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Title: GEOGRAPHICAL VARIATION IN APPARENT NET PHOTOSYNTHESIS OF ROCKY MOUNTAIN DOUGLAS-FIR, PSEUDOTSUGA MENZIESII VAR. GLAUCA

Abstract approved: Signature redacted for privacy.
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Rates of apparent net photosynthesis were measured on a sample of Rocky Mountain Douglas-fir consisting of eleven one-year-old seedlings from each of the thirteen different seed sources. Secondary observations of needle length, needle weight, and needle number were also obtained. A formula derived through step-wise multiple regression of the secondary observations on the rate of photosynthesis controlled 87% of the variation. The significance of the regression formula indicated the importance of the process of mutual shading. Analysis of variance of the un-adjusted rates of photosynthesis was significant between and within seed sources. After adjustment only seed source differences were significant. Analysis of variance of the regression formula was significant between and within seed sources. By the process of contrasting sums of squares, climatic

factors at the origin of the seed sources were found significantly to effect only the morphological factors. Therefore adaptation of the photosynthetic process by morphology to an environment is probable. The importance of this study is that the processes used to determine the adjusted rate of photosynthesis enable a better estimate of the apparent net photosynthesis than has been possible before with Douglas-fir.

Geographical Variation in Apparent Net Photosynthesis of Rocky Mountain Douglas-fir, *Pseudotsuga menziesii* var. *glauca*

by

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INTRODUCTION

Rocky Mountain or inland Douglas-fir (Pseudotsuga menziesii var. glauca (Beissn.) Franco) has an immense distribution extending from 20° North latitude in north-central Mexico to 55° North latitude in British Columbia. Within this range it is limited to the microthermal temperature province, mostly within the humid to subhumid subdivision, in which rainfall is sufficient in all seasons or limiting only in the summer (U. S. Forest Service 1965).

The rate of photosynthesis of a plant is a function of its physiological status as affected by past and present evironment and its genetic complement. Each of these factors is not an entity within itself but each is confounded with the other. The present study attempts to relate the variation in the rate of apparent net photosynthesis to possible genetic differences as well as possible adaptations suggested by the history of the species.

The genus Pseudotsuga enters the fossil record during the early Tertiary in Europe (Arnold, 1947), Asia, and North America (Cain, 1944), already evolved into a variety of types. In the Eocene the genus was associated with the Arcto-tertiary Flora with a temperate mesophytic nature, suggesting that it ranged from within 8° of

the North Pole to central Colorado in the newly formed Rocky Mountains. The genus was but a minor component of the temperate upland forest at that time. The forest with which it was associated was probably composed of Sequoia, Abies, Ulmus, Fagus, and Lithocarpus (Chaney, 1947; 1938; Daubenmire, 1969). During the Oligocene the Rockies limited eastward migration of many of the coniferous forest species, but a general cooling trend permitted migration southward. The temperate latitudes continued to support mixed-coniferous forests with the genus Pseudotsuga a minor component not only on the North American continent (Stebbins, 1947), but also in the Asian mixed-coniferous forests (Daubenmire, 1969). In the Miocene epoch, many geological and climatic changes occurred which imposed intense selective pressures on the western forests. The Coast Range emerged from the ocean and the Cascades and Sierra Nevadas became high mountain ranges. The rain shadow resulting from these ranges halted east-west interchange of genetic material between the Rockies and the Cascades and Sierras, where broad valleys had permitted such migration in the Oligocene (Whittaker, 1961). Floristic provincialism was more pronounced than in previous epochs. The western forest became five distinct forest provinces with Pseudotsuga present in each. It occupied slightly different sites in these provinces and had distinct morphological differences as shown by the Trapper Creek Flora (Axelrod, 1964), Mount Eden

Beds (Axelrod, 1938), and Deschutes Flora (Chaney, 1938), to name but a few. Later during this epoch a much drier climate developed accompanied by progressive retreat of the forest toward the coast and higher elevations, which increased differentiation of the forest types. This climate along with extremes in temperatures continued well into the Pliocene causing widespread replacement of the Arcto-tertiary forests. Within the next epoch, the Pleistocene, minor glaciation further fractionated the forests of the West, eliminating certain components.

This brief sketch of the history indicates the possible cause of some of the variation that may exist in Douglas fir. It is hoped that the photosynthetic response of diverse seed sources may be indicative of their past history. Adaptations to differences in climate and selective pressure may be important in differentiating phenotypes within these seed sources with respect to photosynthesis. Probably more important is the possibility that selection of genotypes based on the photosynthetic response may become applicable in the future.

LITERATURE REVIEW

The literature dealing with photosynthetic responses of ecological races within a species is scant with only a few species being represented. Much of the earlier work is contained in a short review by Hiesey and Milner (1965). Among the herbaceous species, Solidago virgaurea from shaded and exposed habitats was found to have differential photosynthetic responses to saturating light. This effect was attributed to differences in the amount of carboxydismutase (Björkman, 1968; 1968a), differences in leaf structure affecting stomatal and mesophyll resistance, and possible hormonal control (Holmgren, 1968). Thus, populations of S. virgaurea within close proximity of each other had become genetically adapted to two differing environments, enabling survival under different light intensities.

Mooney and Billings (1961) measured apparent net photosynthesis in Oxyria digyna in an effort to determine how that species was able to grow so successfully in such a wide array of habitats. They first showed that alpine types had a greater affinity for CO_2 than arctic lowland plants. They later found that arctic plants had a lower optimum temperature, and could utilize light of lower intensity more efficiently (Mooney and Billings, 1961; Billings, Clebsch and Mooney, 1961).

Genetic adaptations to diverse habitats in these two herbaceous species resulted partly from differences in responses to light, affinity for CO_2, enzymatic efficiency, and responses to temperature. Therefore, it seems reasonable to assume that selection has resulted in similar adaptations of the photosynthetic apparatus in a variety of forest tree species. Support for such a notion is not readily found in the literature.

Bourdeau (1963) found differences in photosynthesis and respiratory responses of six ecotypes of Pinus strobus, but only under certain conditions. Seedlings grown out-of-doors had higher rates of photosynthesis than greenhouse-grown seedlings, except at low light intensity. The extrapolated light compensation point was highest for outdoor grown seedlings. Reduction in temperature caused greater depression in rates of photosynthesis in seedlings grown indoors while having little effect on seedlings grown outdoors. Since plants from both treatments had the same water content and outdoor grown seedlings had significantly less chlorophyll and more starch grains, the significantly higher rates of the outdoor grown seedlings could not be attributed to greater hydration, higher chlorophyll content or accumulation of starch as an end product of photosynthesis. Therefore, it seems reasonable to assume that pre-conditioning greatly modified the response of all ecotypes. It was concluded that racial differences in photosynthesis does exist in this species, and that the

southern race was more shade tolerant.

McGregor, Allen and Kramer (1961) found differences between two ecotypes of Pinus taeda from Georgia and Florida when rates of photosynthesis were expressed as the CO_2 exchange rate per seedling. If the rate was expressed on a per-fascicle-length basis no difference was found. Therefore the differential response was due to differences in the size of the fascicle of the two ecotypes, with one having more photosynthetic tissue on a per-plant basis.

Zavitkovski and Ferrell (1968; 1970) studied the effect of drought on photosynthesis, respiration, and transpiration of a xeric and a mesic ecotype of Pseudotsuga menziesii seedlings two to three months and two years old. They found distinct behavioral differences in these physiological parameters. Specifically, the rate of photosynthesis at high soil moisture and high relative turgidity was greater for the xeric type while at low soil moisture and turgidity the mesophytic type had greater apparent assimilation. However, when the soil moisture and relative turgidity is correlated with the rate of photosynthesis, the seedlings from the xeric area were more efficient with respect to conserving water and maximizing photosynthetic production. Also, as the total weight of the needles of the plant increased the photosynthetic rate decreased. Cause of this phenomenon was attributed to self-shading or increased boundary-layer resistance.

