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Title: PLANT-WATER RELATIONS IN DOUGLAS-FIR SEEDLINGS AND

SCREENING SELECTED FAMILIES FOR DROUGHT RESISTANCE

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Abstract approved: _____

William H. Emmingham

This study was divided into two parts. Part I was a quantitative description of some aspects of plant-water relations in Douglas-fir seedlings, and part II was tests of several techniques of screening selected families of Douglas-fir seedlings for drought resistance.

Part I.

During 20 mild summer days in 1976 at Corvallis, Oregon, diurnal measurements were taken on two-year-old Douglas-fir seedlings for xylem water potential, leaf conductance of water vapor, air temperature, soil temperature, dew point temperature, and solar radiation. Leaf conductance was at a maximum early in the morning as soon as the seedlings were illuminated. It dropped rapidly in the morning and more gradually in late afternoon. There was little correlation between leaf conductance and radiation.

At low moisture stress (high pre-dawn xylem water potential) there was a good linear relationship between leaf conductance and

absolute humidity deficit; leaf conductance decreased as evaporative demand increased. As plant moisture stress increased, the level of absolute humidity deficit necessary for stomatal closure decreased.

The relation between leaf conductance and plant moisture stress was made clearer by separating the data into six ranges of absolute humidity deficit. Leaf conductance decreased as plant moisture stress increased, rapidly at first and more gradually later as stomata closed. As the level of absolute humidity deficit increased, the degree of moisture stress causing stomatal closure decreased.

Transpiration was estimated by the product of leaf conductance and absolute humidity deficit, and daily estimates were calculated by integrating diurnal values over the day. Daily transpiration decreased with increasing moisture stress with the greatest decrease being between -3 and -5 bars pre-dawn zylem water potential.

Daily depression in xylem water potential was greatest between -8 and -12 bars pre-dawn xylem water potential while daily transpiration, a direct cause of changes in xylem water potential, was greatest at higher pre-dawn xylem water potential. An index of plant resistance to water flux was calculated which suggested plant resistance increased with moisture stress.

Plant-water relations was suggested as a possible basis for screening seedlings for drought resistance.

Part II.

Sixteen full-sib families of seedlings from three sites in the Coast Range of Westcentral Washington were studied for differences in drought resistance. Pre-dawn xylem water potential of understory

western hemlock indicated small differences among sites in plant moisture stress during the 1976 summer drought.

Families from the driest site burst bud five days earlier than those from the wettest site, which may indicate a drought adaptation. Neither bud burst nor origin of family were correlated with root: shoot ratio, root area:leaf area ratio or seedling productivity.

Seedlings from each family were measured at four levels of moisture stress (mean pre-dawn xylem water potentials were -3.7, -6.3, -10.8 and -12.3 bars) for diurnal leaf conductance of water vapor, xylem water potential, air temperature, dew point temperature and solar radiation. Diurnal transpiration was calculated and daily transpiration was estimated as in Part I. There were no differences among families in daily transpiration at any level of moisture stress. However, two families were always high and two were always low in transpiration. Daily transpiration was not correlated with date of bud burst or seedling productivity. Daily transpiration was correlated with root:shoot ratio at -3.7 and -12.3 bars pre-dawn xylem water potential and with root area:leaf area ratio at -3.7, -10.8, and -12.3 bars pre-dawn xylem water potential.

The lack of any indications of large differences in drought resistance among families probably resulted from the narrow range of seed resources. The good correlation between daily transpiration and root:shoot ratio suggests transpiration may be a good way to evaluate nursery seedlings for their root:shoot ratio.

PLANT-WATER RELATIONS IN DOUGLAS-FIR SEEDLINGS AND

SCREENING SELECTED FAMILIES FOR DROUGHT RESISTANCE

by

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INTRODUCTION

The advent of genetic selection of Douglas-fir in the Pacific Northwest necessitated appropriate testing procedures for evaluating tree performance in the field. Plant water economy was of prime concern because of summer drought. The project reported here had the objectives of quantifying some aspects of plant-water relations in Douglas-fir seedlings and developing screening techniques for drought resistance in selected families of Douglas-fir seedlings.

The thesis is composed of two somewhat autonomous chapters-- each with an introduction, materials and methods, results, discussion and conclusions--and an appendix on measurement of leaf conductance of water vapor. The bibliography is cumulative. Chapter I is a quantitative description of plant-water relations in Douglas-fir seedlings with suggestions for its use in screening for drought resistance. Chapter II is an attempt to test for drought resistance among sixteen families of Douglas-fir seedlings and to establish guidelines for screening for drought resistance in seedlings. The chapters are written this way so they can be easily rewritten for publication as separate documents.

Chapter I

THE EFFECTS OF EVAPORATIVE DEMAND AND PLANT MOISTURE STRESS ON

LEAF CONDUCTANCE OF WATER VAPOR IN DOUGLAS-FIR SEEDLINGS

Introduction

Most physiological work on drought resistance has been based on plant water potential and transpirational water loss because these are easily measured and correlations with environmental variables are good. Development of the pressure chamber technique for measuring plant water potential (Scholander et al. 1965) was an important step in describing differences in plant responses to water stress. Waring and Cleary (1967) showed pressure chamber measurements could be used to describe changes in plant water potential in the field and to explore the relationship between plant distribution and plant moisture stress.

Some of the first transpiration measurements with Douglas-fir found definite responses to moisture stress. At high soil water potentials of -1 to -2 bars, transpiration calculated from pot weighing of Douglas-fir seedlings began to decline and at -10 bars transpiration was still 37% of the maximum (Lopushinsky and Klock, 1974). A sharp drop in transpiration, indicating stomatal closure, was observed at -19 bars xylem water potential (Lopushinsky, 1969). Genetic variation in Douglas-fir has been demonstrated in resistance to dessication (Ferrell and Woodard, 1965; Pharis and Ferrell, 1966) and in transpiration (Zavitkovski and Ferrell, 1970; Unterschuetz et al. 1974).

Jarvis (1976) reported a model to predict stomatal conductance from empirically determined relations between stomatal conductance and five driving variables, quantum flux density, ambient CO_2 concentration, leaf-air vapor pressure difference, leaf temperature and leaf water status. The parameters which determine these relations must be evaluated at intervals during the year because these relations depend upon the physiological conditions of the plant which changes with season and previous weather. In application the model accounted for 51% of the variation in data on Sitka spruce and 73% of the variation in Douglas-fir data. It was believed that broader distribution of the data in time would have improved the prediction. Jarvis said the model is only descriptive and an improved mechanistic model awaits a more thorough understanding of stomatal action on a cellular level.

Running (1976) also felt capable of predicting leaf conductance of water vapor in Doublas-fir from measurements of environmental variables and plant water potential. Radiation opening of stomata was apparently saturated above 0.1 ly min^{-1}. As absolute humidity deficit increased beyond 6 g m^{-3} stomata began to close. Pre-dawn zylem water potential determined morning maximum leaf conductance. The threshold xylem water potential for stomatal closure was -20 bars. These relations were used in a computer simulation model to predict transpirational water loss on a daily basis (Running et al. 1975), but no test of the model such as that done by Jarvis (1976) was presented.

The objective of this study was to quantitatively describe the leaf conductance response of selected Douglas-fir seedlings to several environmental variables. Refined knowledge of plant water relations of Douglas-fir seedlings could be applied in screening techniques for

drought resistance of genetically improved stock. For example, quantifying the water potential threshold for stomatal closure could be a test for drought resistance, since stomata are the most important plant control of water loss. The plan was to study the relationships between leaf conductance of water vapor (k_1), xylem water potential (Ψ_x) and several environmental variables over a range of soil moisture conditions. Development of the diffusion porometer to measure k_1 and the pressure chamber to measure Ψ_x made diurnal measurements of these variables possible.

Materials and Methods

The plant material consisted of 16 full-sib families of Douglas-fir seedlings grown from seed collected from superior trees found on three similar sites in the Coast Range near Centralia, Washington. These superior trees were selected for outstanding form and growth. The seedlings were grown for 11 months in 164 cm^3 plastic containers in a 1:1 peat and vermiculite mixture at a spacing of about 270 trees m^{-2}. Efforts were made to produce large healthy seedlings by controlling the greenhouse environment and supplying abundant moisture and nutrients. At the time of outplanting the seedlings had a mean oven dry weight of 4.4 g, mean diameter 1 cm below the cotyledon scar of .38 cm, mean height of 31.08 cm and a mean root:shoot ratio of .34.

The seedlings were outplanted in a raised bed of soil at Corvallis, Oregon at 10 cm spacing during March 6 to 8, 1976. The beds were 2 m wide, 5 m long and 0.6 m deep. Two types of soil were in the bed; 45 cm of sandy loam river terrace soil was the bottom

layer and 15 cm of sandy loam forest soil was the top layer. Underlying the bed was 15 cm of coarse gravel. Seedlings were placed in the bed according to the experimental design for another study of family differences in drought resistance (see Chapter II). Families were randomly assigned row positions in a block (a row was eight seedlings long). Each family was represented in five blocks which were randomly placed in the bed.

Until June 1, 1976 the soil was kept moist in order to ensure survival. It was assumed that the first flush of growth, which elongated during this period, had conditions conducive to normal leaf and shoot growth. Drought in the Willamette Vally normally does not begin until June. After June 1 drying began, and a plastic transparent sheet was used to cover the seedlings during periods of rain to prevent wetting of the soil. Drying conditions were continued until September 13 when the soil was rewatered to field capacity to observe the plant response to renewed high soil moisture.

Twenty days of diurnal measurements of plant water relations and environmental variables were accomplished between July 13 and September 27, 1976. Pre-dawn xylem water potential ranged from -2 bars to -35 bars for some seedlings. Measurements were taken only on cloudless days except for August 4 and 5 which were partly cloudy. The days were generally mild; the maximum temperature was 32° C and the minimum was 9°C.

Xylem water potential (Ψ_x) was measured with a pressure chamber according to the methods described by Scholander et al. (1965) and Waring and Cleary (1967). The sample twig was excised from a

lateral branch 5 to 10 cm above the ground and immediately measured for Ψ_x. Ψ_x was measured twice during the day, just before dawn and between 1200 and 1400 hours Pacific Standard Time (PST). The pre-dawn measurement represented an equilibrated plant-soil water potential, and thus, it reflected the gradual progression of soil drought and plant moisture stress (Waring and Cleary, 1967; Waring, 1969; Richie and Hinckley, 1975). The midday reading minus the pre-dawn reading represented the maximum depression of Ψ_x for cloudless days (Richie and Hinckley, 1975), and it was influenced by the level of drought as well as daily weather.

k_l was measured with a null-balance diffusion porometer (Beardsell et al. 1971) until August 5, 1975 and with an aspirated diffusion porometer (Turner and Parlange, 1976) after that date. The null-balance porometer was preferred because it was faster, but it had to be used elsewhere. Data from the two instruments was comparable because they were both calibrated to give absolute values of k_l.

Leaf samples measured for k_l were on the tips of lateral branches in the highest whorl of branches on a seedling. Most of the foliage in the sample was fully elongated for the first measurement, and after that it was hardened. Measurements began as soon as there was light and the dew was evaporated, and they were repeated on the same needles every two to three hours until dark. There were three to seven measurements taken on each seedling each day and 12 to 32 seedlings were sampled depending on which porometer was used.

In order to prevent dew formation the seedlings were covered by a plastic sheet at night. The cover allowed air circulation and was

believed to not affect Ψ_x and k_1 measured during the next day.

At the end of the day the leaf conductance sample was clipped and taken to the laboratory for surface area determination. Planar surface area was measured with an optical planimeter and corrected to actual surface area by a factor of 2.36 (Gholz et al. 1976). k_1 was calculated for total leaf surface area.

Transpiration (g cm^{-2} sec^{-1}) was estimated as the product of k_1 (cm sec^{-1}) and absolute humidity deficit (g cm^{-3}) which was measured nearby (Comacho-B. et al. 1974; Elfving et al. 1972; Running et al. 1975). It was considered a good estimate of transpiration when leaf to air temperature difference and boundary layer resistance were low (Comacho-B. et al. 1974). These conditions were likely to exist for the small needles of Douglas-fir. Daily transpiration (mg cm^{-2} d^{-1}) was estimated by integrating diurnal estimates over the day.

Family differences were ignored and a completely randomized design was assumed in the data analysis. Data from each measurement day were grouped according to level of pre-dawn Ψ_x and means were calculated. The means represented 7 to 15 measurements. Linear regression techniques were applied to these means to analyse relations between k_1, Ψ_x, and environmental variables.

