AN ABSTRACT OF THE THESIS OF

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Title: THE EFFECT OF SEED SOURCE ON TRANSPIRATION RATES OF SEEDLINGS OF DOUGLAS-FIR (PSEUDOTSUGA MENZIESII (MIRB.) FRANCO)

Abstract approved: _____
W. K. Ferrell

In this study transpiration rates were measured on Douglas-fir seedlings from five seed sources. The seedlings were grown under two environments, a growth chamber and outside in cold frames. Transpiration was measured at two ages, 4 and 16 weeks. Comparison of transpiration rates was made at low soil moisture stress.

Transpiration was measured using humidity sensing elements to measure water vapor before and after passing the seedling. A dual bath system was used to control the humidity.

Seedlings grown outside transpired significantly more (1% level) than seedlings grown in a growth chamber. For all seed sources combined (outside and growth-chamber-grown) the 16-week-old seedlings transpired less than 4-week-old seedlings, however seedlings from some sources showed increasing transpiration rates with

age. This was expressed in a highly statistical significant seed source-environment-age interaction.

Among the outside grown seedlings the mesic seed sources had higher transpiration rates than the seedlings from xeric sources. When grown in the growth chamber the xeric sources had higher transpiration rates. Transpiration rates of seedlings from mesic sources were affected more by the growing conditions than seedlings from xeric sources.

The Effect of Seed Source on Transpiration Rates of Seedlings of Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco)

by

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INTRODUCTION

Regeneration of Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) on south slopes is a severe problem in portions of the Pacific Northwest. Prolonged summer drought, often lasting from mid-June until September, causes high mortality among seedlings in their first year. Encroaching weed and brush species magnify the problem in later years.

Survival during these periods of stress can be a function of drought hardiness or drought avoidance. In this study the concern was with one aspect of the latter. Drought avoidance might include such factors as extensive tap root growth, early germination or possible differences in the rate of transpiration. A high transpiration rate certainly would be undesirable in any area where the trees would be subject to moisture stress. Thus the objective of this study was to measure transpiration variation in Douglas-fir seedlings.

Very pronounced differences in plant behaviour sometimes occur between plants grown under different environments, especially between plants grown under low-light, high-moisture indoor environments and those grown outside. Since much work is done with plants grown in growth chambers, there is a need to know just what

influence the conditions in the growth chamber have on transpiration rates. Consequently, seedlings grown outside and in growth chambers were compared.

It was also of interest to know how genetics affected transpiration rates, so seedlings from the five provenances were compared under well watered conditions. A further comparison was made between seedlings of two seed sources under drought stress. This was done to determine if certain transpiration differences might appear only under moisture stress.

REVIEW OF LITERATURE

A voluminous literature has been written about transpiration. It deals with transpiration in many species of plants and many methods of transpiration measuring devices (Kramer and Kozlowski 1960, p. 298-303). Only the literature most pertinent to this study on Douglas-fir seedlings will be reviewed here.

The apparatus used for measuring transpiration was modeled after the one suggested by Bierhuizen and Slatyer (1964). With such an apparatus the seedling environment could be closely controlled while transpiration measurements were made.

Differences in drought resistance between seedlings of different sources of Douglas-fir, when grown in the laboratory, have been shown by Ferrell and Woodard (1962, 1966) and Pharis and Ferrell (1966). Irgens-Moller (1968) noted the higher mortality in outplanted coastal seedlings, as compared with the seedlings from other areas, during the severe drought in Corvallis in the summer of 1967.

In a later paper Zavitkovski and Ferrell (1968) suggested that transpiration differences appear to account for some of this seed source diversity. However up to this time no closely controlled transpiration measurements had been made on Douglas-fir seedlings.

In comparing inside and outside-grown seedlings, de Keijzer

and Hermann (1966) found a much greater tolerance to heat among those grown outside. Changes in cuticle thickness between growth chamber-grown and outside-grown seedlings of Douglas-fir have been noted by Tucker (1966). These findings suggested that possibly differences in transpirational response could also be found between plants grown in the growth chamber and those grown outside.

Differences in transpiration rate between species, when subjected to drought, were shown by Lopushinsky (1968) using a weighing technique. Using a similar method Zavitkovski (1964) found a significantly higher transpiration rate among seedlings of Douglas-fir from a coastal source as opposed to those from an interior source. However the weighing technique lacks the sensitivity to obtain useful quantitative transpiration values, especially when the plants are small, growing seedlings (Kramer and Kozlowski 1960, p. 298).

MATERIALS, METHODS AND PROCEDURES

Seed Sources

Douglas-fir seed from five locations was used in this study. Selection of the seed sources was made so that a variety of the environments found in the range of Douglas-fir was represented. Figure 1 lists the sources and depicts the seasonal precipitation distribution in the areas from which the seed was collected. Since the seed collections were not made by the author it was impossible to measure any other site factors such as soil moisture.

The highest rainfall area was represented by Forks, Washington (denoted (F)), where some 117" of precipitation fall annually. It is also a low elevation area with mild temperatures. The seed was furnished by the Manning Seed Company of Seattle.

The Corvallis, Oregon seed came from areas of equal precipitation, approximately 40" annually, but from two very different sites. The south slopes from which the Corvallis-South 700' elevation seed (CS) was collected represent a much more severe environment with respect to drought than do the north slopes, from which the Corvallis-North 1,300' elevation seed (CN) was collected. These two sources were chosen to see if local selection favored a tree with low transpiration.

