AN ABSTRACT OF THE THESIS OF

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

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The objective of this study was to determine the effect of age on rates of transpiration by Douglas-fir seedlings. Seeds were collected from a xeric site (Goldendale, Washington) and a mesic site (Forks, Washington). The seedlings were grown in a cold frame and in a growth chamber for periods of two, four, eight, and sixteen weeks.

Although much interaction occurred between age, seed source, and growing condition, significant differences were delineated for each of the three variables. For both the Forks and Goldendale seedlings, the transpiration rates were not found to decrease significantly with increasing age. At a given age, four, eight, or sixteen weeks, the Forks seedlings grown in the cold frame, in general, had the highest rates of transpiration.

The effect of seed source was found to be significant when the

seedlings were grown in the cold frame. Under these conditions the seedlings from Forks, Washington, had a significantly higher rate than the seedlings from Goldendale, Washington.

The effect of conditioning on transpiration resulted in the seedlings grown in a cold frame having a significantly higher rate than those grown in a growth chamber.

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

by

Rollin Roger Geppert

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THE EFFECT OF AGE ON TRANSPIRATION RATES OF DOUGLAS-FIR SEEDLINGS (PSEUDOTSUGA MENZIESII (MIRB.) FRANCO)

INTRODUCTION

Survival of newly germinated Douglas-fir seedlings is a critical problem in the forested regions of the Pacific Northwest between the months of June and September. High soil surface temperatures and prolonged periods of drought are the two most important factors contributing to the mortality of seedlings. Young seedlings which have not developed sufficient root systems for taking up moisture from the deeper soil layers suffer from soil moisture deficiencies during the first summer. Although morphological factors and adaptations are of major concern, physiological processes must be considered also to gain a more comprehensive understanding of drought hardiness, drought evasion and seedling mortality.

Trees of different species and trees of different ecotypes of the same species vary in their ability to survive and grow under drought conditions. Although many studies have been conducted the reasons for these differences are not clearly understood. Additional studies are thus necessary for understanding these differences in seedling behavior.

This study was conducted to determine how Douglas-fir seedlings from different habitats vary in their transpiration behavior.

When supplied with adequate soil moisture, how does the transpiration rate vary with advancing age? Are transpiration rates different between seedlings from xeric and mesic sites? Does the transpiration rate of seedlings grown under artificially controlled conditions differ greatly from seedlings grown outside. These were questions investigated in this research.

REVIEW OF LITERATURE

The significance of transpiration, as an important physiological process in plant development and growth, has been argued for many years. Barnes (1902) and Clements (1934) viewed transpiration as being necessary to bring about the influx of mineral salts to the leaves and beneficial in maintaining a reduced leaf temperature. Curtis (1926), however, considered transpiration unnecessary and harmful to plants. Kramer (1959) suggested that transpiration, as influenced by light, temperature, wind, humidity, and soil moisture, was an "unavoidable evil" due to the structure of the plant. Transpiration produced water deficits, which may inhibit photosynthesis, reduce growth, and if too severe, may cause death from dessication (Kramer, 1959).

High surface temperatures and prolonged periods of drought limit the species of plant occupying the site and survival rates. Roeser (1932) found that Douglas-fir and Engelmann spruce increased their transpiration rates more under severe heat exposure than either western yellow pine or lodgepole pine. Pharis (1966), following leaf water content, found ponderosa pine, incense cedar, and Douglas-fir to be more drought resistant than sugar pine and grand fir. While

working with alpine plants in the Sierra Nevada Mountains of California, Mooney, Hillier, and Billings (1965) showed that Ivesia, coming from a dry site, was more drought resistant with its low transpiration rate than Caltha, a moist site species.

Although variation between species is expected, ecotypic variation within species has also been found. Ferrell and Woodard (1965) showed that interior mountain Douglas-fir seedlings had more drought hardiness and drought avoidance than the Pacific coastal seedlings located west of the Cascade Mountains. Pharis and Ferrell (1967) experimented with Douglas-fir seedlings from eight seed sources ranging from interior British Columbia to Arizona. They found that the five inland sources were more drought resistant than the three coastal sources. The significance of seed source was further studied by Zavitkovski (1964). He showed that Douglas-fir seedlings from a relatively xeric site in northwestern Washington had a significantly lower transpiration rate than seedlings from a mesic site near Valsetz, Oregon.

Numerous techniques have been employed to measure transpiration but few have considered the microenvironment of the plant during the period of measurement (Franco and Magalhaes, 1965). This study employed a method of measurement similar to that used by Bierhuizen and Slatyer (1964) in that such factors as light, wind, humidity, temperature and soil moisture were controlled during the period of

measurement. Available soil moisture has been varied in past studies of Douglas-fir and its response to drought. In this experiment soil moisture was maintained near field capacity.

MATERIALS, METHODS, AND APPARATUS

Selection and Growth of Material

In the fall of 1966, Douglas-fir seeds were collected from a mesic site in the Coast Range[1] and from a xeric site in the Cascade Mountains.[2] The mesic site, near Forks, Washington, was located at an elevation of 107 meters (350 feet) and averaged 297 cm (177 inches) of annual precipitation. The xeric site, near Goldendale, Washington, was located at an elevation of 793 meters (2600 feet) and averaged 41 cm (16 inches) of annual precipitation.

Seeds from both sources were stratified for seven days and germinated on filter paper in petri dishes at room temperature. When the radicles attained a length of about two centimeters, the seeds were planted in pint pots, painted black, perforated for drainage, and containing surface soil obtained from the McDonald Forest located in the Coast Range west of Corvallis, Oregon.

Equal numbers of seedlings from both sources were grown in a cold frame and in a growth chamber between June 1 and November 24, 1967. The cold frame environment had an average day-night

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[1] Courtesy of Manning Seed Company.

[2] Collected by the author in cooperation with the U. S. Forest Service, Pacific Northwest Range and Experiment Station.

temperature of 35 and 8 C, respectively, with ranges from 44 to 0 C at 25 cm above the ground. The mean radiation load received during the daylight period at 20 cm above the plant level was 0.43 langleys min^{-1}, between 400 and 700 millimicrons. Light mesh screen, providing 55% shade, was placed over the seedlings until they attained the age of four weeks. To conserve moisture and reduce seedling mortality, caused by excessive heat during the extremely dry summer, the pots were covered to the rim with moistened wood chips.

The growth chamber was maintained at a day-night temperature of 28 and 17 C, respectively, with a twelve hour photoperiod. Artificial lighting was provided by warm-white fluorescent lamps supplemented by incandescent lamps giving a light intensity of 0.033 langleys min^{-1} (approximately 720 foot candles), between 400 and 700 millimicrons, at the plant level.

Five replications of each seed source at four different ages were grown under the two growing conditions. For the two-week old transpiration measurements only growth chamber seedlings were used because cold frame plants were unavailable at the time of the experiment.

Treatment of Seedlings Immediately Prior To, During and After Transpiration Measurements

Transpiration measurements were determined for the Forks (F) and Goldendale (G) seedlings at three different ages (four, eight, and sixteen weeks) for those seedlings grown in the cold frame and at four ages (two, four, eight, and sixteen weeks) for the seedlings grown in the growth chamber.

Twenty-four hours prior to measurement, each plant was transferred to the constant temperature room, watered, and placed under fluorescent lamps providing a light intensity of 680 foot candles. Transpiration rates were determined in the following manner: two of the five seedlings planted in each pot were selected for exhibiting healthy foliage and similar height. The tops of the two potted seedlings were sealed into the cuvette base by using a slotted rubber polymer stopper, coated with silicone or lanolin grease. Care was taken to prevent the needles from making contact with the grease by placing a band of masking tape, adhesive side turned out, around the foliage. The tape was removed after the seedlings were sealed into the cuvette. Depending on the size of the seedlings, each pot remained sealed into the cuvette until a constant transpiration rate, over a ten minute period, was attained. Two-week-old seedlings reached a constant rate within fifteen to twenty minutes whereas the larger sixteen-week-old seedlings required two to four hours.

Upon completing each individual transpiration measurement, the seedlings were removed from the cuvette, an unslotted rubber stopper inserted, and the system allowed to equilibrate to base rates. All needles were then removed from the seedlings, weighed, and measured for surface area (Appendix I).