The papers cited above allude to the idea that structural as well

as physiological adaptation of plants to their environment strongly influences their ability to carry on photosynthesis. If we are to account for these modifying factors between different ecotypes then we must devise a base for comparison of the rates of photosynthesis of the various ecotypes. Decker (1955) and Watson (1952) question whether this will ever be possible on an ecologically meaningful basis. Ledig (1969) produced a growth model based on the rate of photosynthesis, photosynthate distribution, and seasonal duration of the photosynthetic maximum. This indicated the importance of different genotypes or similar genotypes in different environments affecting the proportion of photosynthate allocated to the various parts of the plant causing differences in morphology. These morphological differences would in a secondary way affect the total photosynthesis of the plant. For instance, the more photosynthate allocated to the growth of the needles the more the total photosynthesizing surface area is increased. This increased surface area may increase the plants photosynthetic production or it may in fact cause a decrease due to self-shading and boundary-layer resistance.

Since it is difficult to measure the rate of photosynthesis of one needle, the rate of an individual plant part must be adjusted for its morphological characteristics in order to compare it with a part from another plant. In making this adjustment both the

morphological characters most influential in modifying the rate of photosynthesis as well as a better estimate of the rate are obtained.

METHODS AND MATERIALS

Plant Material

The experimental plant material was selected from one-year-old stock growing in four inch pots in the cold frames at the Oregon State University Forest Research Laboratory. They were derived from seed collected in 1961 by Dr. Frank Sorenson from various localities within the Rocky Mountains. An effort was made to select samples evenly distributed throughout the Rocky Mountain area. However, due to frost damage to some of the plants, the selection was limited to eleven seedlings from each of the thirteen seed sources (Table 1; Figure 1).

The plants were moved from the cold frames March 3, 1970 and placed in two growth chambers. Lights were on for sixteen hours daily at an intensity of 12,056 Lux. Temperature was held constant at 20° C. The photosynthetic measurements of these plants began July 17, 1970 and ended September 5, 1970.

Experimental Apparatus

The apparatus for the photosynthetic measurements was modeled after that used by Sorenson (1964) and Krueger (1963). It consisted of a L.I.R.A. infra-red gas analyser linked in a closed system to a cylindrical cuvette 6.35 cm. in diameter and 8.89 cm.

Table 1. Locations and elevation of seed sources.

No.	Seed source	Elevation
725	Shoshone Pass, Idaho	1, 200 m.
753	Troy, Montana	1, 160 m.
833	Alder, Montana	2, 380 m.
923	La Garita, Colorado	2, 530 m.
930	Grants, New Mexico	2, 620-2, 710 m.
951	Grants, New Mexico	2, 830-2, 900 m.
969	Cloudcroft, New Mexico	2, 410 m.
1003	Santa Catalina Mts., Arizona	2, 410-2, 560 m.
1027	Pinal Mts., Arizona	2, 230-2, 380 m.
1035	San Francisco Mts., Arizona	2, 410 m.
1046	San Francisco Mts., Arizona	2, 740 m.
1068	Kaibab, Arizona	2, 160-2, 230 m.
1094	Delmar, Idaho	1, 1770 m.

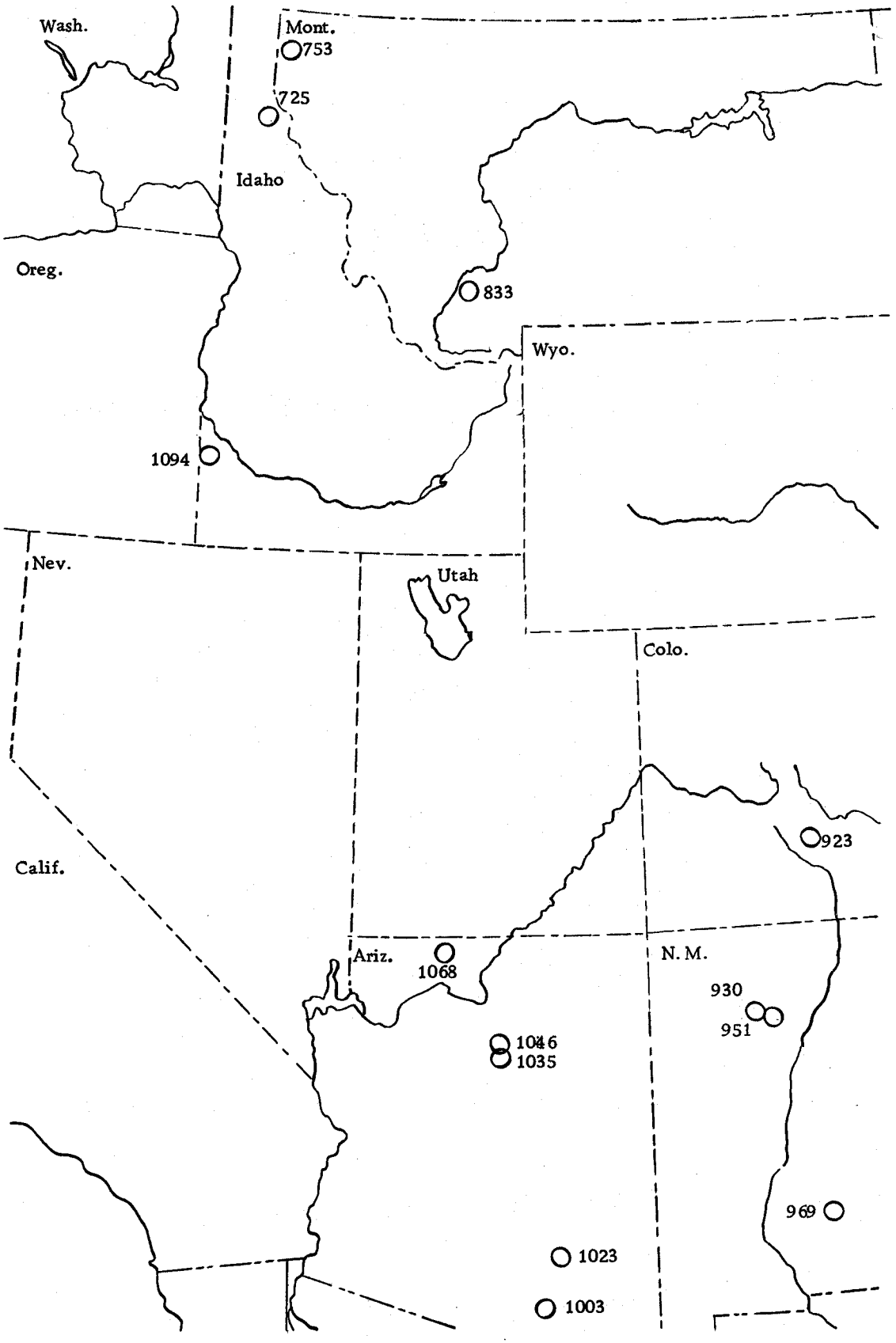

Figure 1. Location of seed sources.

high. The volume of the entire system was 1.245 liters as determined by measuring the CO_2 depression after a known amount of CO_2-free air was injected into the system; from this a ratio estimate of the volume was made. The light was a 1,200 W., 120 V. Radiant "Hi Spot" incandescent lamp producing 60,278 Lux as measured through the cuvette. Temperature in the cuvette was maintained by circulating water at 4° C. through a jacket around the cuvette producing a constant 20 ± 2° C. temperature within the cuvette. A water bath 50 cm. above the cuvette served as an infra-red filter reducing the heat load from the lamp. The temperature was monitored continuously by a thermocouple inside the cuvette. A rubber stopper cut in half and having a groove in the center provided the seal between the plant and the cuvette. Due to the size difference of the plants, modeling clay was used to improve this seal. The relative humidity in the cuvette was maintained at 50±8% by passing the gas of the system over a 10% solution of phosphoric acid and water immediately before it entered the cuvette. A diagram of the apparatus is found in the appendix.