Soil temperature at 20 cm in the bed and air temperature at 100 cm above the bed were measured throughout the experiment by Parlow 30 day continuous recording mercury thermometers. At a more complete meteorological station 100 m from the seedlings, continuous measurements were made of air temperature with a thermister and dew point temperature with a heated lithiumchloride sensor (Interface Instruments Inc., Corvallis, Oregon). From air and dew point temperature measurements,

absolute humidity deficit ($g\ m^{-3}$) was calculated. Air temperatures measured at the station and the bed were very similar, so the station measurements were considered adequate for further analysis. Solar radiation ($ly\ min^{-1}$) was measured by an Epply pyranometer.

Results

Data from the null-balance and aspirated diffusion porometers were analyzed together. I felt justified in doing this because the two instruments were designed and calibrated to measure absolute values of k_l. The relation between k_l and absolute humidity deficit was compared for data taken by the two instruments on similar days at low plant moisture stress (pre-dawn Ψ_x was -2 to -4 bars). The F-test, based on the extra sum of squares principle, for differences in the regression models for the two sets of data was not significant ($P \leqslant .01$). I felt this result further justified the type of analysis performed.

Typical diurnal patterns for radiation, air temperature, absolute humidity deficit, k_l and transpiration are presented in Figure 1. On this date, August 3, 1976, the seedlings were under low stress, pre-dawn Ψ_x was -3 bars. k_l was at a maximum in the early morning as soon as there was sufficient light for stomatal opening. The maximum was maintained for only a short time because as quickly as temperature and absolute humidity deficit began to rise, k_l decreased. Apparently transpiration caused a reduction in leaf water potential, and stomata responded by closing. k_l decreased rapidly in the morning, dropped slowly in the afternoon and finally reached a constant low level at darkness and stomatal closure. Transpiration decreased at a much slower rate than k_l during the day.
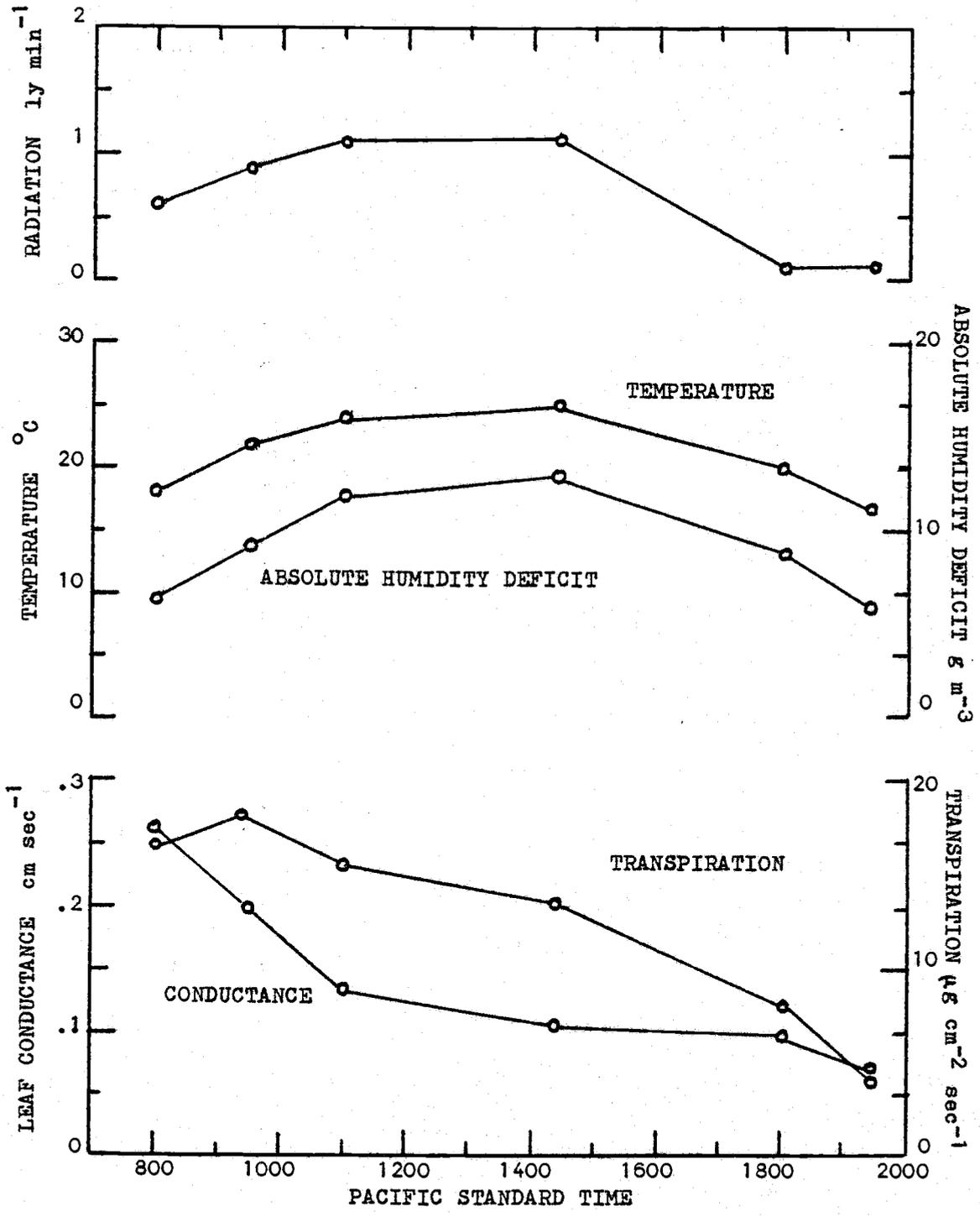
Fig. 1. Daily course of leaf conductance and environmental variables for Douglas-fir seedlings taken August 3, 1976 at Corvallis, Oregon. Each leaf conductance point represents the mean of fifteen measurements. Seedlings were at -3 bars pre-dawn Ψ_x.
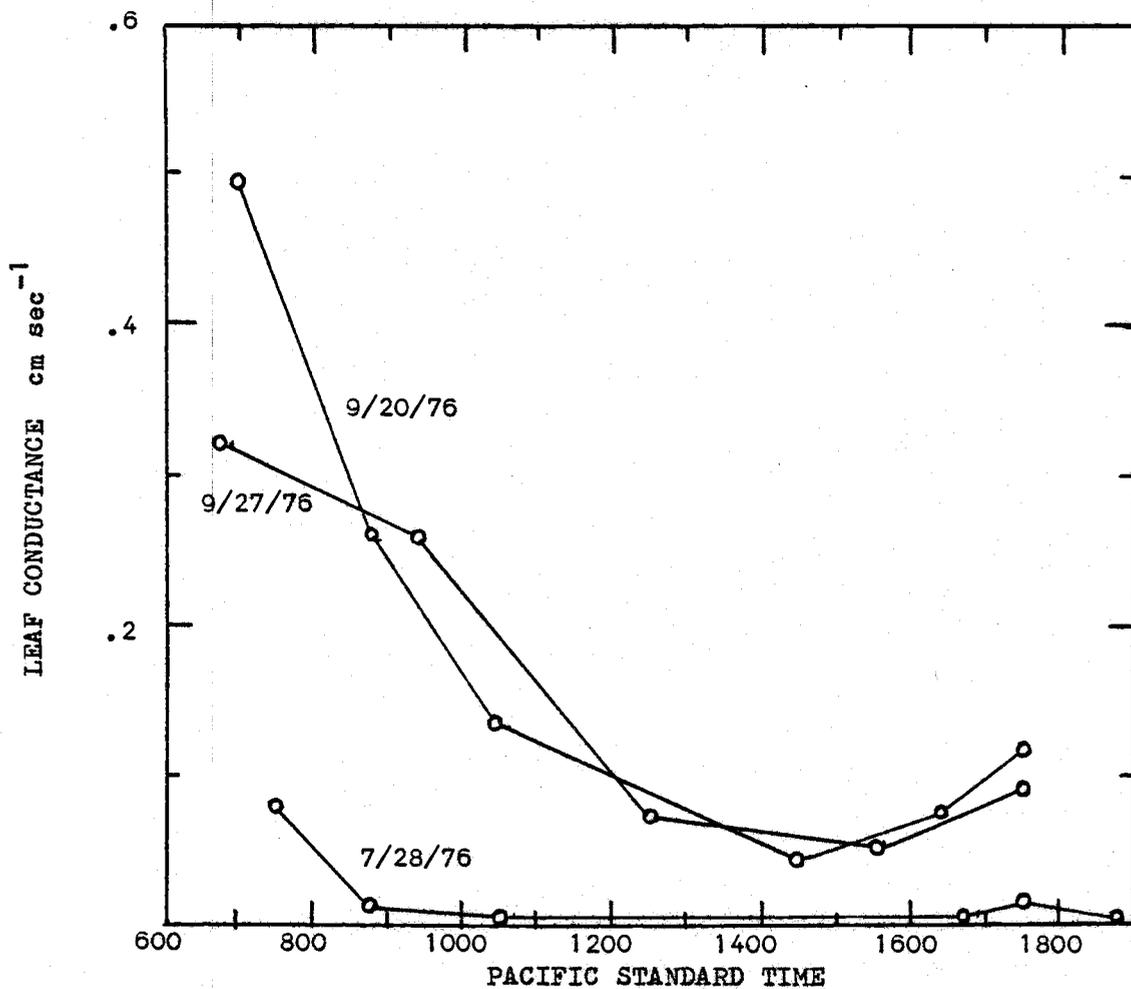
Fig. 2. Daily course of leaf conductance showing late evening recovery at low moisture stress, pre-dawn Ψ_x -4 bars(9/20/76 and 9/27/76) and moderate stress, pre-dawn Ψ_x -14 bars (7/28/76). Each point represents the mean of 7 to 12 measurements.

For seven of the twenty days measurements were taken, absolute humidity deficit decreased by at least 2 g m^{-3} in the late afternoon. On three of these days there was a recovery of k_1 from the afternoon minimum (Figure 2). The recovery values were significantly higher ($P \leq .05$) than the afternoon minimum values. The recoveries were observed at low moisture stress (pre-dawn Ψ_x -4 bars) on September 20 and 27, and at moderate stress (pre-dawn Ψ_x -14 bars) on July 28. The recovery continued until darkness, at low stress, but was reversed by low evening light at moderate stress.

Soil temperature reached a peak of 20° C in late August and was at a minimum of 14° C in late September. Diurnal fluctuation was less than 1° C at 20 cm. Because of the low variation in soil temperature, it was not used as a variable in modelling k_1 response.

The relation between k_1 and radiation is reported to be hyperbolic for Douglas-fir (Jarvis, 1976) and the opening response of stomata is apparently saturated at low levels, about 0.1 ly min^{-1} (Running, 1976). All my measurements of k_1 were taken when there was some light. There was no clear relation between k_1 and radiation (R = .03). However, a slight drop in k_1 in the late evening (Figure 1) seemed to be related to dim light.

Due to the strong correlation between air temperature and absolute humidity deficit (R = .98), I decided to concentrate analysis on the relation between the latter and k_1. Furthermore, it was felt that absolute humidity deficit acted more directly on transpiration and in turn k_1 than did air temperature, especially under the mild summer weather.

The relation between k_1 and absolute humidity deficit was not clear (Figure 3) until the data were separated by level of pre-dawn Ψ_x, -2 to -4 bars, -5 to -9 bars, -10 to -12 bars, and -14 to -16 bars. Linear regression models were fitted to these data for each level of moisture stress.

Above 18 g m^{-3} absolute humidity deficit stomata were closed ($k_1 <$ .01 cm sec^{-1}) and k_1 did not change thus these values were eliminated from the regression analysis so that a simple linear model would fit the data. Also, late evening data were eliminated from the analysis ($>$ 1700 hours PST) because light could have been limiting. The F-test based on the extra sum of squares principle for differences among these models (Figure 3) was highly significant (P $\leq$ .01). The poor fit of the models at high moisture stress may be due to experimental error which become more important as k_1 decreased.

A large drop in k_1 was found as pre-dawn Ψ_x decreased below -4 bars expecially at low evaporative demand. Stomatal closure ($k_1 <$ .01 cm sec^{-1}) occurred sooner at low pre-dawn Ψ_x; closure occurred at 16 g m^{-3} at -2 to -4 bars and -5 to -9 bars pre-dawn Ψ_x and at 13 g m^{-3} at -10 to -12 and -14 to -16 bars pre-dawn Ψ_x.