The Missoula, Montana seed (M) was collected in 1961 in the

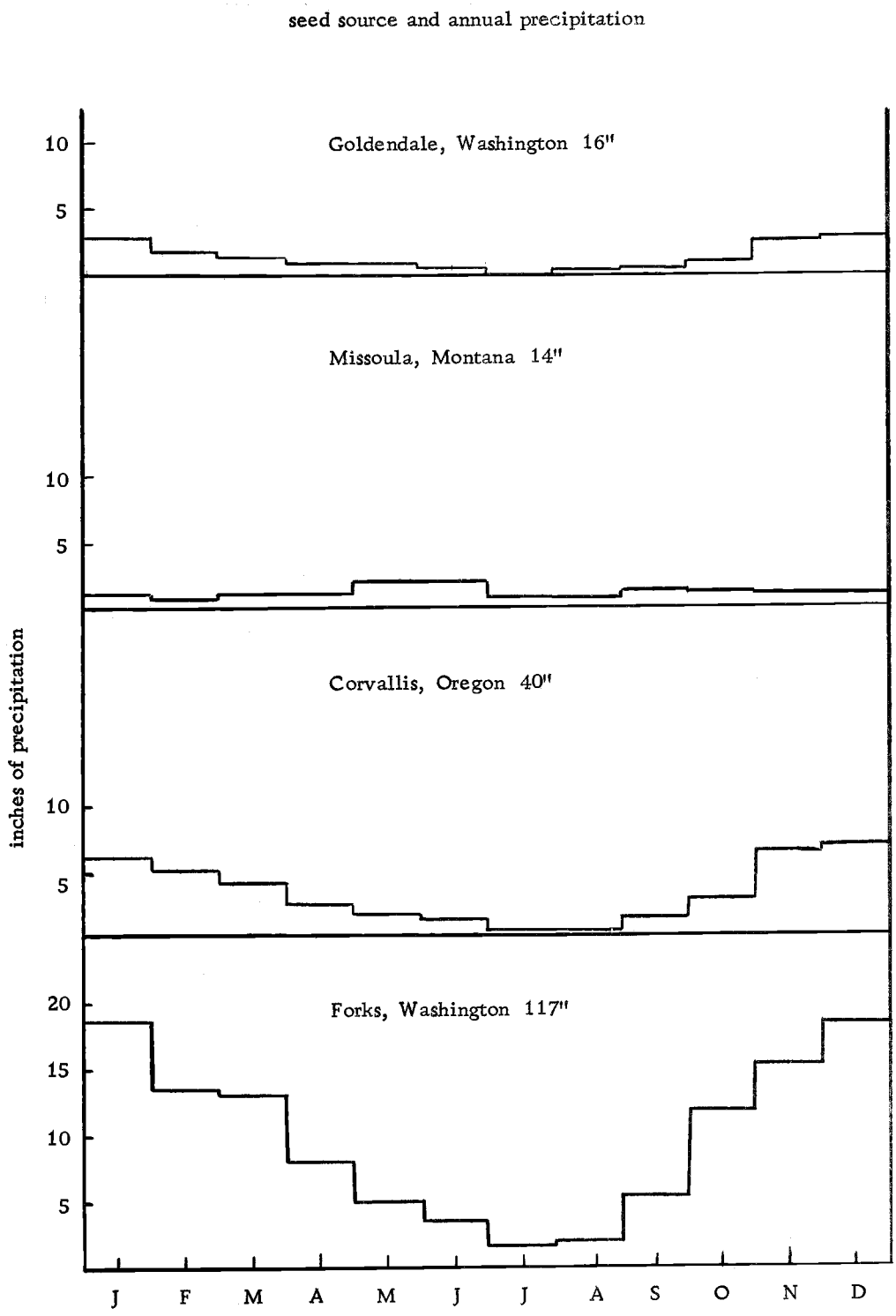

Figure 1. Distribution of monthly precipitation in inches for the seed collection areas. (U. S. D. A. Yearbook, 1941)

foothills of the Garnet range at an elevation of 4, 300'. The nearest meteorological station is found at Missoula, for which the precipitation figures are shown (Figure 1). This source was chosen because it represents an area far removed from the Northwest and because the precipitation is very low and evenly distributed throughout the year with no pronounced seasonal variation, as is found in the Northwest.

Possibly the driest area was Goldendale, Washington. The average precipitation at Goldendale, which is somewhat lower in elevation than the 2, 600' from where the seed was collected, is 16" annually. This source was chosen because the trees grow near the limit of the range of Douglas-fir on the eastern slope of the Washington Cascades. The Goldendale and Missoula seed was collected by Dr. Frank Sorensen of the Pacific Northwest Forest and Range Experiment Station at Corvallis.

Growth of Seedlings

Seed from the five provenances was soaked in water for 24 hours and then drained and placed in a refrigerator at 2°C for 7 days. Twenty to forty seeds were placed in petri dishes, containing moist filter paper, for germination. When a minimum root length of one cm had been attained, the seeds were planted in black, pint plastic pots containing Wren silt loam - A_1 horizon. Five seeds were planted per pot; these were thinned to two seedlings per pot before measurement.

All seedlings remained in the growth chamber for one week following planting to assure better survival. After this one week period the seedlings which were to be grown outside were transferred to cold frames.

Growth Chamber

The seedlings grown inside remained in the growth chamber from the time of germination until measurement. Conditions were kept constant at 28°C day and 17°C night with a twelve hour photoperiod. Humidity was near 100% at night and 60-70% during the day. Light intensity was 970 f. c. (0.033 Langleys/min), the source being fluorescent tubes supplemented with incandescent bulbs. The seedlings were watered regularly so that at no time were they under high moisture stress.

Cold Frames

The seedlings which were to be grown outside were randomly chosen from all the planted seedlings and placed in cold frames in late June and July. More pots were placed outside than inside to assure that the seedlings in the necessary five pots would survive. It was expected that higher mortality might occur outside. This expectation was realized when it was found that a variegated cutworm had severely defoliated several seedlings.

The pots were randomly placed in the cold frame and set flush with wet sawdust. This was done to assure that soil conditions, especially temperature, inside the pot would resemble natural conditions more closely. Screening of the seedlings was necessary to provide some shade (55%) and also to protect the seedlings from possible animal damage. Temperatures at the seedling level ranged from 49°C to 6°C during the summer. Measurement was done with a portable hygrothermograph. All seedlings were adequately watered and occasionally weeded.

Description of Apparatus for Transpiration Measurements

The apparatus was based on a warm and cold water bath system to control humidity of the air surrounding the seedling. Humidity was measured using a pair of Aminco-Dunmore electric hygrometer sensing elements (Figure 2).

Air was pumped (AP)[1] through a fritted glass bubbler submerged in water (B_1) to saturate the air at a temperature of 23°C in the warm water bath. The air was then bubbled through a second bubbler (B_2) located in the cold water bath (11°C). The warm bath flask was necessary since it was found that one flask in the cold bath did not assure saturation at the cold bath temperature.

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[1] Symbols refer to Figure 2.

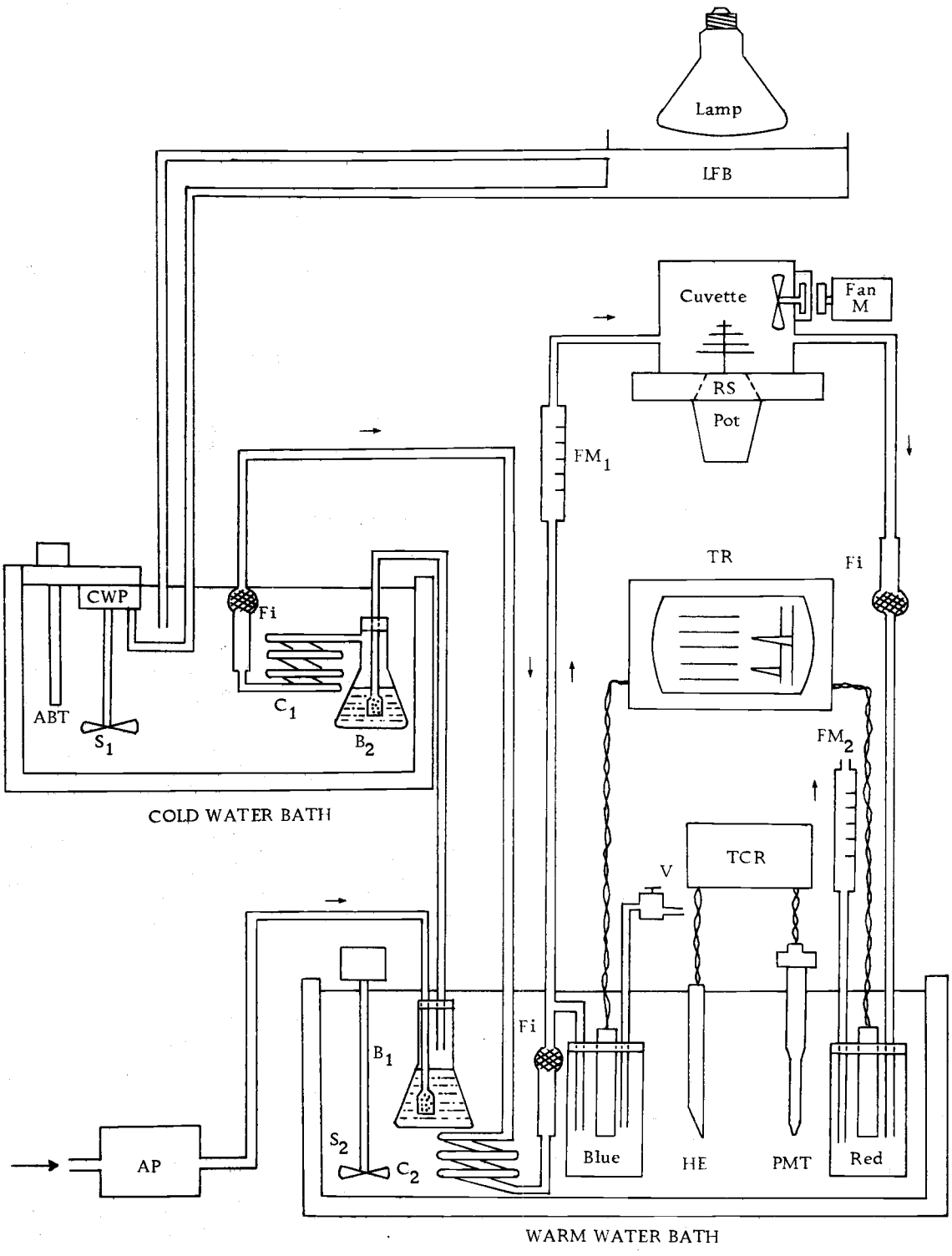


Figure 2. Transpiration Measurement Apparatus (Description of symbols appears on p. 11-12.)