Once the plant was sealed into the cuvette it was allowed to equilibrate for approximately five minutes before measurements began. The time the plant required to depress the CO_2 concentration of the system from 330 ppm. to 290 ppm. was then measured. A sample of the calculations of the photosynthetic rate appears in

the appendix.

Preparation of the Plant Material

The plants were removed from the growth chambers just prior to their photosynthetic measurement. Only the recent years growth of the main leader of the intact plant was used for measurement. This procedure was used so that the maximum rate under the experimental conditions could be achieved. Others had found decreasing photosynthesis with increasing age of needles (Woodman, 1971; Sësták, 1972).

Since large variation was expected within each seed source (Krueger, 1963; Sorenson, 1964), every effort was made to control the amount of variation through experimental means. From visual observations it was evident that the needle length, shape, number, and density about the stem was different among, as well as within the seed sources. These morphological factors have an effect on the photosynthesis by causing differences in the efficiency of the absorption of light energy so that the expression of the photosynthetic rate in terms of CO_2 mg. /g. dry weight/hour becomes an inadequate measure of the physiological process. Also, due to these morphological traits an adequate method had to be developed to determine the appropriate amount of plant material to place into the cuvette at any one time. Too much plant material in the cuvette would depress

the CO_2 content of the system too fast for accurate measurement, and too little material would be time consuming as well as allowing certain artifacts to become evident by removal of photosynthetic sinks (Wareing, Khalifa and Treharne, 1968). A compromise determining how much plant material to use for measurement based on these morphological differences was reached by using the following procedure: An index card was placed with its edge against the stem forming a plane parallel to the stem, and all the needles and lateral branches below the first three fully expanded needles touching the card along most of their length were removed. This procedure was repeated on the opposite side of the leader. Every effort was made to remove the needles carefully to reduce injury to the stem. Severe injury would affect the movement of water, nutrients, and photosynthate. Since this procedure was done immediately before photosynthetic measurements started, any possible water loss, respiration increase, or effects on movements of nutrients and photosynthate were assumed negligible in relation to the measurement.

After measurement of the CO_2 exchange rate was completed the remaining needles were removed, counted, dried for forty-eight hours at 70° C. and weighed to the nearest 0. 1 mg. After the needles were dried a random sample of ten needles was measured to the nearest 0. 1 mm. Multiple analysis of covariance was then employed to evaluate the results.

RESULTS

Analysis of variance tests (ANOVA) were conducted on the concomitant observations (Tables 2, 3, 4; Figures 2, 3, 4). This procedure was done to establish that the apparent differences observed were in fact statistically significant.

The number of needles on the stem contained within the cuvette at the time of measurement was an estimate of the density of needles about the stem. This is evident since the index card method of determining the amount of material to be contained within the cuvette is density dependent (see Methods). There were significant differences in needle number at the 99% level between seed sources as well as within seed sources (Table 2). The degree of variability within the seed sources was also markedly different among sources as shown by the Student's "t" confidence interval and range (Figure 2). The average length of needle was significantly different at the 99% level between but not within seed sources (Table 3). The difference in variability is also evident from the Student's "t" confidence interval and range (Figure 4). The average needle dry weight was not significantly different between or within sources (Table 4; Figure 4).

Because differences were apparent in the needle length and needle number (density) but not in needle dry weight, the possibility that there was an effect of needle length and number upon the rate

Table 2. ANOVA of needle number.

Source	df	SS	MS	F
Between source	12	5366.91	447.24	12.77**
Within source	10	1274.06	127.41	3.64**
Error	120	4201.40	35.01	
Total	142	10,842.36		

Table 3. ANOVA of needle length.

Source	df	SS	MS	F
Between source	12	784.02	65.33	2.95**
Within source	10	336.71	33.67	1.52NS
Error	120	2,662.03	22.18	
Total	142	3,782.76		

Table 4. ANOVA of needle weight.

Source	df	SS	MS	F
Between source	12	76,032.60	6,336.05	1.46NS
Within source	10	37,214.42	3,721.44	.86NS
Error	120	522,157.73	4,351.31	
Total	142	635,404.75		

** = .01 level * = .5 level NS = not significant

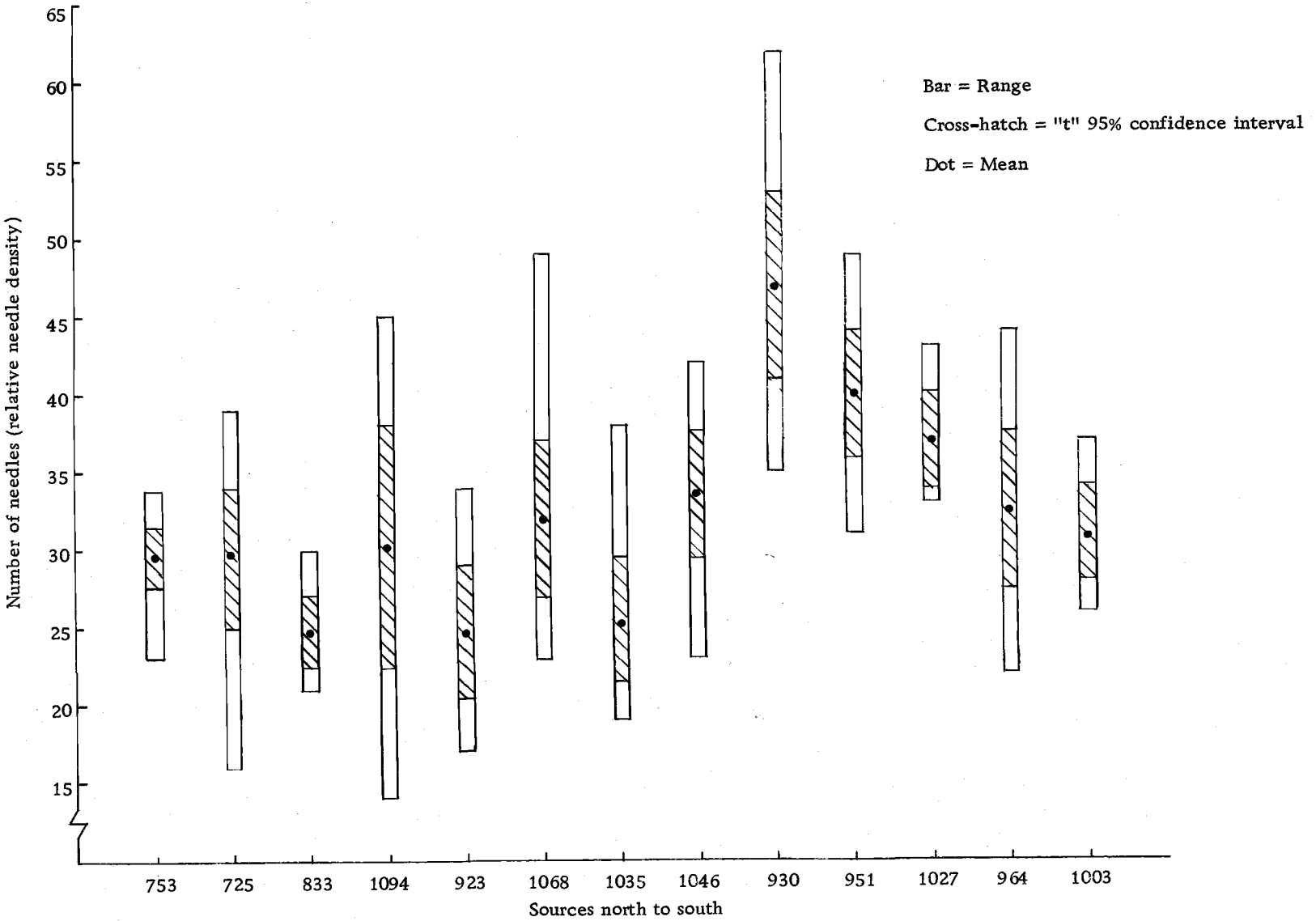

Figure 2. Number of Needles.