The relation between k_1 during the day and pre-dawn Ψ_x was also examined (Figure 4). The interaction between moisture stress and absolute humidity deficit in their effects on k_1 made this relation unclear until the data were separated into six levels of absolute humidity deficit. Late evening data were excluded because light could have been limiting.
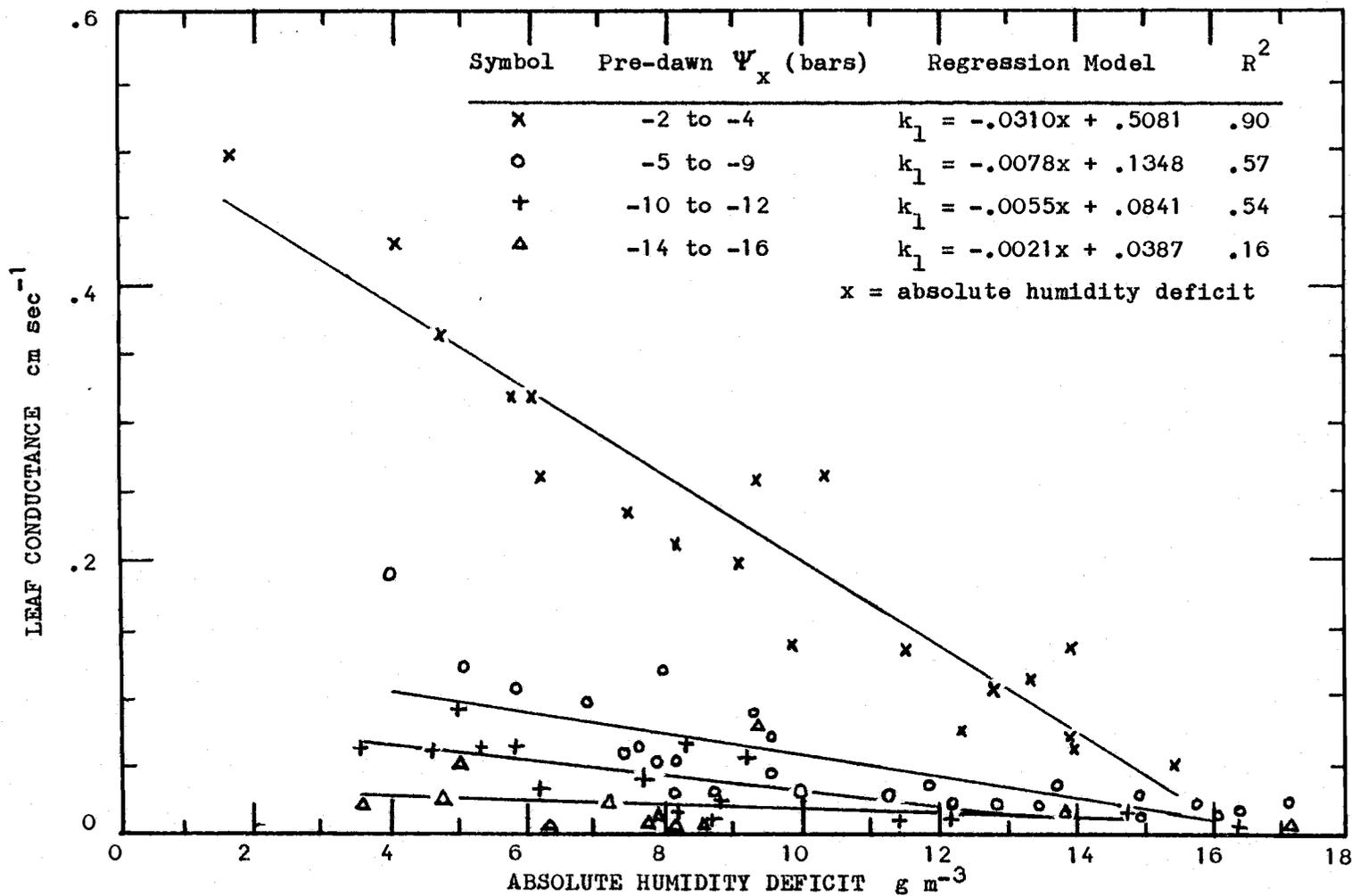
Fig. 3. Relationship between leaf conductance and absolute humidity deficit. The data were separated into four ranges of pre-dawn Ψ_x and were collected on 20 days. Each point represents the mean of 7 to 15 measurements. Curves were fitted by least squares method.
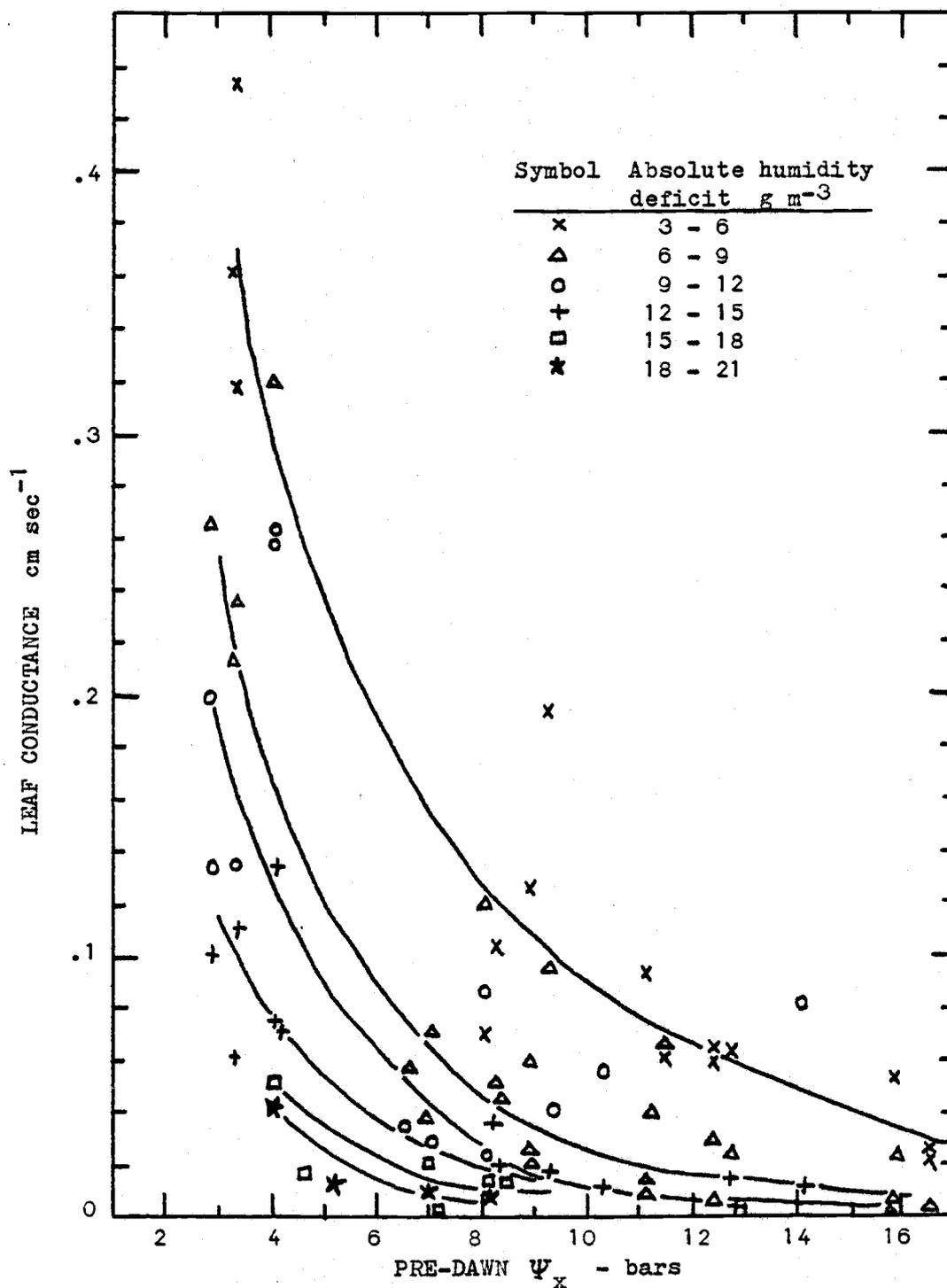
Fig. 4. Relationship between leaf conductance and pre-dawn Ψ_x. The data were separated into six ranges of absolute humidity deficit and were collected on 20 days. Each point represents the mean of 7 to 15 measurements. Curves were drawn by hand.

k_1 dropped rapidly with increasing moisture stress (decreasing pre-dawn Ψ_x). Most of the drop in k_1 occurred before the seedlings reached pre-dawn Ψ_x of -8 bars. Absolute humidity deficit had a marked influence on k_1 even at low moisture stress. There was a large reduction in k_1 when the absolute humidity deficit increased from 3-6 g m^{-3} to 6-9 g m^{-3} over the range of pre-dawn Ψ_x observed. As absolute humidity deficit increased stomatal closure occurred at lower levels of moisture stress: 3-6 g m^{-3}, -16 bars; 6-9 g m^{-3}, -15 bars; 9-12 g m^{-3}, -9 bars; 15-18 g m^{-3}, -8 bars, and 18-21 g m^{-3}, -7 bars.

Transpiration is probably a better measure of plant response to the environment because it is the quantity of water the plant is loosing to the atmosphere. The loss of water is what the plant should be controlling. On August 3 when k_1 dropped rapidly, early in the day, transpiration actually increased. Transpiration began to decrease later in the day when absolute humidity deficit decreased. At 1420 hours PST k_1 was only 39 % of maximum while transpiration was still 82% of the early morning maximum.

Daily transpiration greatly decreased as moisture stress increased (Table 1). As pre-dawn Ψ_x decreased from -3 bars to -13 bars, transpiration dropped from 53.3 mg cm^{-2} d^{-1} to 5.3 mg cm^{-2} d^{-1}. The greatest decrease in daily transpiration, as pre-dawn Ψ_x decreased, occurred when pre-dawn Ψ_x was still high, -3 to -5 bars.

The maximum depression of Ψ_x (midday Ψ_x minus pre-dawn Ψ_x) was highest at pre-dawn Ψ_x of -8 to -12 bars (Table 1 and Figure 5). At -26 bars pre-dawn Ψ_x, maximum depression of Ψ_x was only 2.6 bars.

Resistance to liquid flow in the plant can be expressed as the ratio of water potential gradient to water flux. As an index to the plant resistance, I used the ratio of maximum depression of Ψ_x to calculated daily transpiration flux (units were bars mg^{-1} cm^{-2} d^{-1}). The index values increased greatly with moisture stress, 0.2 at pre-dawn Ψ_x or -3 bars, 1.6 at -5 bars, 1.0 at -8 bars, 1.5 at -13 bars, and 5.3 at -17 bars.

Discussion

The close relationship among k_1, Ψ_x and absolute humidity deficit for Douglas-fir seedlings was clearly shown by the data presented in Figures 3 and 4 and was helpful in explaining diurnal patterns. As soon as light was adequate in the morning, stomata opened and k_1 quickly reached a maximum (Figure 1) which was largely dependent on pre-dawn Ψ_x and absolute humidity deficit. Running (1976) also found morning maximum k_1 to be dependent on pre-dawn Ψ_x. High transpiration rates due to high k_1 resulted in decreased leaf water potential, and stomata began to close early in the day. Minimum k_1 during daylight hours was reached late in the day when Ψ_x should have been lowest and absolute humidity deficit was highest. Sometimes there was a recovery of k_1 in the evening when radiation, temperature, and evaporative demand decreased. A similar recovery was reported by Tan and Black (1976). Finally, minimum k_1 was maintained as light became limiting at night.

Light causes a response in stomata only indirectly through light induced changes in CO_2 concentration in the stomatal cavity (Raschke, 1975) and normally, stomata open during the day and close

Table 1. Pre-dawn Ψ_x, maximum depression of Ψ_x, daily transpiration, daily radiation, maximum air temperature, and maximum absolute humidity deficit for five levels of pre-dawn Ψ_x.