DETAILS OF EQUIPMENT INDICATED IN FIGURE 2

COLD WATER BATH	An Aminco Wide Range Laboratory Bath containing 38 gallons of water maintained at 11.0 C ± 0.05 C.
WARM WATER BATH	A styrifoam cooler containing 9 1/2 gallons of water maintained at 23 C ± 0.05 C.
Lamp	A mercury-incandescent lamp providing a maximum light intensity of 5800 f.c. (560 f.c., 0.023 ly./min between 400-700 nm inside the cuvette).
LFB	A three inch deep lamp filter bath connected to the cold water bath.
Cuvette	A plant chamber constructed from transparent plexiglass and sealed to the base by an "O-ring".
Fan M	An electric motor with a bar magnet used to power the self-contained propeller. The rpm was controlled by an attached rheostat.
Pot	A black plastic pint container used to grow the seedlings.
AP	A Dyna-Vac Air Pump (pressure vacuum) made by Cole-Parmer Instrument and Equipment Company.
RS	A rubber seal made from two-part liquid polymer rubber and used to seal the seedling into the cuvette.
FM_1	A Monostat flowmeter used to monitor the air flow (0.66 liters per minute) entering the cuvette.
FM_2	A RGI flowmeter momentarily connected to the air exiting the Red sensing element bottle for the purpose of determining a leak with seedlings in the cuvette.
B_1	A flask containing 800 milliters of distilled water and a fritted glass bubbler.
B_2	A flask containing 400 milliters of distilled water and a fritted glass bubbler.
C_1	Six feet of coiled copper tubing, 1/4 inch inside diameter, submerged in the cold water bath.

C_2	Six feet of coiled copper tubing, 1/4 inch inside diameter, submerged in the warm water bath.
S_1	A stirring motor that circulates the water in the cold water bath.
S_2	A stirring motor that circulates the water in the warm water bath.
Fi	A glass wool filter used to remove foreign air particles before the air is exposed to the sensing element.
HE	A heating element connected to the temperature control relay and used to heat the warm water bath.
TR	A Taylor Transcope Electronic Recorder (model 701JE2) to record the response of the humidity sensing elements, Red and Blue.
Red	An Aminco-Dunmore electric hygrometer sensing element to measure the relative humidity of the air after passing over the seedling.
Blue	Same as above only used to measure the relative humidity of the air before passing over the seedlings.
PMT	A Precision Mercury Thermoregulator (model # 2151) used to control the warm water bath temperature.
TCR	A Versatherm Electronic Temperature Control Relay (model #2149) used to monitor the HE and PMT.
CWP	The cold water bath pump used to circulate water up to the water bath above the cuvette; the water returns to the cold bath via gravity.
ABT	An Aminco bimetal thermoregulator positioned in the cold water used to regulate the temperature to $\pm 0.05^{\circ}C$.

By using the two flasks, the nearly saturated warm air was condensed to the saturation point of air at the cold bath temperature. To assure complete condensation the air was cooled in a condensing coil (C_1).

A filter (F_i) containing spunglass was put in the line at this point to block any water droplets from getting into the line, since droplets outside the cold bath would cause further evaporation as the air warmed.

The air was then brought up to warm bath temperature (C_2) and passed through a filter (F_i). The line divided at this point, one branch continuing on to the cuvette and the other passing into a flask containing a humidity sensing element. This was connected to a blue pen on a Taylor dual pen recorder (TR).

A valve (V) was attached at the air outlet from this flask. It was used to regulate air flow in the entire system.

By varying the cold bath and warm bath temperature a base humidity of 38.5 - 39% was obtained in the system. Transpiration of the largest seedlings caused the humidity to increase to a maximum of 60%. For this range, the appropriate narrow-range humidity sensing element was used. Other humidity ranges could have been used and measurements made using different range elements; however it was felt this range was closest to what might be found under field conditions. A calibration curve between pen reading and relative

humidity at the cuvette temperature was determined using magnesium perchlorate in drying tubes (Appendix II, Figure 9).

The other branch of the line went to the cuvette. A flow meter (FM_1) was attached at this point to measure air flow. The air then passed into the cuvette where a magnetically driven fan circulated the air. A turbulence value equivalent to 0.4 mph was obtained in the cuvette. Measurement was done using an Alnor thermo-anemometer. It was hoped this turbulence would partially break the boundary layer resistance. However, as has been suggested by Waggoner and Zelitch (1965), the increase in turbulence can cause a decrease in leaf transpiration due to the lowering of leaf temperature; this decrease might partially compensate for any gain in transpiration due to the breaking of the boundary layer.

The air was then filtered (Fi) before entering the flask containing the sensing element which was connected to a red pen on the recorder. A flowmeter (FM_2) was attached at the outlet before each measurement to check for possible leaks.

A thermocouple was placed in the cuvette to measure any temperature changes in the air surrounding the seedling. However, since the temperature inside the cuvette remained constant (23°C±1°) it was not necessary to monitor temperature at all times.

A mercury incandescent lamp was used as a light source. A reading of 560 foot candles (0.023 langleys/min) was obtained at

seedling level inside the cuvette.

The entire apparatus was set up in a standard room where the temperature was maintained at 21°C ± 1° and a relative humidity of 60%.

Experimental Design

The main study involved three variables, the five seed sources, two ages (4 weeks and 16 weeks) and two environments (growth-chamber-grown and outside grown). Five replications were made for each individual treatment (Table 1).

Table 1. Experimental Variables[a]

Seed Source and Environment[b]	Age		
	Measurements under low moisture stress		Measurements under high stress
Forks (GC)	4 weeks	16 weeks	8 weeks
Forks (O)	4 weeks	16 weeks	-------
Corvallis-North (GC)	4 weeks	16 weeks	-------
Corvallis-North (O)	4 weeks	16 weeks	-------
Corvallis-South (GC)	4 weeks	16 weeks	-------
Corvallis-South (O)	4 weeks	16 weeks	-------
Missoula (GC)	4 weeks	16 weeks	-------
Missoula (O)	4 weeks	16 weeks	-------
Goldendale (GC)	4 weeks	16 weeks	8 weeks
Goldendale (O)	4 weeks	16 weeks	-------

[a]Five replications per treatment with two seedlings per replication.
[b](GC) denotes growth chamber grown and (O) outside grown.

A further comparison was made between seedlings of two seed sources subjected to moisture stress. They were grown in a growth chamber and transpiration was measured at an age of 8 weeks. Again five replications were made for each seed source.

Treatment Prior to Measurement

The seedlings were transferred from their respective locations to the standard room 24 hours before measurement. Here they were well watered and kept under fluorescent lighting, 680 f. c. Care was taken to assure that no droplets of water were on the needles prior to the time when the seedlings were inserted into the cuvette. At this time the seedlings were also thinned so that only two remained per pot.

Thirty minutes before the plants were to be placed in the cuvette, a slit rubber stopper was sealed around the stem of the seedlings. Lanolin was used around the base to make an air tight seal around the stem. The sealed plants were then ready for insertion into the cuvette.