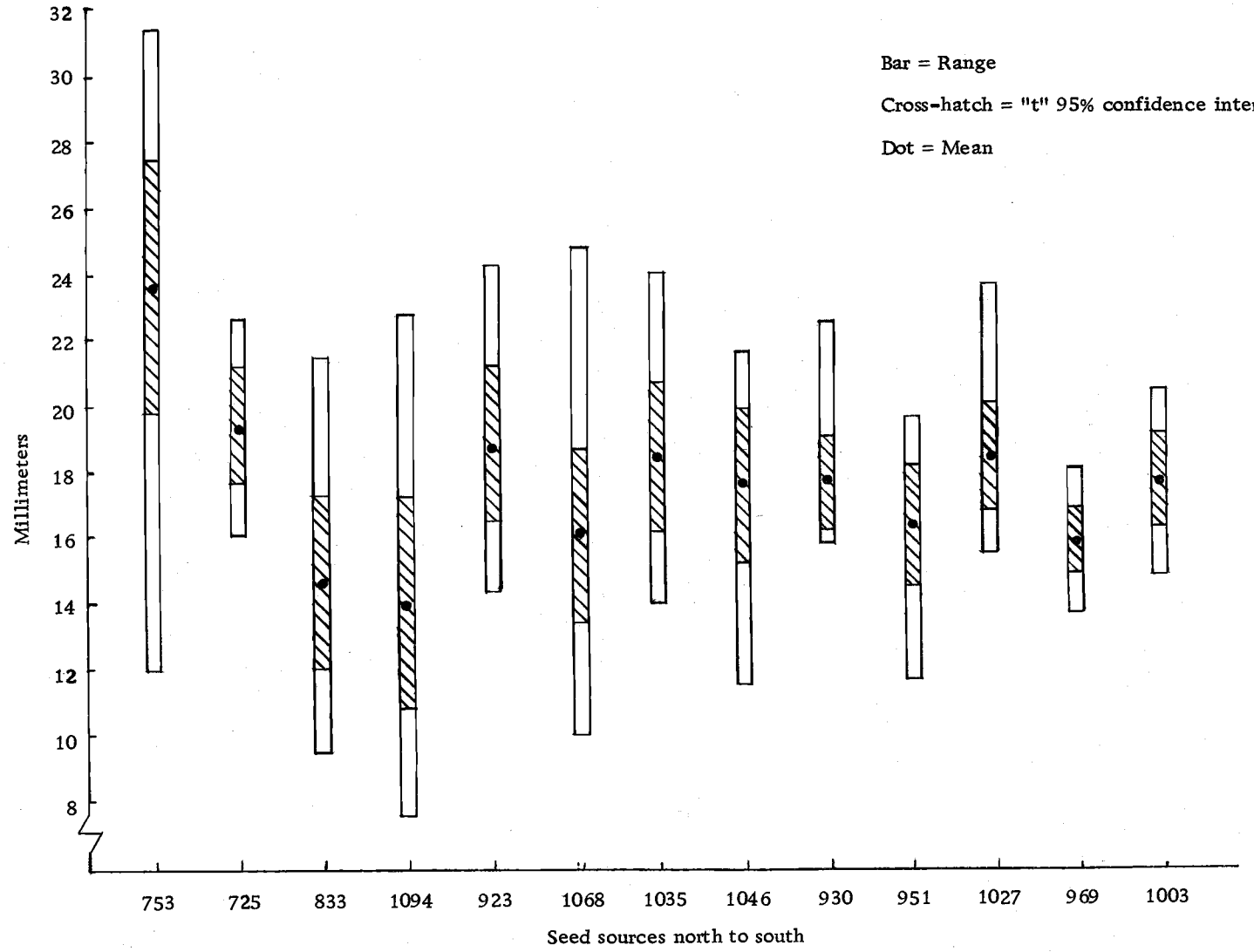

Figure 3. Average needle length.

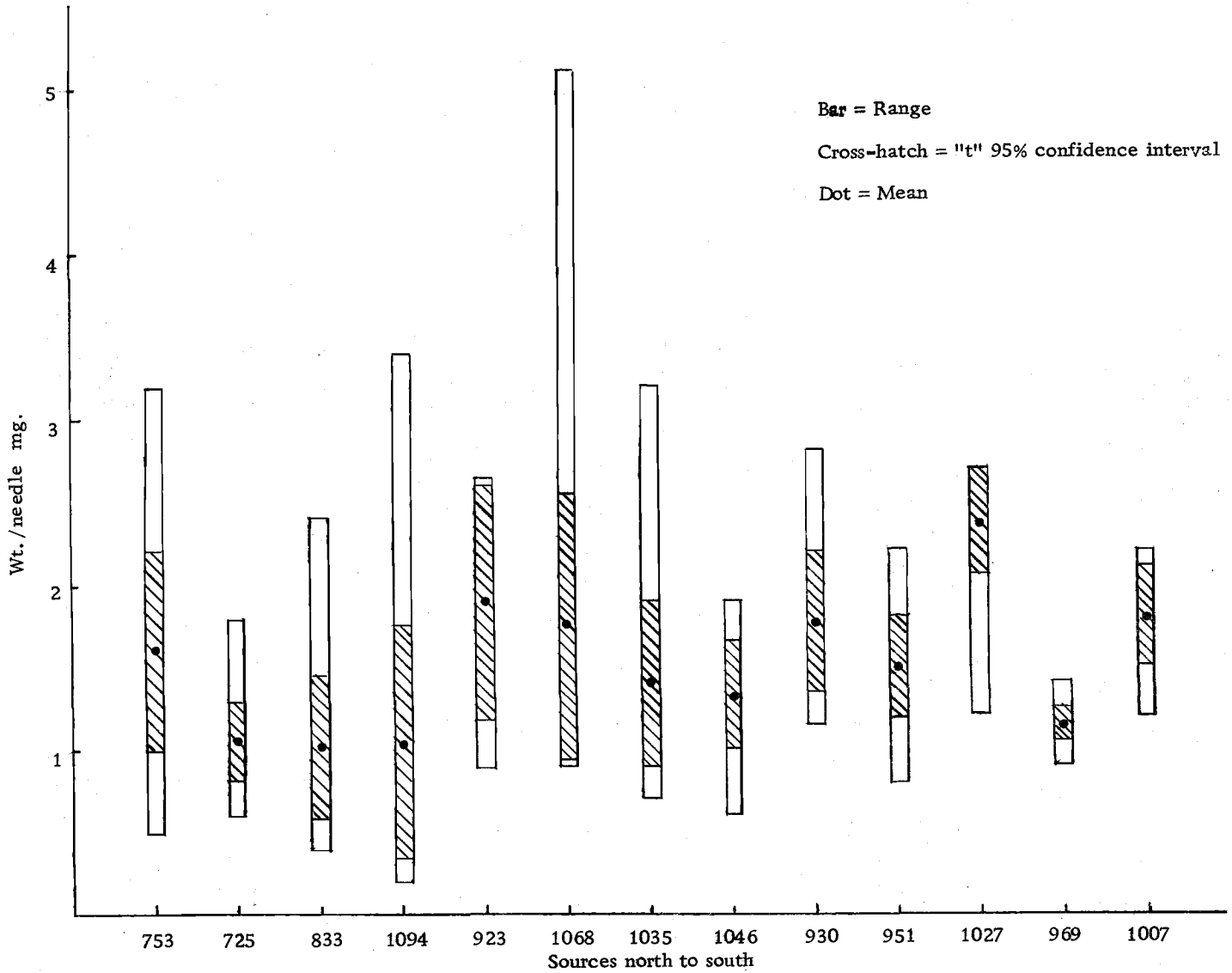

Figure 4. Average weight of needle.

of photosynthesis was examined. The primary effect of these differences on the rate of photosynthesis would be through mutual shading, i. e., the shading of the lower needles by those above resulting in differences in light energy available for absorption among the needles of the plant. It is also likely that the width and thickness of the needle may change with the length to account for the absence of weight differences. However, due to the relative difficulty in obtaining needle width and thickness measurements, the needle dry weight was assumed to be a function of the dimensions of the needle. Since the length was known the width and thickness could be statistically accounted for collectively.

