

AN ABSTRACT OF THE THESIS OF

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Title: THE EFFECT OF INTERNAL AND EXTERNAL MOISTURE
STRESS ON THE TRANSPIRATION RATE OF DOUGLAS-
FIR SEEDLINGS (PSEUDOTSUGA MENZIESII (MIRB.)
FRANCO)

Abstract approved: Signature redacted for privacy.
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The objective of this study was to examine the transpirational behavior of Douglas-fir seedlings from two origins, each grown in two environments, and subject to a range of moisture stress. Forks and Goldendale, Washington were the mesic and xeric seed origins respectively. One-half of the seedlings from each origin was grown in a growth chamber and the other half in a cold frame. The transpiration rate, soil moisture stress, and plant moisture stress were measured on each seedling at the age of six weeks. Tri-variate response surfaces were developed for each seed origin-preconditioning combination of seedlings.

There were no apparent differences in the transpirational response of the seedlings of the seed source-preconditioning combinations to soil moisture stress. There were highly significant

differences in transpirational behavior among seedlings of the several factor combinations in response to plant moisture stress.

Those seedlings grown in a cold frame had consistently higher subsequent transpiration rates regardless of the plant moisture stress. Xeric origin seedlings had higher subsequent transpiration rates at moderate plant moisture stress than did the mesic origin seedlings. The transpiration rates of the seedlings from the two origins were at parity between ten and 12 atmospheres plant moisture stress. Above 12 atmosphere plant moisture stress, the xeric source seedlings transpired less than the mesic source seedlings. The observed transpirational behavior of the xeric source seedlings was seen to be a distinct ecological advantage.

The Effect of Internal and External Moisture Stress on
the Transpiration Rate of Douglas-fir Seedlings
(Pseudotsuga menziesii (Mirb.) Franco)

by

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THE EFFECT OF INTERNAL AND EXTERNAL
MOISTURE STRESS ON THE TRANSPIRATION
RATE OF DOUGLAS-FIR SEEDLINGS
(PSEUDOTSUGA MENZIESII
(Mirb.) Franco)

INTRODUCTION

The post-harvest regeneration of Douglas-fir constitutes a problem of particular importance to the forester of the Pacific Northwest. One of the major factors which contributes to the difficulty of obtaining satisfactory regeneration is the annual drought condition which develops each summer. Considerable effort is being focused on methods for avoiding the deleterious effects of drought. For example, studies with agricultural species have demonstrated the effectiveness of using genetic strains with drought-resistant characteristics.

Genetic strains or ecotypes are products of marginally severe local environmental conditions that selectively alter the gene pool in favor of characteristics compatible with those conditions. If a species exists over a wide geographical range, it is necessarily subject to a great variety of local environments and may be expected to exhibit pronounced ecotypic variability. Such a species is Douglas-fir. In fact the genetic plasticity of Douglas-fir is considerable, and the existence of drought-resistant ecotypes is well established (Ferrell and Woodard, 1966; Pharis and Ferrell, 1966).

Drought resistance may be characterized as the combination of two factors. It may be due to the avoidance of dry internal conditions and to the tolerance of dry internal conditions. The relative drought avoidance capacities of different ecotypes may be strongly influenced by their respective transpirational response to droughty environments. The objective of this study was to investigate the differences in the transpiration rates of seedlings of two contrasting ecotypes of Douglas-fir over a range of internal and external moisture conditions.

Much of the research of Douglas-fir seedlings has been done with material grown in growth chambers, while other studies have utilized material grown in cold frames. Ruetz (1968) and Geppert (1968) found that the manner in which seedlings are grown affects their transpirational behavior. Consequently, the study was designed to include an investigation of the effects of prior growth in a growth chamber and a cold frame respectively on the subsequent transpiration rate of the seedlings of both ecotypes.

REVIEW OF LITERATURE

It is generally accepted that plant growth is inversely related to the energy required to remove water from the soil. As the soil dries, water becomes increasingly difficult to remove. If the trend continues, the plant eventually succumbs to desiccation. Resistance to drought is variable within a species, a fact which has led to the energetic search for drought resistant strains. The work of Meuli and Shirley (1937) with green ash (Fraxinus pennsylvanicum Marsh) indicated the possibility of intraspecies variability with respect to drought resistance of forest trees. Squillace and Bingham (1958) reported ecotypic variation in drought resistance of western white pine (Pinus monticola Douglas) as did Brix (1959) for loblolly pine (Pinus taeda L.).

A number of studies in recent years have been concerned specifically with the drought resistance of Douglas-fir seedlings. Seedlings from a number of interior sources were compared with seedlings from several maritime sources by Ferrell and Woodard (1966). They suggest that "Apparently there has been natural selection favoring drought resistance in local populations, and the differences are in drought avoidance, probably in transpiration." Pharis and Ferrell (1966) demonstrated that differences in leaf and soil moisture content at the death point occur among Douglas-fir seedlings. They

concluded that interior ecotypes are more drought hardy than those from coastal sources.

Zavitkovski (1964) monitored the transpiration rates of Douglas-fir seedlings from two sources over a range of soil moistures. His data, collected on growth chamber grown seedlings, indicated a depression in transpiration rate caused by excessively high soil moisture content. The rate increased slightly to a brief plateau and then rapidly decreased as the soil dried. The seedlings from the more mesic source had higher transpiration rates than those from the drier source over the entire soil moisture range. He argued that a lower shoot/root ratio, characteristic of the xeric source seedlings, may explain the ecological mechanism responsible for the selection of drought-resistant ecotypes.

Ruetz (1968) examined the transpiration rates of well-watered Douglas-fir seedlings from five sources at the ages of four and 16 weeks. He compared the effect of growing these seedlings in a growth chamber against the effect of growing them in a cold frame outside. He found significantly higher transpiration rates among seedlings grown outside, as did Geppert (1968) in a concurrent study. Furthermore, "When grown outside the most mesic source had the highest transpiration rate and the two xeric sources the lowest" (Ruetz, 1968). When grown inside this relationship was not evident. The two ecotypes used in this study were also studied by Geppert

(1968). He showed that between the ages of two and 16 weeks the transpiration rate of either source is independent of age.

The foregoing is representative of the published history leading up to this study. Many notable publications have appeared that bear upon the understanding of the mechanisms involved in transpiration studies. Among them are the more extensive treatments of the subject such as the texts of Kramer (1949), Slatyer (1967), and Kozlowski (1968).

MATERIALS AND APPARATUS

Materials

Douglas-fir seed was collected from two locations selected primarily for their extreme differences in total annual precipitation. One area is located in the wet coastal mountains of western Washington near the city of Forks. The area is at an elevation of 107 meters (350 ft) and receives annually 297 cm (177 in) precipitation. The other area is located on the relatively dry eastern slope of the Washington Cascades near the city of Goldendale. The area is at an elevation of 792 meters (2600 ft) and receives annually 41 cm (16 in) precipitation.

The clay loam soil used for the study was obtained from McDonald Forest on the eastern side of the coast range west of Corvallis, Oregon. It was collected from the top 15 cm (6 in) after the organic layer was removed. A measure of soil uniformity was achieved by twice sifting it through a quarter-inch mesh screen and mixing it thoroughly in a small soil homogenizer.

The soil was used to fill pint-sized plastic pots which had been perforated at the bottom for drainage and painted black to exclude light from the root medium. One hundred of these pots were filled to the brim and allowed to settle before the seeds were sown.

The stratification procedure consisted of soaking the seed for 24 hours in tap water, draining and storing for seven days in a refrigerator at 5°C. After stratification the seed was placed in petri dishes between wet filter paper and allowed to germinate at growth chamber temperatures. When the radicles reached approximately one centimeter in length, three seeds were planted per pot with their seed coats at the soil surface. Eventually, 50 pots were sown with seed from Forks, Washington and 50 with seed from Goldendale, Washington. One-half of the material from each source was grown in a growth chamber; the other half was grown out-of-doors in a cold frame.

Geppert (1968) demonstrated no consistent effect of age on the transpiration rates of Douglas-fir seedlings between the ages of two and eight weeks; it seemed desirable, however, to work with material of the same age and approximate size. Since each transpiration observation was expected to require one to two hours to complete, it was necessary to plant only four pots per day so that they could be measured as the seedlings reached the desired age of six weeks. Consequently, small quantities of seed were being stratified, germinated, and planted each day.

The growth chamber was set for a 24 hour cycle with an 18 hour day and a six hour night. A light intensity of 2300 uw cm^{-2} (720 foot-candles) was achieved with a combination of incandescent

and fluorescent lamps. The temperature was maintained at 25°C during the light periods and gradually diminished to 18°C during the dark periods.

A cold frame was established in a field near the laboratory. A quarter-inch mesh screen was positioned about 12 inches above the cold frame to provide enough shade to prevent mortality from excessive heat loads during the summer. The screen reduced the solar intensity by approximately 50% when the sun was overhead. The value varied considerably depending on the angle of incidence of the sun.

All the pots were set in moist sawdust to help reduce rapid fluctuations in soil temperature. The seedlings were well watered as uniformly as practical and kept free of weeds. The position of the pots was rotated periodically in both the cold frame and in the growth chamber so that positioning effects would not confound the factors of interest.

When the seedlings in each pot reached an age of six weeks they were brought into the laboratory for measurement. One of the three seedlings was removed to create an experimental unit of two seedlings growing in a pot of soil. All data were obtained from this experimental unit. Hereafter, reference to an experimental unit, or more simply a unit, will comply with the above definition.

Apparatus

The problem required the assessment of several variables; the transpiration rates of the seedlings, in $\text{mg H}_2\text{O cm}^{-2} \text{ hr}^{-1}$; the plant moisture stress, in atmospheres; and the soil moisture tension, in atmospheres. The device used to assess the transpiration rate was originally adapted from a design of Bierhuizen and Slatyer (1964) and was later described by Geppert (1968) and Ruetz (1968). The apparatus consists of two water baths for temperature and humidity control, a cuvette to accommodate the experimental unit, two lithium chloride humidity sensing elements, and a dual pen strip chart recorder. A small pump forces air through the open system at a constant rate of 0.66 liters per minute. The air is saturated at 11°C and warmed to 23°C resulting in 48.3% relative humidity (10 $\text{mg H}_2\text{O}$ per liter of dry air). The air is pumped in this condition through the cuvette and then over the sensing element; the absolute humidity of the air stream is recorded on the strip chart. A base level reading is taken when the moisture content of the air stream is at an equilibrium. The experimental unit is inserted in the cuvette, and a new equilibrium is allowed to establish before a second reading is taken. The difference between the two readings yields a reliable measure of the amount of water transpired by the seedlings in the cuvette.

The cuvette was equipped with a small externally driven fan. The wind speed created by this fan was measured with a calibrated commercial hot-wire anemometer at different fan speeds (Figure 1). The effect of different wind speeds on the boundary layer resistance was determined.

The boundary layer is a "skin" of air immediate to the surface of the plant. Water moves across this layer by vapor diffusion only. Consequently, the boundary layer presents a resistance to water movement that is directly proportional to the thickness of the layer. The thickness of the layer is profoundly affected by the wind speed. To ascertain the effect of the wind speeds that could be obtained with the apparatus on the boundary layer resistance (r_a), a crude ceramic model of a seedling of known surface area was wetted and sealed in the cuvette. The evaporation rates from this "seedling" at various wind speeds were measured and the boundary layer resistances calculated by a method presented by Slatyer and Bierhuizen (1964).

$$r_a = \frac{\Delta e}{E}$$

$$\Delta e = e_1 - e_a$$

where: r_a = boundary layer resistance

Δe = vapor pressure gradient from seedling to air in

g H₂O cm⁻³.