Date	Pre-dawn Ψ_x	Maximum depression of Ψ_x	Transpiration	Radiation	Maximum Temperature	Maximum absolute Humidity Deficit	N
	bars	bars	mg cm^{-2} d^{-1}	ly d^{-1}	°C	g m^{-3}	
8/3/76	-3	10	53.3	610	25	13	15
7/14/76	-5	11	7.0	690	29	20	14
7/29/76	-8	11	11.1	640	28	16	7
7/29/76	-13	8	5.3	640	28	16	8
8/4/76	-17	9	1.7	170	20	8	8
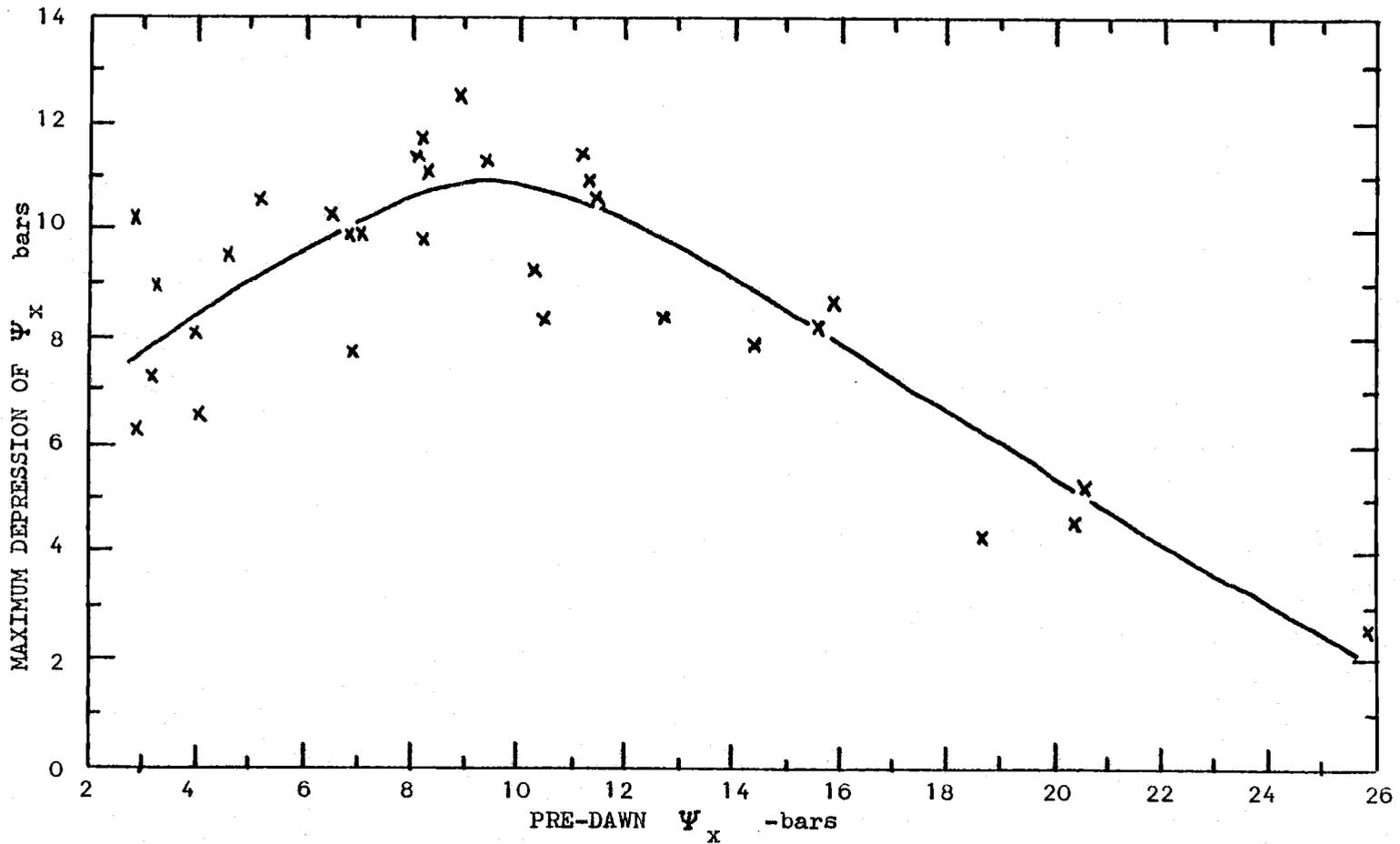
Fig. 5. Relationship of maximum daily depression of Ψ_x to pre-dawn Ψ_x. Each point represents the mean of 7 to 15 measurements. Data were collected on 20 days. The curve was drawn by hand.

at night (Meidner and Mansfield, 1968). However, Douglas-fir stomata have been found to open day and night during periods of high moisture in late fall, winter, and early spring (Emmingham, 1974; Running, 1975). During drier periods they close at night and open during the day, but very little light is needed for opening (0.1 ly min^{-1}). Any response of k_1 to light above the threshold is probably hidden by the more important controls of Ψ_x and atmospheric demand.

I found that k_1 normally decreased through the morning (Figure 1) while radiation increased. On July 27 and August 4, 1976, k_1 increased slightly between 0630 and 0900 hours PST as light increased. In the late evening dim light ($<$ 0.1 ly min^{-1}), k_1 was usually at a minimum, even though absolute humidity deficit decreased, which probably indicated a light response. However, on September 20 and 27, 1976 when pre-dawn Ψ_x was high, there was a recovery in k_1 which lasted until darkness.

The relation between k_1 and absolute humidity deficit was strongly influenced by plant moisture stress. Different linear regression models were used to fit the relation at four levels of pre-dawn Ψ_x. k_1 decreased as absolute humidity deficit increased from 2 to 16 g m^{-3}. I feel that Running (1976) over simplified the relation when he stated stomatal closure began at an absolute humidity deficit of 6 g m^{-3}. Stomatal closure ($k_1 <$ .01 cm sec^{-1}) occurred sooner at low pre-dawn Ψ_x than at high pre-dawn Ψ_x. Thus, as drought stress increased a lower evaporative demand caused stomatal closure. Black et al. (1975) plotted stomatal resistance against vapor pressure deficit for three levels of soil matric potential and found the level of vapor pressure deficit at which resistance began to increase,

decreased as soil moisture decreased.

The least squares fit of the data was not good at low pre-dawn Ψ_x (high moisture stress). At -14 to -16 bars R^2 was .159. This may be due to experimental error which became more important as k_1 decreased. However, similar results were reported by Hinckley and Scott (1971). They found a high correlation between radiation and sap flow at low moisture stress but little correlation between radiation, air temperature or both and transpiration at high stress.

The relation between k_1 and absolute humidity deficit should be compared among groups of plants with different levels of drought resistance. It may be possible to associate differences in this relation with differences in drought resistance, and thus, use it as a test for drought resistance. For example, drought resistance may be linked with high k_1 at low absolute humidity deficit and a very rapid decrease in k_1 as the absolute humidity deficit increases.

The relation between k_1 during the day and pre-dawn Ψ_x was made clearer when the data were separated into six levels of absolute humidity deficit (Figure 4). As pre-dawn Ψ_x decreased, k_1 dropped rapidly at first then more gradually as stomata closed completely. At all levels of absolute humidity deficit the greatest decrease in k_1 was seen before pre-dawn Ψ_x dropped below -8 bars.

As absolute humidity deficit increased, the level of pre-dawn Ψ_x necessary for stomatal closure ($k_1 < .01$ cm sec^{-1}) decreased. At 3-6 g m^{-3} closure did not occur even below -16 bars while at 6-9 g m^{-3} closure was seen at -15 bars. At 18-21 g m^{-3} stomata appeared to close at -7 bars. Stomatal closure is a mechanism to conserve water and thus a drought avoidance adaptation. If the level of pre-dawn Ψ_x and absolute humidity deficit at which closure occurs were found to be correlated with drought resistance, that correlation could be used as a test for drought resistance.

Tan and Black (1976) found a great deal of scatter in the relation between soil water potential and stomatal resistance which they felt was caused by the effect of humidity. The relation was made

clearer by dividing the data into six ranges of vapor pressure deficit. They went on to say (Black et al. 1975) that there was no clear relation between xylem water potential and soil matric potential, but vapor pressure deficit had a significant effect on xylem water potential during the day.

Transpiration was very high in the morning when k_1 was high and absolute humidity deficit was low (Figure 1). It remained high as k_1 decreased because absolute humidity deficit increased during the day. Seedlings at high pre-dawn Ψ_x showed less control of water loss than those at lower pre-dawn Ψ_x. At -4 bars the minimum transpiration was 23 to 44% of the daily maximum. Once the seedlings dropped below -8 bars, the minimum transpiration was only 1 to 12% of the maximum. It was possible that at high Ψ_x the guard cells were so turgid that they could not close tightly.

Leaf water potential can be considered a function of transpiration flux (T) and plant resistance to water movement (r_{plant}) by the following relation:

$$\Psi_{leaf} = \Psi_{soil} - r_{plant} (T)$$

(Elfving et al. 1972)

Pre-dawn Ψ_x represents an equilibrated soil-plant water potential (Waring and Cleary, 1967) and at least in small seedlings midday Ψ_x is not too different from midday leaf water potential. Thus, I substituted and rearranged the equation as follows:

$$\text{pre-dawn } \Psi_x - \text{midday } \Psi_x = r_{plant} (T)$$

According to this relation, if r_{plant} remains constant then maximum

midday depression of Ψ_x (pre-dawn Ψ_x - midday Ψ_x) is directly related to transpiration. However, the greatest depression of Ψ_x occurred between -8 and -12 bars pre-dawn Ψ_x while the highest transpiration came at much higher pre-dawn Ψ_x. Perhaps r_{plant} was much lower at higher pre-dawn Ψ_x as indicated by the index of plant resistance calculated above. Low r_{plant} would not allow a large midday depression of Ψ_x because the plant could quickly resupply water to meet demand. If r_{plant} increased with moisture stress as the index of plant resistance suggested, then differences between Ψ_{soil} and Ψ_{leaf} would develop more quickly at high stress, stomata would close sooner, transpiration would decrease, and the maximum depression of Ψ_x would be diminished. At pre-dawn Ψ_x of -26 bars daily fluctuation in Ψ_x was only 2.6 bars and transpiration was very low (Figure 5). Cleary and Waring (1967) and Ritchie and Hinckley (1975) reported that no daily change in Ψ_x occurred below -25 bars Ψ_x.

In further studies it may be possible to associate differences in the relation between r_{plant} (Or an index to plant resistance) and pre-dawn Ψ_x with differences in drought resistance. For example, drought resistance may be associated with a steep increase in r_{plant} as pre-dawn Ψ_x decreases.

Conclusions

These conclusions are based on data collected for Douglas-fir seedlings on k_1, Ψ_x and several environmental variables during twenty mild summer days in 1976 at Corvallis, Oregon. Pre-dawn Ψ_x, considered a measure of plant moisture stress (Waring and Cleary, 1967; Ritchie and Hinckley, 1975), ranged from -2 to -35 bars.

There was a typical diurnal pattern of k_1 for illuminated seedlings. k_1 was highest early in the morning; it declined rapidly during the morning and more slowly during the afternoon, and finally reached a low constant level late in the day which was maintained until darkness. There can be a recovery in k_1 late in the day, as temperature and evaporative demand decrease, for seedlings under low to moderate stress. Seedlings under low stress may continue the recovery until darkness.

At low moisture stress (pre-dawn Ψ_x was -2 to -4 bars) there was a good linear relation between k_1 and absolute humidity deficit. At higher stress the relation was not as clear. As moisture stress increased, the level of evaporative demand to cause stomatal closure decreased.

The relation between k_1 and pre-dawn Ψ_x was made clearer by separating the data into six groups of absolute humidity deficit. Apparently there was a strong interaction between pre-dawn Ψ_x and evaporative demand in their effects on k_1. At low to moderate levels of absolute humidity deficit, the greatest drop in k_1 with increasing stress occurred before pre-dawn Ψ_x dropped below -8 bars. As evaporative demand increased stomatal closure occurred at lower and lower levels of moisture stress.

Maximum transpiration was early in the morning when k_1 was also high, and it remained high even as k_1 decreased. Transpiration decreased to a minimum late in the day at a much slower rate than k_1. Calculated daily transpiration decreased with increasing moisture stress, with the greatest drop coming before -5 bars pre-dawn Ψ_x.

The highest diurnal depression of Ψ_x was found at moderate stress (pre-dawn Ψ_x was -8 to -12 bars). Daily transpiration was much higher at lower stress, but diurnal depression of Ψ_x was lower possibly because plant resistance to water flow was lower. At high moisture stress diurnal fluctuation in Ψ_x became very small.

The relations between k_1 and absolute humidity deficit, between k_1 and moisture stress and between r_{plant} and moisture stress should be compared among groups of plants with differences in drought resistance to see if differences in these relations can be associated with differences in drought resistance.

Chapter II

SCREENING FAMILIES OF DOUGLAS-FIR SEEDLINGS FOR DROUGHT RESISTANCE

Introduction

There are many programs in the Pacific Northwest for the development of genetically improved stock for Douglas-fir (Campbell, 1972; Campbell and Sugano, 1975; Ching, 1965; Ching and Bever, 1960; Irgens-Moller, 1957; Irgens-Moller, 1967; Hermann and Lavender, 1967; Lavender and Overton, 1972). These programs necessarily include testing of selected stock for superior productivity under various environments. Moisture supply is a major environmental factor controlling survival and productivity in the Pacific Northwest (Waring, 1969; Zobel et al. 1976). Therefore, we need to know the relative drought resistance of selected seedlings.

Differences in drought resistance between Douglas-fir seedlings of different origins has been demonstrated. Inland Douglas-fir were more drought resistant than coastal sources (Ferrell and Woodard, 1966; Pharis and Ferrell, 1966), and, in a small area, seedlings from a south slope source were more drought resistant than those from a north slope source (Ferrell and Woodard, 1966). Differences in drought resistance were expressed in drought hardiness and drought avoidance, but tests for drought avoidance gave greater differences (Ferrell and Woodard, 1966).

Drought avoidance mechanisms are adaptations which act to maintain a high water potential in the plant when it is exposed to

external water stress. For example, a plant under drought conditions can maintain a favorable internal water balance by closing its stomata to reduce transpiration. A large root:shoot ratio can have the same effect by increasing the ability of the plant to resupply moisture and meet demand (Levitt, 1972).

