are identical to those of the seed sources. Such a differential response to outplanting site has been demonstrated by Squillace and Bingham (1958), who found that progenies of western white pine from high elevations grew more rapidly when outplanted at a high elevation then progenies of low-elevation sources.

Seasonal Growth Rates.--Many investigators have studied the periodicity of seasonal growth and have found much variability among species (Gregory, 1957; Walters and Soos, 1963; Williams, 1968). Except for ponderosa pine (Fowells, 1941; Hanover, 1963) and Douglas-fir (Hermann and

Table 10.--Analysis of variance of total height of western white pine progeny after four seasons of growth.

Source of Variation	Degrees of Freedom	Mean Squares	Variance Ratio (F)
Source	26	16.095	N.S.
Blocks	3	331.120	
Error	78	16.920	
Total	107		

Lavender, 1968; Irgens-Moller, 1968), however, little is known of the variation existing within most forest tree species.

The average growth curve pattern of the 240 seedlings studied is shown in Figure 7. Analyses of variance indicated common beginning (April 29) and ending (August 4) dates of growth for all sources, as measured by the number of days from January 1 required to complete five percent and 95 percent of seasonal growth. Seed source did not affect the number of days needed to complete 75 percent of growth, but did influence the number of days to 25 and 50 percent of seasonal growth (Table 11).

Differences among progenies in relative rapidity of growth were also analyzed using Duncan's new multiple range test. The differences were not consistently related to the latitude or elevation of the seed source (Table 12). Fowells (1941) found that even in natural populations, the minimum