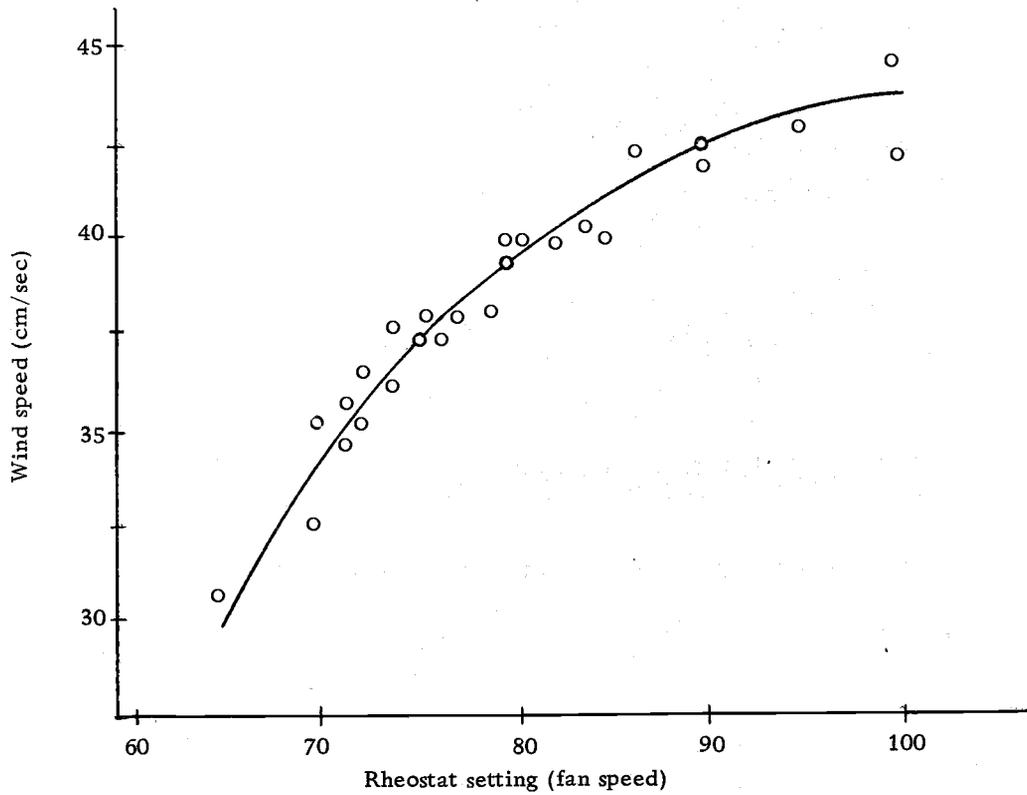


Figure 1. Wind speed in the cuvette as a function of fan speed.

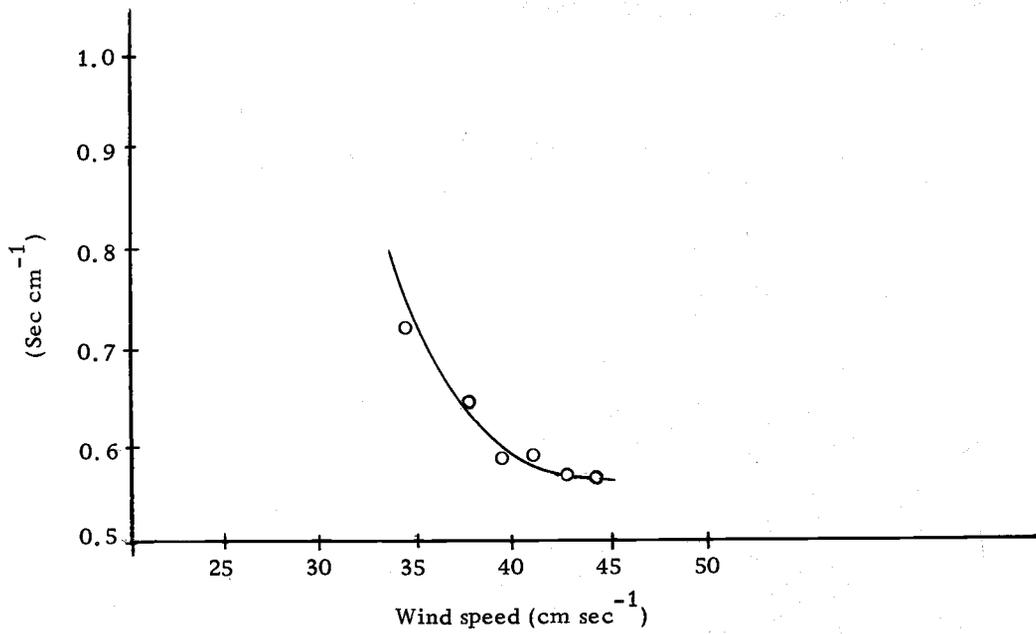


Figure 2. The effect of wind speed on boundary layer resistance.

E = evaporation rate ($\text{mg H}_2\text{O cm}^{-2} \text{sec}^{-1}$)

e_1 = saturation vapor pressure at 23°C (20.6 mg l^{-1})

e_a = vapor pressure of cuvette atmosphere at the time
of measuring E .

A wind speed of 40 centimeters per second was chosen for the experiment because it was reliably reproducible with the equipment used and seemed to have near maximum effectiveness reducing the boundary layer resistance (Figure 2).

A 1250 watt combination mercury vapor-incandescent lamp was used over the cuvette. The spectral intensity was measured using an integrating spectral radiometer; an output of $8,000 \text{ uw cm}^{-2}$ (1850 foot-candles) was measured between 400 and 700 nanometers (Appendix C).

Since the transpiration data were to be expressed per unit of needle area, it was necessary to measure the needle area of each seedling. To accomplish this, an optical planimeter was calibrated within the expected range of areas. The optical planimeter that was used has been described by Davis (1966) and by Geppert (1968). Geppert reports considerably more error inherent in the planimeter than reported here (Appendix C). The difference was probably due to the fact that the range of areas he was measuring was several times greater than that measured during this study. Areas obtained from the planimeter are not the true surface area of the needles

being measured, but rather the area of the image of the needles projected onto a plane surface. This corresponds roughly to the stomated surfaces in the case of Douglas-fir needles. No attempt was made to convert this value to a true needle area, and this fact should be kept in mind when making comparisons in the literature.

Plant moisture stress was evaluated using a pressure bomb after the principle advanced by Scholander et al. (1965) and as modified by Cleary (1968). Each seedling was severed at the soil surface and placed in the pressure bomb immediately after being removed from the cuvette. This procedure gave two values of plant moisture stress for each experimental unit which were averaged and recorded as a single measurement. Of the 94 units measured, only 11 produced two values which were significantly different from each other. The causal factors behind these differences are open to some speculation. They may include differences in root development, unexpected variation in the soil moisture distribution within a pot, and the high genetic variability of Douglas-fir.

A partial physical analysis of the soil used in the study was performed by the Forestry Sciences Laboratory of the Pacific Northwest Forest and Range Experiment Station in Corvallis, Oregon. The texture analysis indicated that the soil belonged to the silty clay loam class. The soil moisture tensions at several different percent moisture contents were determined using a pressure plate technique.

The logarithmic transformations of the results of that analysis were found to be linearly related (Appendix C). After the transpiration measurements were completed, the soil moisture percentage of each unit was determined on an oven dry weight basis. These values were converted to moisture tensions using the moisture tension curve of Appendix C).

The shape of the soil moisture tension curve depends on the relative size distribution of the pore fraction. The moisture tension curve of this particular soil is indicative of a high degree of uniformity in the distribution of the pore sizes responsible for the moisture tensions between 0.1 and 15 atmospheres. Whether or not this uniformity is maintained for smaller pore sizes, and the linearity apparent in Figure 14 is continued for tensions greater than 15 atmospheres is a moot question. This difficulty notwithstanding, however, the moisture tension curve was extrapolated to cover the range of percent moisture contents observed during the study. On a theoretical basis Temperley (1947) calculates the order of tensile strength of water to be between 500 and 1000 atmospheres. Extreme moisture tension values during this study were estimated at 1000 atmospheres. While more will be said concerning the consequences of this extrapolation in the discussion of the results of the experiment, I can say here that they were negligible.

DESIGN AND ANALYSIS

As described above, the initial measurement of transpiration was obtained from the difference between a base reading and a final reading. The quantity so obtained was an absolute humidity in mg H₂O per liter of moist air. This value was converted to a transpiration rate per unit leaf area by multiplying by the rate of air flow (39.60 liters per hour) and dividing by the projected needle area.

Ordinarily the atmosphere behaves as an enormous sink, absorbing the transpirational input with scarcely an effect on the ambient vapor pressure. In the cuvette, however, the continuous transpirational input of the seedlings is confined to a relatively small volume. Consequently, the greater the transpiration rate the greater the amount of water vapor that will accumulate in the cuvette, resulting in a high "ambient" vapor pressure. Transpiration is movement of water in response to pressure gradients; if the vapor pressure of the cuvette atmosphere is increased, the transpiration rate is decreased. Since the experimental units were all transpiring at different rates, the observed rates were all decreased differently. To overcome this effect, each transpiration rate was corrected to a common vapor pressure gradient. An evaporation rate-vapor pressure gradient relationship was obtained using wetted filter paper disks of known area (Figure 3). Observations on evaporation rates

from discs of different area were made following the same procedure used to obtain the transpiration data.

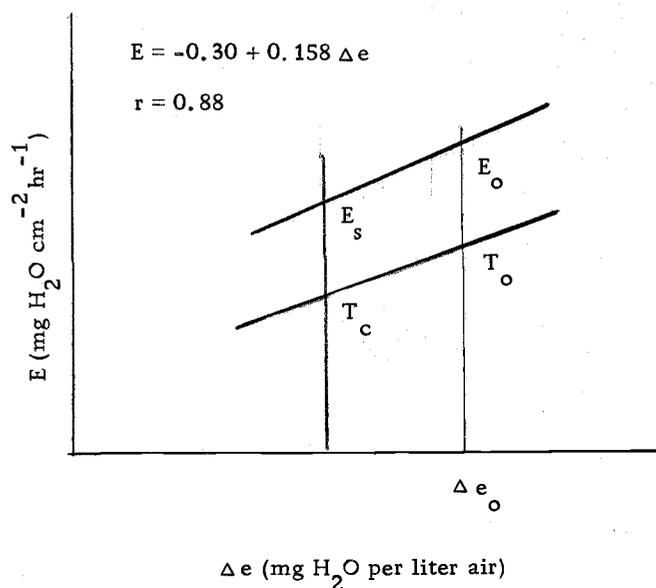


Figure 3. Schematic representation of Δe correction computation.

Δe equals the saturation vapor pressure at 23°C minus the observed vapor pressure at evaporation rate E . These values are expressed in terms of mg H₂O per liter moist air and are in fact saturation deficits of water vapor concentration at a stable temperature of 23°C.

A Δe value of 11 mg H₂O per liter was arbitrarily chosen as a standard, and each transpiration rate was adjusted to this common condition by proportionality.

$$\frac{T_c}{E_s} = \frac{T_o}{E_o} \quad T_e = \frac{(T_o)(E_s)}{E_o}$$

T_c = adjusted transpiration rate

T_o = observed transpiration rate

E_s = standard evaporation rate at standard Δe (1.238 mg H_2O
 $cm^{-2} hr^{-1}$)

E_o = evaporation rate at Δe_o calculated from regression

Δe_o = observed saturation deficit

Design and Analysis

The study design conformed naturally to a two by two factorial form. Seed source constituted the first factor, having two levels; Forks and Goldendale. Preconditioning constituted the second factor, also with two levels: growth chamber and cold-frame. Four source-preconditioning combinations are thusly created: Forks--growth chamber, Forks--cold frame, Goldendale--growth chamber, Goldendale--cold-frame. For each of these factor combinations three variables were measured; transpiration rate, soil moisture tension, and plant moisture stress. A trivariate response surface was generated statistically for each of the factor combinations using the transpiration rate as the dependent variable of the Y axis and soil moisture stress and plant moisture stress as the two independent variables of the horizontal axes.

Preliminary examination of the data suggested transforming each of the three variables to base ten logarithm and fitting the

following linear model:

$$y_j = \beta_0 x_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_1^2 + \beta_4 x_2^2 + \beta_5 x_1 x_2 + e$$

where:

y_j = the observed transpiration rate of jth seed source-preconditioning combination

β_0 = the effect common to all observations

β_i = the effect of the ith variable (i=1, 2, 3, 4, 5)

$x_0 = 1$

x_1 = the observed values of plant moisture stress in atmospheres

x_2 = the observed values of soil moisture stress in atmospheres

x_1^2 = the quadratic expression of x_1 allowing for curvature on the x_1 axis

x_2^2 = the quadratic expression of x_2 allowing for curvature on the x_2 axis

$x_1 x_2$ = the interaction between plant moisture stress and soil moisture stress

e = error

A response surface can be generated for each of the source-preconditioning combinations using this model; however, making comparisons among the four surfaces represented in this form would be prohibitively time-consuming. It was desirable to condense the

four models into one expression in such a way that the source-preconditioning combinations could be treated separately and also grouped in relevant combinations. This was achieved by merging the four models in the following manner:

$$\begin{aligned}
 y = & \beta_{01}x_{01} + \beta_{02}x_{02} + \beta_{03}x_{03} + \beta_{04}x_{04} + \beta_{11}x_{11} + \beta_{12}x_{12} \\
 & + \beta_{13}x_{13} + \beta_{14}x_{14} + \beta_{21}x_{21} + \beta_{22}x_{22} + \beta_{23}x_{23} + \beta_{24}x_{24} \\
 & + \beta_{31}x_{11}^2 + \beta_{32}x_{12}^2 + \beta_{33}x_{13}^2 + \beta_{34}x_{14}^2 + \beta_{41}x_{21}^2 + \beta_{42}x_{22}^2 \\
 & + \beta_{43}x_{23}^2 + \beta_{44}x_{24}^2 + \beta_{51}x_{51} + \beta_{52}x_{52} + \beta_{53}x_{53} + \beta_{54}x_{54} \\
 & + e
 \end{aligned}$$

where:

y = the predicted transpiration rate

β_{0j} = the effect common to all observations on the j th source-preconditioning combination ($j=1, 2, 3, 4$)