Transpiration is both a passive and an active process; however, within limits it is controlled by the plant through stomatal and mesophyll resistances. The most important short term control is by stomatal resistance (Slatyer, 1967). Douglas-fir are known to close stomata below a plant water potential of -20 bars (Lopushinsky, 1969; Running, 1976). If this threshold is not reached during the day, then leaf conductance of water vapor is strongly correlated with pre-dawn plant water potential. There is also a good correlation ($R^2 = .71$) between daily maximum leaf conductance and pre-dawn plant water potential (Running, 1976). I hypothesized that family differences may exist for Douglas-fir seedlings in the water potential threshold for stomatal closure and in the effect of water potential on diurnal patterns of leaf conductance.

Douglas-fir seedlings from a mesic provenance maintained a higher transpiration rate than those from a xeric provenance under high plant moisture stress (Unterschuetz et al. 1974). Closed stomata, while favorable to water conservation, are unfavorable to photosynthesis (Levitt, 1972). Therefore, this drought avoidance mechanism may enhance survival by preventing dessication but at the same time reduce growth. On the other hand, estimates of photosynthesis by CO_2 diffusion measurements have usually been weakly correlated with growth

(Ledig, 1969). I attempted to uncover relationships between diurnal transpiration at different levels of moisture stress and growth.

Levitt (1972) reported that drought increased the root:shoot ratio, thereby causing a drought adaptation, while Zahner (1968) stated that findings were not consistant and that some results showed no such response. It is clear that the development of a large root: shoot ratio would allow higher transpiration rates in trees under moisture stress, provided soil moisture is available (Levitt, 1972). In Douglas-fir results have been confusing, and Heiner and Lavander (1972) found no correlation between root:shoot ratio and seedling survival during drought. In contrast seedlings from a xeric provenance which should have been resistant to drought were shown to develop a higher root:shoot ratio than less resistant seedlings from a mesic provenance (Hermann and Lavender, 1967; Lavender and Overton, 1972). Therefore, I studied the interrelationships between transpiration, growth, and root:shoot ratio.

Two recent developments prompted the study. First, new instrumentation made possible field measurement of leaf conductance of water vapor and plant water potential for diurnal patterns. These data coupled with biomass data allowed me to test interrelationships among transpiration, plant water potential, root:shoot ratio, and growth. Second, high quality full-sib seedlings were readily available.

The objectives of this study were: 1) to determine if there were differences among several families of Douglas-fir seedlings from different provenances in adaptations to drought, and 2) to

determine if it was possible to easily screen families of seedlings for their level of drought resistance. It was reasoned that if seedling success was found to be correlated to the morphological-anatomical adaptations to drought, it could be possible to formulate a screening method for relative drought resistance in seedlings. Emphasis was placed on determining the proper time and conditions for making a few simple measurements.

Materials and Methods

The Weyerhaeuser Company supplied Douglas-fir seedlings grown from seed collected from trees selected for superior form and growth on three sites in the Coast Range near Centralia, Washington. The three sites were on very productive forest land within 25 kilometers of each other. Sixteen full-sib families were used including crosses between sites (Table 2).

Table 2. Code numbers for sixteen full-sib families resulting from controlled crosses between trees from three sites.

♂ \ ♀	Absher Creek	White Star	Eaton Creek
Absher Creek	137	107 115	129 134
White Star	104	106 117	109 116
Eaton Creek	126 138	103 110	128 132

Twice, during the summer drought of 1976, pre-dawn xylem water potential was measured by the pressure chamber technique (Scholander et al. 1965) on understory western hemlock at the three seed sources. This was done in order to compare the severity of drought experienced at the sites with the idea that the sites with the most drought would yield the most drought resistant seedlings (Emmingham, 1977).

Prior to outplanting the seedlings were grown for eleven months in 164 cm^3 plastic containers in a 1:1 peat-vermiculite mixture at a spacing of 270 trees m^{-2}. Efforts were made to produce large healthy seedlings by controlling the greenhouse environment and supplying abundant moisture and nutrients. At the time of outplanting the seedlings had a mean oven dry weight of 4.4 g, mean diameter 1 cm below the cotyledon scar of .38 cm, mean height of 31.08 cm, and a mean root:shoot ratio of .34.

The seedlings were planted in a raised bed of soil at Corvallis, Oregon at 10 cm spacing during March 6 to 8, 1976. The beds were 2 m wide, 5 m long, and .6 m deep. Two types of soil were in the bed; 45 cm of sandy loam river terrace soil was the bottom layer, and 15 cm sandy loam forest soil was the top layer. Underlying the bed was 15 cm of coarse gravel.

To facilitate summer measurements of plant water relations, seedlings to be measured during one day had to be planted close together. I decided to measure eight families per day, so eight families were randomly selected from the total sixteen and randomly assigned row positions in a block. The balance of families were randomly assigned row positions in a second block. This procedure

was repeated five times, and thus, each family was represented by a row of eight seedlings in five blocks in the bed. These blocks were randomly placed in the bed.

Until June 1 the soil was kept moist in order to ensure survival. It was assumed that the first flush of growth, which elongated during this period, had conditions conducive to normal leaf and shoot growth. Drought in the Willamette Valley normally begins in June. After June 1 drying began and a plastic transparent sheet was used to cover the seedlings during periods of rain to prevent wetting of the soil. Drying conditions were continued until September 13 when the soil was rewatered to field capacity to observe the plant response to renewed high soil moisture.

Bud condition was noted for forty seedlings per family for terminal and most advanced lateral bud every three and one-half days until all seedlings had burst bud. Bud burst was defined as new needles exposed. These data were used to calculate the family mean number of days from January 1, 1976 until bud burst of either terminal or lateral bud.

Plant size and biomass distribution were determined for a sample of ten trees per family at the beginning of the experiment. Stem height was measured from the first lateral root to the tip of the terminal bud, and stem diameter was determined by caliper 1 cm below the cotyledon scar. The seedlings were severed at the first lateral root, and needles were stripped from the top. Surface area of roots and needles was measured. Oven dry weight was measured for roots, stems, and needles. At the end of the experiment, September 27, 1976, 15 seedlings were carefully removed from the soil and analysed for

size and biomass distribution in the same way.

Surface area of roots and needles was measured while they were fresh at the beginning and end of the experiment. I used an optical planimeter which gave planar surface area that had to be corrected to actual surface area. Roots were considered cylinders, and the correction factor was taken to be π or 3.1415. Needle area was corrected by a factor of 2.36 which was determined by cross section analysis (Gholz et al. 1976). At first I measured all roots and needles, but this was too time consuming. It was decided to determine a weight to surface area ratio for roots and needles for each tree from subsamples. These ratios were then used to calculate total leaf and root surface area by tree from oven dry weights.

A sample of three trees per family was measured for diurnal changes in plant water potential (Ψ_x) and leaf conductance of water vapor (k_1) at four different times during the period, July 13 to September 27, 1976. Drought was allowed to increase during this period until many of the seedlings had pre-dawn Ψ_x below -20 bars, before rewatering on September 13, 1976. Measurements were taken only on cloudless days except for August 4 and 5, 1976 which were partly cloudy. The measurement days were generally mild. The maximum temperature observed was 32° C and the minimum was 9° C.

Ψ_x was measured by the pressure chamber technique described by Scholander et al. (1965) and Waring and Cleary (1967). A sample twig was excised from a lower branch five to ten cm above the ground and immediately measured for Ψ_x. Ψ_x was measured twice a day, just before dawn and midday (1200 to 1400 hours Pacific Standard Time (PST)).

k_l was measured with a null-balance diffusion porometer (Beardsell et al. 1971) until August 5, 1976 and with an aspirated diffusion porometer (Turner and Parlange, 1976) after that date. The null-balance porometer was preferred because it was faster, but it had to be used elsewhere. Data from the two instruments were comparable because they were both calibrated to give absolute values of k_l. In any case, instrument differences did not affect the data analysis because I studied differences within diurnal runs and not between.

Leaf samples measured for k_l were on the tips of lateral branches in the highest whorl of branches on a seedling. Most of the foliage in the samples was fully elongated for the first diurnal run, and after that it was well hardened. Measurements began as soon as there was light and the dew was evaporated, and they were repeated on the same branch every two to three hours until dark. Measurements were taken three to seven times on each day. In order to prevent dew formation the seedlings were covered by a plastic sheet at night. The cover allowed air circulation and was believed to not affect Ψ_x and k_l measured during the next day. Two days were required to measure all sixteen families, eight families per day, with the null-balance porometer. With the aspirated diffusion porometer four days were required, four families per day. At the end of each measurement day, the leaf conductance sample was clipped and taken to the laboratory for surface determination. Planar surface area was measured with an optical planimeter and corrected to actual surface area by a factor of 2.36 (Gholz et al. 1976). k_l was calculated for total leaf surface area.

Transpiration ($g\ cm^{-2}\ sec^{-1}$) was estimated as the product of k_1 ($cm\ sec^{-1}$) and absolute humisity deficit ($g\ cm^{-3}$) which was measured nearby (Comacho-B. et al. 1974; Elfving et al. 1972; Running et al. 1975). It was considered a good estimate of transpiration when leaf to air temperature difference and boundary layer resistance were low (Comacho-B. et al. 1974). These conditions were likely to exist for the small needles of Douglas-fir. Daily transpiration ($mg\ cm^{-2}\ d^{-1}$) was estimated by integrating diurnal estimates over the day.

Daily transpiration estimates were used to test for differences among families in water use at four levels of pre-dawn Ψ_x, -3.7 bars (September 20, 23, 24, 27), -6.3 bars (July 13, 14), -10.8 bars (July 28, 29) and -12.3 bars (August 4, 5). To remove differences between days at each level of moisture stress, the difference between the daily mean transpiration and the run mean was added to the value for each tree. Further analysis was done on the adjusted values. Pre-dawn Ψ_x was used as a covariable in the analysis of covariance to test for family differences.

Soil temperature at 20 cm in the bed and air temperature at 100 cm above the bed were measured throughout the experiment by Partlow 30 day continuous recording mercury thermometers. At a more complete meteorological station 100 m from the seedlings, continuous measurements were made of air temperature with a thermistor and dew point temperature with a heated lithium chloride sensor (Interface Instruments Inc., Corvallis, Oregon). From the air and dew point temperature measurements, absolute humidity deficit ($g\ m^{-3}$) was calculated. Solar radiation ($ly\ min^{-1}$) was measured by an Epply pyranometer.

Results

Drought Stress at Seed Sources

Pre-dawn xylem water potential of understory vegetation during peak drought was considered an index of the severity of drought for comparing forest environments (Waring, 1969). The Absher Creek site had the lowest readings, Eaton Creek had the highest, and White Star was intermediate (Table 3). Thus, Absher Creek was the driest, Eaton Creek the wettest, and White Star was in between.

Table 3. Pre-dawn Ψ_x in bars for summer 1976 for understory western hemlock at three seed sources (Vertical bars indicate values which were not significantly different ($P \leqslant .05$)).

Source	N	Date 8/10/76	10/12/76
Absher Creek	11	-5.8	-7.9
White Star	6	-5.0	-4.4
Eaton Creek	11	-4.3	-4.1

Date of Bud Burst

There was a fifteen day span between extremes in family mean number of days from January 1, 1976 until bud burst (Table 4). The F-test for family differences was highly significant ($P \leqslant .01$). The multiple range test for differences between pairs of families based on the Studentized Range was Q = 3 days ($P \leqslant .05$) (Snedecor and Cochran, 1967).

Table 4. Ranked family mean number of days from January 1, 1976
until bud burst (Absher Creek = A, White Star = W,
Eaton Creek = E).

Family Code	Origin	Number of Days from January 1, 1976
129	A x E	95
137	A x A	98
138	E x A	99
128	E x E	100
126	E x A	101
107	A x W	101
104	W x A	101
103	E x W	103
115	A x W	104
134	A x E	104
110	E x W	104
132	E x E	104
109	W x E	106
117	W x W	107
116	W x E	108
106	W x W	110

Plant Size and Biomass Distribution

The mean total dry weight per seedling almost tripled from 4.4 g to 11.8 g. Mean diameter increased from .38 cm to .59 cm which meant an increase in cross sectional area from .11 cm^2 to .27 cm^2. Mean height increased by a factor of 1.7 from 31.1 cm to 51.2 cm.

It was expected that date of bud burst would be correlated with plant size and growth. I did find family mean date of bud burst was highly correlated (R = .74) with family mean initial weight. However, date of bud burst was not correlated with family mean final weight (R = .28), growth (R = .02) and ratio of growth to initial weight (R = -.47). Date of bud burst was also not correlated with family mean root:shoot ratio at the beginning (R = .15) and end (R = .13) of the experiment.