Transpiration Measurements

Transpiration rates were recorded by using two Aminco-Dunmore electric hygrometer sensing elements connected to a dual-pen Taylor transcope electronic recorder (TR).[3] Both elements were corrected and standardized using anhydrous magnesium perchlorate (Appendix II). The transpiration rates were determined by measuring the relative humidity of the air before and after it passed over the two seedlings enclosed in the plant chamber (cuvette) which provided controlled temperature and light conditions. The temperature and relative humidity of the air were controlled by means of two water baths. All measurements were conducted in a constant temperature and relative humidity room, 21 ± 1 C and 60 ± 2% respectively. Standard room air was taken in through a pressure vacuum air pump (AP) and raised to the saturation point by first bubbling the air

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[3] Parenthetical symbols refer to the transpiration measurement apparatus in Figure 1.

through 800 ml of distilled water contained in a glass flask (B_1) submerged in a 36 liter warm water bath and bubbled a second time through 400 ml of distilled water contained in a glass flask (B_2) submerged in a 144 liter cold water bath (Figure 1). The saturated air was circulated through 183 cm of coiled copper tubing (C_1), a glass wool filter (F_i) and back through the warm water bath where it passed through 183 cm of coiled copper tubing (C_2) and another glass wool filter (F_i). Prior to leaving the warm water bath, a portion of air was drawn off through a by-pass into a glass bottle (Blue) containing one of the humidity sensing elements. The air exited the bottle through a needle valve (V) used to maintain an air flow of 0.66 liters per minute in the system. The remainder of the air from the by-pass was passed through an air flow meter (FM_1), calibrated with a standard gas-test meter (Appendix III, Figure 10), and over the two enclosed seedlings. Thorough mixing of the air in the cuvette was achieved by using a self enclosed plastic blade fan with a metal bar attached to the opposite end of the shaft. The fan was powered by an electric motor (Fan M) having a bar magnet attached perpendicular to the end of the shaft. The turning of the bar magnet rotated the fan blade providing a wind speed of 17.7 cm per second. After passing over the seedlings, the air passed out of the cuvette, opposite the point of entry, and forced through a glass wool filter (F_i) to remove foreign particles. The increased relative humidity value was

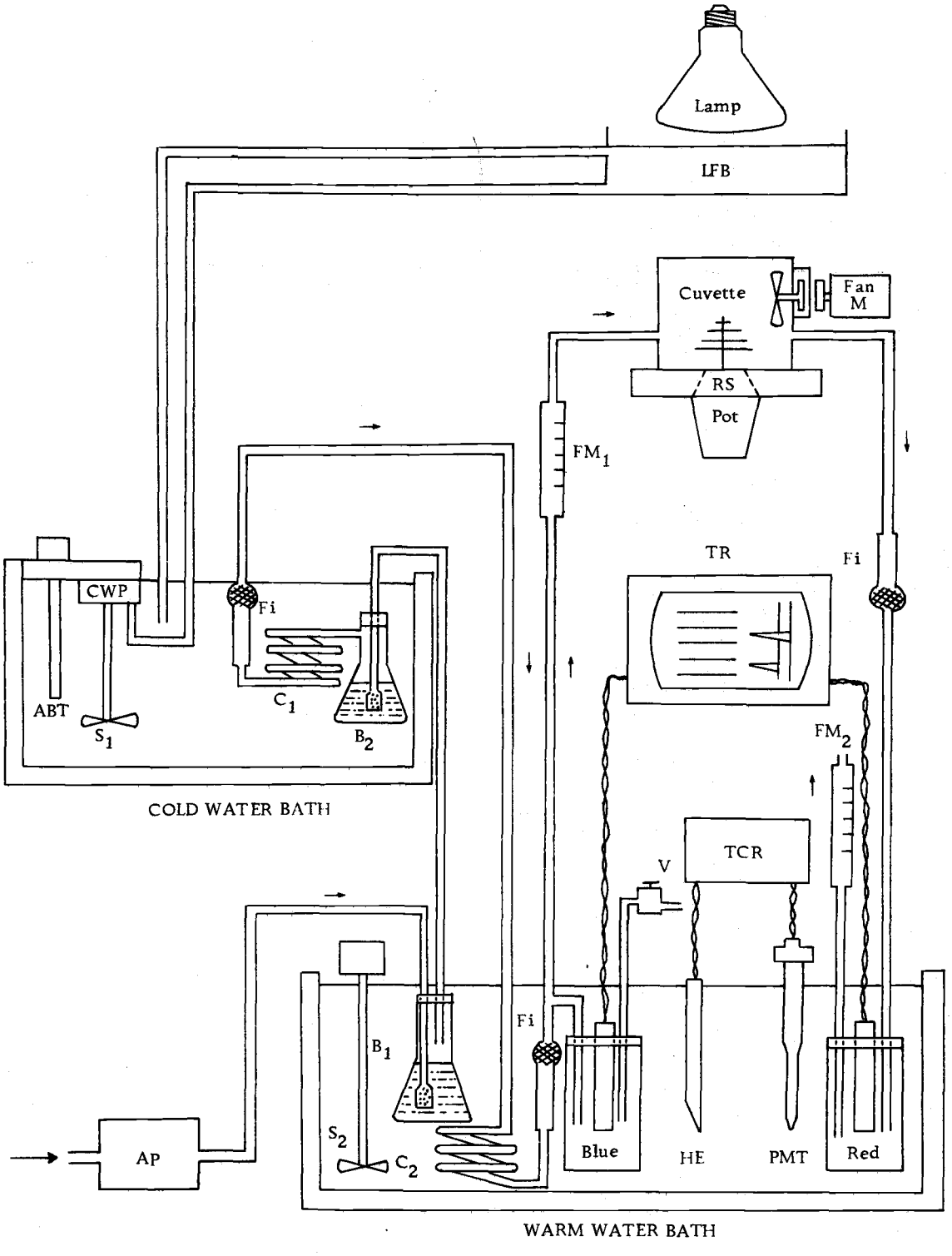


Figure 1. The transpiration measurement apparatus.

DETAILS OF EQUIPMENT INDICATED IN FIGURE 1

COLD WATER BATH	An Aminco Wide Range Laboratory Bath containing 144 liters of water maintained at 11.0 ± 0.05 C.
WARM WATER BATH	A styrifoam cooler containing 36 liters of water maintained at 23 ± 0.05 C.
Lamp	A mercury incandescent lamp providing a light intensity of 0.023 langleys min^{-1} at the plant level.
LFB	A 7.5 cm 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-enclosed fan. The rpm was controlled by a 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 (A and B) 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 l min^{-1}) 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 filter.
C_1	A coil of copper tubing 183 cm long, 6.35 mm inside dia. (1/4 inch), submerged in the cold water bath.
C_2	A coil of copper tubing 183 cm long, 6.35 mm inside dia. (1/4 inch), 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) recorded the response of the humidity sensing elements, Red and Blue.
Red	An Aminco-Dunmore electric hygrometer sensing element measured 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 (LBF); 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 ± 0.05 C.

recorded on the sensing element submerged in the warm water bath (Red). Relative humidity readings in the system ranged from a base rate of 38%, recorded by both Red and Blue sensing elements, to as high as 60%, recorded by the Red sensing element.

The temperature of the warm water bath was maintained to within ± 0.05 C by using a Versatherm electronic temperature relay (TCR) in connection with a precision mercury thermoregulator (PMT) and a heating element (HE). The cold water bath was maintained to within ± 0.05 C by using an Aminco bimetal thermoregulator (ABT) and a supersensitive relay. Transport of the air stream to all sensing, cooling, and heating devices was through tygon hose, 6.35 millimeters (1/4 inch) in diameter.

The plant chamber was constructed of plexiglass in the shape of a cylindrical cuvette, 16.5 cm in diameter, 10.2 cm high and sealed to a plexiglass base by means of a rubber "O-ring". An air tight seal around the stems and cuvette base was assured by momentarily measuring the air flow after the air had exited the cuvette thus seeing if the air flow through the cuvette containing a pot corresponded to the air flow through the cuvette without a pot.

The light source (Lamp) was provided by a mercury incadescent lamp having an output of 0.023 langleys min^{-1} (about 560 foot candles) at the plant level inside the cuvette. The lamp was positioned 50 cm above the cuvette and 12 cm above a 7.5 cm deep circulating water

bath (LBF) used to filter out the longer wave lengths of light. All light intensity measurements were made with an ISCO Spectro-radiometer Model-SR. The air temperature inside the cuvette was 23 ± 0.5 C determined with a thermocouple. The wind speed inside the cuvette was measured with an Alnor thermo-anemometer.

Treatment of Data

Transpiration rates were determined from the measured differences in relative humidity obtained before and after the seedlings were enclosed in the cuvette. The recorder reading from the sensing element measuring the relative humidity of the air leaving the cuvette was used for all calculations. From the conversion curve (Appendix II, Figure 9) the percent humidity was found for the respective recorder readings. With the temperature inside the cuvette maintained at a constant 23 C ± 0.5 C, the saturation would be 20.6 mg H_2O l^{-1}. The air flow through the system was maintained at a constant 0.66 liters min^{-1}. The transpiration rates were expressed in mg H_2O cm^{-2} hr^{-1} and calculated as follows:

1. First the evaporation rate was calculated by the following formula:

$$E = 0.66 (H_2 - H_1) = \text{mg } H_2O \text{ min}^{-1}$$

H_1 = % Relative humidity of air stream before passing over the seedlings multiplied by 20.6 mg H_2O $liter^{-1}$.

H_2 = % Relative humidity of air stream after passing over the seedlings multiplied by 20.6 mg H_2O l^{-1}.