Measurement of Transpiration

The seedlings were placed inside the cuvette when the pen readings reached a constant value, indicating constant humidity inside the system. The bath temperatures which gave a base humidity of

38.5% were 23°C and 11°C. At that temperature combination the pen readings were near the bottom range of the scale, thereby allowing full range use (38% - 60% R.H.).

Air flow through the cuvette was kept constant at 0.66 liters per minute throughout the duration of the study. Higher flow rates caused pressure to build up, which forced the lid off the top of the cuvette. The fan inside the cuvette was also kept at a constant rpm.

By checking the flow-meter before the cuvette with the one after, it was possible to determine any leaks in the system. If such occurred the seedlings were resealed.

The seedlings were left in the cuvette for a minimum of 30 minutes to equilibrate, after which time the readings were taken if they had attained a constant rate. For the small seedlings a steady transpiration rate was reached in 20 minutes, while in the large seedlings it sometimes took two to four hours. When the pen readings were constant for ten minutes, indicating equilibrium conditions had been obtained, readings were made. The seedlings were then removed, the cuvette resealed and the apparatus allowed to equilibrate at base flow again.

The foliage was then removed from the seedlings and fresh weight determined. The seedlings were then ready for foliage area determination.

In some cases seedlings were measured two or three times for

the purpose of checking the reproducibility of the measurements. Apparently the constant conditions under which the seedlings were kept before measurement caused transpiration to vary little during the day. In a few instances the temperature baths were found to fluctuate, making it necessary to repeat some measurements.

Determination of Foliage Area

Two methods were used in the measurement of foliage area. The first involved simply placing the plucked needles on glass plates along with one cm squares and obtaining an enlargement of the needles on contact print paper. The outlines of the needles and the squares were then cut out, weighed and the area obtained by proportion. This method was used for all the 4-week-old seedlings and the majority of the 16-week-old seedlings.

The second method involved the use of an optical planimeter. Area was determined by measuring the amount of light passed through a glass plate containing the needles. The decreased light transmission due to the presence of the needles on the glass plate allowed one to determine foliage area from a meter reading - area calibration curve (Davis et al. 1966; Geppert 1968). The latter method took only 15 - 30 minutes per two seedlings, whereas the former took up to three hours for two 16-week-old seedlings.

Evaporation Correction Factor

A humidity correction term had to be computed for each of the transpiration values. This factor, denoted Δe, (Slatyer and Bierhuizen 1964) accounts for the fact that transpiration varies with the humidity of the surrounding air. As the seedling transpired water into the surrounding air, the gradient of humidity from plant to air decreased, causing a constantly decreasing transpiration rate until some equilibrium had been reached. The gradient would be influenced by the transpiration rate in a sort of feedback relationship.

This factor was computed as follows:

$$e_s - e_a = \Delta e$$

e_s = Water vapor content of air at 100% humidity, expressed in mg H_2O/liter air, at temperature T.

e_a = Water vapor content of ambient air, expressed in mg H_2O/liter air, at temperature T.

Thus the larger the gradient (Δe), the greater the transpiration rate would be.

To obtain the relationship between Δe and transpiration, circular discs (0.66 cm^2) were cut out of blotter paper, saturated with water and sealed into the cuvette for evaporation measurement. It was assumed that the evaporation from the blotter paper would resemble evaporation from a leaf surface. In measuring the

evaporation, the discs were placed on pins (Figure 3). By varying the number of discs (area of evaporating surface), various equilibrium evaporation rates and Δe values could be obtained.

A regression for the various values of evaporation and Δe was calculated. All transpiration values were corrected to a Δe value of 11 mg H_2O/liter air, which was the mode for all Δe values obtained from the individual plant measurements. The corresponding evaporation rate for a Δe of 11 was 0.513 mg $H_2O/cm^2/min$.

The corrected transpiration value was computed as follows, with the symbols referring to Figure 4.

By proportion:

$$\frac{E_i}{E_c} = \frac{E_r}{E_s} \qquad \text{or} \qquad E_c = \frac{E_i E_s}{E_r}$$

Drought Study

In this study only Forks and Goldendale seedlings, grown in the growth chamber, were used. The same procedure was followed as before except that six sunflower seeds were planted in each pot of two seedlings. When the sunflower roots had grown throughout the pot (usually after two sets of secondary leaves had appeared), moisture was withheld from the pot. As soon as wilting of the sunflower leaves was apparent, the sunflowers were cut and transpiration of the seedlings was measured. The water stress of the seedlings

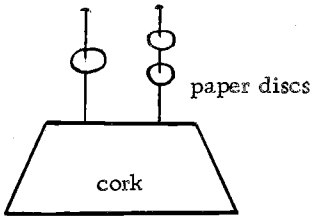
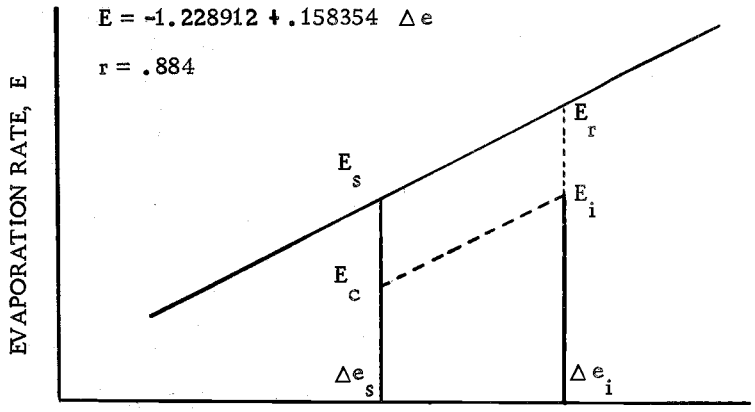


Figure 3. Discs for obtaining Δe - evaporation regression.



Δe_s = Standard Δe, 11 mgH_2O/liter air.

Δe_i = Observed Δe at transpiration rate E_i.

E_s = Standard E at $\Delta e = 11$, 0.513 $mgH_2O/cm^2/min$.

E_i = Observed transpiration rate in $mgH_2O/cm^2/hr$.

E_r = Evaporation value at Δe_i, computed from the regression.

E_c = Corrected transpiration value.

Figure 4. Schematic representation of Δe correction computation.

was then measured by using a pressure bomb (Scholander, et al. 1965).

Statistical Analysis

An analysis of variance using an F test was used to test the three main variables, seed source, location, age and their interaction. A least significant difference (L. S. D.) was then computed to test individual differences.

RESULTS AND DISCUSSION

The results of the study can be divided into three parts. These are the effect of environment on transpiration rates of seedlings from five seed sources, transpiration for the five sources at two ages (4 and 16 weeks) and the effect of drought on transpiration for the Goldendale and Forks seedlings grown in a growth chamber. The average transpiration rates are listed in Table 2.

Table 2. Corrected Transpiration Values[a]

Source	4 Weeks old		16 Weeks old	
	Growth Chamber	Outside	Growth Chamber	Outside
Forks	7.4[b]	11.9	5.9	12.4
Corvallis North	5.4	10.7	4.5	12.1
Corvallis South	8.4	12.3	2.1	9.8
Goldendale	8.8	9.8	6.2	9.9
Missoula	6.5	11.4	7.8	8.0

Drought Study:

Source	Transpiration rate	Moisture stress
Goldendale	1.2 mg$H_2O/cm^2/hr$	225 - 300 psi
Forks	0.9 mg$H_2O/cm^2/hr$	280 - 420 psi

[a]Average of 5 pots (10 seedlings)

[b]mg $H_2O/cm^2/hour$

The Effect of Environment on Transpiration

For all the seed sources, the outside grown seedlings transpired significantly more (1% level) than the growth-chamber-grown seedlings (Figure 5). The transpiration rate for all growth-chamber-grown seedlings (4 and 16 weeks) was 6.3 $mgH_2O/cm^2/hr$, while for the outside-grown seedlings it was 10.9 $mgH_2O/cm^2/hr$.