Family mean root:shoot ratios are presented in Table 5. The F-test based on analysis of variance was highly significant (P $\leq$ .01) for differences among families for initial and final measurements. Variation in the ratio decreased between the first and second measurements as evidenced by the decreased range of values, .186 to .582 compared to .275 to .400. Generally, the high values decreased and the low values increased. Mean root area: leaf area ratios are also presented in Table 5 by family for the final measurement. The F-test found family differences to be highly significant (P $\leq$ .01).

Because root:shoot ratio was dependent on plant size (Ledig, 1969) and in order to determine the changes in allocation of plant resources during the summer, the allometric equation was applied to these data.

$$\ln (\text{root weight}) = b (\ln (\text{shoot weight})) + a$$

Slope (b) and intercept (a) were calculated for each family for a line between the initial and final mean values for root and shoot weight. The slope represented the ratio of the relative rate the roots were increaseing to the relative rate the shoots were increasing. The values of b ranged from .666 for family 134 to 1.459 for family 132 (Table 5). Low values indicated less was being devoted to roots than shoots, and high values meant roots were being favored (Pearsall, 1927).

Biomass data were further reduced in two different indexes of productivity. First, the ratio of family mean growth to family mean initial weight showed quite a range among families from 1.32 to 2.30. Second, net assimilation rate (NAR) (Hughes and Freeman, 1967) was calculated by the following equation:

$$NAR = \left[(\ln (\text{weight})) / \triangle t \right] / \left[\text{antln} (\ln (\text{leaf area}) -\ln \text{weight})) \right] \ g \ cm^{-2} \ yr^{-1}$$

where t equals time, and family means were used. In the denominator the / averages of initial and final values were used for leaf area and weight. The family values of NAR ranged from .0136 to .01888 g cm^{-2} yr^{-1}. In this experiment the year was March to September.

Daily Transpiration

Family means of calculated daily transpiration (mg cm^{-2} d^{-1}) were examined for four levels of pre-dawn Ψ_x, -3.7 bars, -6.3 bars, -10.8 bars, and -12.3 bars. At -10.8 bars only ten families were compared, otherwise there were 16 families compared at each level.

Table 5. Family mean values for initial and final root:shoot ratio,
          final root area: leaf area ratio and values of slope (b)
          from the allometric equation.

Family Code	Root:Shoot Ratio		Root area:Leaf area	Slope(b)
	Initial	Final	Final	
103	.263	.307	.249	1.133
104	.305	.322	.276	1.056
106	.316	.306	.226	0.964
107	.302	.354	.301	1.140
109	.398	.335	.270	0.807
110	.311	.292	.223	0.931
115	.335	.342	.316	1.023
116	.354	.342	.255	0.962
117	.432	.395	.364	0.932
126	.343	.312	.292	0.909
128	.346	.312	.263	0.887
129	.299	.337	.239	1.125
132	.186	.275	.255	1.459
134	.582	.400	.355	0.666
137	.415	.333	.267	0.805
138	.280	.302	.200	1.068

On the days transpiration was measured, the seedlings varied slightly from the stated level of pre-dawn Ψ_x. For this reason and because there was a significant linear regression between pre-dawn Ψ_x and transpiration, pre-dawn Ψ_x was used as a covariable in the analysis of variance. The F-test for family differences was not significant at any of the four levels.

Families were ranked by calculated daily transpiration at each level of moisture stress, and it was found that two families, 106 and 138, always had the lowest transpiration. Also, families 117 and 134 consistently had the highest transpiration. There were no obvious trends for families which fell in the middle range. Figure 6 shows the daily course of transpiration at pre-dawn Ψ_x of -3.7 bars for the highest and lowest families. The three runs (levels of pre-dawn Ψ_x) which had sixteen families were used to calculate a mean ranking of transpiration for all families (Table 6).

To compare water use response to increasing moisture stress among families, I calculated transpiration at -6.3, -10.8, and -12.3 bars pre-dawn Ψ_x as a percent of transpiration at -3.7 bars pre-dawn Ψ_x (Table 6). At the highest stress, -12.3 bars pre-dawn Ψ_x, five families, 106, 126, 129, 132, and 138, had transpiration rates less than ten percent of the maximum. Families 103, 106, 109, 110, 132, and 138 had important decreases in transpiration as pre-dawn Ψ_x decreased from -6.3 bars to -12.3 bars. Families 104, 107, and 116 actually increased transpiration over the same range of moisture, and there was not much change in the percentage for families 115, 117, 126, 128, 129, 134, and 137.
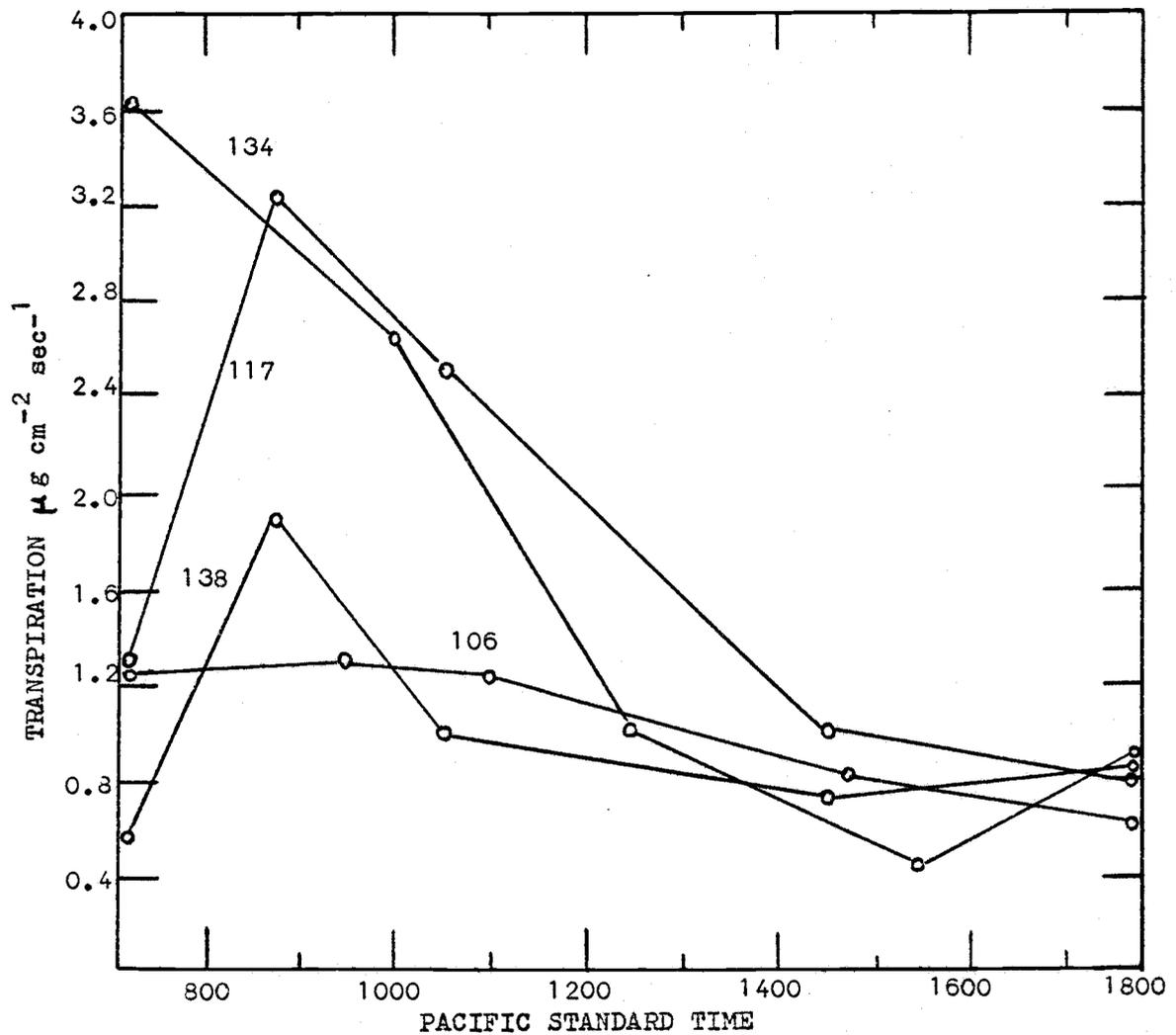
Fig. 6. Daily course of transpiration for families with high daily transpiration(134 and 117) and low transpiration(138 and 106). Each point represents the mean of three measurements. Average pre-dawn Ψ_x was -4 bars.

Table 6. Family mean ranks for transpiration, mean calculated daily transpiration at -3.7 bars pre-dawn Ψ_x, transpiration at -6.3 bars, -10.8 bars, and -12.3 bars pre-dawn Ψ_x as a percent of transpiration at -3.7 bars pre-dawn Ψ_x.

Family Code	Transpiration Rank	Mean Daily Transpiration at -3.7 bars ($mg\ cm^{-2}\ d^{-1}$)	Percent Transpiration (bars)		
			-6.3	-10.8	-12.3
103	8	52.88	17	--	11
104	14	43.36	8	16	15
106	15	35.60	21	16	8
107	11	47.81	12	--	20
109	6	52.97	24	--	12
110	13	43.24	17	--	14
115	3	64.00	13	18	10
116	9	53.01	9	--	19
117	2	63.05	15	21	17
126	7	58.56	8	7	5
128	12	46.09	15	15	16
129	5	62.53	8	--	8
132	4	59.60	16	17	9
134	1	68.62	16	11	16
137	10	51.45	15	18	12
138	16	38.04	9	11	4
$\bar{x}$		52.51	14	15	12

There was no significant correlation between midday depression Ψ_x (pre-dawn Ψ_x minus midday Ψ_x) and transpiration for any of the runs.

The seed sources were ranked by their level of drought stress measured by pre-dawn Ψ_x of understory western hemlock as follows: Absher Creek (1), White Star (2), and Eaton Creek (3). Family indexes of drought stress experienced by the parents were calculated as the product of the ranks of the sources of both parents. For example, family 137 was Absher Creek by Absher Creek, so it was ranked 1 (1 x 1), and family 128 was Eaton Creek by Eaton Creek, so it was ranked 9 (3 x 3). These family rankings were not correlated with transpiration (-3.7 bars, R = -.06 bars; -6.3 bars, R = .26; -10.8 bars, R = -.01; and -12.3 bars, R = .01).

There were found to be no significant correlations between family mean daily transpiration and final weight, growth:initial weight ratio, and slope of allometric equation. NAR was significantly correlated with transpiration at -10.8 bars pre-dawn Ψ_x only. Root:shoot ratio and root area:leaf area ratios were significantly correlated with transpiration for two out of four and three out of four levels of moisture stress, respectively (Table 7).

Leaf shape was tested for its effect on transpiration. The family mean ratio of leaf area to leaf weight varied widely for the final biomass measurement from 156.6 $cm^2 g^{-1}$ to 177.0 $cm^2 g^{-1}$ and was considered a good measure of leaf shape. This ratio was not correlated with daily transpiration for any run (correlation coefficients were -.15 to .10).

Table 7. Correlation matrix for family means of calculated daily
transpiration at four levels of pre-dawn Ψ_x by final
weight, growth:initial weight, slope of allometric
equation, NAR, root:shoot, and root area:leaf area.

	Daily transpiration at four levels of pre-dawn Ψ_x			
	-3.7	-6.3	-.0.8	-12.3
Final weight	-.029	.137	.136	.144
Growth: Initial weight	.022	-.199	-.097	-.006
Slope of Allometric Equation	-.072	-.301	.100	-.314
NAR	.287	.150	.674[*]	.374
Root: Shoot	.563[*]	.261	.457	.760[**]
Root Area: Leaf Area	.705[**]	.473	.704[*]	.788[**]
Number of Pairs	16	16	10	16

[*] $p \leqslant .05$.
[**] $p \leqslant .01$.

Discussion

Pre-dawn moisture stress measurements indicated Absher Creek had the most severe drought during 1976, Eaton Creek experienced the least moisture stress, and White Star was intermediate. Topographic features at the sites supported these data. Absher Creek source was two sloping ridge tops, one northwest facing and one southwest facing; White Star was a broad ridge,and Eaton Creek was a level bottom. Accordingly, I expected that Douglas-fir from Absher Creek would be best adapted to drought, Eaton Creek least adapted, and White Star intermediate (Emmingham, 1977). I acknowledge that one year's data are scant information to base conclusions on. Furthermore, these sites were never under severe moisture stress (pre-dawn Ψ_x, -4 to -8 bars) compared to the range of stress (pre-dawn Ψ_x, -5 to -20 bars) found in Douglas-fir forests in western Oregon (Zobel et al. 1976).