0.66 = Air flow rate in liters min^{-1}.

2. Second, the evaporation rate was converted to mg H_2O cm^{-2} hr^{-1}.

$$E_i = \frac{60\ (E)}{A}$$

E = Evaporation rate in mg H_2O min^{-1}.

60 = Conversion to hourly basis.

A = Area of foliage expressed in cm^2.

3. Due to the effect of humidity upon transpiration a humidity correction factor was calculated for all transpiration values. This was done as follows:

$$\Delta e_i = e_s - e_a$$

Δe_i = Deficit in water vapor concentration below saturation.

e_s = Water vapor content of air at 100% relative humidity expressed in mg H_2O $liter^{-1}$ of air at temperature T of 23 C.

e_a = Water vapor content of the ambient air expressed in mg H_2O $liter^{-1}$ of air at temperature T of 23 C.

The larger the value for Δe_i, the higher the potential evaporation rate. All transpiration rates were standardized to a Δe_s of 11 mg H_2O liter^{-1} having a corresponding evaporation rate, E_s, of 0.513 mg H_2O cm^{-2} min^{-1} by using the equation $E = -1.228912 - 0.158354 \Delta e$ (Ruetz, 1968). The final transpiration value, T_s, was calculated from the following formula:

$$T_s = \frac{(E_i)\,(0.513)}{E_r} = \text{mg } H_2O \text{ cm}^{-2} \text{ hr}^{-1}$$

E_r = Evaporation rate at Δe of 11 mg H_2O liter^{-1}.

E_i = Observed evaporation rate in mg H_2O cm^{-2} hr^{-1}.

RESULTS AND DISCUSSION

A total of seventy measurements (Appendix IV, Table 1) were made on Douglas-fir seedlings under controlled conditions and tested by one-way classification (Ramsey, 1967), t-test for unpaired plots (Freese, 1967), analysis of variance and least significant difference (Mendenhall, 1965 and Ramsey, 1967) to determine the single and multiple interaction of three variables, age, seed source, and conditioning.

By analysis of variance (Appendix IV, Table 2) a significant difference was found between seed sources and between the two growing conditions. No significant differences were found in transpiration rates with increasing age; however, at a given age, significant differences were found between seed sources. Significant interactions were found between seed sources and growing conditions and among all three variables taken collectively.

The Effect of Seed Source

For outside-grown seedlings at four, eight, and sixteen weeks, the Forks source had a significantly higher transpiration rate than the Goldendale, averaging 12.3 and 8.9 mg H_2O cm^{-2} hr^{-1}, respectively (Appendix IV, Table 3). The outside-grown Forks had a significantly higher transpiration rate than either growth-chamber Forks or Goldendale

(Appendix IV, Table 4). No significant differences were found between the transpiration rates by the Forks and Goldendale seedlings grown in the growth chamber when the data from all ages were pooled.[4] For seedlings of both sources, less than sixteen weeks old, it appeared that differences were evident only when the seedlings were grown in the cold frame. The reasons for the higher transpiration rates by the Forks seedlings grown in the cold frame were not clearly understood; however, there were several possible explanations. The Forks seedlings may have had wider stomatal openings or they may have had more stomata per unit area of leaf surface. It is also possible that the Forks retained open stomata for a longer period of time under the low light intensity during measurement than the Goldendale seedlings under the same conditions. Testing these possibilities was not within the scope of this study.

The Effect of Age

Prior studies of transpiration have been concentrated on angiosperm species (Hygen, 1953) or gymnosperms older than one year (Jarvis and Jarvis, 1963; Oksbjerg, 1961). In the gymnosperm studies, transpiration was reported to decrease with advancing age (Roeser, 1932). On very young Douglas-fir seedlings investigated in this study no significant decrease in transpiration was apparent. At

[4] Tested by using the t-test for unpaired plots. All results were nonsignificant at the 5% level and not included in Appendix IV.

a given age, however, significant differences were found between seed sources when grown in a growth chamber and in a cold frame.

At the age of two weeks no significant differences were found in rates of transpiration between the Forks and Goldendale seedlings (Appendix IV, Table 5).

At the age of four weeks the Forks seedlings, grown in a cold frame, had a significantly higher transpiration rate than all of the Goldendale seedlings and all of the Forks seedlings grown in the growth chamber. The rate of transpiration for Goldendale seedlings grown in the cold frame was significantly greater than the Forks seedlings grown in the growth chamber (Appendix IV, Table 6).

For seedlings eight weeks old, the Forks seedlings grown in the cold frame had a higher transpiration rate than all of the Goldendale seedlings (Appendix IV, Table 7).

For seedlings sixteen weeks old, the Forks seedlings grown in the cold frame had a significantly higher rate of transpiration than the Forks and Goldendale seedlings grown in the growth chamber (Appendix IV, Table 8).

In general, the Forks seedlings grown in the cold frame, between the ages of four and sixteen weeks, exhibited a higher and more constant rate of transpiration than all of the Goldendale seedlings and all of the Forks seedlings grown in the growth chamber (Figure 2). It was apparent that the Goldendale seedlings exhibited a decrease in

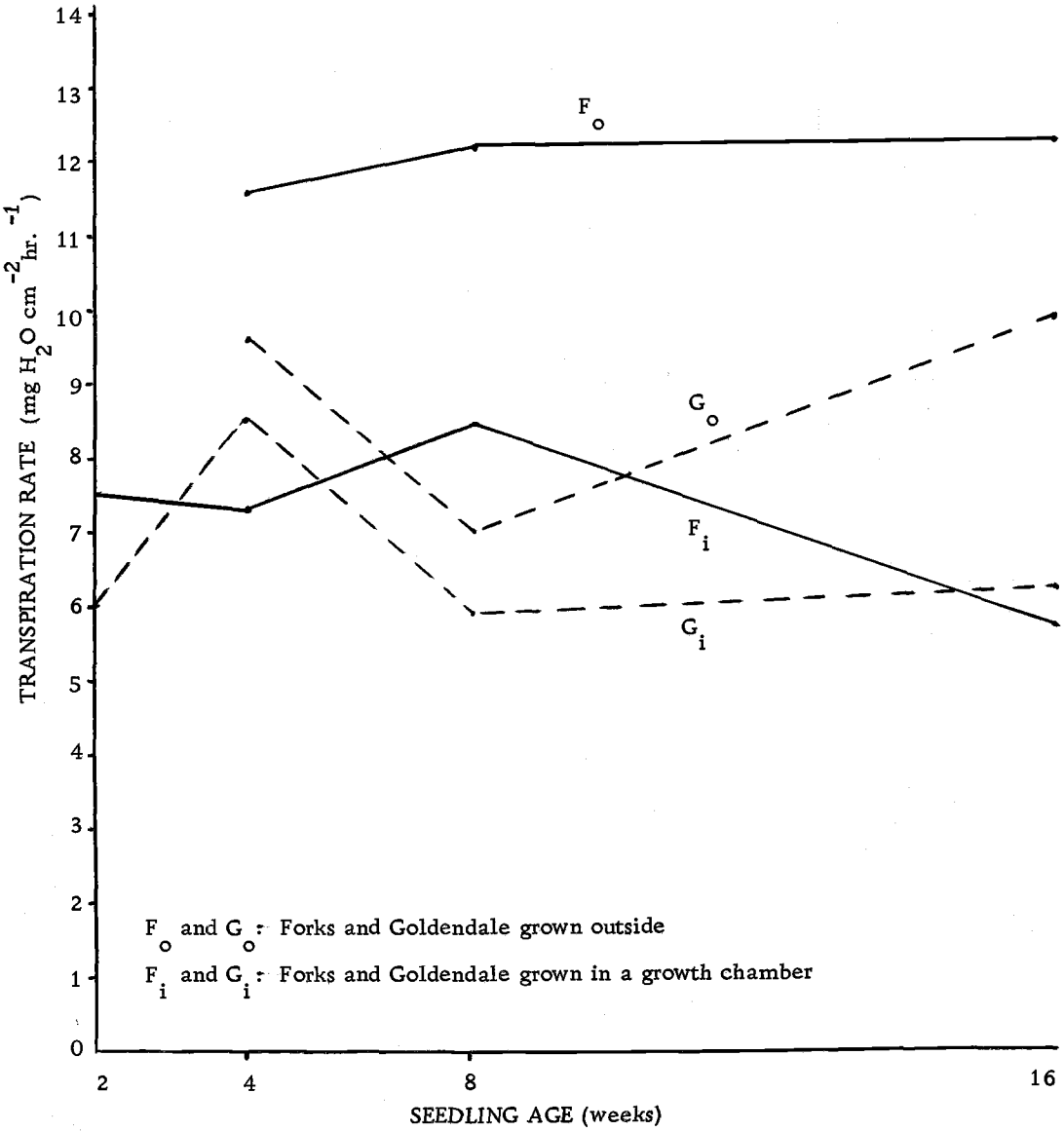
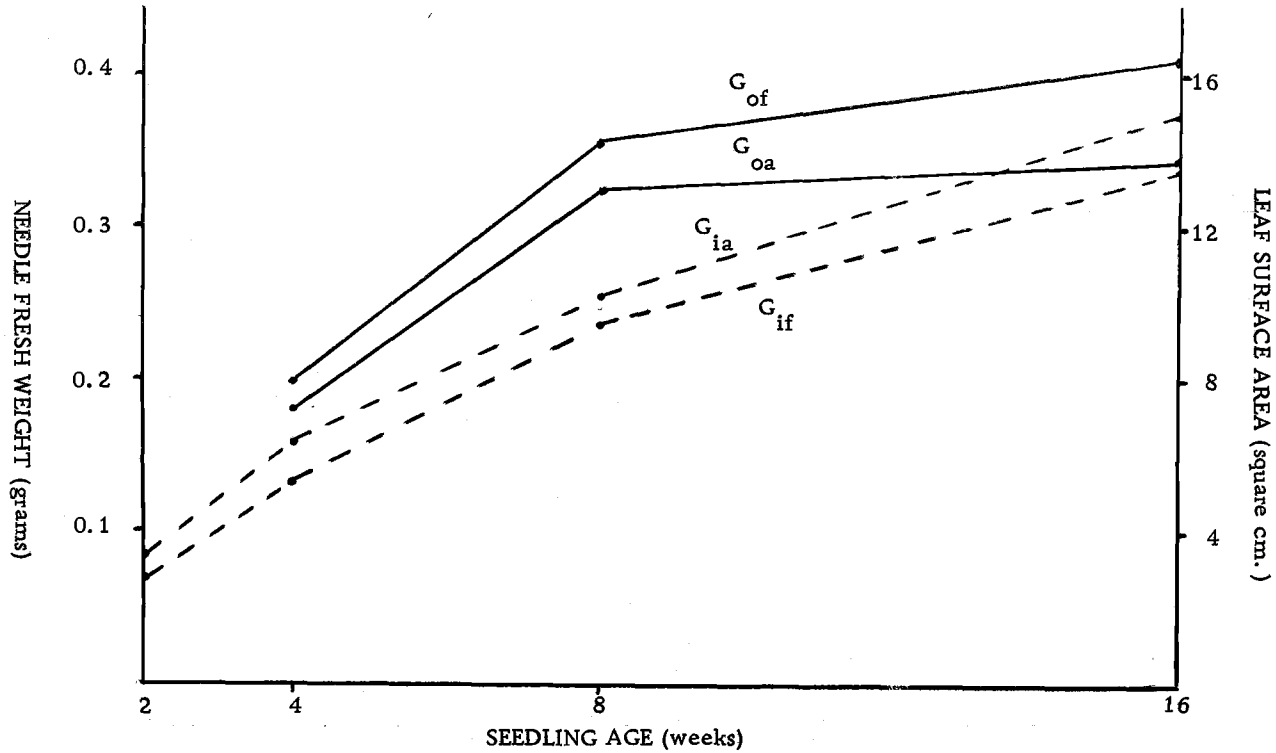