β_{ij} = the effect of the i th variable on the j th source-preconditioning combination ($i=1, 2, 3, 4, 5$; $j=1, 2, 3, 4$)

x_{01} = 1 for all Forks-cold-frame data; 0 otherwise

x_{02} = 1 for all Goldendale-cold-frame data; 0 otherwise

x_{03} = 1 for all Forks-growth chamber data; 0 otherwise

x_{04} = 1 for all Goldendale-growth chamber data; 0 otherwise

x_{1j} = the observed values of plant moisture stress on the j th source-preconditioning combination

x_{2j} = the observed values of soil moisture stress on the j th source-preconditioning combination

x_{1j}^2 = the quadratic expression of x_{1j} allowing for curvature on the x_1 axis

x_{2j}^2 = the quadratic expression of x_{2j} allowing for curvature on the x_2 axis

x_{5j} = the interaction between soil moisture stress and plant moisture stress (x_{1j}, x_{2j}) for the j th source-preconditioning combination

The full model consisting of 24 parameters was fitted to the complete collection of data points using the "canned" computer program known as the Stepwise Multiple Linear Regression. Only two statistics were used from each resulting printout, the residual sum of squares (SSE) and the total sum of squares (SST). The full model was then subjected to various degrees of restraint, and the residual sums of squares for each restrained model was recorded. An F test was performed to determine whether the increase in error sum of squares due to the restraint was significant. For example: the test for significance of the effect of seed source would be:

$$F = \frac{(SSE_r - SSE_f)/d.f.}{SSE_f/d.f.}$$

$$F = \frac{3.41420 - 2.50686/12}{2.50686/70}$$

$$F = 2.11* \text{ with 12 degrees of freedom}$$

The source of the numerical values is the analysis of variance table of Appendix A. The SSE_f is that of the full model; the SSE_r is

the residual sum of squares obtained by fitting a 12 parameter model to the complete set of data points. This 12 parameter model is created by grouping the data from Forks-cold-frame and Goldendale-cold-frame together and from Forks-growth chamber and Goldendale-growth chamber together for each of the six "i" variables. The F test is the comparison of the residual sums of squares of the two models. The full model makes a distinction between seed sources, whereas the restrained model does not. If the residual sums of squares for the two models are not significantly different, the distinction between seed sources contributes nothing to the explanation of the variation in the data. If, as in this case, the F test is significant, the distinction between seed sources does explain a portion of the variation in the data. The effect of seed source being significant, the conclusion follows that the response of transpiration to plant and soil moisture stress is not the same for Forks and Goldendale seedlings. For each test of significance, the model was subject to the appropriate restraint after the fashion described above.

The recognition of seed source and preconditioning as factors in a factorial experiment as distinct from the variables (PMS, SMS) on which the transpirational response surfaces were based is essential to understanding the results of this experiment. When the effect of a variable is said to be significant, the meaning is that the change in transpiration rate in response to that variable is not the same for

the factor combinations being compared.

For example, it can be seen from the analysis of variance that the effect of soil moisture stress was not significant. This means simply that the transpirational responses of the seedlings of each factor combination were indistinguishable with regard to soil moisture stress. The significance of both the seed source and preconditioning effects are seen not to be explained in terms of soil moisture stress alone.

RESULTS AND DISCUSSION

Aside from the analysis of variance, the statistical analysis provided numerical values for the regression coefficients. Using these values in the model resulted in equations for the three dimensional surfaces that best conformed to the sets of data points used to calculate the coefficients. The solution of these equations defined the three-dimensional surfaces which were then plotted on isometric paper. Eight tri-variate graphs were plotted in this manner and are presented in Appendix B. A visual abstract of Appendix B is presented in Figure 4 so that comparisons among the transpirational responses can be made conveniently. Frequent referral to Figure 4 should be useful as the following discussion is based on the relationships shown there and found to be significant in the analysis of variance.

A preliminary analysis of variance revealed the effect of both seed source and preconditioning to be significant (Appendix A). The question was then asked, "How were the response surfaces different?" As a starting point, the homogeneity of the surfaces was tested with 18 degrees of freedom. Since the transpirational response surfaces for the various factor combinations were not the same, their 18 degrees of freedom were partitioned into six groups each with three degrees of freedom. The tests for the significance of each of these

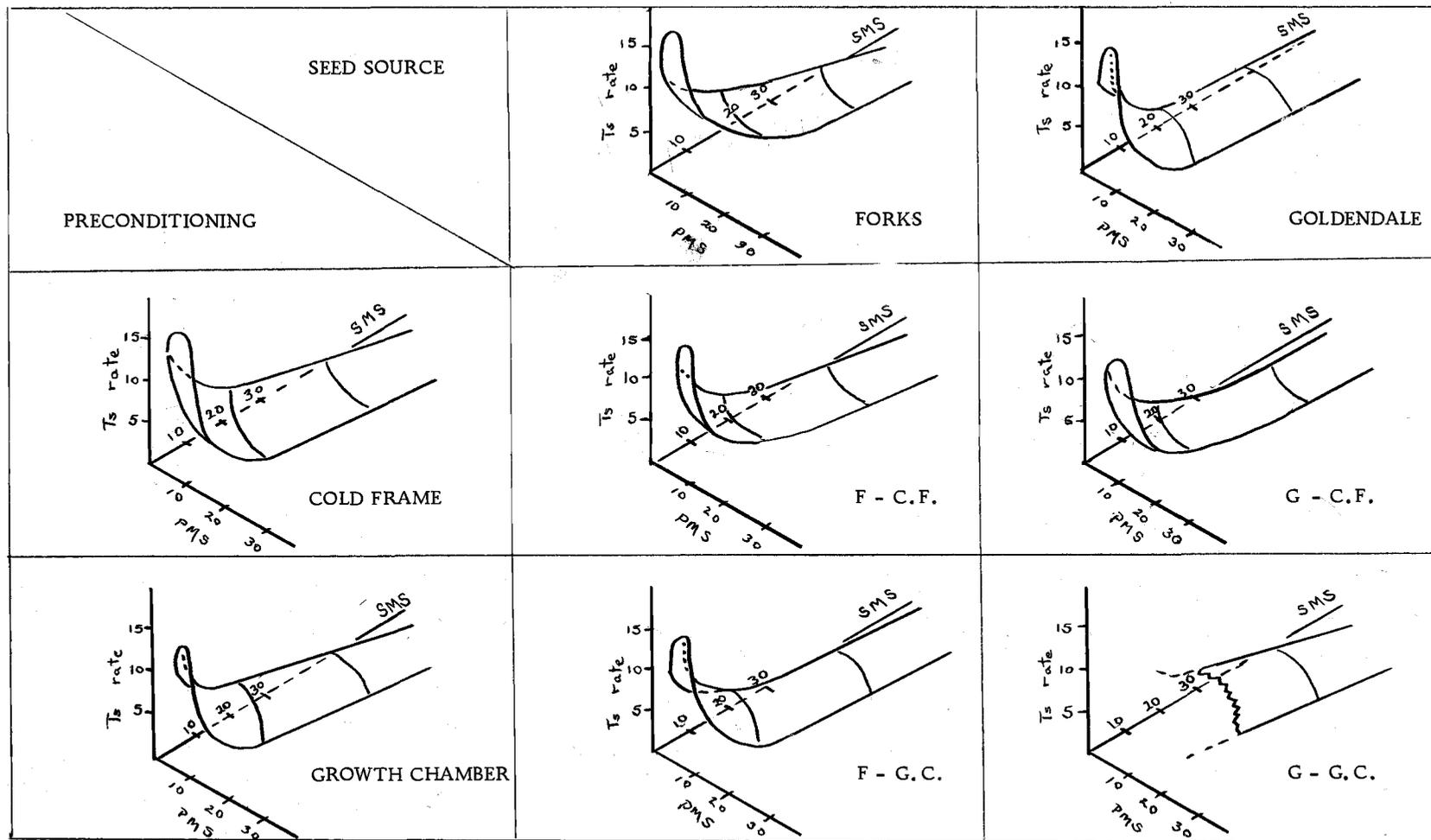


Figure 4. Diagrammatic presentation of the experimental design showing the response of transpiration rate to increasing soil moisture stress (SMS) and plant moisture stress (PMS) for each factor combination (Sketched from Appendix B).

groups disclosed no apparent differences among the surfaces with regard for their intercepts with the Y axis or their response to soil moisture stress (SMS). The differences among the surfaces were due to differences in response to plant moisture stress (PMS). Since the seedlings of the four factor combinations exhibited the same transpirational changes in response to changes in soil moisture stress and their response to changes in PMS was different, the correlation between SMS and PMS was different for the factor combinations. This shows up as a significant SMS-PMS interaction.

The description of the apparatus includes a brief discussion of the resistance offered to the loss of water across the boundary layer. The seedling itself also offers considerable resistance to the movement of water along the transpirational pathway. This total resistance has been analyzed as a composite of many resistances distinguishable as to their location within the plant or as to their physical or chemical characteristics. This concept is frequently expressed as a formula:

$$r_{\text{total}} = r_{\text{root}} + r_{\text{stem}} + r_{\text{leaf}} + r_a$$

where r is resistance to the movement of water in that part of the plant indicated, and r_a is the external boundary layer resistance. The resistance of the leaf (r_1) is often further partitioned into two parallel resistances, cuticular resistance (r_c) and stomatal

resistance (r_s), in the following manner:

$$\frac{1}{r_l} = \frac{1}{r_c} + \frac{1}{r_s}$$

The stomatal resistance is often thought of as the sum of three components: pore resistance (r_p), the mesophyll cell wall resistance (r_w), and the resistance afforded by the intercellular spaces (r_i). When the soil is near saturation and the plant moisture stress is very low, the total resistances to water loss may be considered equal to the sum of the diffusive resistances within the wet leaf (r_l) and the boundary layer resistance (r_a). From previous considerations, the magnitude of r_a was calculated to be about 0.55 sec cm^{-1} . In a similar manner, a representative magnitude for r_l can be calculated for the moist conditions described above.

$$(r_l + r_a) = \frac{e_1 - e_a}{E}$$

$$r_l = \frac{e_1 - e_a}{E} - r_a$$

$$r_l = \frac{9.5 \times 10^{-3} \text{ mg cm}^{-3}}{4.2 \times 10^{-3} \text{ mg cm}^{-2} \text{ sec}^{-1}} - 0.55 \text{ sec cm}^{-1}$$

$$r_l = 2.28 \text{ sec cm}^{-1} - 0.55 \text{ sec cm}^{-1}$$

$$r_l = 1.73 \text{ sec cm}^{-1}$$

e_1 = the saturation vapor pressure of the leaf at 23°C (20.6 mg l^{-1})

e_a = the ambient vapor pressure

E = expected transpiration rate

This study was not designed to directly explore quantitative changes in diffusive resistances, and this calculation is offered only for comparison with other values of r_1 reported in the literature. Slatyer (1967) lists some typical open stomata values of r_s : 0.5 sec cm^{-1} for wheat, 0.8 sec cm^{-1} for corn, 2.0 sec cm^{-1} for cotton and 4.0 sec cm^{-1} for tomato. As the stomata closes r_s presumably approaches infinity; it seems reasonable to conclude that the stomata of the seedlings used in this study were open during periods of low moisture stress.

When subject to high PMS, the stomata are probably closed, and continued transpiration is then cuticular. A value for r_c could be calculated; however, the value would be a reflection of the total resistances to transpiration when the seedlings are under high PMS, and as such would not represent a pure cuticular resistance.

The fact that transpiration rates of the various factor combinations did not differ from one another in response to soil moisture stress indicates that the total resistance at any particular SMS was the same for each of the groups of seedlings. The pronounced effect of PMS is probably understandable as the result of differences in the relative importance of the individual resistances along the transpiration pathway.

If this analysis of variance is indicative of the general case, if these relationships hold among species as well as within species, then the common practice of reporting transpiration as a function of soil moisture might be misleading. The availability of soil moisture is only one of several environmental factors that exert drought stresses on plants. Internal moisture stress appears as an integrated result of the environmental stresses and the inherent drought avoidance capacities of the plant.

It should be remembered that drought resistance is a result of the capacity to tolerate internal moisture stresses as well as to avoid them. Satoo (1956) for instance demonstrated that a drought-resistant species could tolerate higher internal stresses than less drought-resistant species. Differences in drought tolerance among ecotypes of Douglas-fir were shown by Pharis and Ferrell (1966), and the evidence indicates that greater PMS is tolerated by seedlings from xeric environments. If relative drought resistance among ecotypes or among species is to be evaluated using PMS as an indicator, some accounting should be made for the tolerance factor.