Step-wise multiple regression was combined with analysis of variance forming a multiple analysis of covariance to correct for the morphological differences. Existing step-wise multiple regression in the O. S. U. -3 computer program library tests the concomitant observations as well as a number of transformations of these variables. Since these morphological characters were thought to operate in a similar manner irrespective of seed source a one-way multiple regression was employed. From this operation the model,

$$\text{photosynthetic rate} = 9.55 - 0.10\ \text{needle length}^2 + 2.61\ \text{needle length} + 0.03\ \text{needle length} \times \text{needle number} + 0.08\ \text{needle number} - 0.66\ \text{needle weight},$$

explained 87% of the variation of the photosynthetic response. This model is in the form $\hat{y}_{ij} = b_o + b_1x_1^2 + b_2x_1 + b_3x_1x_2 + b_4x_2 + b_5x_3$ with $\hat{y}_{ij}$ being the photosynthetic response, $b_1, \ldots, b_5$ the coefficients of the first through fifth terms, x_1 the needle length, x_2 the needle number, x_3 the needle weight. Using the analysis of variance, expansion of this model is possible. This allows testing the morphological traits in the regression formula that may vary among seed sources as well as comparing the photosynthesis between seed sources without the variation caused by the morphological traits. The model for the ANOVA of the predicted values from the regression (Table 6) is, $\hat{y}_{ij} = \hat{\bar{y}} + T_i + B_j + E_{ij}$, with T_i being the seed source effect, B_j the block or within source effect, and E_{ij} the random error effect. Using the appropriate "F" test, significance of the variation of the morphological factors in the regression model can be evaluated. The model for the ANOVA of deviations from predicted (Table 7) is, $y_{ij} - \hat{y}_{ij} = \bar{y} + T_i + B_j + E_{ij}$, which is the analysis of variance of the rate of photosynthesis minus the effect of the morphological factors in the regression model.

Figure 5 shows the actual rate of photosynthesis with the morphological factors not accounted for. Note that the mean rate of photosynthesis of source 833 is higher than those previously reported for one-year-old seedlings of Douglas-fir (Larcher, 1969). Analysis of variance of these observations produced significant

differences between as well as within sources, as shown in Table 5, Figure 5. After the variation due to the regression on the morphological factors was removed only the differences between the sources were significant (Table 7). The analysis of variance of the predicted values from the regression indicates the regression factors are significantly different within and between the seed sources (Table 6). This was expected from visual observations and the analysis of variance of the individual morphological factors.

Ecological Implications

Climatic data were averaged over the weather bureau district from which the mother trees were located (U. S. Weather Bureau, 1960) (Figure 7). The four climatic considerations felt most important in the evolution and migration of Douglas-fir are:

a) More than 150 days with temperatures above 32° F.

b) More May-June rainfall than July-September rainfall.

c) Greater than 2 inches of summer rainfall.

d) Distribution along a North-South gradient.

Table 6 expresses the importance of the climatic considerations on the morphological variables within the regression formula using contrasting sums of squares. Each is significant at the 95% level except summer rainfall greater than two inches. The same contrasts

Table 5. ANOVA of unadjusted photosynthetic rate.

Source	df	SS	MS	F
Between source	12	555.19	46.27	11.65**
Within source	10	91.42	9.14	2.30*
Error	120	478.75	3.97	
Total	142	1,123.35		

Table 6. ANOVA of values predicted from the regression.

Source	df	SS	MS	F
Between source	12	555.37	46.28	11.65**
Days above 32°F.	1	25.04	25.04	6.26*
N. to S.	1	17.56	17.56	4.39*
More June rain	1	19.33	19.33	4.83*
Summer rain	1	12.99	12.99	3.25NS
Within source	10	91.44	9.14	2.29*
Error	119	476.15	4.00	
Total	141	1,122.96		

Table 7. ANOVA of the deviations from predicted or the adjusted rate.

Source	df	SS	MS	F
Between source	12	501.32	41.78	3.58**
Days above 32°F.	1	10.71	10.71	.91NS
N. to S.	1	10.66	10.66	.91NS
More June rain	1	11.08	11.08	.94NS
Summer rain	1	15.13	15.13	1.29NS
Within source	10	201.17	20.12	1.72NS
Error	119	1,400.36	11.78	
Total	141	2,102.84		

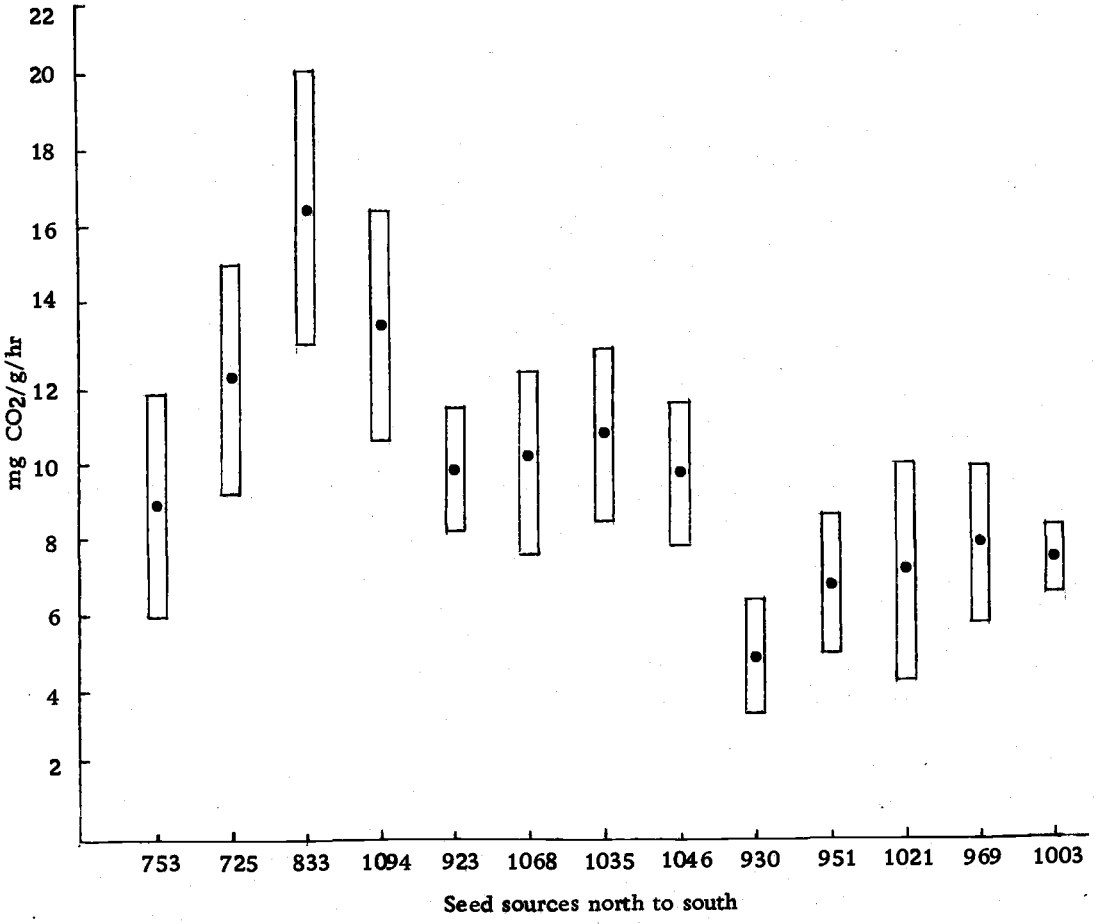

Figure 5. Observed apparent net photosynthesis.