Figure 2. The average transpiration rates of outside-grown and growth-chamber-grown seedlings from Forks and Goldendale at two, four, eight, and sixteen weeks.



G_{of} and G_{oa}: Goldendale seedlings grown outside (o), fresh weight (f) and leaf surface area (a)

G_{if} and G_{ia}: Goldendale seedlings grown in a growth chamber (i), fresh weight (f) and leaf surface area (a)

Figure 3. A comparison of fresh weight and leaf surface area as influenced by growing condition for Goldendale seedlings at four ages.

growth from eight to sixteen weeks (Figure 3) whereas, the Forks seedlings (Figure 4), during the same period, exhibited continuous growth. The more active growth by the Forks seedlings may account for their higher transpiration rates.

The Effect of Conditioning

Seedlings grown in the cold frame had a significantly higher mean rate of transpiration than seedlings grown in the growth chamber, 10.6 and 7.2 mg H_2O $cm^{-2}hr^{-1}$, respectively (Appendix IV, Table 9).

A change in the transpiration rate can be attributed to anything that affects the steepness of the water vapor pressure gradient between the outside air and the evaporating surface of the needle. Recent research with Douglas-fir seedlings may help to explain the effect of different environmental growing conditions. The effect of temperature and relative humidity was demonstrated by Fry (1965). He concluded that Douglas-fir seedlings, not under soil moisture stress, decreased in both photosynthesis and transpiration when the needle temperature was increased from 24 to 28 C and the relative humidity lowered from 83 to 54%. He also demonstrated that light had only a minor influence on the stomatal movement. Tucker (1966) concluded that this same species, when grown under high light intensities and long photoperiods, produced mesophyll cells of increased

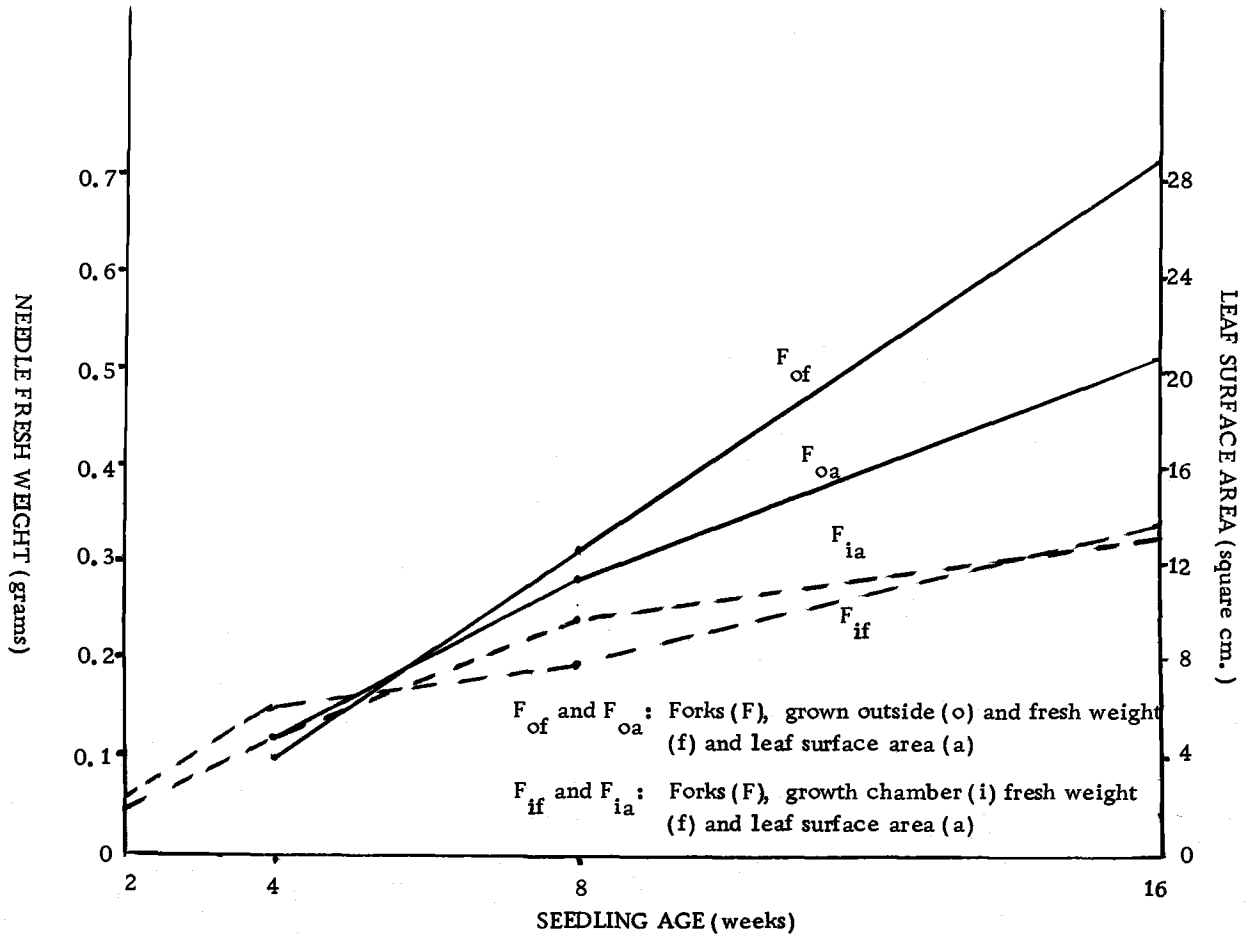


Figure 4. A comparison of fresh weight and leaf surface area as influenced by growing condition for Forks seedlings at four ages.

lengths and needles of increased width as compared with seedlings grown under low light intensities. Although the cuticle was significantly thicker under the conditions mentioned above, it was not considered important in transpirational losses unless the stomata were closed (Slatyer, 1966). From these studies it can be seen that seedlings grown under different environmental conditions exhibit characteristic differences. The existence of these differences may be related to transpiration differences.