Since the linear response to PMS, the curvilinear response to PMS, and the interaction between PMS-SMS were significant, each of their three degrees of freedom was partitioned to three single degrees of freedom: One for the effect of seed source, one for the effect of preconditioning, and one for the effect of the interaction

between them. There were similarities between the effects of seed source and preconditioning; thus, as far as is possible duplication of the discussion of these will be avoided.

The Effect of Preconditioning

The effect of preconditioning was found to be significant at the 1% level. The cold frame seedlings had consistently greater transpiration rates than the growth chamber seedlings. This is consistent with the data for the same seed sources reported by Ruetz (1968) and by Geppert (1968). It is clear that for these ecotypes at least there is something inherent in the growth chamber environment that preconditions the subsequent transpirational response of Douglas-fir seedlings to drought conditions.

The shape of the response surfaces clearly illustrates the differences. The surface for cold frame seedlings is strongly concave, indicating a decreasing rate of change in transpiration rate along both axes (PMS, SMS). On the other hand, the response surface for growth chamber seedlings, while not differing with respect to soil moisture stress, shows an increasing rate of change in transpiration rate in response to PMS. These differences indicate that the mechanisms of control of transpiration is complex and that preconditioning affects the components of this mechanism differently.

The results for growth chamber seedlings is comparable to that

reported by Zavitkovski (1964) who worked with Douglas-fir seedlings grown in a growth chamber. His transpiration data is presented as a response to decreasing soil moisture percentages; however, it appears that what he was observing was the indirect effect of soil moisture stress influencing the transpiration rate through plant moisture stress.

Environmental influences on the anatomy of Douglas-fir seedlings were investigated by Tucker (1966). He compared several anatomical characteristics of two ecotypes under a number of growth chamber environments and included a control group from each ecotype grown outdoors. Those seedlings subject to low light intensities exhibited typically mesomorphic characteristics. They had only one layer of shortened palisade parenchyma, which resulted in a thinner needle. Their cuticle was thinner and could be stained with Sudan IV which is fat specific, but not with safranin which is cutin specific. The effect of the cuticle differences are generally considered to be negligible if the stomata are open. However, increasing PMS eventually exerts an influence on the turgidity of the stomata. At PMS above 20 atmospheres, the transpiration rates were generally less than 10% of the maximum. The continued decline in transpiration rate was exceedingly slow indicating that the stomata had closed and the resulting transpiration was by way of the cuticular pathway.

The development of a thinner cuticle under low light intensities

notwithstanding, the cuticular transpiration for growth chamber seedlings was apparently less than that for cold frame seedlings. This phenomenon might be explained by the apparently greater proportion of fats to cutin making up the cuticle.

Transpiration is profoundly affected by the stomata, and it seems reasonable to expect differences in this regard. Observations made with a dissecting microscope have revealed no differences in number and distribution of the stomata of the seedlings. Perhaps differences in stomatal behavior exist between the two groups of seedlings. Perhaps less obvious characteristics of the stomata are affected during their development by the relatively low humidity and low light intensity to which growth chamber seedlings are usually subject.

The Effect of Seed Source

The effect of seed source was found to be significant with respect to the response to plant moisture stress. There were differences both in the magnitude of the transpiration rate and the shape of the response surfaces. Seedlings from the more xeric seed source (Goldendale, Washington) exhibited an almost linear decrease of the transpiration rate along the PMS axis, while the seedlings from the mesic seed source had a more concave transpirational response surface. The greatest transpiration rates attained by the

Goldendale seedlings were between six and eight atmospheres, and were considerably greater than those reached by the Forks seedlings. However, the rate of change of the transpiration rate as PMS increased was much more rapid for the Goldendale seedlings than for the Forks seedlings. Between nine and ten atmospheres PMS their transpiration rates were at parity. At plant moisture stresses above ten atmospheres the Goldendale seedlings transpired at rates considerably below those of the Forks seedlings.

There is a marked similarity between the form of the transpirational response of the Forks seedlings and that of the cold frame seedlings, and likewise between the responses of the Goldendale and growth chamber seedlings. This suggests an overriding interaction between these two factors. However, the statistical analysis indicates that, while an interaction does exist, the main effects of both seed source and preconditioning are even more important.

Some of the differences in the transpirational response of the seedlings from the two seed sources might be understood in the light of previous studies. The thickness of the tracheal cell wall and the size of the lumen were found by Tucker (1966) to be inversely related, and to respond to definite ecotype-environment interactions. His work suggests that an ecotype from a more xeric environment may exhibit tracheal characteristics differing from those of an ecotype from a more maritime environment in that they would present less

resistance to transpirational movement up the stem. As long as adequate soil moisture was available, relatively high transpiration rates could be maintained without the rapid development of limiting plant moisture stresses. Once PMS exceeds a limiting magnitude, mesophyll resistances, possibly due to differences in anatomical structure, and more probably stomatal behavior, react to the internal moisture stress, rapidly reducing the transpiration rate of seedlings of xeric ecotypes below those of seedlings of mesic ecotypes. Tucker was unable to demonstrate a seed source effect on the anatomical structure of the mesophyll of the two seed sources of Douglas-fir that he studied. Although this does not preclude the possibility that there are differences in mesophyll resistance among seedlings of ecotypes in general, it seems that further investigation into the ecological mechanisms involved here might profitably focus on the stomatal response.

The moisture stress in the mesophyll has a definite and direct effect on the stomata aperture. There is some evidence that very low stresses may result in higher turgor pressures in the mesophyll cells relative to that in the guard cells causing their closure (Meidner, 1965; Slatyer, 1967). For example, Slatyer states the hypothesis that ". . . adjacent epidermal cells tend to exchange water more readily with both the vascular system and the atmosphere than the guard cells themselves." A release of tension in the xylem

elements may lead ". . . to a water flow into the adjacent cells, slightly increasing their turgor relative to that of the guard cells and causing partial stomatal closure." This hypothesis is offered to explain observed changes in stomatal aperture when a transpiring shoot is detached or placed in an osmotic solution. This effect is a brief one, and as such would not explain a sustained depression of the transpiration rate at low PMS. However, a similar argument might be evoked for that purpose. Stomatal aperture is sensitive to changes in the intercellular CO₂ concentration. Below a certain concentration the stomatal aperture widens, and above a certain level it is reduced. The particular CO₂ concentration is different among species and is affected by changes in internal moisture stress. The differences in transpiration rate of the seedlings from the two origins at low PMS may well involve differences in stomatal behavior.

At soil moisture stresses greater than 15 atmospheres, Zavitkovski (1964) reported greater transpiration rates for mesic source seedlings than for xeric source seedlings. If this can be explained as a response to high PMS then the higher transpiration rates of Forks seedlings at high PMS compared to those of Goldendale seedlings are consistent with his findings. This result must surely be a reflection of the relative cuticular resistances of the seedlings from the two seed sources.

In summing up, it is evident that seedlings from xeric sources

transpire more than mesic source seedlings when plant moisture stresses are moderate. As PMS becomes more critical, the transpiration rates of xeric source seedlings are reduced below those for mesic source seedlings. This effect is a distinct advantage for the survival of seedlings regularly subject to severe drought. It provides a mechanism for the avoidance of lethal internal moisture conditions to which mesic source seedlings would succumb (Pharis and Ferrell, 1966).

The Effect of Interaction Between Factors

It has been stated that when seedlings are grown in growth chambers under low light intensities they typically exhibit mesomorphic characteristics such as one layer of shortened palisade parenchyma and thin cuticle. One might suspect from this that the transpiration rates of growth chamber seedlings are typical of mesomorphic ecotypes of Douglas-fir. There is some difficulty on this point, however, since the response of these seedlings compares with that for the seedlings from the xeric seed source (Goldendale) and not with that for the mesic source seedlings (Forks). The reason for this is unclear. There was a seed source-preconditioning interaction that was significant at the 5% level. It appears that the Forks seedlings were affected more radically by the preconditioning treatments than were the Goldendale seedlings. However, the

relative importance of this interaction was small compared to the importance of the main effects. The evidence leads to the conclusion that the effects of both seed source and preconditioning were indeed real. The differences between ecotypes, being genetic in nature, would have developed under the selective pressure of the entire environmental complex, drought conditions being only one. One should appreciate the probable complexity of the causal relationships responsible for the interaction discussed here.

SUMMARY

The study objectives were to investigate the differences in the transpiration rates of seedlings of two ecotypes of Douglas-fir subject to a range of internal and external moisture conditions. The seedlings were grown in a growth chamber and in a cold frame from seed that was collected from a mesic environment (Forks, Washington) and from an xeric environment (Goldendale, Washington). They were well watered during the six week growing period. At various intervals during the sixth week, watering was stopped allowing various degrees of soil moisture stress to develop. The transpiration rates were measured using a cuvette-humidity sensor technique. Plant moisture stress was measured with a pressure bomb, and the soil moisture stress was estimated from soil moisture percentage. These three variables formed the basis for the generation of tri-variate response surfaces for each of the seed sources-preconditioning combinations.

A study of the analysis of variance and comparisons of the response surfaces revealed no significant differences among the factor combinations in response to soil moisture stress. That is to say that increasing soil moisture stress affected the transpirational behavior of the seedlings of each factor level in the same way and to the same degree. The differences in transpirational response to

plant moisture stress was highly significant. These differences are shown diagrammatically in Figure 5. The cold-frame seedlings had consistently higher transpiration rates than the growth chamber seedlings. Goldendale seedlings transpired at a greater rate at moderate plant moisture stresses, but the rate decreased below that of Forks seedlings as plant moisture stress increased above ten atmospheres. This effect was seen as a decided ecological advantage for Goldendale seedlings which are as an ecotype regularly subject to drought conditions.

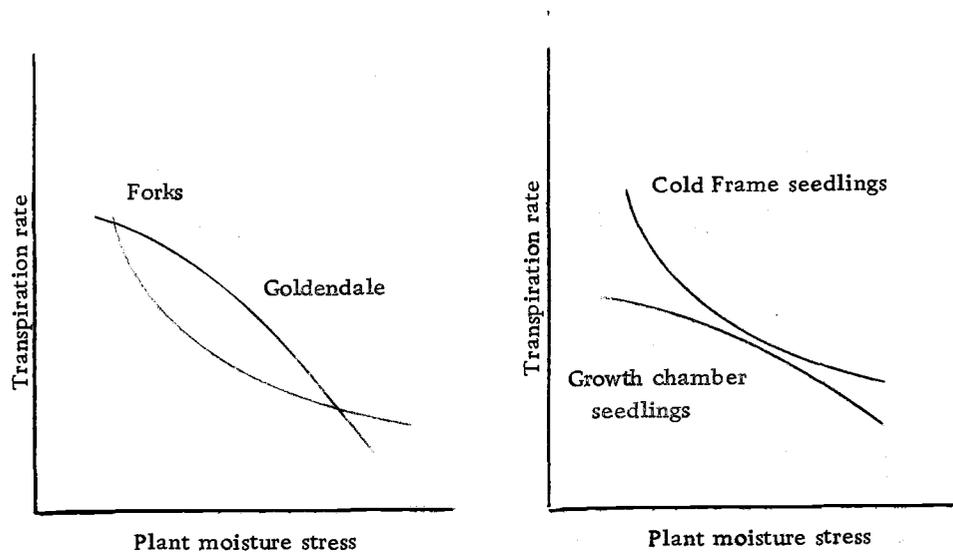


Figure 5. Schematic representation of the factor effect on the transpirational response to plant moisture stress.

It was felt that the lack of effect of soil moisture stress in causing differences in transpiration rate among the groups of seedlings indicated that the total resistance to water loss of the seedlings

of each factor group was equal. The significant effect of plant moisture stress was considered to be indicative of differences in the relative importance of the individual resistances that make up the total resistance to transpiration. The transpiration rate is determined by a complex of influences that can result in quite different transpirational behavior among ecotypes. Since preconditioning also affects the transpirational behavior in a profound way, the interpretation of experimental results and their extrapolation to natural phenomenon should be attempted with the greatest of caution.

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APPENDICES

APPENDIX A

ANALYSIS OF VARIANCE TABLE

An investigation of the variation of the surface defined by the basic model (page 19) on the four treatment combinations.

SOURCE OF VARIATION	df	SS	MS	F
Preliminary Analysis				
Seed source	12	0.90734	0.07561	2.11*
Preconditioning	12	1.13929	0.09494	2.65**
Full Model (SSR)	24	77.57903	3.23246	
Detailed Analysis				
Due to fitting model	6	73.53824	12.25637	
Homogeneity of surfaces	18	3.94079	0.21893	6.11**
Equality of intercepts	3	0.08256	0.02752	0.77
PMS	3	0.34122	0.11374	3.18*
Seed source	1	0.03833		1.07
Preconditioning	1	0.11973		3.35
Interaction	1	0.07234		2.02
SMS	3	0.14794	0.04931	1.38
PMS ²	3	2.28367	0.76122	21.26**
Seed source	1	0.31481		8.80**
Preconditioning	1	0.29212		8.17**
Interaction	1	0.25255		7.05*
SMS ²	3	0.09520	0.03173	0.89
SMS x pMS	3	0.35893	0.11964	3.34*
Seed source	1	0.03977		1.07
Preconditioning	1	0.18113		5.06*
Interaction	1	0.16506		4.61*
Residual (SSE)	70	2.50686	0.03581	
Total (SST)	94	80.08589		

*Significant at 5%

**Significant at 1%

The multiple correlation coefficient (R^2) for the full model was 96.86.