It can also be seen in Figure 5 that the 4-week-old seedlings (all sources) transpired more than the 16-week-old seedlings in their respective locations. However, the difference in transpiration rates between 4 and 16-week-old seedlings is not as great in those from the outside as in those from the growth chamber. The difference was 2 $mgH_2O/cm^2/hr$ for the growth-chamber-grown and 0.7 mgH_2O for the outside-grown seedlings. Thus prolonged growth under growth chamber conditions lowers the transpiration rates in Douglas-fir seedlings.

Figure 6 depicts the transpiration rates for all seed sources grown in the growth chamber and grown outside. In this graph the two ages were averaged together. For every seed source the outside-grown seedlings transpired significantly more (1% level) than those grown in the growth chamber.

The Forks source seedlings, which came from the most mesic area, transpired more than 12 $mgH_2O/cm^2/hr$ when grown outside.

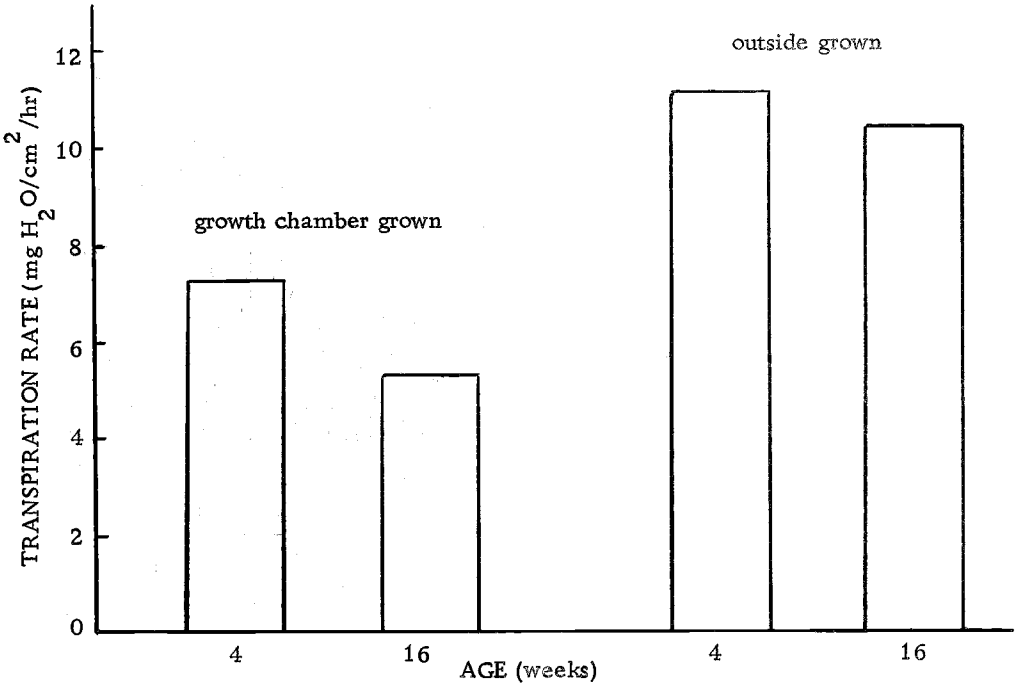


Figure 5. Comparison of transpiration rates between outside and growth chamber grown seedlings; and between 4 and 16-week-old seedlings (average of all seed sources).

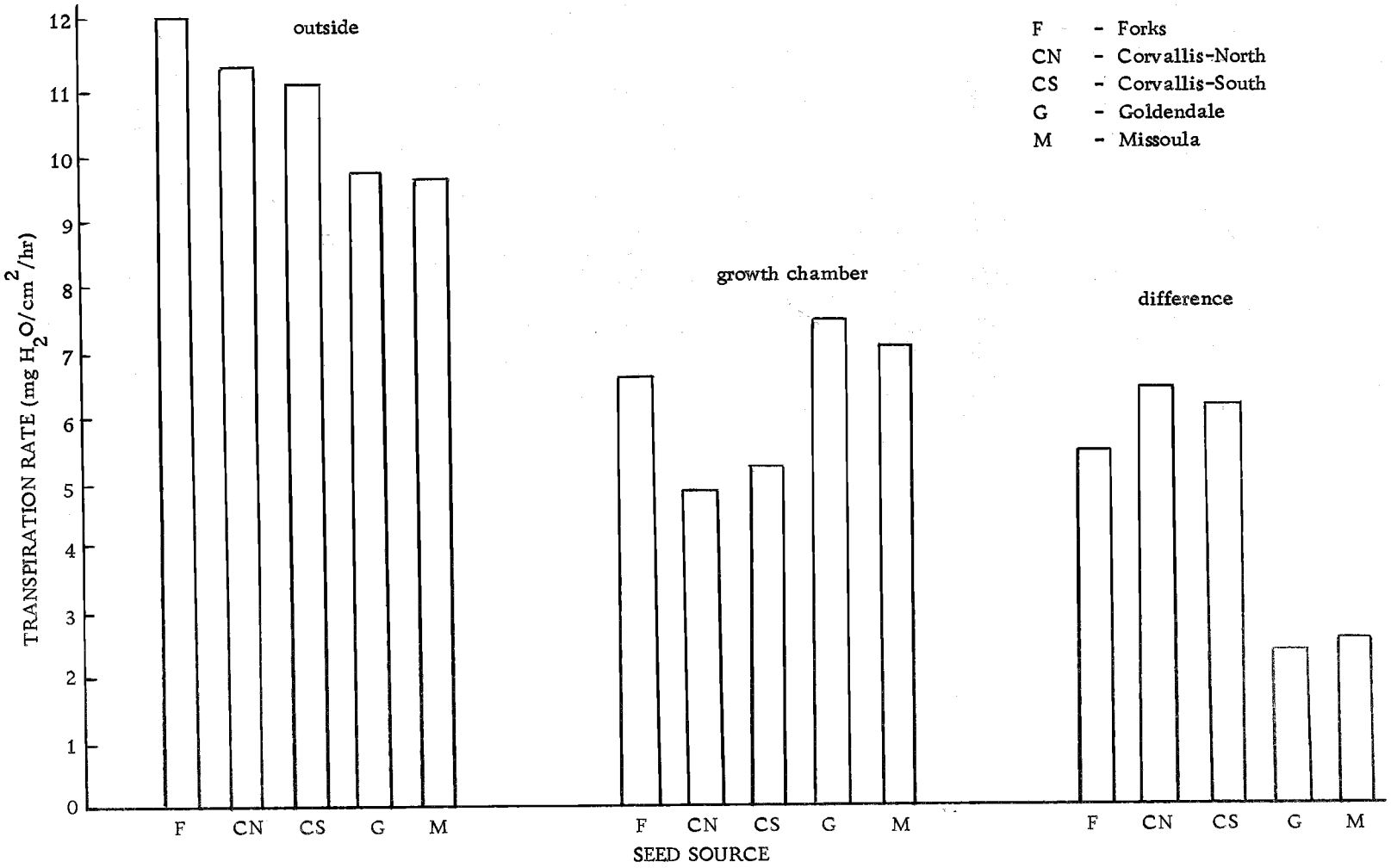


Figure 6. Transpiration rates of the five seed sources, average of 4 and 16-week-old seedlings, comparison between growth chamber and outside grown.

This was significantly more than the outside-grown Goldendale and Missoula seedlings which transpired 9.8 and 9.7 $mgH_2O/cm^2/hr$ respectively.