An increase in cell length and needle width may result in the needle having a greater water holding capacity. This larger reservoir of water and perhaps lower water tension in the leaf may account for the higher transpiration rate. It may also help to explain why both Forks and Goldendale seedlings grown in the cold frame had a higher fresh weight-to-area ratio than seedlings grown in the growth chamber (Figures 3 and 4).

Two possible explanations for the differences in rates of transpiration by Douglas-fir seedlings between environments, may lie in the function of the cell wall and membrane. Seedlings grown outside may have a cell wall that is more efficient in conducting water along a gradient and a membrane more efficient in permitting rapid water exchange than seedlings grown in the growth chamber.

SUMMARY AND CONCLUSIONS

The effect of age and growing condition on the transpiration rates by Douglas-fir seedlings from two seed sources was studied.

A significant difference in transpiration rates was found between seed sources when the seedlings were grown in a cold frame. When the transpiration data at four, eight, and sixteen weeks were pooled, the seedlings from the mesic site at Forks, Washington had a higher transpiration rate than the seedlings from the xeric site at Goldendale, Washington.

No significant decrease in rates of transpiration were found in either the Forks or Goldendale seedlings as age advanced from two to sixteen weeks. At a given age, however, a significant difference between seed sources was found when the seedlings were grown under two different conditions. In general, the Forks seedlings, when grown in the cold frame, exhibited a higher transpiration rate than all of the Goldendale and all of the Forks seedlings grown in the growth chamber.

The effect of conditioning on rates of transpiration resulted in the seedlings grown in the cold frame having a significantly higher rate than those seedlings grown in the growth chamber.

One of the objectives of the study was to determine how early genetic differences in transpiration rates could be detected. The

results with the seedlings grown outside suggest that these differences can be detected very early when they are grown under these conditions.

BIBLIOGRAPHY

Barnes, C. R. 1902. The significance of transpiration. Science, new ser., 15:460.

Bierhuizen, J. F. and R. O. Slatyer. 1964. An apparatus for the continuous measurement of photosynthesis and transpiration under controlled environmental conditions. (Canberra, Australia, 16 p. Commonwealth Scientific and Industrial Research Organization. Division of Land and Regional Survey. Technical Paper no. 24)

Clements, H. F. 1934. Significance of transpiration. Plant Physiologia 9:165-171.

Curtis, O. F. 1926. What is the significance of transpiration? Science, new ser., 63:267-271.

Davis, R. G., G. M. Roberson, W. C. Johnson and A. F. Wiese. 1966. A modified optical planimeter for measuring leaf area. Agronomy Journal 58:106-107.

Ferrell, W. K. and S. Woodard. 1965. Effect of seed origin on drought resistance of Douglas-fir. Ecology 47:499-503.

Franco, C. M. and A. C. Magalhaes. 1965. In: Methodology of plant ecophysiology: Proceedings of the Montpelier Symposium, Montpelier, France, 1962. Paris, p. 211-224. (United Nations Education Scientific and Cultural Organization. Air Zone Research. Vol. 25)

Frear, D. E. H. 1935. Photoelectric apparatus for measuring leaf area. Plant Physiology 10:569-574.

Freese, F. 1967. Elementary statistical methods for foresters. Washington, D. C. 85 p. (U. S. Department of Agriculture. Agriculture Handbook 317)

Fry, K. 1965. A study of transpiration and photosynthesis in relation to the stomatal resistance and internal water potential in Douglas-fir. Ph. D. thesis. Seattle, University of Washington. 192 numb. leaves.

Hygen, G. 1953. Studies in plant transpiration. II. Physiologia Plantarum 6:106-133.

Jarvis, P. G. and M. S. Jarvis. 1963. The water relations of tree seedlings. III. Transpiration in relation to osmotic potential of the root medium. Physiologia Plantarum 16:269-275.

Kramer, P. J. 1937. An improved photoelectric apparatus for measuring leaf area. American Journal of Botany 24:375-376.

_____ 1959. Transpiration and the water economy of plants. In: Plant physiology, a treatise. Vol. 2. New York, Academic, p. 105-192.

Mendenhall, W. 1965. Introduction to statistics. Belmont, California, Wadsworth, 305 p.

Miller, E. F., C. A. Shadbolt and L. G. Holm. 1956. Use of an optical plainmeter for measuring leaf area. Plant Physiologia 31:484-486.

Mooney, H. A., R. D. Hillier and W. D. Billings. 1965. Transpiration rates alpine plants in the Sierra Nevada of California. American Midland Naturalist 74(2):374-386.

Oksbjerg, E. 1961. Transpiration of growing shoots in Norway spruce (Picea abies) with some notes on drought phenomena. Svensk Botanisk Tidskrift 55(2):397-415.

Pharis, R. 1966. Comparative drought resistance of five conifers and foliage moisture content as a viability index. Ecology 47: 211-221.

Pharis, R. and W. K. Ferrell. 1967. Differences in drought resistance between coastal and inland sources of Douglas-fir. Canadian Journal of Botany 44(3):1651-1659.

Ramsey, F. 1967. Assistant Professor, Oregon State University, Dept. of Statistics. Lecture notes from statistical inference for biological and sociological science, St. 452. Corvallis.

Roeser, J. J. 1932. Transpiration of coniferous seedlings and the problem of heat injury. Journal of Forestry 30:381-395.

Ruetz, W. F. 1968. The effect of seed source on transpiration rates of seedlings of Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco). Master's thesis. Corvallis, Oregon State University, 46 numb. leaves.

Slatyer, R. O. 1966. Some physical aspects of internal control of leaf transpiration. Agricultural Meteorology 3:281-292.

Tucker, R. E. 1966. An anatomical study of the needles and hypocotyles of Douglas-fir seedlings grown under various environments. Master's thesis. Corvallis, Oregon State University. 73 numb. leaves.

Zavitkovski, J. 1964. The effect of drought on the rate of photosynthesis, respiration, and transpiration in two ecotypes of Douglas-fir. Master's thesis. Corvallis, Oregon State University. 65 numb. leaves.

APPENDICES

APPENDIX I

LEAF AREA MEASUREMENT

Two methods were used to measure the foliar surface area after making transpiration measurements on the Douglas-fir seedlings. The first method described has been widely used in the past and was assumed to be one of the most accurate known.

Two pieces of opaque paper, of known area, were placed between two silicone-grease-coated glass plates along with the needles. The paper and foliage together were photographed on uniform density Kodabromide paper at two to three times their normal size. The needle images were carefully cut out with a scissors and weighed on a Meetler scale. By finding the weight of the opaque squares, the area of the foliage could be calculated by proportion. The use of this method was very slow, often requiring one hour for a two-to-eight-week old seedling.

In search of a faster and possibly a more accurate way of finding foliar surface, a second method (optical planimetery) was developed for measuring the larger sixteen-week old seedlings.[5] The basic principles of operation are similar to those advanced by Davis, et. al.

[5] Assembled and tested by the author in cooperation with Dr. Kenneth Krueger, plant physiologist for the U. S. Forest Service, Pacific Northwest Range and Experimental Station, Corvallis, Oregon.

(1966). Frear (1935), Kramer (1937), and Miller (1956). Light emitted from a 12-volt automotive bulb passes through the collimator lens and emerges below in parallel rays (Figure 5). These rays are then either absorbed by the foliage or transmitted through the glass plate where they are then condensed and collected on the photocell (Figures 6 and 7). From a standardized curve (Figure 8) the fraction of light absorbed by the foliage is expressed as area on the microammeter by noting the deviation from a maximum reading of 25 microamps when the glass plates contained no foliage.

A standardized curve (Figure 8) was constructed by using both the photographic method and the optical planimeter on twenty sixteen-year-old seedlings. Due to the limited working area on the glass plates, one to five separate readings were taken on each seedling with the sum of their deviation from 25-microamps being plotted against the area determined by the photographic method on the same seedlings.

Although much more work needs to be done in perfecting this planimeter, present findings indicate that this method is much faster, requires only 10 to 15 minutes for a sixteen-week old seedlings, and may be more accurate than the photographic method.