ORIGIN OF THE SUMS OF SQUARES LISTED IN THE
ANALYSIS OF VARIANCE

Source of Variation	df	Hypothesis Tested or Calculation of Sums of Squares
Seed source	12	The parameters for Forks - cold frame = those for Goldendale - cold frame, and the parameters for Forks - growth chamber = those for Goldendale - growth chamber. $\beta_{i1} = \beta_{i2} \quad \text{and} \quad \beta_{i3} = \beta_{i4}^*$
Preconditioning	12	The parameters for Forks - cold frame = those for Forks - growth chamber, and the parameters for Goldendale - cold frame = those for Goldendale - growth chamber. $\beta_{i1} = \beta_{i3} \quad \text{and} \quad \beta_{i2} = \beta_{i4}^*$
Full model regression SS	24	SSR = SST - SSE
Due to fitting	6	SS _{fitting} = SST - (SSE + SS _{homogeneity of surfaces})
Homogeneity of surfaces	18	The parameters for the Forks - cold frame = those for Forks - growth chamber = those for Goldendale - cold frame - those for Goldendale - growth chamber. $\beta_{i1} = \beta_{i2} = \beta_{i3} = \beta_{i4}^*$
Equality of intercepts	3	The parameters for the main effects of the factors (seed source and preconditioning) are equal. $\beta_{01} = \beta_{02} = \beta_{03} = \beta_{04}^*$
PMS	3	The parameters for the interaction between PMS and each of the four factor combinations are equal. $\beta_{11} = \beta_{12} = \beta_{13} = \beta_{14}^*$
SMS	3	The parameters for the interaction between SMS and each of the four factor combinations are equal. $\beta_{21} = \beta_{22} = \beta_{23} = \beta_{24}^*$
PMS ²	3	The parameters for the interaction between PMS ² and each of the four factor combinations are equal. $\beta_{31} = \beta_{32} = \beta_{33} = \beta_{34}^*$
SMS ²	3	The parameters for the interaction between SMS ² and each of the four factor combinations are equal. $\beta_{41} = \beta_{42} = \beta_{43} = \beta_{44}^*$
SMS x PMS	3	The parameters for the interaction between SMS x PMS and each of the four factor combinations are equal. $\beta_{51} = \beta_{52} = \beta_{53} = \beta_{54}^*$

Source of Variation	df	Hypothesis Tested or Calculation of Sums of Squares
Residual (SSE)	70	Residual sums of squares for the full model of 24 parameters.
Total (SST)	94	Total sums of squares for the full model of 24 parameters (n = 94).

*Refer to page 19 for explanation of the model.

The partitioning of the sums of squares for PMS, PMS^2 , and (PMS)(SMS), each with three degrees of freedom, was done differently. For example, the sums of squares for PMS, with three degrees of freedom, was partitioned into three sums of squares each with one degree of freedom. A single degree of freedom sum of squares was obtained for the effect of PMS on each of the two factors (seed source and preconditioning) and for the interaction between them. To accomplish this, a 24 parameter model was constructed. Only the four parameters for the effect of PMS are shown below.

$$y = \dots + \beta_0 + \beta_1 + \beta_2 + \beta_{12} + \dots$$

where:

y = the predicted transpiration rate

β_0 = the effect of PMS common to the factor combinations

β_1 = the effect of PMS on seed source

β_2 = the effect of PMS on preconditioning

β_{12} = the effect of PMS on the factor interaction.

The single degree of freedom sum on squares for the effect of PMS on the interaction was obtained by deleting the interaction parameter

before fitting the model to the data points. The sum of squares for the effect of PMS on seed source was obtained by deleting both the seed source parameter and the interaction parameter before fitting the model, and subtracting the sum of squares for the interaction. The sum of squares for the effect of PMS on preconditioning was calculated similarly.

APPENDIX B

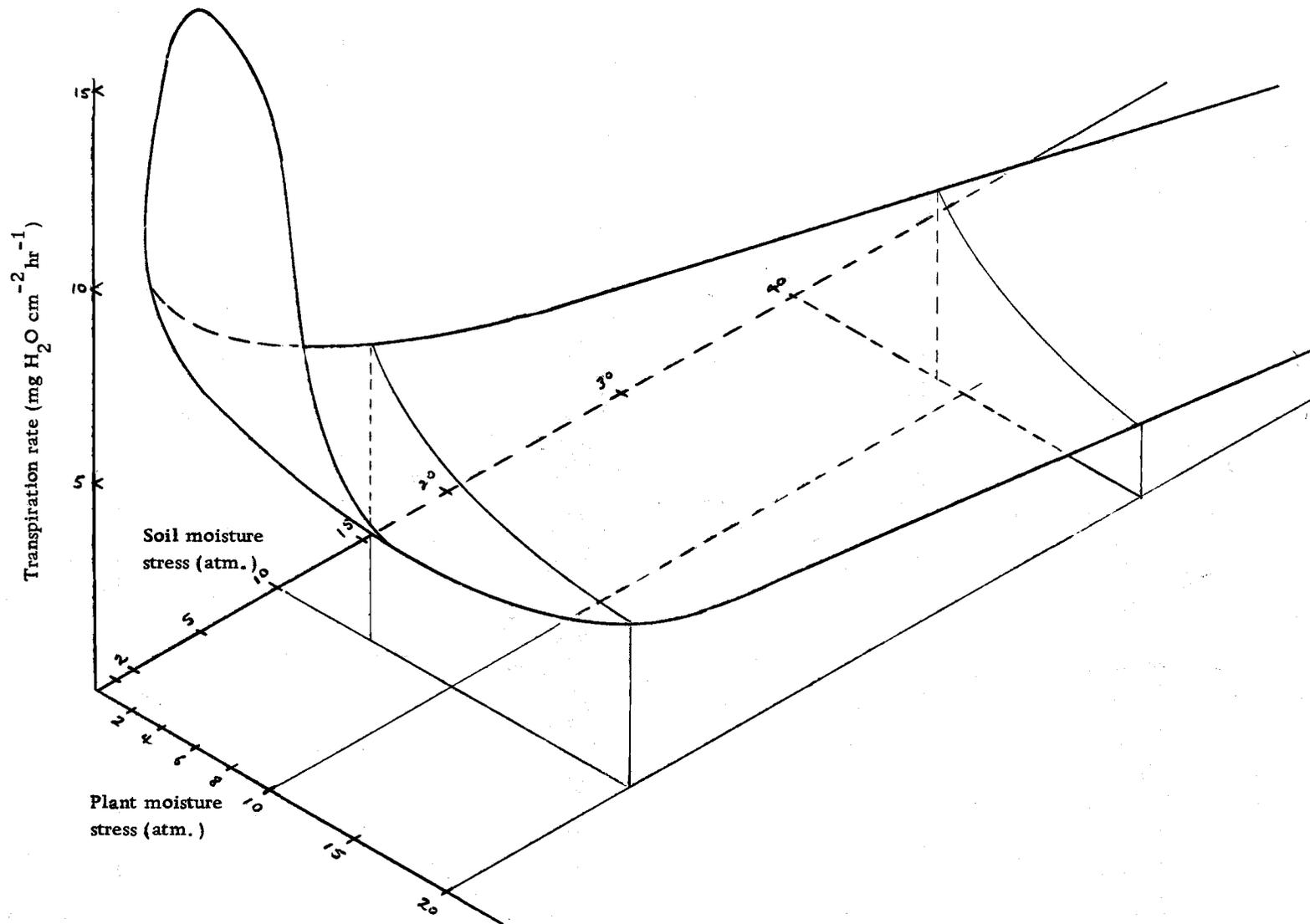


Figure 6. FORKS: Transpirational response of the mesic seed source seedlings to plant and soil moisture stress.

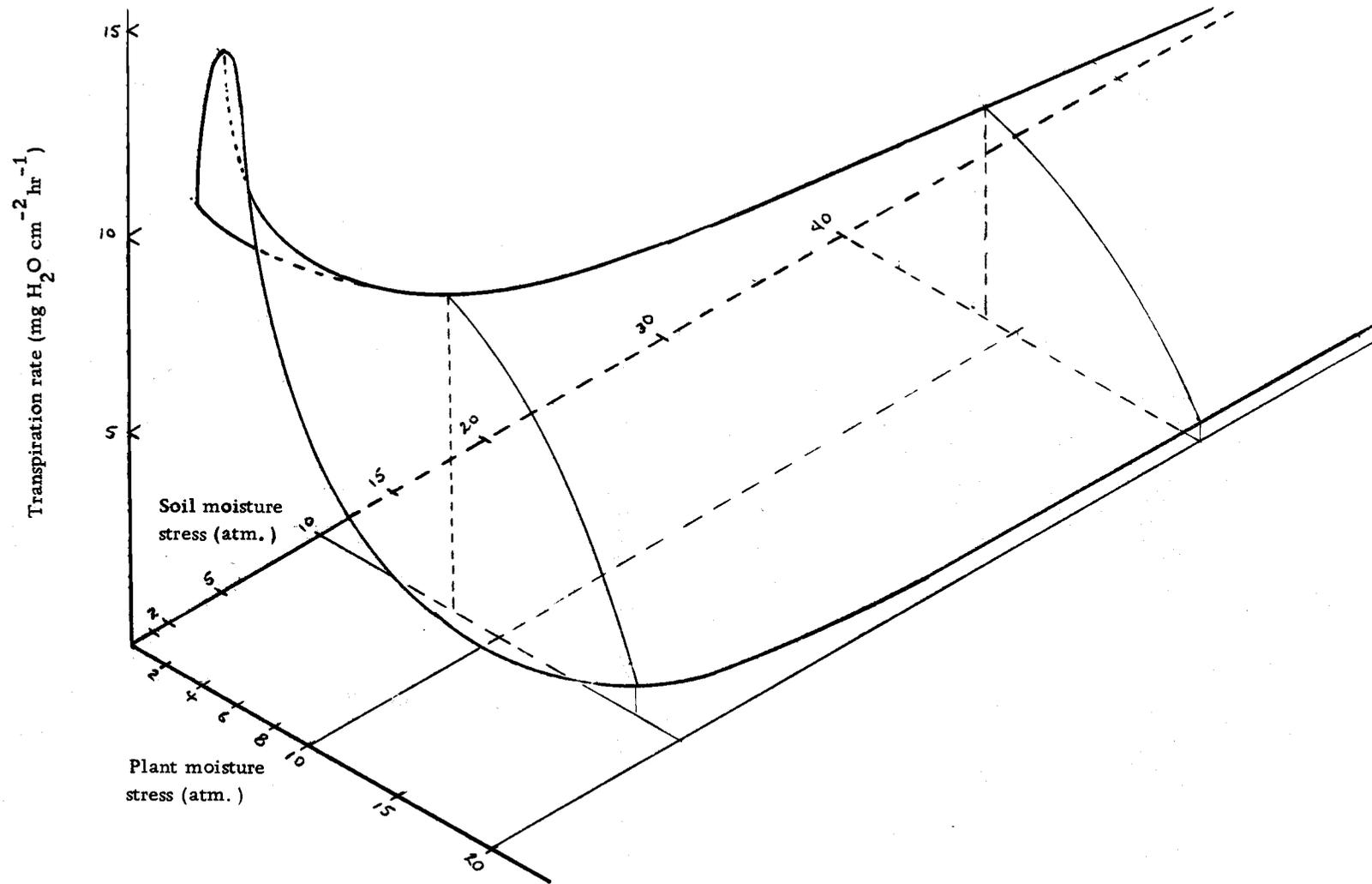


Figure 7. GOLDENDALE: Transpirational response of the xeric seed source seedlings to plant and soil moisture stress.