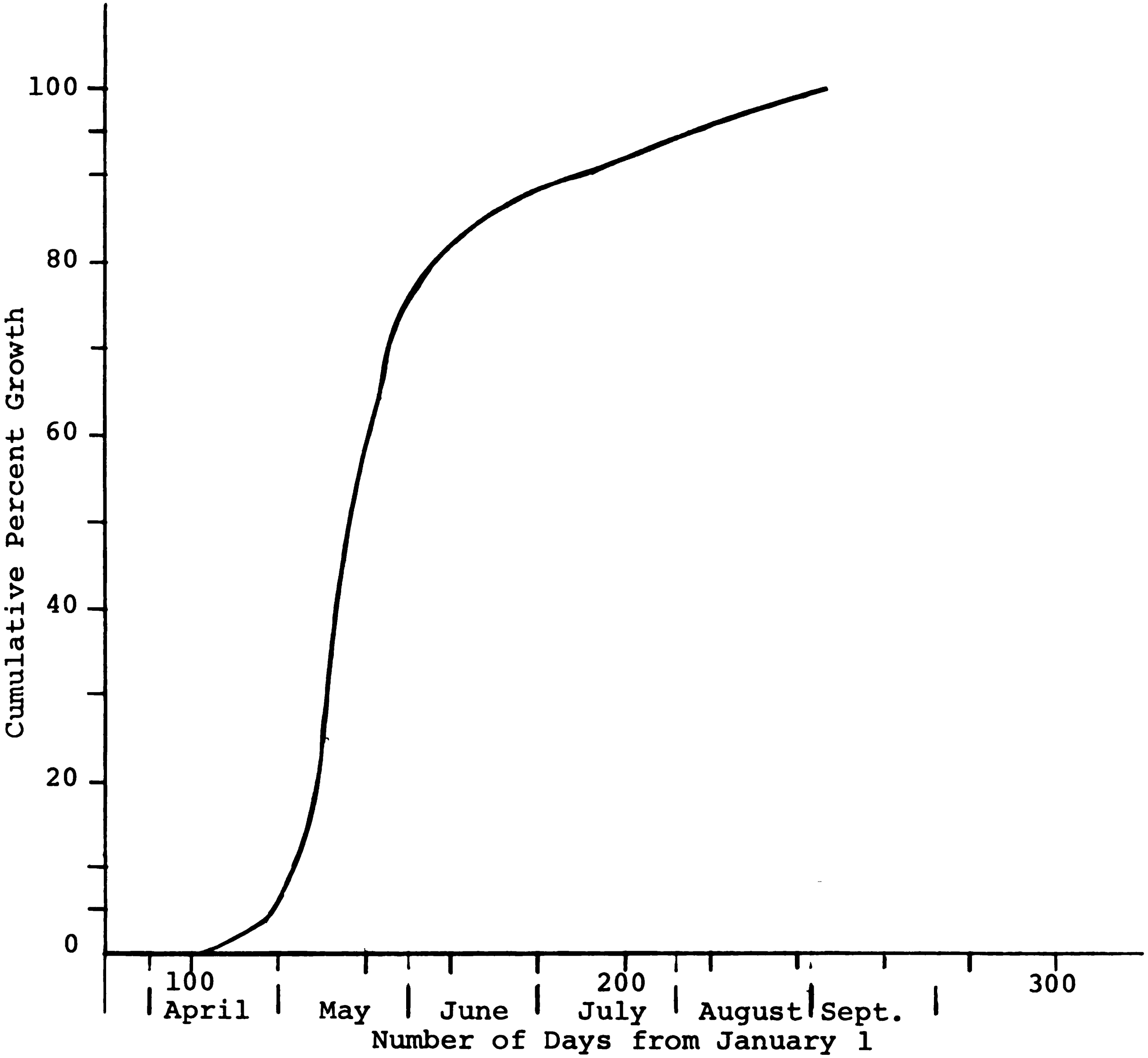

Figure 7.--Average cumulative leader growth of 240 western white pine seedlings grown at MSU, 1969.

Table 11.--Analyses of variance of (1) number of days from January 1, 1969, to complete five, 25, 50, 75, and 95 percent of growth and (2) the number of trees in each source expressing lammas growth.

Source of Variation	Degrees of Freedom	Variance Ratio (F)					
		5%	25%	50%	75%	95%	Lammas Growth
Seed source	14	N.S.	4.08**	2.82**	N.S.	N.S.	N.S.
Blocks	3						
Error	42						
Total	59						
Average Date		4/29	5/10	5/18	5/30	8/4	

**Significant at the 1% level.

Table 12.--Ranking of western white pine seed sources by the number of days from January 1, 1969, to 25 percent and 50 percent of seasonal growth.[1]

25 Percent		50 Percent	
Source[2] (Hundreds of Feet)	Mean Number of Days	Source[2] (Hundreds of Feet)	Mean Number of Days
C 15	125.7	C 30	133.7
T 46, SJ 30, SJ 40	128.2	C 15	136.0
T 52	129.7	SJ 30	137.0
T 30	130.0	SJ 40, T 52, T 46	137.2
T 35, C30	130.2	K 25, T 30	137.7
C 52	130.5	T 35	138.0
K 25, C 25, T 21	130.7	C 52, C 25	138.2
K 40	133.0	T 25, T21	139.2
T 25	134.0	K 40	142.0
T 40	134.2	T 40	142.2

[1] Means included within the same line are not significantly different.

[2] Symbols used to denote seed sources: T = Trestle Creek; K = Kaniksu; C = Clearwater; SJ = St. Joe.

number of days required for ponderosa pine to complete 50 percent of its growth did not vary with altitude. Fowells did find a delay in growth initiation with an increase in altitude, but the delay was significant only when the increase in elevation was 3000 feet.

The Trestle Creek seed sources 2100', 3500', and 5200', which were used in the photosynthetic study, all appeared homogeneous in growth patterns (Table 12). The 4000' population within the Trestle Creek transect was significantly slower in relative growth rapidity compared to its neighbors at 3500' and 4600'. This small difference provides very tenuous support to the idea that natural selection may cause localized differentiation within a few miles, even where the species is continuously distributed.

The Kaniksu 4000' and Clearwater 1500' sources, which probably possess the greatest dissimilarity in environments also appear to have distinctly separate growth periods, at least during the grand period of growth (Table 12). The warm, moist climate of the Clearwater 1500' source apparently has promoted the evolution of a population which can take advantage of good growing conditions in early May by beginning growth earlier. With regard to rapidity of growth, the Clearwater 3000' source is unique for its fast growth rate; little more than three days were required for it to complete the increase from 25 to 50 percent of total growth.

Lammas shoots resulting from bursting and elongation of current-year, terminal buds were observed in 64 percent of the 240 trees. Seed source was not a significant factor in determining the frequency of lammas shoots, however (Table 11). Other investigators have shown appreciable genetic variation in lammas growth. Studies at the Lake States Forest Experiment Station disclosed that frequency of lammas growth, prolepsis, and combinations in both types in *Pinus banksiana* varied significantly at the 0.01 level between seed sources (Kozlowski, 1964). Wright and Bull (1962) found strong genetic control of lammas shoot growth in *Pinus nigra*.