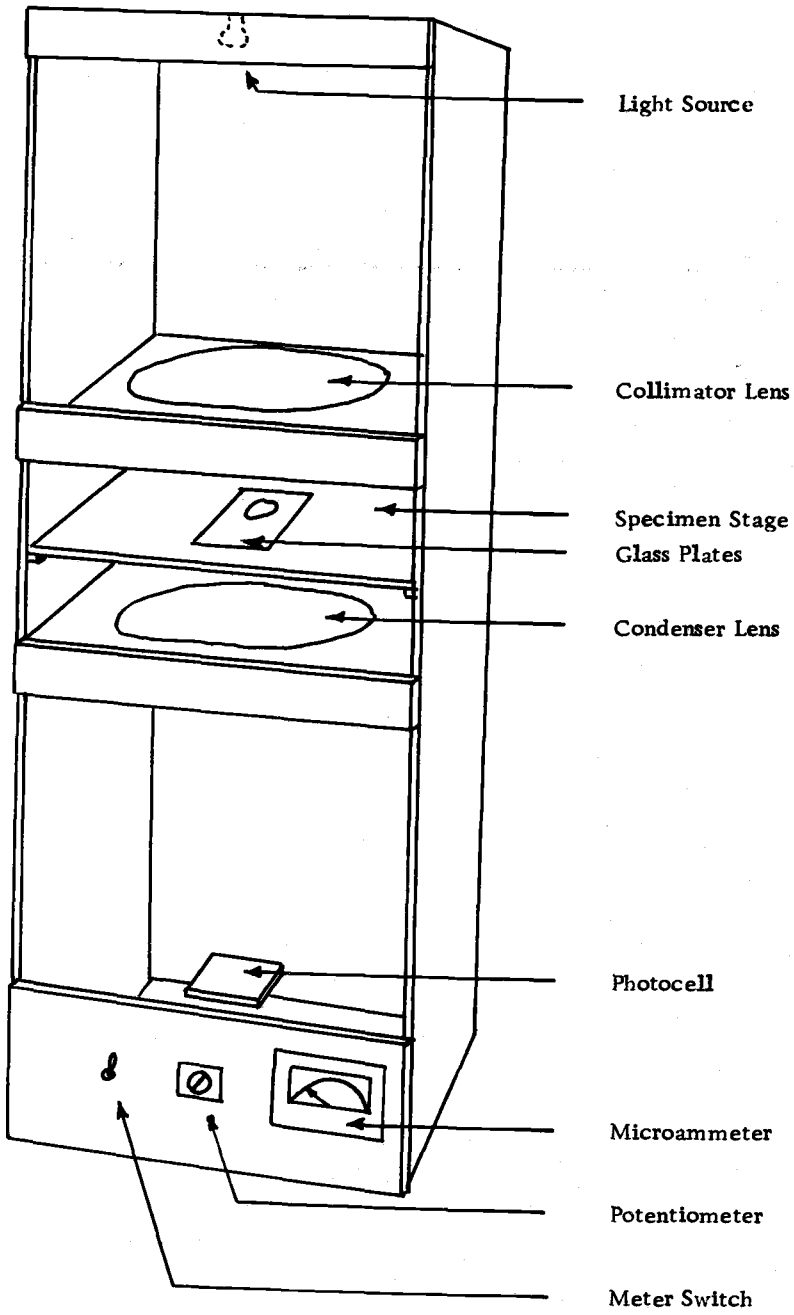


Figure 5. The optical planimeter with doors removed.

List and Definitions of Optical Planimeter

Light Source	A 12 volt automobile light bulb, powered by a 12 volt car battery, providing a light intensity of 32 f. c.
Collimator Lens	A Kodak Ektalite field lens (#HE-40412) 14.5 inches in diameter and 1/16 inch thick, having a 15 inch focal length and used to collimate the light emitted from the bulb.
Specimen Stage	A platform that supports the glass plates.
Glass Plates	Two hinged glass plates, 8 × 5 × 1/8 inches each, with a 6 cm. diameter circle offset 1 1/2 inches from lens center.
Condenser Lens	Same as the collimator lens only used in reverse position to condense the light rays upon the photocell.
Photocell	An International Rectifier Selenium Photovoltaic cell having 9.41 square inches of photosensitive area with an illumination range of 0.01 to 10,000 f. c.
Microammeter	A Simpson microammeter with a 0 to 25 microamp range.
Potentiometer	A variable rheostat used to adjust the microammeter to 25 before making each reading.
Meter Switch	A two-way switch, off and on, used to control the microammeter.

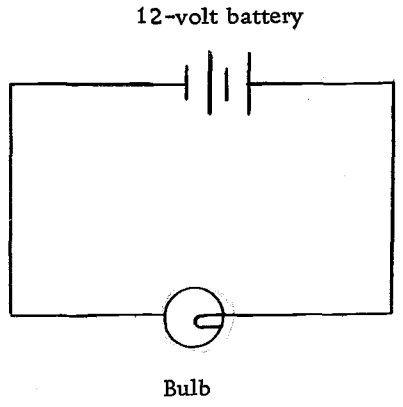


Figure 6. The illumination circuit for the optical planimeter.

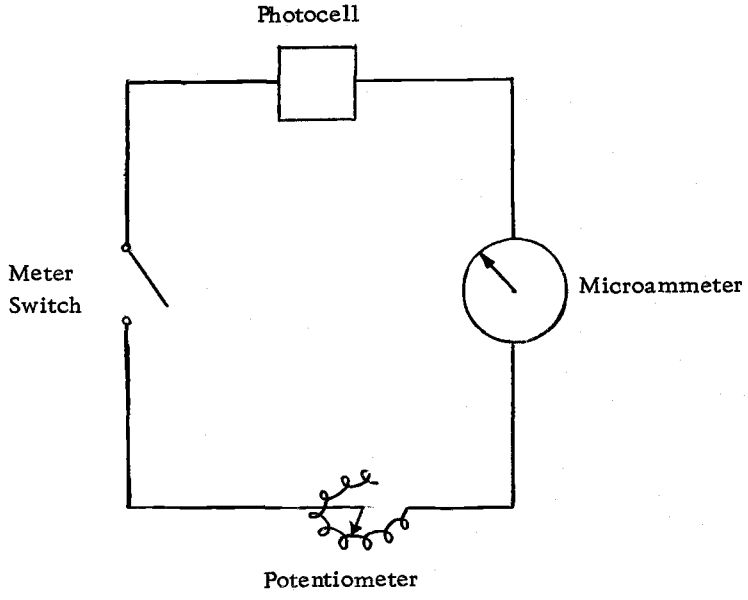


Figure 7. The sensing circuit for the optical planimeter.

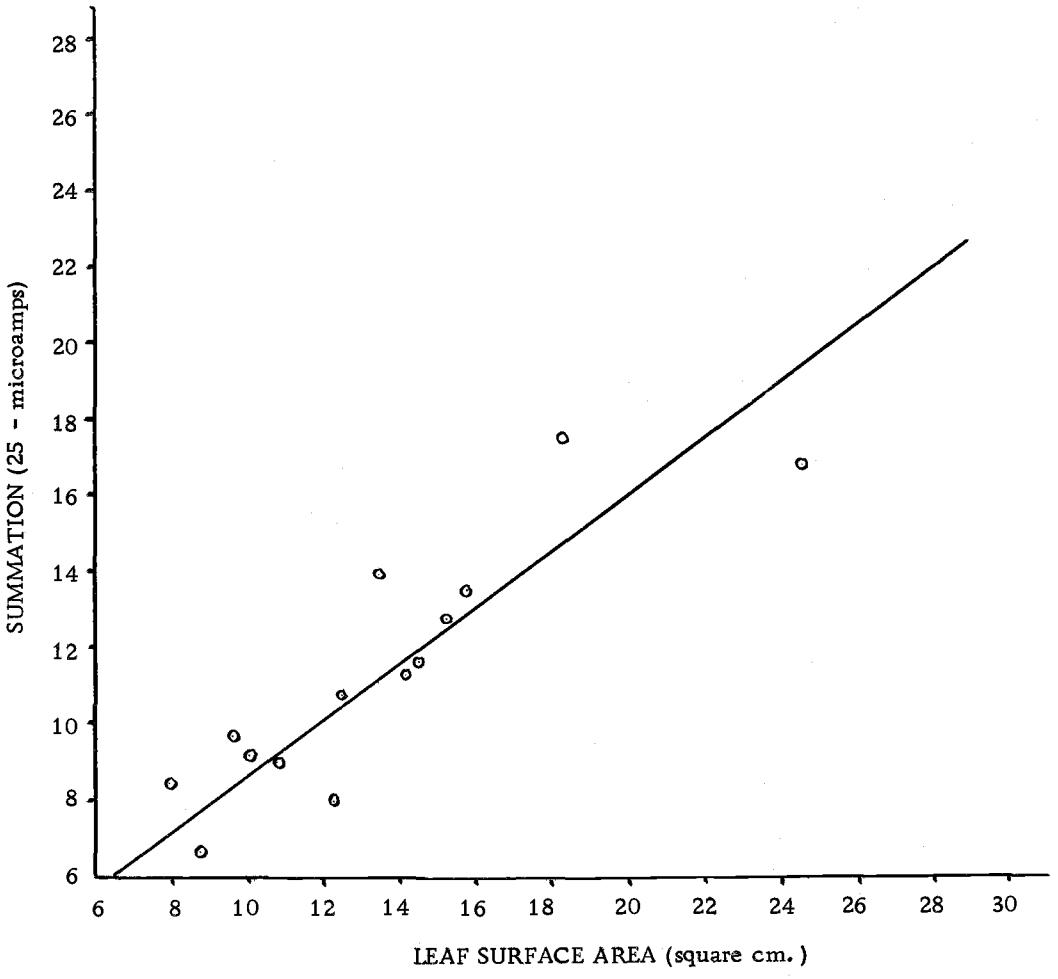


Figure 8. A standardized curve for finding leaf surface area using the optical planimeter.