The seedlings from the two Corvallis sources transpired slightly more than 11 mg of water when grown outside, thus also being greater than the Missoula and Goldendale seedlings. At the 5% probability level transpiration of Corvallis-North seedlings was significantly higher than in the Missoula seedlings.

If one looks at the behaviour of the seedlings from the five seed sources grown in a growth chamber one finds an almost complete reversal in the relative magnitude of the transpiration rates (Figure 6). This reversal is also manifested as a seed source-environment interaction effect (Table 3, Appendix I). The seedlings from Goldendale transpired significantly more than the two Corvallis sources and the Missoula seedlings transpired more than the Corvallis-North seedlings (1% level). The Forks seedlings were significantly higher than the Corvallis-North seedlings at the 5% probability level.

The magnitude of the change in transpiration between growth-chamber-grown and outside-grown seedlings (Figure 6), suggests that seedlings from mesic or nearly mesic sources were affected by the growth chamber conditions much more than the seedlings from the xeric sources.

There are several possible explanations for the lower transpiration rates in the growth chamber seedlings. For one thing, the soil temperature in the growth-chamber-grown seedlings was closer to ambient air temperature since the pots were not set in any cooling bath. This might have promoted more rapid drying of the soil, thus putting the seedlings under more stress and more drought adaptation, even though watering of growth-chamber-grown and outside-grown seedlings was done regularly.

Changes in the cuticle composition (Tucker 1966) between growth-chamber-grown and outside-grown seedlings could also contribute to the lower transpiration rate for the growth-chamber-grown seedlings. Although the cuticle among outside-grown seedlings is thicker, Hull and Shellhorn (1966), in their studies with mesquite, suggest that cuticle thickness in itself may have little relationship to water loss. Crisp (1966), in his studies found marked differences between cuticular composition of a xerophyte, mesophyte and hydrophyte. Thus possibly there is a difference between cuticle composition of the various provenances. The differential staining of the cuticle between outside and growth-chamber-grown seedlings would certainly suggest a different composition.

Whiteman and Koller (1965) found cuticular transpiration rates in Pinus halepensis as high as 1.6 mg$H_2O/cm^2/hr$; thus the magnitude of cuticular changes could be influential in reduced rates among the

growth-chamber-grown seedlings.

Another factor to be considered is the differential stage of development of the seedlings. The earlier onset of dormancy in the growth chamber seedlings (Lavender 1968), with a subsequent increase in lignification of the leaves, would cause transpiration to decrease (Oksbjerg 1961). This of course would be true only for the 16-week-old seedlings.

The much higher humidity as well as the reduced light intensity inside the growth chamber would cause lower transpiration rates as opposed to a high light, low humidity environment. Possibly such preconditioning could have an effect on transpiration which would take longer than the 24 hour adjustment period to be overcome.

Variation in the size, number and structure of the stomata could also account for the different transpirational rates. However, preliminary investigations did not reveal any significant differences.

Certainly one cannot overemphasize the difference between growth chamber and cold frame conditions. Similarly one could ask what is the difference between cold frame grown and field grown seedlings in their transpiration rates. Such things must be known before extrapolation from laboratory to field can be made.

Transpiration at 4 and 16 Weeks

In Figures 7 and 8 one can see how transpiration varied with age for the five seed sources. The differential effect of age on seedlings from different sources is demonstrated as a source-age interaction (Appendix I, Table 3). Only the Corvallis-South seedlings show a consistent trend when grown in the growth chamber and when grown outside. Transpiration rates dropped from 8.4 to 2.1 $mgH_2O/cm^2/hr$ for the growth-chamber-grown seedlings, and from 12.3 to 9.8 $mgH_2O/cm^2/hr$ for those grown outside.

The Corvallis-North seedlings decreased in rate from 4 to 16 weeks when grown in the growth chamber, but transpiration increased with age when grown outside. Similar trends were also found for the Forks and Goldendale seedlings.

The Missoula seedlings were the only ones which showed an increase with age when grown in the growth chamber, while decreasing with age when grown outside.

Although some seedlings showed increased transpiration rates with age when each seed source was taken separately; the transpiration rate was significantly lower (1% level) at 16 weeks than at 4 weeks when taken collectively. The actual values were 7.9 $mgH_2O/cm^2/hr$ at 16 weeks and 9.3 $mgH_2O/cm^2/hr$ at 4 weeks.

An interesting speculation regarding possible inherited

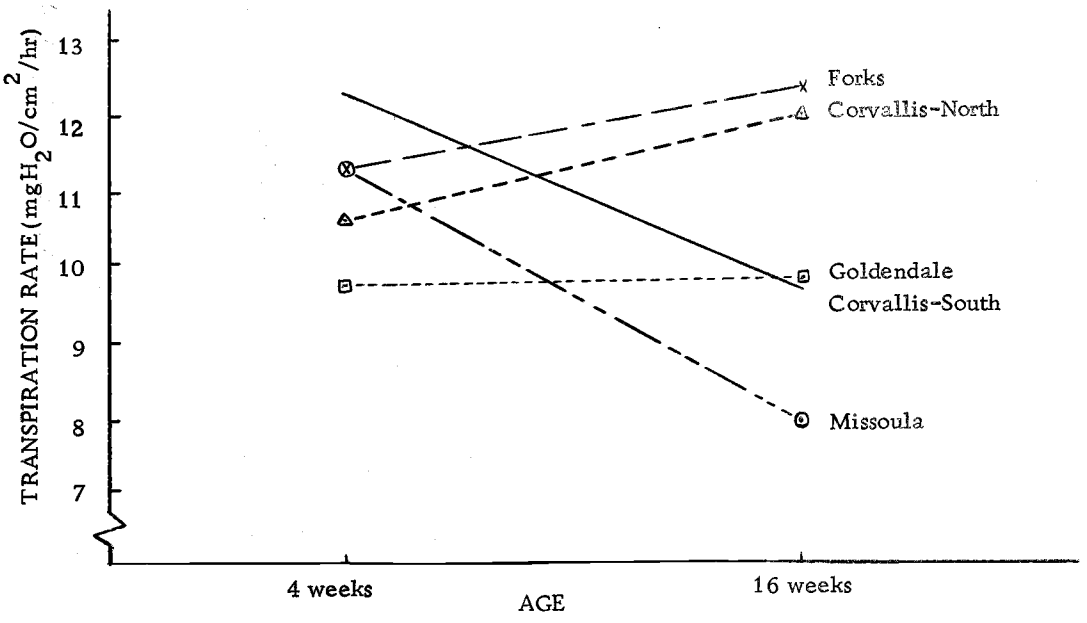


Figure 7. Transpiration rates for outside grown seedlings at two ages.