Bud burst data provided the first indication that family differences existed. Family mean bud burst date ranged 15 days, from 95 days (April 5) to 110 days (April 20) from January 1, 1976. By seed source the mean for families with at least one parent from Absher Creek was 100 days, Eaton Creek 102 days, and White Star 105 days. Only the Absher Creek and White Star means were significantly different ($P \leq .05$). When families were ranked by date of bud burst, none of the families with at least one parent from Absher Creek fell in the last six, and none of the White Star families fell in the first five.

Previous work with Douglas-fir seedlings strongly suggested that early growth of roots and shoots was correlated with superior survival potential under drought stress (Heiner and Lavender, 1972). Also, plants which burst bud early may grow for a longer period, before moisture stress becomes important, than those which burst bud late. Growth under high moisture stress (low Ψ_x) as during drought, is severely curtailed (Emmingham, 1977; Hsiao, 1973). From my data it appeared that Absher Creek seedlings had a five day advantage in beginning growth over White Star seedlings which could be a drought avoidance adaptation. This was consistant with the hypothesis that seedlings fromthe driest site (Absher Creek) were the most drought resistant. However, Absher Creek seedlings did not grow more than White Star seedlings (mean dry weight growth was 7.8 g for Absher Creek and 7.7 g for White Star which were not significantly different.)

I expected the transpiration response to moisture stress to depend on the physiological condition of the seedlings. The relations between stomatal conductance of water vapor and environmental variables change with the physiological condition of the plant in conifers (Jarvis, 1976) and hardwoods (Turner and Heichel, 1977). Date of bud burst may be correlated with the physiological condition of the plant, but it was not correlated with transpiration at any level of moisture stress.

A favorable root:shoot ratio is an important drought adaptation (Levitt, 1972). Drought resistance in wheat was associated with an extensive root system (Hurd, 1974). In order to survive drought a plant should devote more energy to its root system to explore the soil

for remaining moisture at the expense of the above ground parts. Reduction in the transpiring surface reduces demand for moisture and possibly facilitates a better water balance in the plant during drought (Kozlowski, 1976).

There were significant differences among families in root:shoot ratio before and after the 1976 growing season, but these differences decreased. It seemed that the seedlings were converging on a common optimum root:shoot ratio, as the range in values decreased. Large ratios decreased and small ratios increased. In contrast, Lavender and Overton (1972) and Hermann and Lavender (1967) reported seedlings from a xeric provenance which should have been resistant to drought developed a higher root:shoot ratio than less resistant seedlings from a mesic provenance. I found no significant differences among provenance means for final root:shoot ratio (Absher Creek, .338; White Star, .333; Eaton Creek, .321) which may indicate there were small differences in drought experienced at the sites.

Natural selection under drought is known to result in larger roots (Hermann and Lavender, 1967; Hurd, 1974; Lavender and Overton, 1972). My data suggested that not only are the differences in root: shoot ratio small but the seedlings were allocated energy to reduce the differences. Thus, some mechanism in the seedlings was causing them to converge on an optimum root:shoot ratio rather than simply to develop large roots. However, most of the growth occurred before soil moisture became limiting.

Root:shoot ratio is known to change with plant size (Ledig, 1969), and indeed there was a significant correlation (R = .506) between total weight and root:shoot ratio. For this reason I decided to apply

the allometric equation to the biomass data. The slope of the line which is most often determined by the least squares method is the ratio of the relative rates of growth of the root and shoot. It is important to note that this equation is meant to be applied to data representing changes in time and not space (Hughes and Freeman, 1967).

I calculated the slope between initial and ending family means, and the range was .666 to 1.459. According to Pearsall (1927) a slope of 1 means both parts of the plant are growing at the same rate; 1.5 means the roots are increasing as to volume and the shoots as to area, and .67 means the roots are increasing as to area while the shoots are increasing as to volume.

In general the families with low initial root:shoot ratios had large slope values, and those with high initial ratios had low slope values (Table 5). The value of the slope was directly related to the degree to which the initial value deviated from the average. Thus, the propensity to converge on the optimum root:shoot ratio increased with the difference between the actual family ratio and the optimum.

Originally, I had hoped to analyse family differences in maximum morning leaf conductance and diurnal patterns of k_l. However, these analyses were not possible because it was not feasible to achieve the sampling intensity necessary to resolve differences. For example, one to two hours were required to measure all families (eight families were measured each day), but in the early morning k_l changed more rapidly than this, making comparisons among families impossible. Patterns of k_l during the day did not lend themselves to easy interpretation for the same reason.

Daily transpiration is an easily measured plant function which is strongly influenced by drought (Lopushinsky and Klock, 1974, Running, 1976; Emmingham, 1977; Unterschuetz et al. 1974; Zavitkovski and Ferrell, 1970). But understanding drought resistance in plants is accomplished only by studying the interrelationships of transpiration, plant water potential and root:shoot ratio. Fluctuations in Ψ_x change leaf resistance to water flux to the atmosphere, and thus, determine transpiration (Comacho-B. et al. 1974; Elfving et al. 1972; Jarvis, 1976; Lopushinsky, 1969). Root:shoot ratio balances the supply and demand of plant water relations (Kozlowski, 1976; Levitt, 1972) and, at least in part, influences plant water potential and in turn transpiration.

Despite the fact that there were no statistically significant differences in transpiration among families, there were important trends. Two families, 138 and 106, had low transpiration, and families 117 and 134 were always high in transpiration. It seemed that drought resistance should be a transpiration response to Ψ_x. In other words, there should be some change in transpiration with increasing drought. However, these families did not change their relative position in transpiration but were always high or low. This indicated there were no differences in transpiration response to drought.

Drought resistant families might decrease transpiration rate significantly with decreasing Ψ_x in order to ensure survival by conserving water. On the other hand, extreme stomatal closure to conserve water may reduce photosynthesis by reducing gas exchange and result in less growth. The data in Table 6 show some families

decreased transpiration as pre-dawn Ψ_x decreased from -6.3 bars to -12.3 bars; some increased transpiration, and several remained the same. These trends were not correlated with growth, so at least under the conditions of this experiment; differences in growth could not be linked to the transpiration response to drought.

Transpiration was correlated with root area:leaf area ratio for three out of the four runs (Table 7). By regulating the supply and demand components of water flux through the seedlings, the distribution of biomass to roots and leaves can apparently influence the rate of transpiration. This relationship held for root:shoot ratio as well but for only two out of the four runs. These data supported the hypothesis that root:shoot ratio significantly influences plant water relations.

Transpiration did not correlate with any of the other biomass variables such as total weight, growth:initial weight, slope of the allometric equation, and NAR. Ledig (1969) reported that gas exchange measurements of photosynthesis have always been poorly correlated with growth. Thus, it was not surprising that our measurements of transpiration were also poorly correlated with growth.

The proximity of the seed sources and the small range of moisture stress (Table 3) pointed to very little difference in natural selection for drought resistance among these sources. However, differences in drought resistance have been demonstrated among Douglas-fir seed sources of narrow distribution for north versus south slope sources (Ferrell and Woodard, 1966). Because of the tremendous amount of natural genetic variation in Douglas-fir, attested to by its broad

range and plasticity to many ecological variables, I expected to find differences among a large number of families, even if they came from similar seed sources. The fact that seed trees were selected for their superior growth and form also mediated against this hypothesis. The results of this study, clearly limited in scope, did not suggest that families of similar geographic origin could be different in their drought resistance.

Conclusions

The sixteen families studied showed a narrow range in drought resistance. Seedlings from the most xeric provenance burst bud earlier than those from wetter sites which may be a drought adaptation. Root: shoot ratio, productivity and transpiration responses to moisture stress were not related to drought experienced at the seed sources. Plants allocated energy to achieve an optimum root:shoot ratio common to all families. There were no significant differences in transpiration among families over the range of moisture stress tested. There were no interrelationships between bud burst, root:shoot ratio, and productivity.

Transpiration was strongly correlated with root area:leaf area ratio, and root:shoot ratio. It may be possible to use a simple measurement of transpiration to estimate root:shoot ratio of families of seedlings in the nursery. A better understanding of the relationship of planting success to root:shoot ratio for different species would make this test even more valuable.

In order to detect genetic differences in drought resistance, there must be large variation in the genetic material. The best way to achieve this is to select from a broad range in seed source moisture conditions. This study was limited to a narrow range of seed sources, and thus, differences were small and hard to detect.

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APPENDIX

APPENDIX: MEASUREMENT OF LEAF CONDUCTANCE OF WATER VAPOR

General Theory

The two main pathways for water movement from the vascular
system to the external leaf surface are through the stomata and
the cuticle. When stomata are open the predominance of evapora-
tion is through the stomata, and when they are closed no water loss
occurs through them. In the leaf with closed stomata, the only
pathway for water transport is through the cuticle. These path-
ways are in parallel, and the resistances they represent can be
expressed as follows:

$$1/r_1 = 1/r_c + 1/r_s$$

where r_1 is leaf resistance to water vapor conductance, r_c is
cuticular resistance, and r_s is stomatal resistance (sec cm^{-1}).
Stomatal resistance can be further broken into the following
components:

$$r_s = (r_w + r_i + r_p)$$

where r_w, r_i, and r_p are the resistances of the mesophyll walls,
intercellular spaces, and the stomatal pore, respectively (Slatyer,
1967).

All leaves have a certain external or boundary layer resistance
to water vapor diffusion. This external resistance is dependent on
the geometry and size of the leaf and the air turbulence. Thus, the

equation for total leaf resistance becomes:

$$r = r_a + r_l$$

where r_a is the boundary layer resistance, and r is the total leaf resistance (Slatyer, 1967).

Many plants have leaves which are amphistomatous; they have stomata on the upper (u) and lower (l) sides. In this case, r should be represented as follows:

$$r = \frac{(r_{au} + r_{lu})(r_{al} + r_{ll})}{(r_{au} + r_{lu}) + (r_{al} + r_{ll})}$$

In the case where the resistances of both sides of the leaf are equal, the measured total leaf resistance is one-half the resistance of one side. Total leaf conductance would be twice the conductance of one side (Burrows and Milthorpe, 1976). Resistances and conductances are often presented in the literature per unit leaf area (one side), while Douglas-fir data have most often been presented per unit surface area (two sides). I have adapted the convention for Douglas-fir.

The complex arrangement of the components of the water transport system of the leaf has resulted in almost all measurements being reported in resistance values for the last twenty years. This practice is alright when certain resistance components are to be assessed, but it is inappropriate when functional relationships are emphasized because they tend to be obscured. Functional relationships are more likely to be linear with conductance ($1/r$) than with resistance. For example, the relation between conductance and stomatal aperature is

nearly linear while the relation between resistance and stomatal aperature is hyperbolic. Interpretation of the relation with conductance is much easier. Furthermore, statistical analysis of resistance data may require logarithmic transformation because the variance of resistance varies with the mean. The variance of stomatal conductance is reasonably constant over the known range of values making statistical analysis easier (Burrows and Milthorpe, 1976).

Plant-water relations work in the past has concentrated on measurement of stomatal aperature by microscopic or plastic impression techniques. It has become more popular in recent years to measure stomatal response by determining in some way the ability of the whole leaf to conduct gases either by diffusion or mass flow. New techniques have been developed which are faster and more representative of plant processes. One measurement averages the response of many hundreds of stomata and thus, is more representative of general action and more sensitive to small changes. Also, since diffusion resistance to gas flow is a major factor controlling transpiration and CO_2 assimilation, it is more pertinent than stomatal aperature (Kanemasu, 1975).

Mass flow porometry measures stomatal response by forcing air under pressure through the leaf and relating rate of flow to porosity. This technique is only effective with leaves which are amphistomatous (Hsiao and Fischer, 1975) which is not the case with Douglas-fir leaves.

Diffusion porometry is based on the measurement of the rate of diffusion of water vapor in response to concentration gradients. It measures, not only diffusion from the stomata, but also that from the

cuticle and thus, is a better indicator of plant response to the
environment over a wide range of conditions. The diffusion technique
depends on isolating the leaf sample momentarily in a chamber and
measuring the transpiration rate with a humidity sensor. It has the
advantage of being rapid and nondestructive--the sample can be
measured many times (Kanemasu, 1975). In this study I used two
diffusion porometers, the aspirated diffusion porometer (Turner and
Parlange, 1970) and the null-balance diffusion porometer (Beardsell
et al. 1975). Further discussion will be limited to these two
techniques.

The Aspirated Diffusion Porometer

Theory and Calibration

The porometer is simple and compact and thus, has great advan-
tage as a field instrument. It comprises only two units, an acrylic
chamber to which the leaf can be attached (Figure 7) and a meter to
record changes in humidity (Turner and Parlange, 1970). The leaf
sample is isolated in the   chamber, and transit time for a
specified change in humidity is measured. Transit time is shortest
for high conductances. One disadvantage of this porometer is the
complicated calibration.