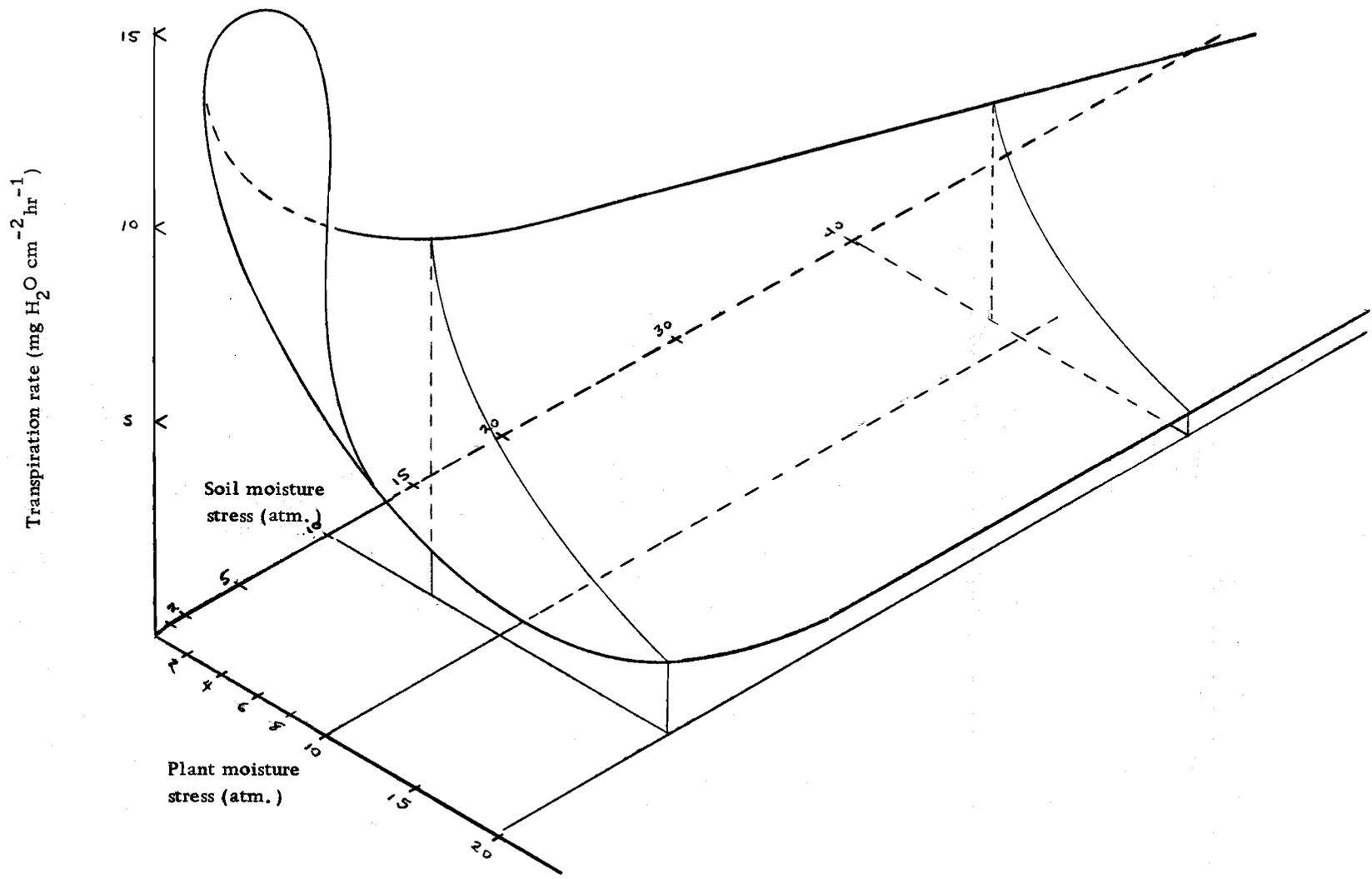


Figure 8. COLD FRAME: Transpirational response of the cold frame seedlings to plant and soil moisture stress.

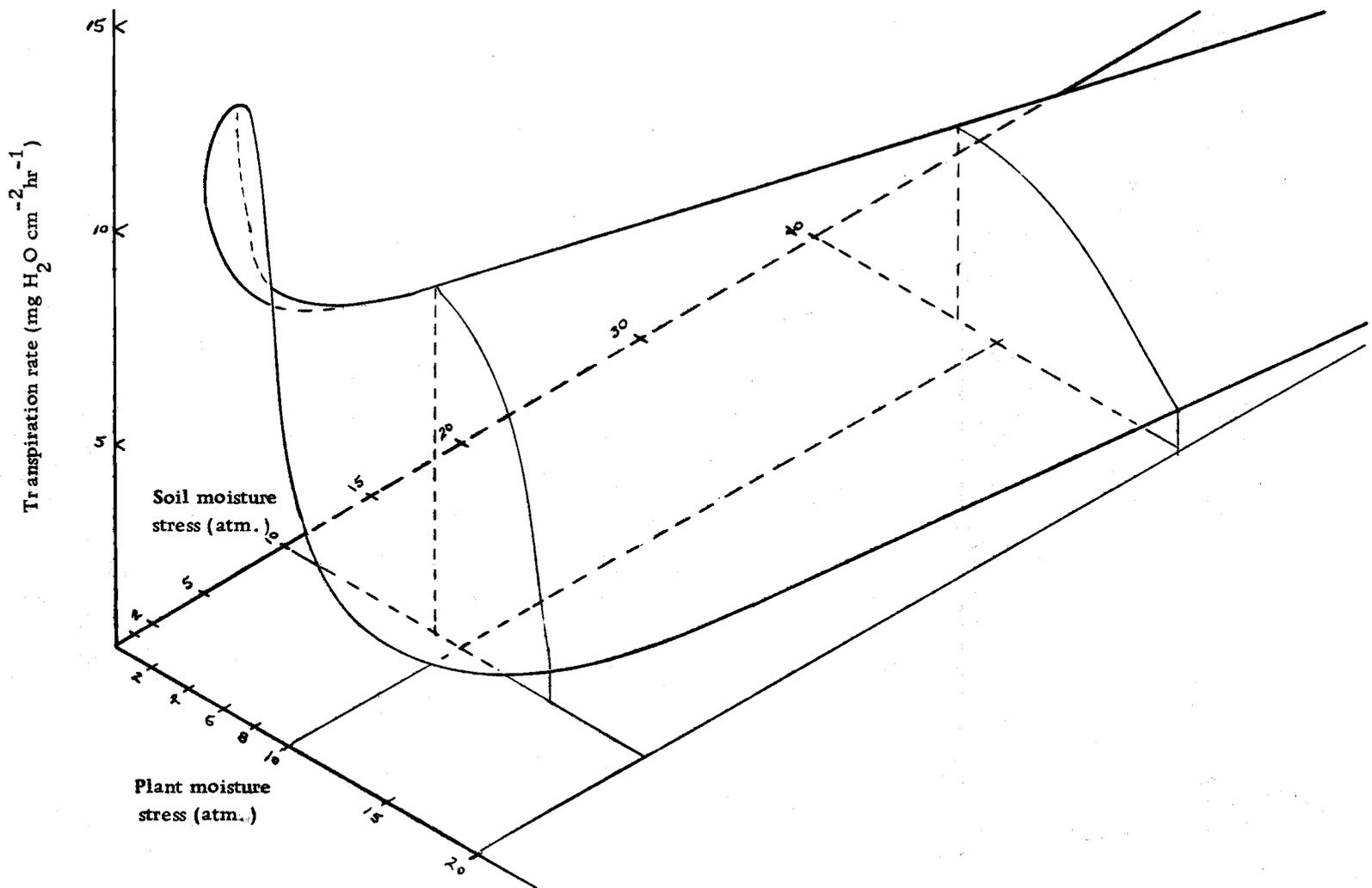


Figure 9. GROWTH CHAMBER: Transpirational response of the growth chamber seedlings to plant and soil moisture stress.

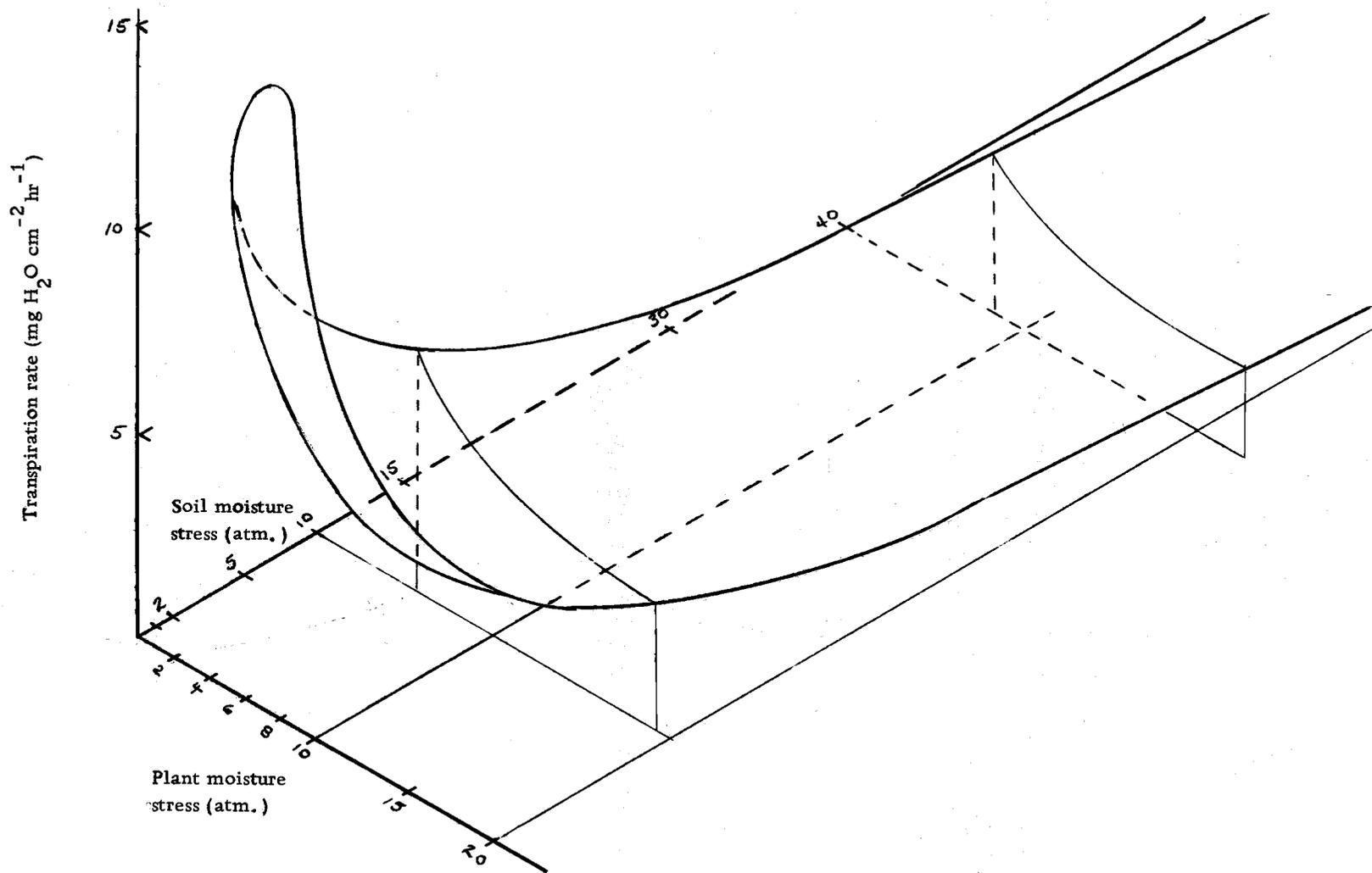


Figure 10. FORKS-COLD FRAME: Transpirational response of the seedlings from the mesic seed source grown in the cold frame, to plant and soil moisture stress.