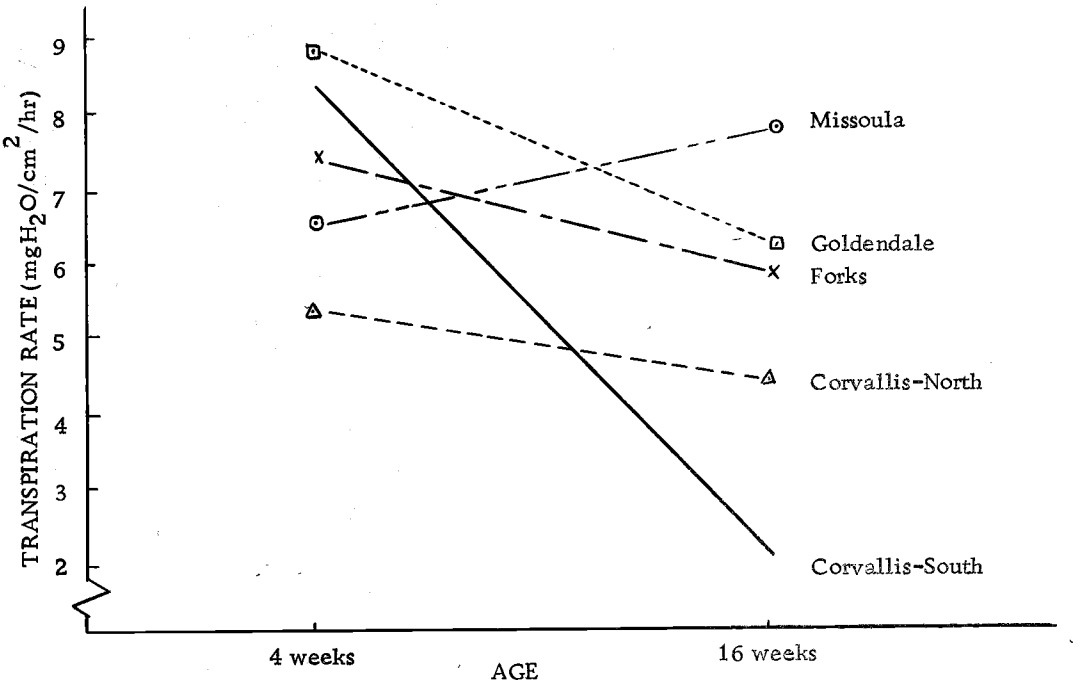


Figure 8. Transpiration rates for growth chamber grown seedlings at two ages.

transpirational response to the environment might be inferred from Figure 7, outside grown. The three sources, Missoula, Goldendale and Corvallis-South, which would probably be under the greatest moisture stress at an age of 16 weeks (approximately the latter part of August) show the lowest transpiration rates at that age (8.0 - 9.8 $mgH_2O/cm^2/hr$). The most mesic source, Forks, transpired 12.4 $mgH_2O/cm^2/hr$, and the Corvallis-North source was only slightly lower at 12.1 mgH_2O. Again one should emphasize that when grown in the growth chamber this relationship did not exist.

The fact that most seed sources showed decreased transpiration at 16 weeks when grown in the growth chamber again points out that long exposure to mild, low-stress conditions may greatly alter a plant's response from that encountered under field conditions.

The Effect of Drought on Transpiration

In this study the stress values were measured using a pressure bomb (Scholander *et al.* 1965). Seedling stress varied from 15 to 28 atmospheres, with the Forks source seedlings under higher stress than the Goldendale seedlings.

Under these conditions of water stress there was no significant difference in transpiration rates between the Forks and the Goldendale seedlings; transpiration rates were 0.9 and 1.2 $mgH_2O/cm^2/hr$ respectively (Table 2). Zavitkovski (1964) found that

transpiration rates of coastal source seedlings to be higher than inland sources at a soil stress of 15 atmospheres.

The higher stress that the Forks seedlings were under makes it difficult to make a valid comparison between the Forks and Goldendale seedlings. To effectively study transpiration rates under drought stress it might be better to make a series of moisture stress and transpiration measurements simultaneously over a wide range of stress values, as did Lopushinsky (1968) and Zavitkovski (1964).

SUMMARY

Seedlings of Douglas-fir from five provenances were grown under two environments. Transpiration rates were measured on these seedlings at two ages (4 and 16 weeks) under conditions of low moisture stress. A comparison was also made between two provenances subjected to drought stress.

It was found that seedlings grown outside transpired significantly more than seedlings grown in a growth chamber for all seed sources. The 16-week-old seedlings transpired less than the 4-week-old seedlings when all seed sources were combined. When taken separately seedlings of some sources showed an increase in transpiration with age. This was expressed statistically as a highly significant seed-source - environment interaction.

When grown outside the most mesic source had the highest transpiration rate and the two xeric sources the lowest. In the growth chamber this relationship did not hold. The transpiration rates of the mesic source seedlings were affected by the growing conditions much more than xeric source seedlings.

Due to different moisture stress values, no valid comparison could be made in the transpiration rates between seedlings of xeric and mesic sources grown under drought conditions.

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APPENDICES

APPENDIX I

Table 3. Analysis of Variance

	Sum Squares	d.f.	M.S.E.	Sample F	Level
Total	1,213.18	99	---	---	---
S-Seed source	21.66	4	5.42	1.59	F^{4}_{80}--
A-Age	48.44	1	48.44	14.21	F^{1}_{80}**
L-Location	514.38	1	514.38	150.84	F^{1}_{80}**
S X A	65.40	4	16.35	4.79	F^{4}_{80}**
S X L	73.83	4	18.46	5.41	F^{4}_{80}**
A X L	9.74	1	9.74	2.86	F^{1}_{80}--
S X A X L	206.78	4	51.70	15.16	F^{4}_{80}**
Error	272.95	80	3.41	---	---

*Significant 5%
**Significant 1%

F^{1}_{80} = 6.96	1% level	
F^{4}_{80} = 3.56		
F^{1}_{80} = 3.96	5% level	
F^{4}_{80} = 2.48		

Table 4. L. S. D. Test for Source and Age

Source & Age[a]	Transpiration mg $H_2O/cm^2/hr$	CS-16	M-16	CN-4	G-16	CN-16
CS-4	10.4	4.5**	2.5**	2.4**	2.3**	2.1*
F-4	9.7	3.8**	1.8*	1.7*	1.6*	1.4
G-4	9.3	3.4**	1.4	1.3	1.2	1.0
F-16	9.2	3.3**	1.3	1.2	1.1	.9
M-4	9.0	3.1**	1.1	1.0	.9	.7
CN-16	8.3	2.4**	.4	.3	.2	---
G-16	8.1	2.2*	.2	.1	---	---
CN-4	8.0	2.1*	.1	---	---	---
M-16	7.9	1.9*	---	---	---	---
CS-16	5.9	---	---	---	---	---

** Significant 1%

CS-4 > CS-16 M-16 CN-4 G-16
F-4 > "
G-4 > "
F-16 > "
M-4 > "
CN-16 > "
G-16 > "

* Significant 5%

CS-4 > CN-16
F-4 > M-16 CN-4 G-16
G-16 > CS-16
CN-4 > CS-16
M-16 > CS-16

$$LSD = t_{\alpha/2}\sqrt{\frac{2mse}{n}}$$

$t_{1\%} = 2.576$ 80 degrees of freedom

$t_{5\%} = 1.960$

LSD = 2.13 (1%)

LSD = 1.6 (5%)

[a] At ages of 4 and 16 weeks.

Table 5. L. S. D. Test for Source and Location

Source & Location[a]	Transpiration mg $H_2O/cm^2/hr$	CN-GC	CS-GC	F-GC	M-GC	G-GC	M-0	G-0
F-0	12.2	7.3**	6.9**	5.5**	5.1**	4.7**	2.5**	2.3**
CN-0	11.4	6.5**	6.1**	4.7**	4.3**	3.9**	1.7*	1.5*
CS-0	11.1	6.2**	5.8**	4.4**	4.0**	3.6**	1.4*	1.2
G-0	9.9	5.0**	4.6**	3.2**	2.8**	2.4**	.2	---
M-0	9.7	4.8**	4.4**	3.0**	2.6**	2.2**	---	---
G-GC	7.5	2.6**	2.2**	.8	.4	---	---	---
M-GC	7.1	2.2**	1.8*	.4	---	---	---	---
F-GC	6.7	1.8*	1.4	---	---	---	---	---
CS-GC	5.3	.4	---	---	---	---	---	---
CN-GC	---	---	---	---	---	---	---	---

** Significant 1%

F-0 > C-GC CS-GC F-GC M-GC G-GC M-O G-O
CN-0 > " " " " "
CS-0 > " " " " "
G-0 > " " " " "
M-0 > " " " " "
G-GC > " "
M-GC > "

*Significant 5%

CN-0 > M-0 G-0
CS-0 > M-0
M-GC > CS-GC
F-GC > CN-GC

$LSD = t_{\alpha/2}\sqrt{\frac{2\ mse}{n}}$

$t_{1\%} = 2.576$ 80 degrees of freedom

$t_{5\%} = 1.960$

LSD = 2.13 (1%)

LSD = 1.6 (5%)

[a] The letters O and GC following the seed source abbreviation denote outside and growth chamber grown respectively.