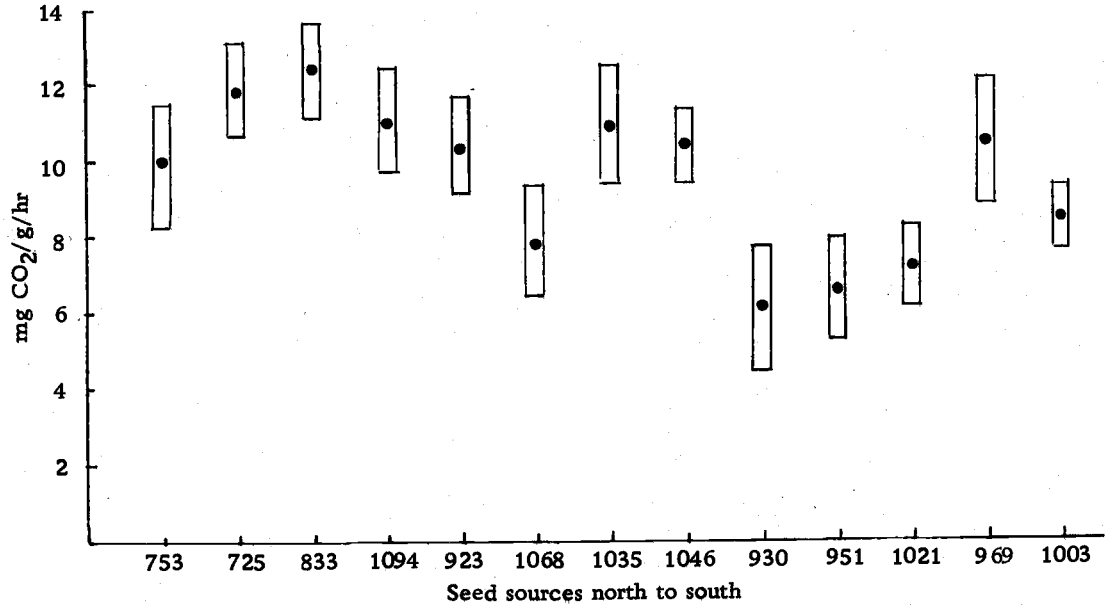

Figure 6. Adjusted apparent net photosynthesis.

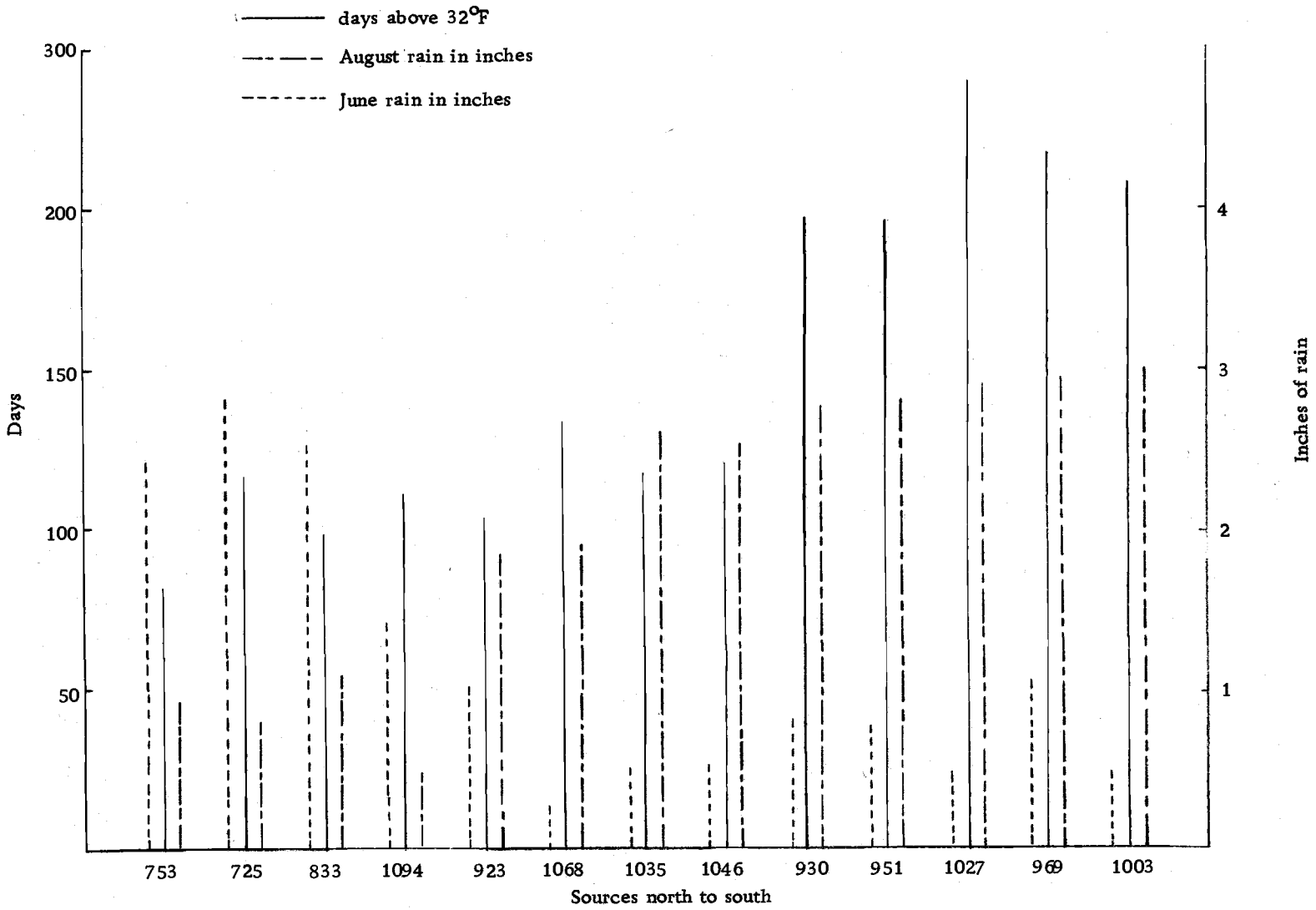

Figure 7. Climatic data of contrasts.

made in the ANOVA of deviations from predicted or the adjusted rate of photosynthesis proved non-significant at the 95% level (Table 7).

DISCUSSION

Explanation of the Regression Formula

Two objectives of the analysis of covariance are to increase precision and give some indication as to how the concomitant observations affect the observed variation (Snedecor and Cochran, 1968). Step-wise multiple regression coupled with the analysis of variance forms a multiple analysis of covariance. Since the process of step-wise regression selects the most significant combination from the set of independent observations, another dimension of knowing which of the observations or their transformations are correlated with the rate of photosynthesis is achieved. In the analysis five transformations of three variables proved to affect the variation in photosynthesis significantly. Although the analysis shown here does not necessarily prove causality, it is helpful in understanding the possible biological processes involved.