The basic expression for the diffusion rate of water vapor
from the leaf interior into the porometer chamber is:

$$dc/dt = \frac{A(c_\infty - c)}{rV}$$

where $A(cm^2)$ is sample surface area, $V(cm^3)$ is chamber volume,

$r(\text{sec cm}^{-1})$ is diffusive resistance, and $c_\infty - c$ (g cm^{-3}) is the difference between saturation water vapor concentration at chamber temperature and the concentration in the chamber. Broad leaves are clamped against the chamber port, so A is simply the area of the port and is constant. Leaf surface area must be determined for conifer needles. r is actually the sum of the resistances of the stomata, porometer, and boundary layer. Instrument calibration requires V and porometer resistance to be determined (Turner and Parlange, 1970).

V is composed of two parts, the volume of the chamber itself (29 cm^3), which is fixed, and the effective capacity of the sensor as it adsorbs water. The adsorbtion of the acrylic chamber can be ignored because it is so small, less than 1 % of the total effective volume. Sensor capacity has been determined for a range of temperatures (29 cm^3 at 35° to 137 cm^3 at 15° C). Porometer resistance has been empirically determined by introducing saturated filter paper to the chamber; the only resistance will be that of the chamber. For broad leaves the the values ranged from 1.01 to 1.14 sec cm^{-1} as temperature decreased, and for conifer needles it was estimated to be 1.5 sec cm^{-1} and constant (Turner and Parlange, 1970).

Stomatal resistance is linearly related to transit time for a fixed increase in humidity. Temperature affects the range in humidity over which lapse time is measured, and the manufacturers' specifications should be used to make necessary calculations. Finally, the appropriate equation for stomatal resistance (r_s) is:
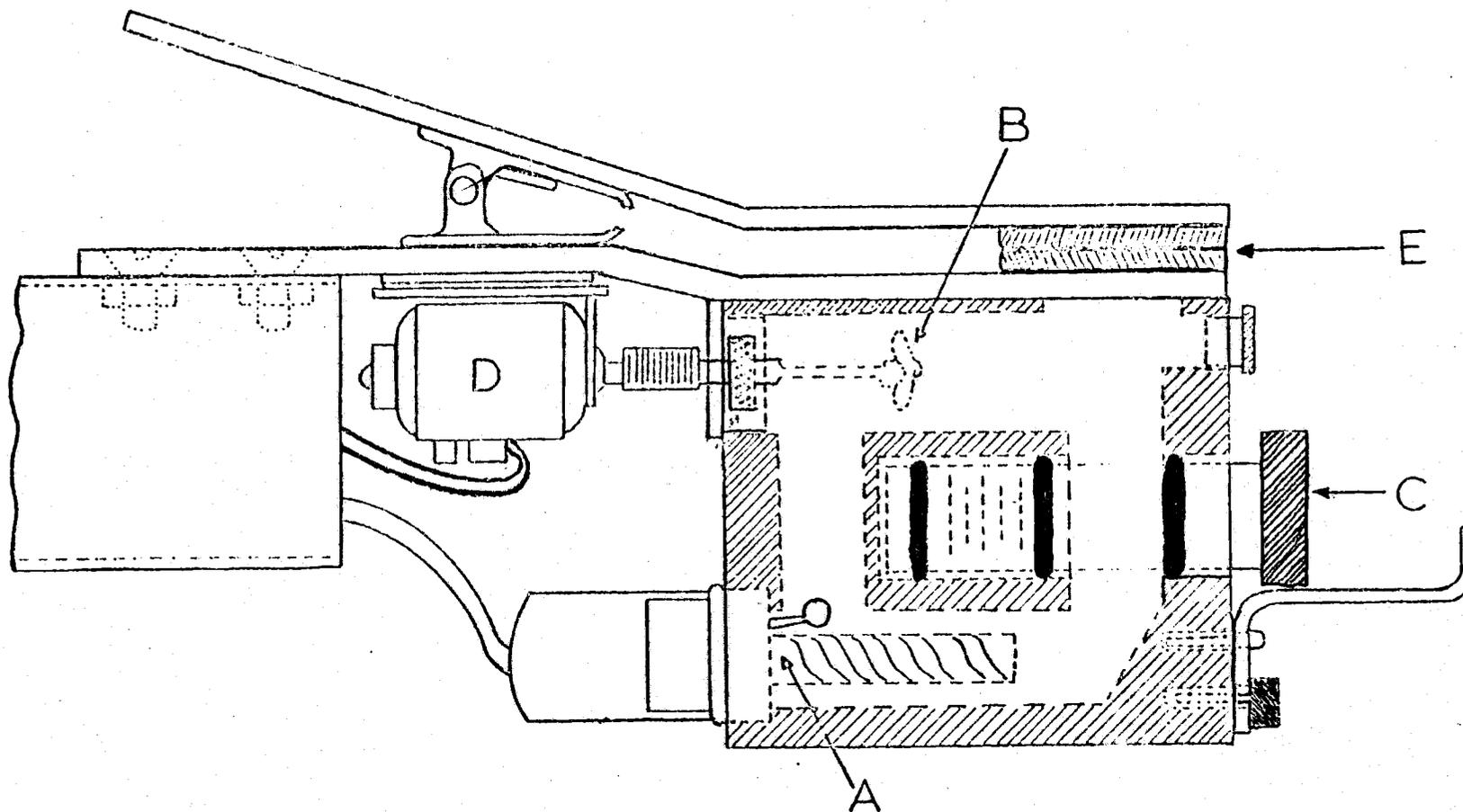
Fig. 7. Aspirated diffusion porometer: A. Humidity sensor and bead thermistor; B. fan; C. plunger containing silica gel dessicant; D. fan motor; E. closed cell sponge rubber for sealing port (Turner et al. 1969).

$$r_s = \frac{At}{V \ln \dfrac{(c_\infty - c_o)}{(c_\infty - c)}} - r_{porometer}$$

where $c_\infty - c_o$ is the change in humidity over which lapse time is measured (Turner and Parlange, 1970).

An empirical calibration is possible using perforated plates of known resistance in front of saturated filter paper, and such experiments have compared well with the calibration outlined above (Turner and Parlange, 1970). The instrument is available commercially (Wren Instruments, Hamden, Connecticut) and a fortran program is available on request to compute r_s according to the above calibration.

The greatest fault with this instrument is the dynamic conditions of humidity measurement. There is a finite response time for all electrical and solidstate hygrometers, and under dynamic conditions of operation of the aspirated diffusion porometer, the relation between meter reading and humidity of its chamber is not precise. To overcome this problem researchers have resorted to empirical calibrations derived under conditions similiar to field situations. However, this requires tremendous effort and does not facilitate routine checks which are necessary with aging of the sensor (Beardsell et al. 1972).

Field Use

The instrument was prepared by testing the humidity sensor before each measurement period by changing the humidity in the chamber and observing the humidity meter. With the fan on, the silica gel decreased the humidity of the chamber, and to increase the humidity the silica gel was removed from the chamber, and outside air was allowed to enter the chamber. Before each sample measurement the humidity was drawn down with the silica gel well below the range of humidities to be measured for lapse time. Then, with the sample inserted in the chamber and the fan on, the silica gel was removed from the chamber, and the lapse time measured as humidity increased.

Early in the morning ambient humidity was high, and care was taken to adequately dry the chamber before each measurement. Late in the day humidity can be very low, and in this case it was possible to dry the chamber too much. Changing field conditions required different perparation procedures.

A sample of 10 to 30 Douglas-fir needles on the tip of a branch were used for this porometer. The port was sealed by two pieces of rubber stopper placed around the twig with sufficient soft clay to fill gaps. With care a tight seal was made without severing the sample, and the sample was used several times. Surface area of the sample was measured with an optical planimeter and corrected to actual surface area by a factor of 2.36 (Gholz et al. 1976).

The Null-Balance Diffusion Porometer

Theory

A null-balance diffusion porometer was developed to solve many of the problems experienced with earlier porometers. Because measurement is made while humidity is held constant, all the problems associated with the difference between the static and dynamic response of the sensor are eliminated. Dry air is fed into the chamber at a measured rate to obtain a balance, at a predetermined humidity, between the water transpired from the leaf sample and the flow of moist air out of the chamber. This method of measurement is not subject to the error resulting from adsorption and desorption of water in the chamber (Beardsell et al. 1972).

The air in the chamber is stirred by a fan which reduces the porometer resistance to nill and the boundary layer resistance of the sample to a minimum. With adequate mixing of the air the placement of the humidity sensor is not important, and the size and shape of the leaf sample do not influence the measurement (Beardsell et al. 1972).

The flux of water vapor from the leaf, q, is equal to the mean water vapor concentration difference between the leaf and sensor divided by total leaf resistance to water vapor flow:

$$q = \frac{C_s(T_1) - C_a}{r}$$

where r is leaf resistance, C_s is the water vapor concentration of the leaf at temperature, T_1, (assumed to be saturation), and C_a is the water

vapor concentration of the balance point. At the balance point the transpiration flux is equal to the outflow of air, $f(cm^3 \ sec^{-1})$, at the balance humidity, $C_a(g \ cm^{-3})$ as follows:

$$q = \frac{f \ C_a}{A} \quad g \ cm^{-2} \ sec^{-1}$$

where A (cm^2) is the total surface area of the leaves (two sides). Combination of the two equations gives:

$$r = \left[\frac{C_s \ (T_1)}{C_a} - 1 \right] A/f \quad sec \ cm^{-1}$$

where r is total leaf resistance to water vapor flow. Under isothermal conditions in the chamber the equation would be:

$$r = \left[\frac{100}{RH} - 1 \right] A/f \quad sec \ cm^{-1}$$

where RH is the relative humidity in the chamber at the balance point (Beardsell et al. 1972).

Calibration

Three measurements made by the null-balance porometer need to be calibrated, air flow, temperature, and humidity of the chamber. The porometer I used had the meters, electronic circuitry, power source, and compressed dry air in a single box. Two meters of wires and plastic hose connected the box to the chamber.

The air flow meter was originally calibrated by Steven W. Running against a standard in the Engineering Department on the Oregon State University Campus. The gas flow system in the porometer

consisted of a capillary tube, a source of dry air at constant 40 psi and a 0 to 10 psi bourdon tube pressure gauge. The relation between air pressure in psi on the down stream side of the capillary tube and air flow was nearly linear over the range 0 to 16 cm^3 sec^{-1}.

The temperature sensor was a diode located behind the humidity sensor in a hole drilled into the chamber body. This was an unfortunate arrangement because air circulation was not enough to reach this spot,and thus, response time was long. Steve W. Running also calibrated the temperature sensor. A quick check of his calibration found it to still be good. The relation between meter reading and temperature was linear from 0° to 40° C.

The humidity sensor was a rectangular waffer located behind a wire gauze parallel to the chamber. Electrical resistance of the sensor decreased as relative humidity increased. I conducted a thorough calibration of the humidity sensor.

The humidity calibration consisted of quantifying the relation between meter reading and absolute humidity (g m^{-3}) over a suitable range of temperatures (5° to 36° C). Isothermal conditions were maintained in the chamber as air of known humidity was introduced to the chamber. Humidity was increased by small increments, and after each increase the meter on the porometer was read. Temperature was read from the porometer also. This procedure was repeated three times at each temperature. The calibration was done at seven temperatures within the range stated above.

The relation between meter reading and absolute humidity was fitted to the following function:

$$y = \frac{B_1}{B_2 + B_3 \, e^{B_1 x}}$$

where y was the meter reading, x was absolute humidity, and B_1, B_2, and B_3 were parameters to be estimated. The curves were fitted for each temperature by the * CURVEFIT program on the Oregon State University OS3 computer. The equations were used to derive the necessary points in the relation between meter reading and absolute humidity. Finally, when a reading was taken in the field, it was necessary to interpolate between these points to find the absolute humidity.

Field Use

The null-balance porometer was carefully prepared for field use. Once the instrument equilibrated with prevailing conditions, the humidity sensor was tested by drying the chamber with a burst of dry air several times. This ensured that the sensor was working and gave an idea of where ambient humidity fell in the range of the humidity scale.

The leaf sample was inserted in the chamber, and the chamber was clamped shut to seal tightly. The most reasonable balance point from biological standpoints was ambient humidity. This reduced the chamber effect on the measurement of leaf conductance. However, the range of humidities covered by the scale was only 20 to 50 % relative humidity. Early in the morning ambient humidity was often above this range, and late in the day it was often below this range.

So it was necessary to artificially change the humidity of the chamber to fall within the range of the meter. Temperature, humidity of the balance point, and the pressure of the gas flow were recorded to use in the calculation of r.

It was found that the range of calibration for the gas flow was not suitable for measuring Douglas-fir. I used only the first two tenths of the pressure gauge scale, and most of my readings fell in the first tenth where measurement error was greatest. Radiation heating on sunny days required that the chamber be shaded to prevent excessive heat build-up. Samples were measured repeatedly during the day and finally measured for surface area with an optical planimeter. Planar surface area was corrected to total surface area by a factor of 2.36 (Gholz et al. 1976).