Table 6. L. S. D. Test for Age and Location

Location	AGE 4 weeks	16 weeks	
Growth chamber grown (GC)	7.3[a]	5.3	6.3
Outside grown (O)	11.2	10.5	10.8
	9.3	7.9	

Age & Location	Transpiration	GC-16	GC-4	0-16
0-4 weeks	11.2	5.9**	3.9**	.7
0-16 weeks	10.5	5.2**	3.2**	---
GC-4 weeks	7.3	2.0**	---	---
GC-16 weeks	5.3	---	---	---

$LSD = t_{\alpha/2}\sqrt{\frac{2\ mse}{n}}$ n = 25 mse = 3.41 80 df. $t_{1\%} = 2.576$

LSD = 1.35 (1%)

**Significant 1%
0 - 4 weeks > GC- 16, GC- 4
0 - 16 weeks > " "
GC - 4 weeks > "

Location	Transpiration	GC
Outside	10.8	4.5**
Growth Chamber	6.3	---

Age	Transpiration	16 weeks
4 weeks	9.3	1.4**
16 weeks	7.9	---

$LSD = t_{\alpha/2}\sqrt{\frac{2\ mse}{n}}$ n = 50 mse = 3.41 80 df. $t_{1\%} = 2.576$

LSD = .951 (1%)

**Significant 1%
Outside grown > Growth chamber grown

4 weeks old > 16 weeks old

[a]Transpiration rates (mg $H_2O/cm^2/hr$)

APPENDIX II

COMPUTATION OF TRANSPIRATION RATES

Computation of transpiration rates was made as follows: From the red pen reading before the tree was inserted in the cuvette (base reading), the base humidity was obtained from the pen reading--humidity graph (Appendix II, Fig. 9). Similarly the pen reading at equilibrium, after the air had passed over the tree was used to get the increased humidity reading. Since the temperature was known, the saturated moisture content of a liter of air was obtained from tables. By multiplying the humidity times the saturated moisture content (e_s), the amount of water in the air before and after passing the seedling could be computed.

The difference was then multiplied by the air flow rate (.66 l air/min), to get transpiration on a mg H_2O/min basis. This value was then multiplied by 60 to get it on an hourly basis and divided by the foliage area. By proportion the Δe correction was made giving the final value in mg H_2O/cm^2 foliage/hour.

Sample calculation: Assume T = 23°C $\therefore$ saturation e_s = 20.6 mg H_2O/1 air

	Pen Reading	% R. H.	mg H_2O/1		
Base	6.0	39.1%	8.055	>	difference
After tree	26.5	44.7%	9.208		1.153 mg/1

$\Delta e = e_s - e_a = 20.6 - 9.2 = 11.4$

$.661/\text{min}\ (1.153\ \text{mg/l}) = .761\ \text{mg}\ H_2O/\text{min}$

Assume area = $5\ cm^2$

$$\frac{60\ \text{min/hr}\ (.761\ \text{mg/min})}{5 cm^2} = 9.1\ \text{mg}\ H_2O/cm^2/\text{hr}$$

Δe correction (See methods p. 19)

$$E_c = \frac{9.1\ \text{mg/cm}^2/\text{hr}\ (.513\ \text{mg/cm}^2/\text{min})}{.577\ \text{mg/cm}^2/\text{min}}$$

$$E_c = 8.1\ \text{mg}\ H_2O/cm^2/\text{hr}$$

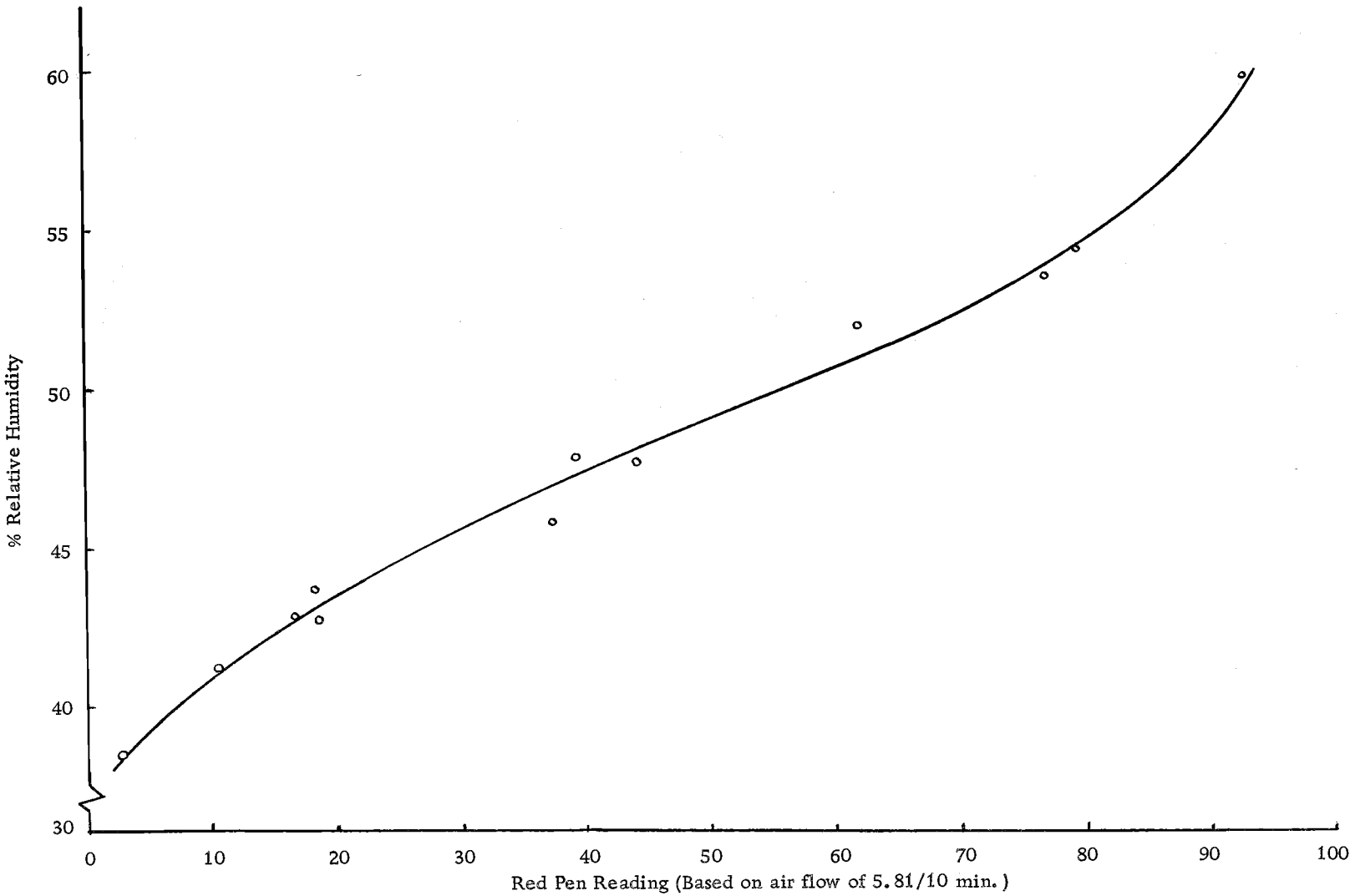

Figure 9. Calibration curve for Taylor Dual-Pen Recorder (Green Element).