The ecological significance of all the growth data analyses is not readily apparent. Few differences among sources were expressed, and those that did exist, at 25 and 50 percent of growth, were relatively small; the difference between the fastest and slowest growing sources was only eight days (see Table 12). It is unlikely that these small differences in growth patterns represent ecological adaptation to seed source habitats, except perhaps to the two sources, Clearwater 1500' and Kaniksu 4000', which are distinctly separate in growth patterns and in habitat environmental factors. The rapidity of growth of most of the sources fails to follow any consistent order related to environmental gradients of altitude and latitude, and therefore cannot be easily explained.

The form of the growth pattern would appear to have adaptive significance, because a population would be required to make sufficient growth within the limits of the growing season at its particular habitat. The intensity of selection against genotypes which fail to make significant height growth during conditions favorable for the grand period of growth could be very high. Those genotypes which prematurely complete their grand period of growth during unfavorable environmental conditions such as cold or dry periods also may be at a competitive disadvantage. Plants that are able to make optimum use of conditions throughout the full growing season would be at a competitive advantage, and would show maximum height growth and dry matter production.

Seasonal height growth often has been represented as a continuous process which can best be characterized by a smooth sigmoid curve. Such a curve was shown for the average of all sources of western white pine (Figure 7). Since this curve was based on averaged observations which were taken on 240 trees and spaced in time, it probably obscures the actual intermittency of height growth, which has been demonstrated by short-time observations of shoot elongation of individual trees (Kozlowski, 1964).

Height growth initially followed a moderate regular weekly increase followed by the grand period of growth in May. A tapering-off of height growth occurred in mid-June, and decreased eventually to zero during September. This pattern differs substantially from the pattern displayed by western white pine in Oregon (Williams, 1968) and in British Columbia (Walters and Soos, 1963). In both of these cases, the grand period of growth occurred later and total growth terminated earlier than the seedlings observed in this study. The Oregon trees took until June 17 in 1963 and until July 7 in 1964 to complete 50 percent of their seasonal growth, and completely terminated growth by the end of August. The environmental conditions of the high elevation site would explain the relatively short growing period of these trees. The British Columbia western white pines also were characterized by a short duration of growth; little more than a month was required to complete from five to 95 percent of total seasonal growth:

	Location	
Cumulative Percentage Growth	Michigan State University	Univ. British Columbia (Walters and Soos, 1963)
	--------Date of Completed Growth-------	
5	April 29	May 23
25	May 10	June 3
50	May 18	June 10
75	May 30	June 18
95	August 4	June 29

With regard to environmental effects on shoot growth patterns, it is important to remember that western white pine possesses buds containing preformed shoots.

Much of the potential of preformed shoots is formed during the year of bud formation. Such shoots begin elongation in the spring using carbohydrate reserves assimilated during the previous season. Because of this dependence on carbohydrate reserves, shoot growth often shows closer correlation with weather of the year of bud formation than with the year of shoot elongation (Kramer and Kozlowski, 1960). However, weather during the period of shoot elongation does influence the degree of realization of shoot growth potential (Hanover, 1963; Williams, 1968).

SUMMARY AND CONCLUSIONS

The most important conclusion that can be drawn from this study is that the western white pine seed sources studied represent a relatively homogeneous population; an overall lack of genetic differentiation in relation to seed source was observed in the physiological, morphological, and biochemical traits chosen for analysis. The concentrations of two of the five monoterpenes were influenced by the location of the seed source. The terpene differences I observed occurred only between widely separated areas, and were unrelated to the elevation or latitude of the parent trees. Seed origin did not appear to be an important determinant of photosynthetic efficiency or CO_2 compensation point. At only one of the three light intensities examined was the seed source important in influencing photosynthetic efficiency. No differences among progeny were observed in the compensation point, at three light intensities and two temperatures. Height growth parameters were characterized by a similar homogeneity. There were no distinctions among sources in total height, and only slight differences in the form of their growth curves.