In the regression formula,

$$\text{Predicted photosynthetic rate} = 9.55 - 0.10\ \text{needle length}^2 + 2.16\ \text{needle length} + 0.03\ \text{needle length} \times \text{needle number} + 0.08\ \text{needle number} - 0.66\ \text{needle weight},$$

the mean needle length squared term can be thought of as being proportional to the area of a plane parallel to the base of a cylinder

representing the shoot enclosed in the cuvette. Plants with the greatest area in this plane would be expected to have the highest rate of assimilation. Contrary to this effect, as the area increases so also does the shading of the plane below, since light was incident mainly from above. Thus this term in the formula represents a contrasting effect of increased absorptive area versus increased shading; the net result being slightly negative as shown in the slight negative slope of its regression coefficient (-0. 10).

Mean needle length × needle number (coefficient = 0. 03) is an expression of the total needle mass enclosed in the cuvette, the positive effect of this term would indicate that the increase in number and length of needles does not increase the amount of shading which might be expected with increased photosynthetic tissue. While shading may increase towards the center of the cylinder (around the immediate vicinity of the stem), it is not enough to off-set the increase in area receiving light at the periphery of the cylinder.

The same argument might also explain the positive effect of needle number which is an expression of the needle density (coefficient=0. 08) and the effect of the mean needle length (coefficient=2. 16). The longer the needles the more area is exposed to light in the less crowded periphery of the cylinder, increasing the total absorption of incident light energy. A note of caution must be expressed as to the possibility of the two length terms being confounded with each

other affecting the significance of the formula only slightly.

The negative effect of needle weight (coefficient=-0.66), a function of the width and thickness of the needles, would also seem biologically meaningful. No increase in exposed area to light is achieved by thicker needles; increased self-shading occurs with wider needles. Since the rate of photosynthesis is expressed on a per gram dry weight basis this assumption appears logical.

The interpretation of the above regression formula is based primarily on the differential absorption of light energy effecting the rate of apparent net photosynthesis. Special importance is given to the phenomenon of mutual shading. Therefore, if this interpretation is true, the method employed to control mutual shading (see Methods) was unsuccessful. Any measure of the amount of material placed in the cuvette may be adequate if it is accompanied with other measurements to characterize mutual shading. This is particularly important with plants such as conifers where photosynthetic measurements on individual needles or leaves is difficult. The above discussion also points out that direct comparisons of photosynthesis based on leaf dry weight of plants differing in needle morphology and arrangement is difficult unless these factors are accounted for in some manner. Statistical adjustment of these differences (accounting for the variation they cause) establishes a base of comparison to evaluate the differences in the photosynthetic responses

between the populations.

The Analysis of Covariance

The regression equation was used to calculate predicted values of the rate of photosynthesis and the deviation from its predicted value. With these values analysis of variance tests were performed.

The ANOVA of predicted values (Table 6), with a model of the form

$$\hat{y}_{ij} = \hat{\bar{y}} + T_i + B_j + E_{ij}$$

(see Results), describes the variation of the observations of the independent variables in the regression formula. In using a randomized block design, blocking by the number of replications of the seed sources and using the seed sources as treatments, variation between (seed source effect) and within source (block effect) can be compared. The within source variation is a function of the variation within the seed sources as well as variation over the time of the experiment, while the variation between the seed sources is attributed to differences in seed source. From Table 6 the between source variation was 99% significant; in other words, the values of the independent variables within the regression varied significantly between seed sources. The differences within the seed sources were 95% significant. This is to be expected since the ANOVA of each of these

variables independently is also significant, except needle weight (Tables 1, 2, 3; Figures 1, 2, 3).

The ANOVA of the deviations from predicted (Table 7), with the model of the form, $y_{ij} - \hat{y} = \bar{y} + T_i + B_j + E_{ij}$, is the analysis of the variation of the photosynthetic rate without the influence of the morphological factors included in the regression formula. In Table 7 the between source variation is significant at the 99% level; however the differences within sources were not significant. Since the within source variation was significant with respect to the regression factors (Table 6), and not significant when these factors were removed (Table 7), the variation of photosynthesis within sources can be attributed to changes of the regression factors within seed sources over the time of the experiment, possibly growth of the needles. The apparent net photosynthesis did not vary significantly over the period of measurement as expected from the work of Negisi (1966) with Cryptomera japonica and Pinus densiflora. In other words the seasonal photosynthetic rate maximum was expected to remain constant over the period of measurement (June to September). Validity of the consistancy of the photosynthetic rate through the period of measurement is shown in the ANOVA Table 7 by the non-significant within source variation.

Ecological Considerations

Geographical variation is known to be highly unpredictable, often having no apparent correlation with environmental variation. Stebbins (1950) cites cases of plant variation in which some of the parameters varied with the environment while others varied independently of environment within the same species. This does not mean that the independent parameters are not adaptive in some unknown manner. Alternatively, regularities in geographical variation do not necessarily represent adaptation (Simpson, 1953). Therefore the following is speculative at best.

Geologic and climatic factors in the Western United States have been the most important factors in governing the evolution of the flora. Many drastic changes in topography and climate have caused rapid evolution. This evolution is characterized by bursts of change followed by periods of relative stability. From the Eocene to the Pleistocene the genus Pseudotsuga has steadily gained ecologic importance. However during most of the periods of selection it was a very minor component of the forests. Specifically within the Miocene, when floristic provincialism was most pronounced, it became ecologically compatible with a variety of forest types. As a progressively drier climate developed, selection pressures reduced the range and composition of the forests. Different selective forces

(both in degree and variety) acted on the genus within each of these floristic provinces. In the Pliocene and early Pleistocene greater fractionation of the forests occurred, caused by minor glaciers, fires, and drought. Probably the most adaptive feature of the species was its capacity for individual modification, in the broadest sense, which includes phenotypic plasticity and genotypic heterogeneity. Different genotypes were most certainly fixed within the different provinces, and individual modification allowed fixation of a large number of these genotypes.

The results of this study seem to confirm the observations above as well as those of Stebbins (1950), cited earlier. The morphological factors used in the regression formula may have been selected by climatic factors while the photosynthetic mechanism varies independently possibly due to isolation during earlier epochs or is in fact adapted to some climatic factor unaccounted for in this study. Since there are significant differences in photosynthesis apparently not attributed to the climatic contrasts (Table 7) it is certain that genetic differences in the photosynthetic rate do in fact exist. However in that the independent observations of the regression formula vary significantly with most of the climatic contrasts (Table 6), and the adjusted photosynthetic rate does not (Table 7), the results would seem to indicate that adaptation of the photosynthetic machinery to various environments has come about by selection of morphological

characters. Those characters proven most significant are needle length, needle density, and needle weight. Thus a physiological mechanism could be adapted by morphology to a given set of conditions. However since the measurements were made under one set of conditions (light intensity, temperature, and pre-treatment) it is not possible to determine the nature of such adaptations.

The existence of the significant variation in the morphological characteristics as well as in the adjusted photosynthetic rate suggests potential interest in terms of tree breeding. Genotypes may be selected, and with growth models based on photosynthesis such as Ledig's (1969) and much further research, growth may be predicted from the interaction of desirable morphological traits, photosynthetic rate, and seasonal duration of the photosynthetic rate maximum comparing these genotypes for optimum growth. Tree improvement based on these criteria may at some later date become feasible.