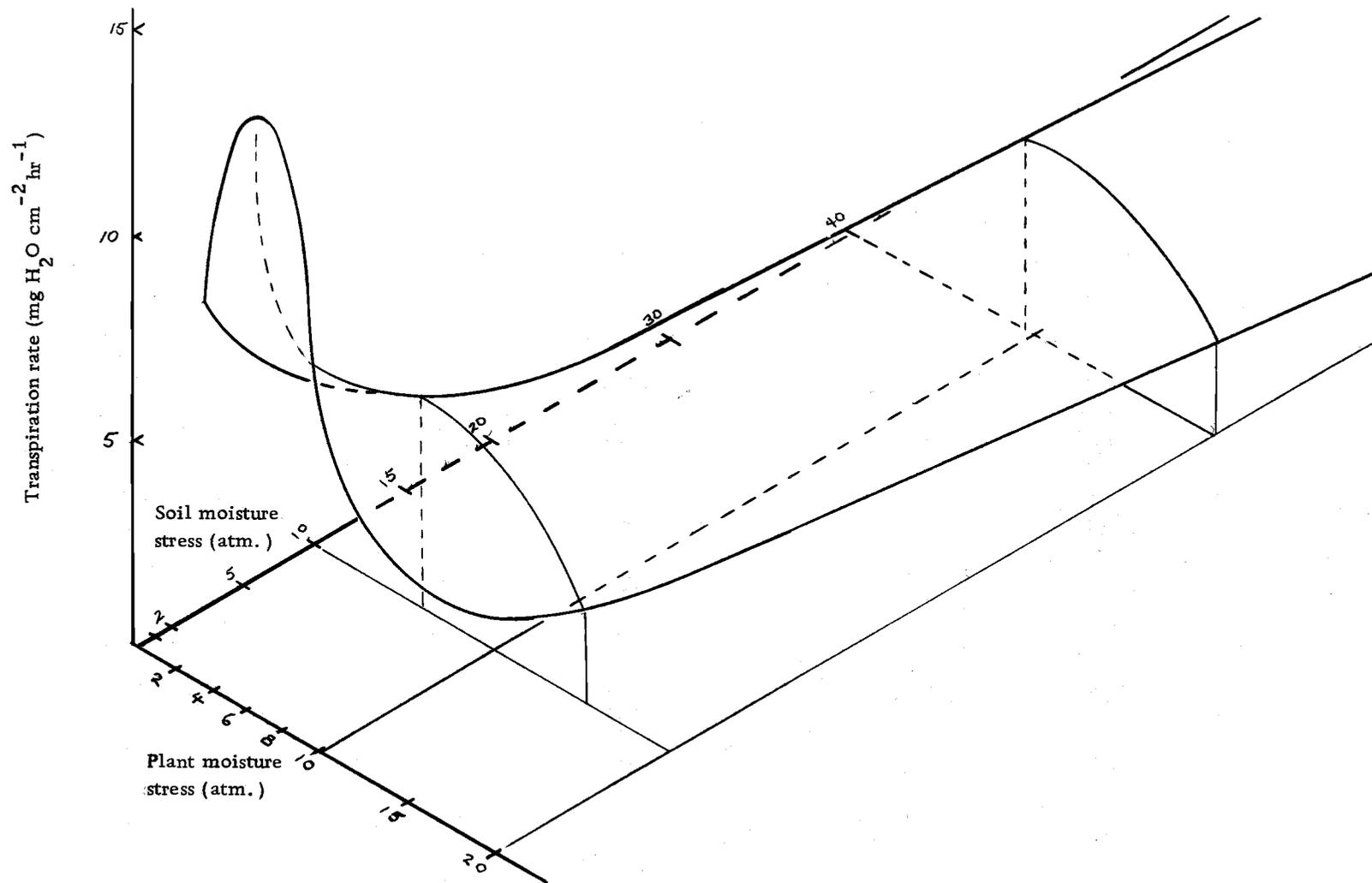


Figure 11. FORKS-GROWTH CHAMBER: Transpirational response of the seedlings from the mesic seed source grown in the growth chamber to plant and soil moisture stress.

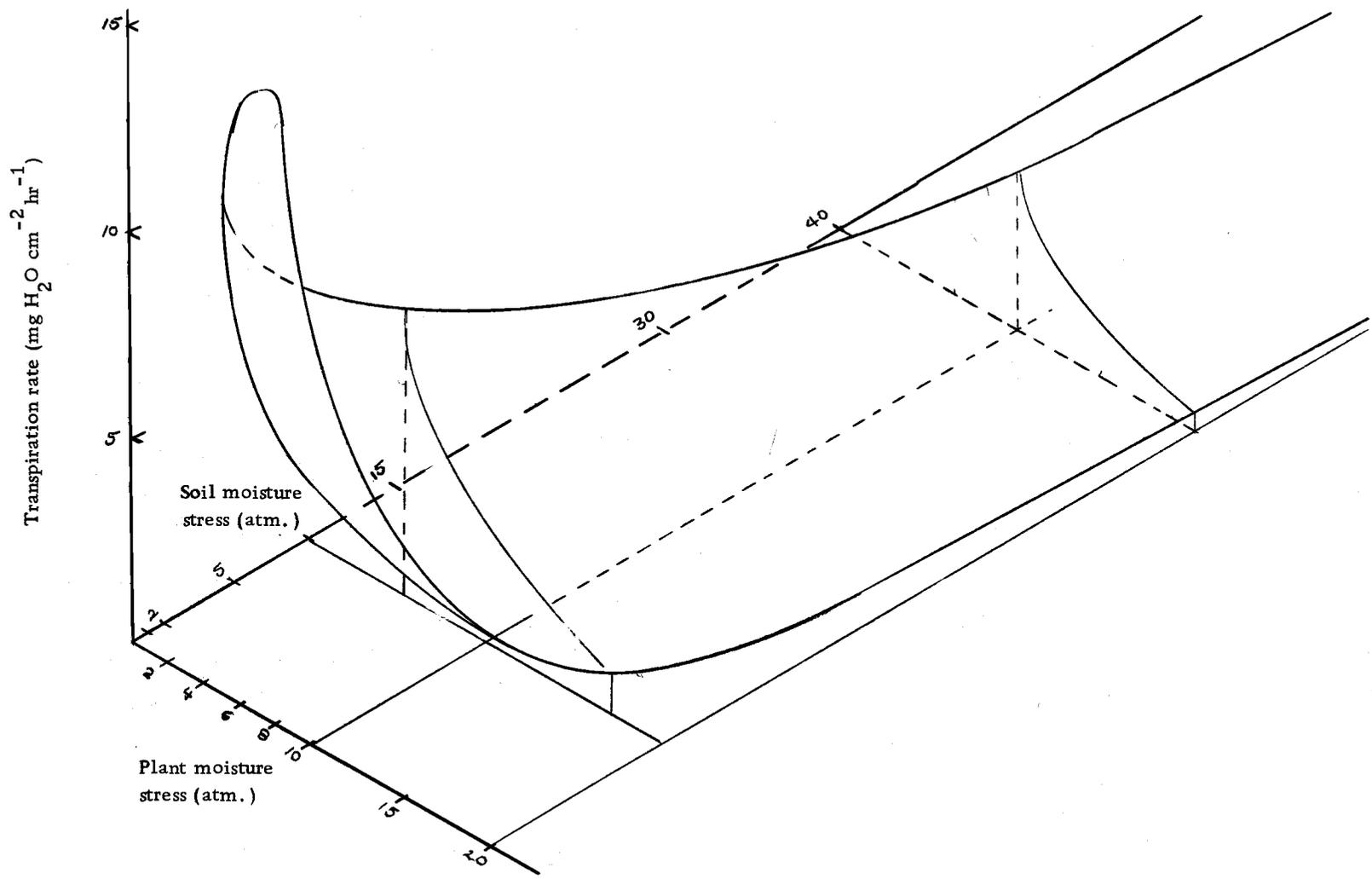


Figure 12. GOLDENDALE-COLDFRAME: Transpirational response of the seedlings from the xeric seed source grown in the cold frame to plant and soil moisture stress.

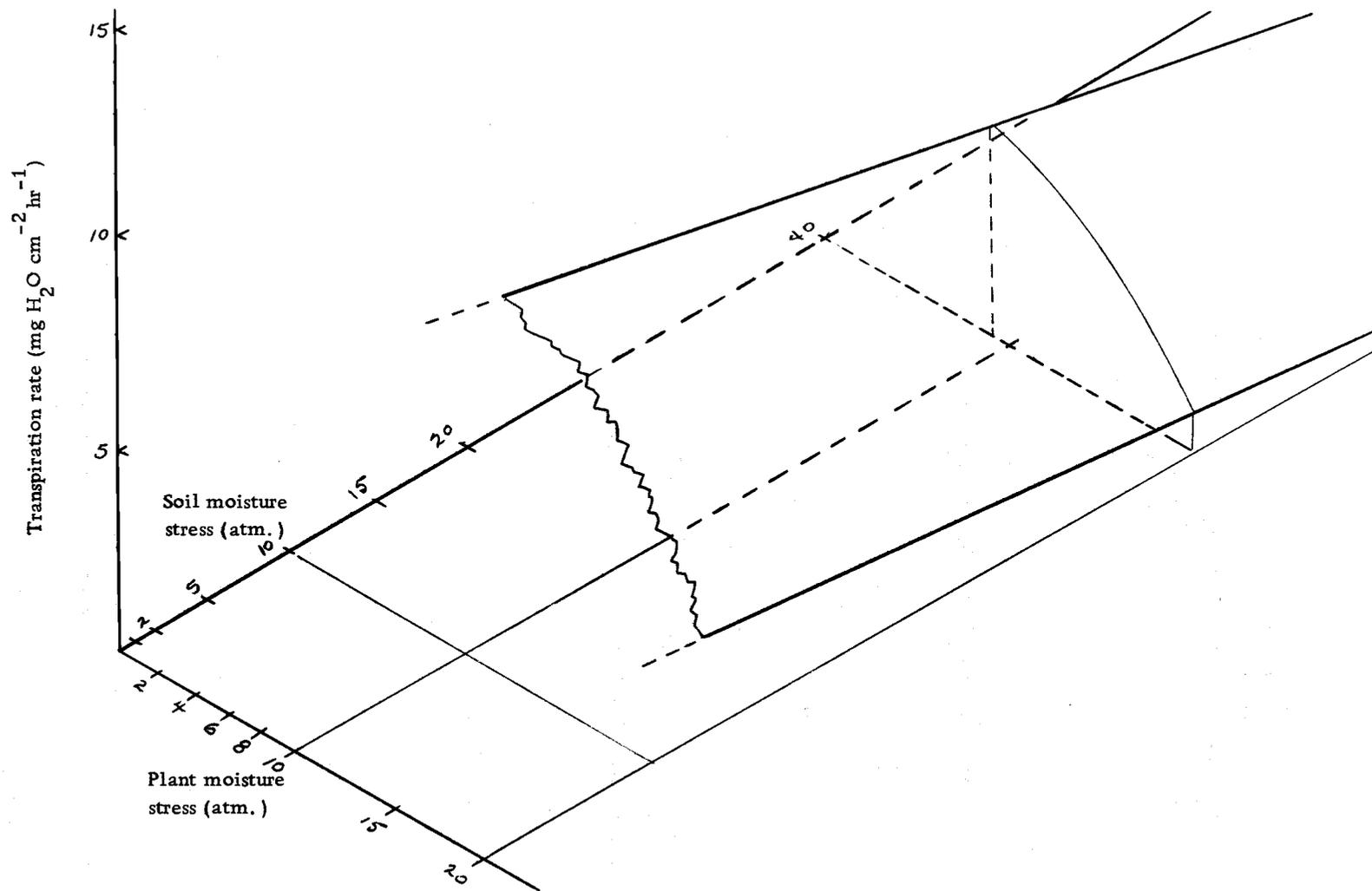


Figure 13. GOLDENDALE-GROWTH CHAMBER: Transpirational response of the seedlings from the xeric seed source grown in the growth chamber to plant and soil moisture stress.

APPENDIX C

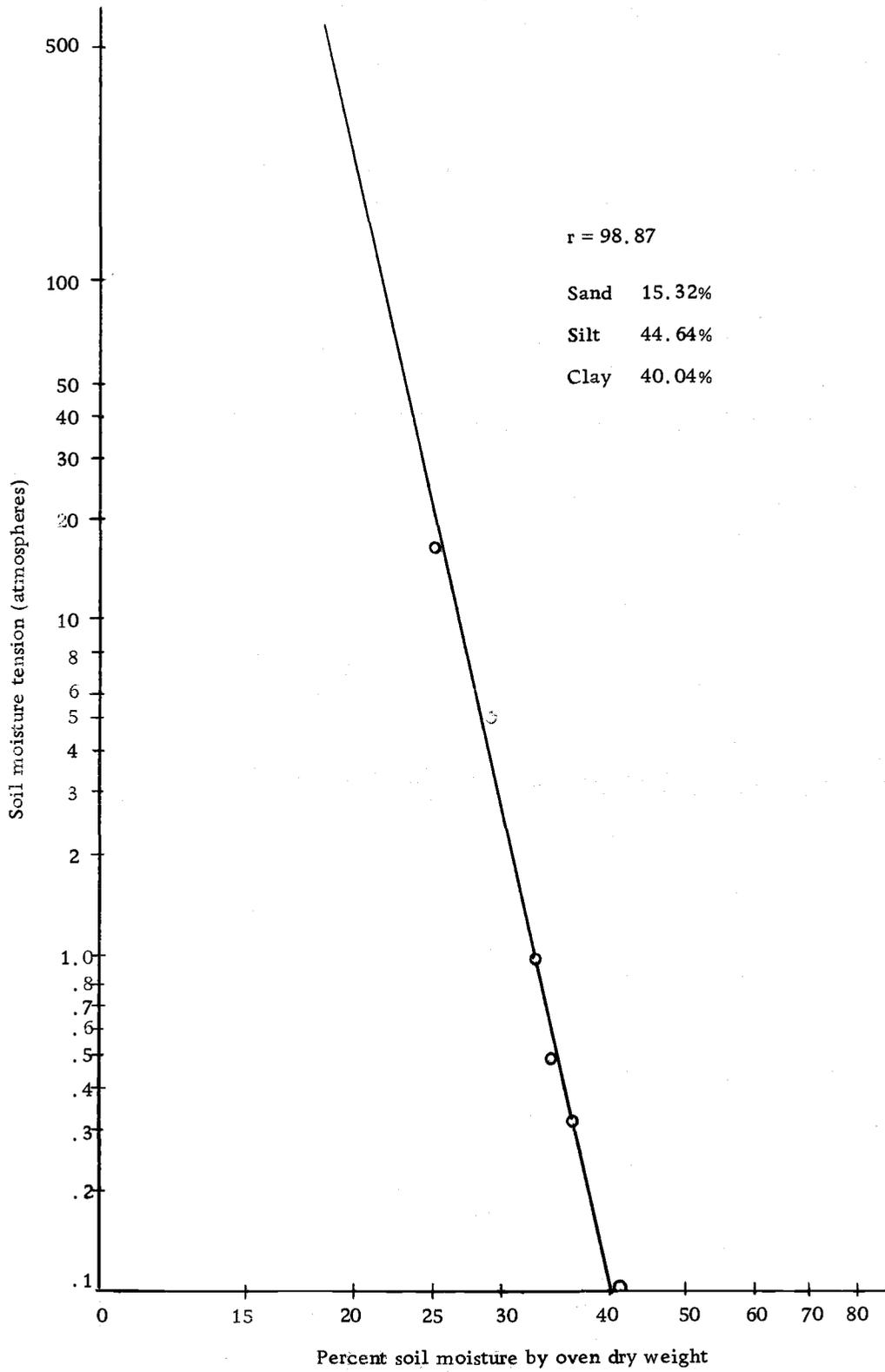


Figure 14. Soil moisture tension curve obtained by pressure plate technique.