The relative homogeneity of the progeny grown at Michigan State University does not substantiate Squillace and Bingham's (1958) evidence of racial variation (within Pinus monticola) occurring over distances as short as half a mile in the mountains of northern Idaho and western Montana. They hypothesized that such variation occurs where topography is highly variable and summer drought prevails. Under these conditions, selection pressures on seedling establishment and growth could discriminate against infiltration of genes from trees adapted to sites that are readically different although adjacent. Hermann and Lavender (1968) have shown that this same hypothesis may apply to Douglas-fir in southern Oregon. They found striking differences in reaction to a long-day cold-night regime between two north and south aspect seed sources, only one mile apart. A recent review by Erlich and Raven (1969) also presents evidence that, in many plant species selection pressures can produce what is effectively a racial divergence along with an apparently high level of intercrossing.

Several reasons can be postulated for the lack of differentiation in the western white pine progeny considered in this study. The breeding system of western white pine may be of major importance in maintaining a uniform range of variability. Cross-pollination promotes the level of heterozygosity and thereby promotes the effect of recombination and release of genetic variation.

Another important consideration is that most adaptive traits in forest trees seem to be controlled by several genes each with small individual effects. Mather has shown that when polygenic control is operative, a constant phenotypic frequency distribution in a population can be maintained by selection with the simultaneous preservation of a high level of genotypic variation (Heslop-Harrison, 1964). Because a given phenotypic expression can be determined by several polygene combinations, the selectively favored trees in each generation may be essentially uniform in adaptive characters, but will always transmit genetic diversity to their progeny. In this way, plasticity and flexibility are retained in the species.

In addition to genetic homeostasis, the developmental flexibility and plasticity of individual trees may enable them to function satisfactorily in a wide range of environments. This attribute may be especially important in this study, in which only <u>one</u> location, at a low elevation, was used for the comparison of high and low elevation genotypes. By eliminating environmental variation normally encountered in the native habitats, the genetically determined differences in the capacity to react adaptively to high-elevation habitats may be obscured. It may be possible, therefore, that only the environment of their native habitat can evoke a unique adaptation by high-elevation progeny.

Another possible reason for a lack of seed source differences is that too few trees were sampled to represent the different populations. As Roche (1968) has pointed out, it is the population which is evolving, not the individual. Variation among individual trees is dominated by gene mutation and genetic recombination, not by natural selection (Stebbins, 1950). It may well be impossible to relate variation in individual trees or small numbers of trees of different populations to factors of their environment at their place of origin.

The practical implications of this study may be important. The U.S. Forest Service maintains separate elevational and geographic seed collection zones for western white pine in Idaho. Because the results of this study indicate that latitude and elevation are relatively unimportant, the validity of these seed collection zones is questionable. However, additional work must be undertaken to determine whether the homogeneity observed in the nursery is retained in outplantings at several elevations and in different geographic regions.

LIST OF REFERENCES

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Alston, R. E., and B. L. Turner. 1963. Biochemical Systematics. Prentice-Hall, Inc., Englewood Cliffs, N.J. 404 pp.

Bannister, M. H., A. L. Williams, I. R. C. McDonald, and M. B. Forde. 1962. Variation of turpentine composition in five population samples of Pinus radiata. New Zealand Jour. Sci. 5:486-495.

Barnes, B. V. 1967. Phenotypic variation associated with elevation in western white pine. Forest Sci. 13: 357-364.

Billings, W. D., E. E. C. Clebsch, and H. A. Mooney. 1961. Effect of low concentrations of carbon dioxide on photosynthesis rates of two races of Oxyria. Science 133:1834.

Bjorkman, O., and P. Holmgren. 1963. Adaptability of the photosynthetic apparatus to light intensity in ecotypes from exposed and shaded habitats. Physiol. Plant. 16:889-914.

Bormann, F. H. 1956. Ecological implications of changes in the photosynthetic response of Pinus taeda seedlings during ontogeny. Ecology 37:70-75.

Bourdeau, P. F. 1963. Photosynthesis and respiration of Pinus strobus L. seedlings in relation to provenance and treatment. Ecology 44:710-716.

________. 1959. Seasonal variations of the photosynthetic efficiency of evergreen conifers. Ecology 40:63-67.

Bravdo, B. A. 1968. Decrease in net photosynthesis caused by respiration. Plant Physiol. 43:479-483.