CONCLUSION AND SUMMARY

Measurements of the rate of apparent net photosynthesis were made on samples of eleven one-year-old seedlings of Rocky Mountain Douglas-fir from thirteen different localities within the Rocky Mountains. Secondary observations on the mean needle length, needle weight, and needle number contained within the cuvette were made. From visual and statistical criteria these secondary observations varied between as well as within the different seed sources. A stepwise multiple regression of these morphological characters as well as a few of their simple transformations was found to control 87% of the variation of the photosynthetic response. From the explanation of the formula produced from this statistical operation it was apparent that the expression of the photosynthetic rate directly from the CO_2 depression as mg. CO_2/needle gram dry weight/hour is not appropriate when making comparisons between populations of Douglas-fir. With a multiple analysis of covariance, adjustment of the rate of photosynthesis based on the morphological observations offers a better comparison between populations. Partitioning the between seed source sum of squares based on climatic data averaged from weather records and a north to south ranking of the seed sources had no significance with respect to the adjusted photosynthetic rate. All the partitioned sums of squares except more

than two inches of summer rainfall had a significance with respect to the morphological factors within the regression formula. This suggested adaptation by way of the modification of photosynthetic rates by the morphology of the plant. Significance of this photosynthetic variation as applied to tree breeding may come about by selecting plants on morphological and photosynthetic rate criteria; and modeling these to predict growth. This requires further investigation.

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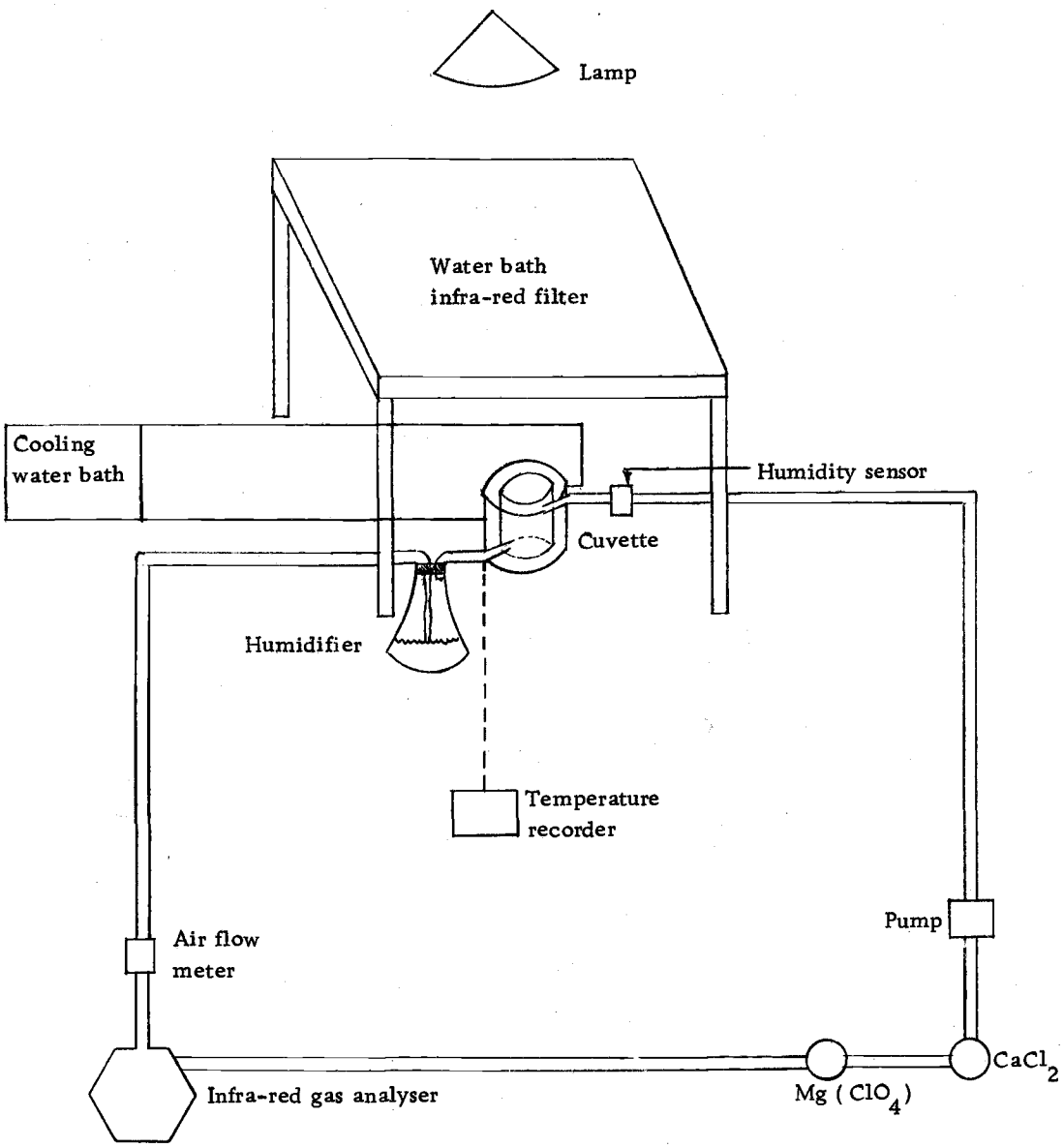
APPENDIX


Lamp
Water bath
infra-red filter
Cooling
water bath
Humidity sensor
Cuvette
Humidifier
Temperature
recorder
Air flow
meter
Pump
Infra-red gas analyser
Mg (ClO4)
CaCl2
Tygon tubing (air)
Rubber tubing (water)
Thermocouple

Apparatus

Calculations for the Rate of Apparent Net Photosynthesis

Example:

14.47 mg dry weight of needles

40 ppm. CO_2 depression

18.52 minutes for the CO_2 depression to occur

40/14.47/18.52 = 0.1492 ppm. CO_2/mg. /min.

24.46 liters is the volume of one mole of air at 22° C.

1.25 liters is the volume of the system

1.25/24.46 = 0.0511 moles of gas in the system.

0.0511×10^{-6} moles/ppm is the number of moles of gas in a ppm.

44.01 g. /mole CO_2 is the gram molecular weight of CO_2

$(5.11 \times 10^{-8})(44.01) = 224.89 \times 10^{-8}$ g. CO_2/ppm. or 0.0022489 mg. /ppm.

To convert ppm. /mg. /min to mg. /g. /hr., the conversion factor is

(.0022489 mg. /ppm.) (60 min. /hr.) (1000 mg. /g.) = 134.9340

Therefore,

0.1492 ppm. CO_2/mg. /min. = 20.1322 mg. CO_2/g. /hr.

Table of Means of the Apparent Net Photosynthesis before and after Statistical Adjustment

Seed source	mg. CO_2/g./hr after	before	deviation
725	11.8406	12.0664	.22583
753	9.9405	8.7166	-1.42771
833	12.4314	17.0443	4.61295
923	10.2834	9.6322	-.64135
930	6.1033	4.6802	-1.42303
951	6.5720	6.5701	-.00585
969	10.4356	7.6608	-2.77560
1003	8.4564	7.2785	-1.17799
1027	7.1354	6.9603	-.17513
1035	10.9282	10.5565	-.37144
1046	10.3559	9.5508	-.80511
1068	7.8713	10.0386	2.16735
1094	11.0857	13.3619	2.27618

Table of Means of the Independent Observations

Seed source	Needle length (mm)	Needle number (density)	Needle weight (mg.)
725	19.29	29.55	1.066
753	20.40	28.36	1.629
833	14.59	24.73	1.026
1094	13.89	30.73	1.062
923	18.77	24.91	1.909
1068	16.01	32.18	1.765
1035	18.42	25.45	1.493
1046	17.53	33.73	1.337
930	17.91	47.17	1.788
951	16.27	40.17	1.581
1027	18.35	37.36	1.890
969	15.95	32.55	1.146
1003	17.64	31.09	1.747