Procedure for Operating the Optical Planimeter

1. Carefully remove all needles from the seedling by grasping the petiole with a small forceps and gently pulling upward.

2. Clean the glass plates with alcohol, or other suitable solutions that do not leave a film on the glass.

3. Place the glass plates, without the needles, in the planimeter, close all doors, turn on the light, and use the potentiometer to adjust the microammeter to read 25 microamps.

4. Remove the glass plates from the planimeter and place the needles between the plates, within the 6 cm diameter circle, without touching one another.

5. Place the glass plates, containing the needles, in the planimeter, close all doors, and read the microammeter.

6. Remove the glass plates, clean with alcohol, replace in planimeter and check for a 25 microammeter reading.

7. Repeat steps 4-6 until all the foliage from one seedling has been measured.

8. Sum the deviations from 25 and read the area in square centimeters off the standardized curve (Figure 8).

APPENDIX II

CALIBRATION OF SENSING ELEMENTS

Although two sensing elements (Blue and Red)[6] were employed in the measuring apparatus, the one (Red) measuring the relative humidity after the air passed through the cuvette was used for interpreting the transpiration rates. A curve (Figure 9), relating relative humidity and the chart reading (TR) was constructed from data collected using the following procedure. With the entire apparatus in operation, gas absorbtion tubes, containing anhydrous magnesium perchlorate, were connected to the air stream passing out of the sensing element container (Red). Various sizes of moist blotter paper were placed in the cuvette, with no enclosed plant, to provide a wide range of humidities. The gas absorbtion tubes were connected to the system for ten minutes and weighed at the end of this period to find the mg of water absorbed. The chart recorder (TR) reading was observed at the end of ten minutes.

$$\% \text{ R. H. } = \frac{W}{6.6\ (S)}$$

W = Milligrams of absorbed water during a 10 minute period.

6. 6 = Constant air flow rate of 6. 6 liters 10 min^{-1}.

S = Saturation point of 20. 6 mg H_2O l^{-1} at temperature T of 23 C.

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[6]Parenthetical symbols refer to Figure 1 on page 11.

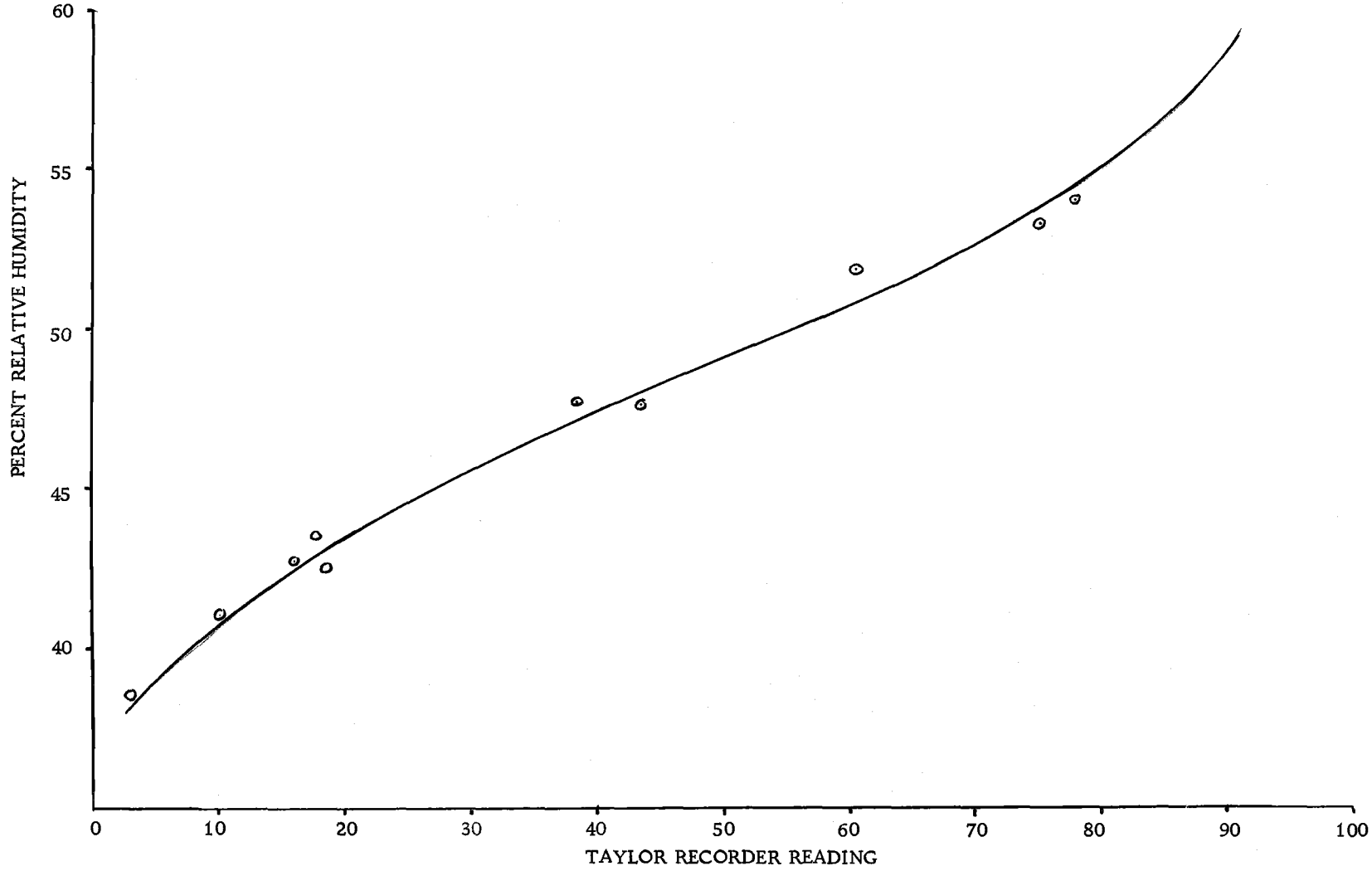

Figure 9. A relative humidity curve for the calibration of the humidity sensing element (RED).