Brix, H. 1968. Influence of light intensity at different temperatures on rate of respiration of Douglas-fir seedlings. Plant Physiol. 43:389-393.

Burbott, A. J., and W. D. Loomis. 1969. Evidence for metabolic turnover of monoterpenes of peppermint. Plant Physiol. 44:173-179.

Callaham, R. F., and A. R. Liddicoet. 1961. Altitudinal variation at 20 years in ponderosa and Jeffrey pines. Jour. Forestry 59:814-820.

Clark, J. 1961. Photosynthesis and respiration in white spruce and balsam fir. State Univ. College of Forestry. Syracuse, N.Y. 72 pp.

Clausen, J., and W. M. Hiesey. 1958. Experimental studies on the nature of species. IV. Genetic structure of ecologic races. Carnegie Inst. Wash. Pub. 615. Washington, D.C. 312 pp.

Clausen, J., D. D. Keck, and W. M. Hiesey. 1947. Heredity of geographically and ecologically isolated races. Amer. Natur. 81:114-133.

Critchfield, W. B. 1957. Geographic variation in *Pinus contorta*. *Maria Moors Cabot Foundation Pub. 3. Cambridge*, Mass. 118 pp.

________. 1947. Effect of air supply on apparent photosynthesis. Plant Physiol. 22:561-571.

________. 1959. Some effects of temperature and carbon dioxide concentration on photosynthesis of *Mimulus*. Plant Physiol. 34:103-106.

Erlich, P. R., and P. H. Raven. 1969. Differentiation of populations. Science 165:1228-1232.

Fowells, H. A. 1941. The period of seasonal growth of ponderosa pine and associated species. Jour. Forestry 39:601-608.

Freeland, R. O. 1945. Apparent photosynthesis in some conifers during winter. Plant Physiol. 19:179-185.

________. 1952. Effect of age of leaves upon the rate of photosynthesis in some conifers. Plant Physiol. 27:685-690.

Gregory, R. A. 1957. A comparison between leader growth of western conifers in Alaska and Vancouver Island. Tech. Note No. 36. Alaska For. Res. Center, U.S. Forest Service, Juneau, Alaska. 2 pp.

Grimes, J. P. 1966. Shade avoidance and shade tolerance in flowering plants, pp. 187-207. In: Bainbridge, R., G. C. Evans, and O. Rackham (eds.). Light as an Ecological Factor. John Wiley and Sons, Inc., New York. 452 pp.

Hanover, J. W. 1963. Geographic variation in ponderosa pine leader growth. Forest Sci. 9:87-95.

________. 1966a. Genetics of terpenes. I. Gene control of monoterpene levels in *Pinus monticola* Dougl. Heredity 21:73-84.

________. 1966b. Environmental variation in the monoterpenes of *Pinus monticola* Dougl. Phytochem. 5: 713-717.

________. 1966c. Inheritance of 3-carene concentration in *Pinus monticola*. Forest Sci. 12:447-450.

Hanover, J. W., and M. M. Furniss. 1966. Monoterpene concentration in Douglas-fir in relation to geographic location and resistance to attack by the Douglas-fir beetle. U.S. Forest Service Res. Paper NC-6:23-28.

Hermann, R. K., and D. P. Lavender. 1968. Early growth of Douglas-fir from various altitudes and aspects in southern Oregon. Silvae Genetica 17:132-151.

Heslop-Harrison, J. 1964. Forty years of genecology. Advances in Ecological Research 2:159-247.

Hilton, R. L. 1968. Genetic variation and interrelationships of the cortical monoterpenes, foliar mineral elements, and growth characteristics of eastern white pine. Ph.D. Thesis, Michigan State Univ. 113 pp.

Holmgren, P. H., and P. H. Jarvis. 1967. Carbon dioxide efflux from leaves in light and darkness. Physiol. Plant. 20:1045-1051.

Irgens-Moller, H. 1968. Geographical variation in growth patterns of Douglas-fir. Silvae Genetica 17:106-110.

Juvonen, S. 1966. Uber die die Terpenbiosynthese beeinflssenden Faktoren in *Pinus sylvestris* L. Acta Botanic Fennica 71. 92 pp.

King, J. L., and T. H. Jukes. 1969. Non-Darwinian evolution. Science 164:788-798.