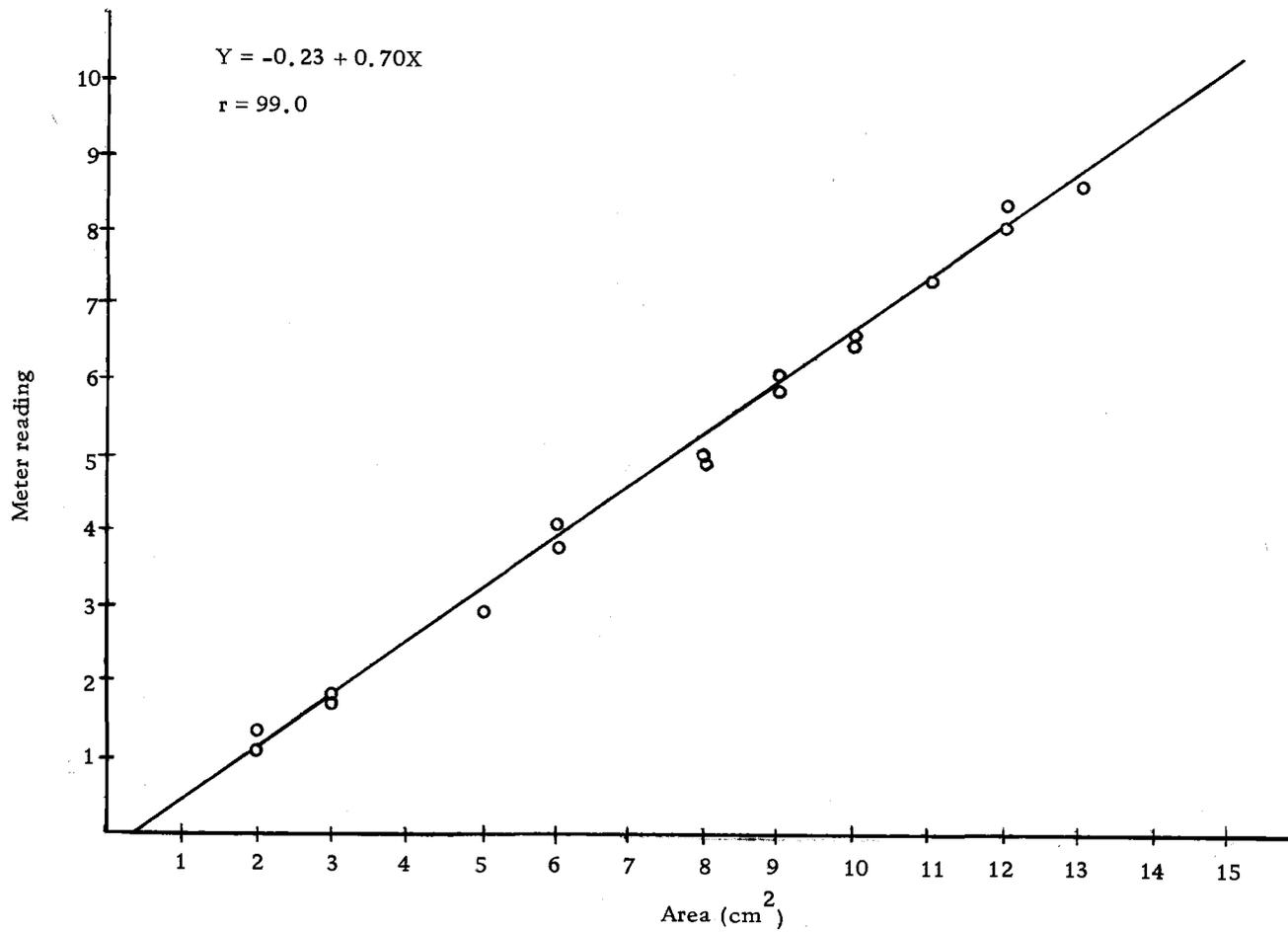


Figure 15. Calibration curve for the optical planimeter.

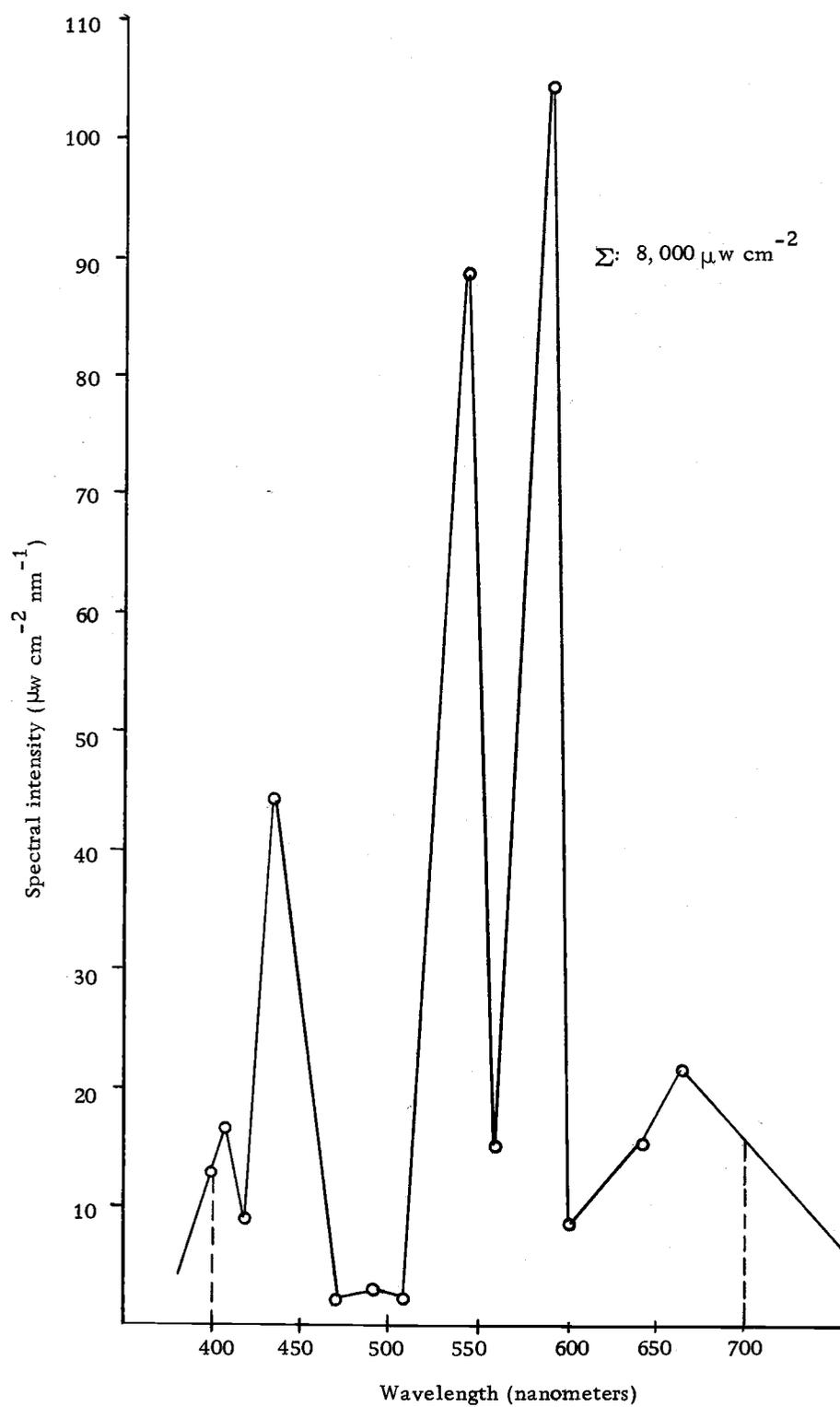


Figure 16. The distribution of spectral energy within the cuvette.

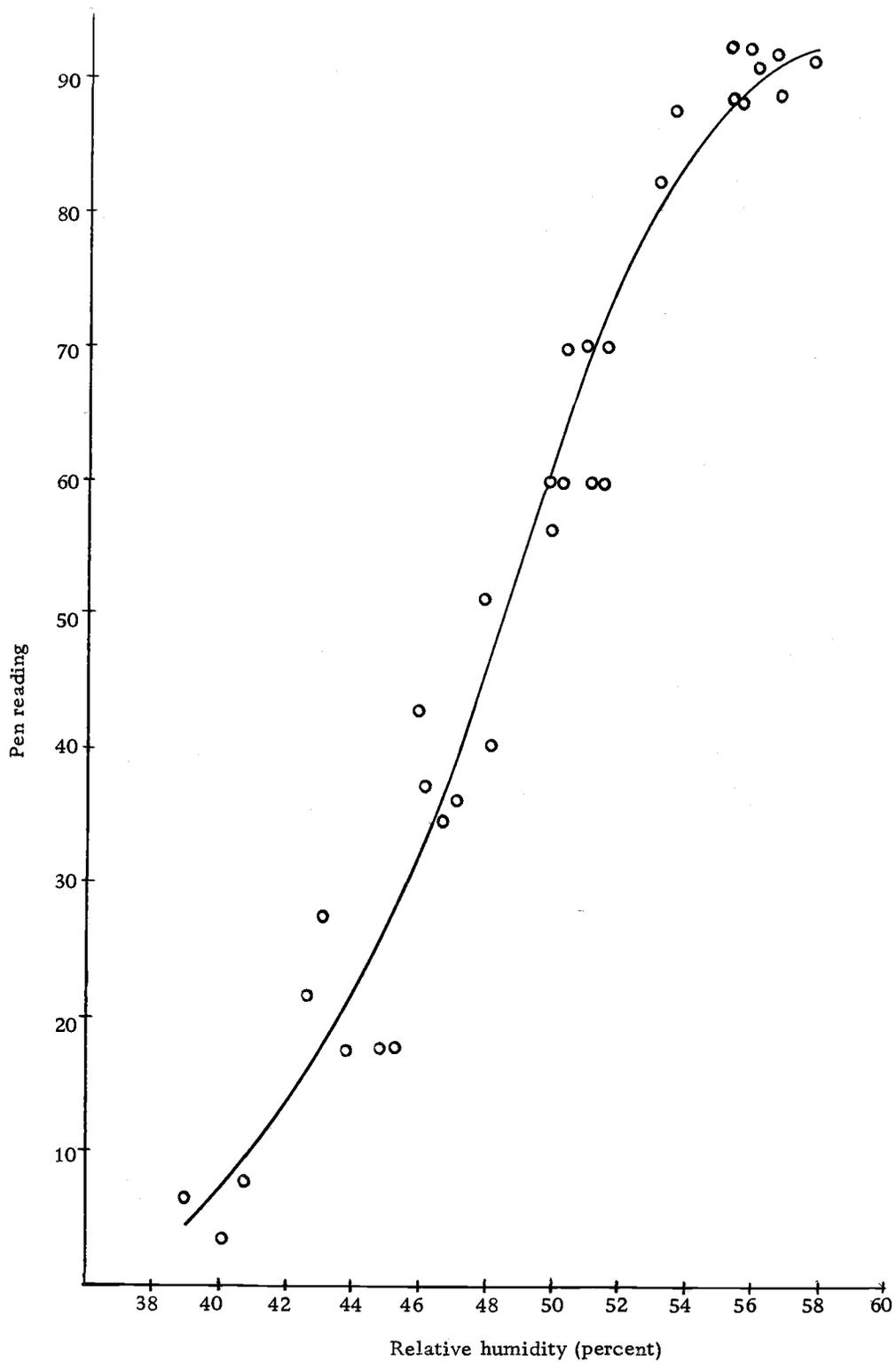


Figure 17. Calibration curve for Taylor Dual-pen Recorder (Green element).