APPENDIX III

MONOSTAT FLOW METER CORRECTION CURVE

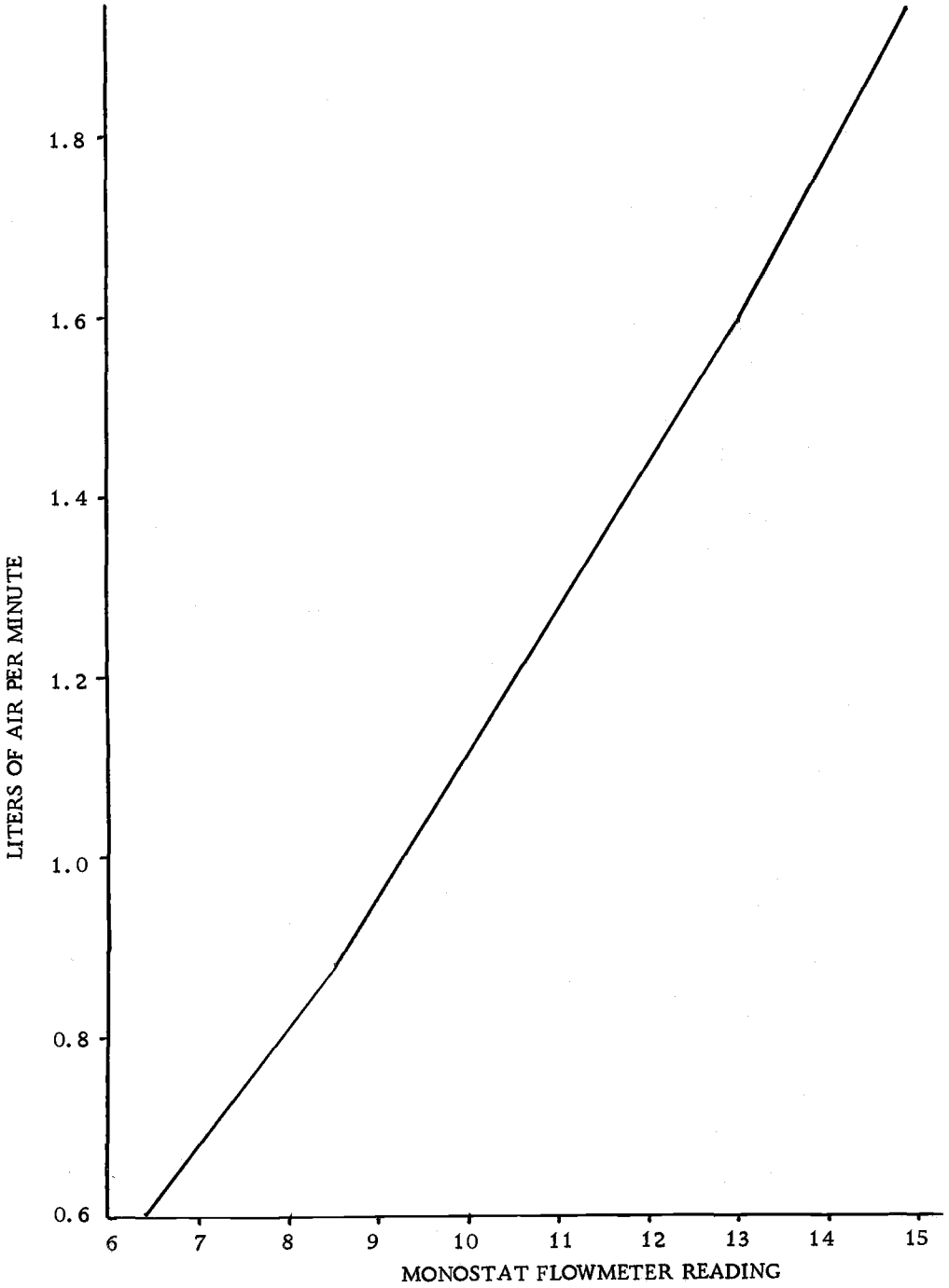

Figure 10. The Monostat flow meter correction curve calibrated with a standard gas-test meter.

APPENDIX IV

STATISTICAL ANALYSIS

Table 1. Transpiration measurements on two seed sources at four ages grown under artificial and natural conditions.

Conditioning and Age	Source	Transpiration mg H_2O cm^{-2} hr^{-1} 1	2	3	4	5	Ave.	Sum X^2
Growth Chamber 2-weeks	F[a]	5.5	7.4	10.2	8.5	6.8	7.7	307.54
	G[b]	7.3	5.8	5.3	6.0	6.2	6.1	189.46
Growth Chamber 4-weeks	F	6.8	5.9	7.3	7.4	9.7	7.4	283.46
	G	10.1	8.8	5.6	9.9	9.7	8.8	402.91
Growth Chamber 8-weeks	F	7.9	9.5	5.2	5.1	15.2	8.6	436.75
	G	3.0	5.1	3.0	11.7	7.2	6.0	232.74
Growth Chamber 16-weeks	F	4.8	5.8	5.0	3.9	10.2	5.9	200.93
	G	2.7	4.6	12.1	6.6	4.9	6.2	242.43
Outside 4-weeks	F	11.4	13.2	11.0	10.0	14.0	11.9	721.20
	G	10.5	11.1	10.7	9.9	6.7	9.8	490.85
Outside 8-weeks	F	17.5	13.4	12.2	10.4	9.2	12.5	827.45
	G	7.6	7.1	6.7	6.4	7.4	7.0	248.78
Outside 16-weeks	F	11.5	17.0	10.0	10.3	13.0	12.4	798.95
	G	8.8	12.1	14.4	6.7	7.6	9.9	533.86

[a]The seed source from Forks, Washington designated as F.

[b]The seed source from Goldendale, Washington designated as G.

Table 2. Analysis of variance for testing transpiration rates of two seed sources (S) at three ages (W) grown under artificial and natural conditions (C).

Source	D. F.	Mean Squares	F-value
Seed source (S)	1	50.59	8.18 **
Age in weeks (W)	2	5.58	0.90 NS
Conditioning (C)	1	117.50	18.92 **
S × W	2	18.83	3.03 NS
S × C	1	34.96	5.63 *
C × W	2	10.30	1.66 NS
S × W × C	2	46.83	7.54 **
Error	48	6.21	

* Significant at 5%
** Significant at 1%
NS Nonsignificant

Table 3. A t-test for unpaired plots for the outside grown Forks and Goldendale at three ages; four, eight, and sixteen weeks.

Forks	Goldendale	Calculation of t
11.4	10.5	$SS_A = \Sigma X_A^2 - \frac{(\Sigma X_A)^2}{n_A} = 85.47$
13.2	11.1	
11.0	10.7	
10.0	9.9	
14.0	6.7	$SS_B = \Sigma X_A^2 - \frac{(\Sigma X_A^2)}{n_B} = 63.89$
17.5	7.6	
13.4	7.1	
12.2	6.7	
10.4	6.4	$s^2 = \frac{SS_A + SS_B}{(n_A-1) - (n_B-1)} = 5.33$
9.2	7.4	
11.5	8.8	
17.0	12.1	
10.0	14.4	$t = \frac{X_A - X_B}{\sqrt{\frac{s^2 (n_A + n_B)}{(n_A)(n_B)}}} = 3.92$
10.3	6.7	
13.0	7.6	
184.1	134.7	
12.27	8.98	Critical value of $t_{.005} = 3.012$
A	B	Forks significantly greater than Goldendale at the 1% level.

Table 4. A test of Least Significant Difference (LSD) for seed source and conditioning between four and sixteen weeks.

Seed source and Conditioning[a]	Transpiration Average	mg H_2O cm^{-2} hr^{-1} G_i	F_i	G_o
F_o	12.3	5.3*	5.0*	3.4*
G_o	8.9	1.9	1.6	-
F_i	7.3	0.3	-	-
G_i	7.0	-	-	-

* Significant at 1%

$F_o > G_i, F_i, G_o$

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

LSD = 2.34

n = 15

D.F. = 48

$t_{.005} = 2.576$

[a]Conditioning designated as Outside (F_o and G_o) and Growth Chamber (F_i and G_i).

Table 5. A statistical test for differences in the transpiration rates of growth chamber grown Forks and Goldendale seedlings at the age of two weeks.

Source	D. F.	Sum Squares	Mean Squares	F-value
Regression	2	482.50		
d.t. H_o	1	476.10		
Difference	1	6.40	6.4	3.55 *
Error	8	14.50	1.8	
Total	10	497.00		

* Significant at 5%

Table 6. A statistical test for differences in transpiration rates of Forks and Goldendale seedlings grown outside and in a growth chamber at the age of four weeks.

Source	D. F.	Sum Squares	Mean Squares	F-value
Regression	4	1849.25		
d.t. H_o	1	1805.00		
Difference	3	44.25	14.75	4.82 *
Error	16	48.90	3.06	
Total	20	1898.15		

* Significant at 5%

Seed source and Conditioning	Transpiration Average	mg H_2O cm^{-2} hr^{-1} F_i	G_i	G_o
F_o	11.9	4.5 *	3.1 *	2.1
G_o	9.8	2.4 *	1.0	-
G_i	8.8	1.4	-	-
F_i	7.4			

* Significant at 5%

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

$n = 5$

$LSD = 2.33$

D. F. = 16

$t_{.025} = 2.12$

Table 7. A statistical test for differences in transpiration rates of Forks and Goldendale seedlings grown outside and in a growth chamber at the age of eight weeks.

Source	D. F.	Sum Squares	Mean Squares	F-value
Regression	4	1576.05		
d.t. H_o	1	1445.00		
Difference	3	131.05	43.7	4.12*
Error	16	169.67	10.6	
Total	20	1745.72		

*Significant at the 5% level.

Seed source and Conditioning	Transpiration Average	mg H_2O cm^{-2} hr^{-1}		
		G_i	G_o	F_i
F_o	12.5	6.5*	5.5*	3.9
F_i	8.6	2.6	1.6	-
G_o	7.0	1.0	-	-
G_i	6.0	-	-	-

*Significant at 5%

$F_o > G_i, G_o$

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

LSD = 4.37

n = 5
D. F. = 16
$t_{.025} = 2.12$

Table 8. A statistical test for differences in transpiration rates of Forks and Goldendale seedlings grown outside and in a growth chamber at the age of sixteen weeks.

Source	D. F.	Sum Squares	Mean Squares	F-value
Regression	4	1625.10		
d.t. H_o	1	1497.20		
Difference	3	127.90	42.63	4.52 *
Error	16	151.07	9.44	
Total	20	1776.17		

* Significant at 5%.

Seed source and Conditioning	Transpiration mg H_2O cm^{-1} hr^{-1}	F_i	G_i	G_o
F_o	12.4	6.5 *	6.2 *	2.5
G_o	9.9	4.0	3.7	-
G_i	6.2	0.3	-	-
F_i	5.9	-	-	-

* Significant at 5%

$F_o > F_i, G_i$

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

$LSD = 4.10$

$n = 5$

D. F. = 16

$t_{.025} = 2.12$

Table 9. A test of Least Significant Difference (LSD) for the effect of growing condition on transpiration.

Conditioning	Average Transpiration mg $H_2O/cm^2/hr.$	Growth Chamber
Outside	10.60	3.44 *
Growth Chamber	7.16	-
* Significant at 1%	$LSD = t_{\alpha/2}\sqrt{\frac{2\ MSE}{n}}$	
		n = 30
Outside > Growth Chamber	LSD = 1.656	D.F. = 48
		$t_{.005}$ = 2.576