Kozlowski, T. T. 1964. Shoot growth in woody plants. The Botanical Review 30:335-392.

Kozlowski, and A. C. Gentile. 1958. Respiration of white pine buds in relation to oxygen availability and moisture content. Forest Sci. 4:147-152.

Kramer, P. J. 1958. Photosynthesis of trees as affected by their environment, pp. 157-186. In: Thimann, K. V. (ed.). The Physiology of Forest Trees. The Ronald Press Company. New York. 678 pp.

Kramer, P. J., and W. S. Clark. 1947. A comparison of photosynthesis in individual pine needles and entire seedlings at various light intensities. Plant Physiol. 22:51-57.

Kramer, P. J., and T. T. Kozlowski. 1960. Physiology of Trees. McGraw-Hill Book Company, Inc., New York. 642 pp.

Krueger, K. W., and W. K. Ferrell. 1965. Comparative photosynthetic and respiratory responses to temperature and light by *Pseudotsuga menziesii* var. *menziesii* and var. *glauca* seedlings. Ecology 46: 794-801.

Leopold, A. C. 1964. Plant Growth and Development. McGraw-Hill Book Company, New York. 466 pp.

Milner, H. W., and W. M. Hiesey. 1964. Photosynthesis in climatic races of *Mimulus*. I. Effect of light intensity and temperature on rate. Plant Physiol. 39:208-213.

Mirov, N. T. 1958. Distribution of turpentine components among species of the genus *Pinus*, pp. 251-268. In: Thimann, K. V. (ed.). The Physiology of Forest Trees. Ronald Press Co., New York. 678 pp.

Moss, D. N. 1966. Respiration of leaves in light and darkness. Crop Sci. 6:351-354.

Parker, J. 1953. Photosynthesis of *Picea excelsa* in winter. Ecology 34:605-609.

________. 1963. Causes of the winter decline in transpiration and photosynthesis in some evergreens. Forest Sci. 9:158-166.

Perry, T. O., and G. W. Baldwin. 1966. Winter breakdown of the photosynthetic apparatus of evergreen species. Forest Sci. 12:298-306.

Roche, L. 1968. The value of short term studies in provenance research. The Commonwealth Forestry Review 47:14-26.

Smith, R. H. 1964. Variations in the monoterpene composition of ponderosa pine oleoresin. U.S. Forest Service Res. Paper PSW-15. 17 pp.

Squillace, A. E., and R. T. Bingham. 1958. Localized ecotypic variation in western white pine. Forest Sci. 4:20-24.

Squillace, A. E., and G. S. Fisher. 1966. Evidences of the inheritance of turpentine composition in slash pine. U.S. Forest Service Res. Paper NC-6: 53-59.

Stebbins, G. L., Jr. 1950. Variation and Evolution in Plants. Columbia Univ. Press. New York. 643 pp.

Tobolski, J. J. 1968. Variation in monoterpenes in Scotch pine (Pinus sylvestris L.). Ph.D. Thesis, Michigan State Univ. 125 pp.

Turesson, G. 1922. The genotypical response of the plant species to the habitat. Hereditas 3:211-350.

Walters, J., and J. Soos. 1963. Shoot growth patterns of some British Columbia conifers. Forest Sci. 9: 73-85.

Wellner, C. A. 1962. Silvics of western white pine. U.S. Forest Service Intermountain Forest and Range Exp. Sta. Misc. Pub. 26. 24 pp.

Williams, C. B., Jr. 1968. Seasonal height growth of upper-slope conifers. U.S. Forest Service Res. Paper PNW-62. 7 pp.

Wright, J. W., and W. I. Bull. 1962. Geographic variation in European black pine--two-year results. Forest Sci. 8:32-42.

Zavarin, E., N. T. Mirov, and K. Snajberk. 1966. Turpentine chemistry and taxonomy of three pines of southeastern Asia. Phytochem. 5:91-96.

Zelitch, I. 1966. Increased rate of net photosynthetic carbon dioxide uptake caused by the inhibition of glycolate oxidase. Plant Physiol. 41:1623-1631.

Zelitch, I., and P. R. Day. 1968. Variation in photorespiration. The effect of genetic differences in photorespiration on net photosynthesis in tobacco. Plant Physiol. 43:1829-1837.