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Title: EFFECTS OF LIGHT PRECONDITIONING ON THE SEASONAL
WATER RELATIONS OF NURSERY-GROWN DOUGLAS-FIR

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Douglas-fir seedlings of a single seed source were grown in a nursery under three levels of light intensity: 9, 44, and 100 percent of full light. Growth over an entire growing season was monitored in terms of proportionate distribution of dry matter to shoot and root systems. This was replicated over two years, 1971 and 1972, with the subsequent effects of prior light treatment upon growth and phenology noted as well as current effects of first-year growth upon seedling water relations.

Water relations analysis was based on the model, $\text{Plant Moisture Stress} = f(\text{Soil Moisture Stress}, \text{Leaf Conductance})$ and data subdivided as to summer, fall, and winter according to differing physiological states of the seedlings. By means of analysis of variance applied to regression models, effects of season of the year and light preconditioning upon water relations were demonstrated.

These were attributed to differing influences of soil moisture stress and leaf conductance upon plant moisture stress.

Water relations analysis indicated that plant moisture stress of seedlings during active shoot growth is lower than after growth has ceased. Decreased water loss in the winter associated with low air temperatures was due to closure of stomata partially independent of moisture stress in the plant. Plant moisture stress was lower in seedlings during the winter than it was during fall at equivalent levels of soil moisture stress.

Low light-preconditioned seedlings possess limited ability to avoid internal desiccation, probably due to a poorly-developed root system relative to their shoot. They are also more subject to spring frost damage. Indications are that low light preconditioning of seedlings' growth the first year may have carry-over effect upon water relations behavior a year later.

Light preconditioning has practical implications for forest nursery management through regulation of dormancy and morphology.

Effects of Light Preconditioning on the
Seasonal Water Relations of
Nursery-grown Douglas-fir

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TABLE OF CONTENTS

	<u>Page</u>
INTRODUCTION	1
LITERATURE REVIEW	6
Acclimation of Physiological Processes	6
Temperature Effects	6
Light Effects	8
Moisture Effects	9
Growth and Morphological Acclimation	12
Temperature Effects	12
Light Effects	13
Moisture Effects	15
MATERIALS AND METHODS	17
Plant and Soil Materials	17
Techniques and Design of Experiment	17
Instrumentation	24
Calculations and Methods of Analysis	35
Growth Analysis	35
Water Relations Analysis	36
RESULTS	41
Climate	41
Seedling Growth	41
Gross Morphology	41
Allometric Analysis	44
Phenology	49
Seedling Water Relations	53
First-year Behavior	53
Second-year Behavior	75
DISCUSSION	82
Growth Analysis	82
Phenology	87
Water Relations	91
Summer and Fall Behavior	91
Winter Behavior	94
Practical Implications	99

	<u>Page</u>
SUMMARY AND CONCLUSIONS	102
BIBLIOGRAPHY	106
APPENDIX	114

LIST OF TABLES

<u>Table</u>		<u>Page</u>
1	Effect of light treatment on seedling dry weight in milligrams measured at seven months for 1971 and 1972.	43
2	Variation in slope, k , of the allometric equation, $\log Y = a + k \cdot \log X$ with level of light preconditioning and year for different combinations of organs in Douglas-fir.	47
3	Sample sizes for water relations analysis.	54

LIST OF FIGURES

<u>Figure</u>		<u>Page</u>
1	Controlled environment apparatus for measurement of transpiration.	25
2	Mean minimum and maximum monthly air temperature at Hyslop Farm, Corvallis, Oregon for the period April to December of 1971 and 1972.	42
3	Decline in shoot/root ratio with size of plant for 100 percent and 9 percent light-grown seedlings.	46
4	Timing of 1972 bud break on seedlings preconditioned to varying light regimes during 1971 and showing amount of 1972 frost damage.	51
5	Timing of 1973 bud break on seedlings preconditioned to varying light regimes during 1971.	52
6	Trivariate response surface representing summer behavior of Douglas-fir seedlings based on the relationship, $PMS = f(SMS, L_c)$.	56
7	Trivariate response surface representing fall behavior of Douglas-fir seedlings based on the relationship, $PMS = f(SMS, L_c)$.	58
8	Leaf conductance as a function of plant moisture stress of Douglas-fir seedlings grown under three light treatments measured during fall and summer.	59
9	Variation in plant moisture stress with time of day on July 30, 1972 for Douglas-fir seedlings growing under three light regimes.	62
10	Variation in plant moisture stress with time of day on August 13, 1972 for Douglas-fir seedlings growing under three light regimes.	63

<u>Figure</u>		<u>Page</u>
11	Variation in leaf conductance with time of day on August 13, 1972 for Douglas-fir seedlings growing under three light regimes.	64
12	The fall response in plant moisture stress as a function of soil moisture stress for six-month-old Douglas-fir seedlings grown under 100 percent and 9 percent light.	67
13	The fall response in leaf conductance as a function of plant moisture stress for six-month-old Douglas-fir seedlings grown under 100 percent and 9 percent light.	68
14	The decline in transpiration rate of Douglas-fir seedlings with low air temperature during the last four months of 1972.	70
15	Fall and winter response of leaf conductance as a function of plant moisture stress for 9 percent light-grown Douglas-fir seedlings.	71
16	Fall and winter response of plant moisture stress as a function of soil moisture stress for 100 percent light-grown Douglas-fir seedlings.	72
17	Winter response of plant moisture stress as a function of soil moisture stress for 100 percent and 9 percent light-grown Douglas-fir seedlings at an age of eight months.	74
18	Winter response of plant moisture stress as a function of soil moisture stress for 44 percent light-grown Douglas-fir seedlings.	76
19	The winter response of leaf conductance as a function of plant moisture stress for 100 percent and 9 percent light-grown Douglas-fir seedlings at an age of eight months.	77

<u>Figure</u>		<u>Page</u>
20	The fall and winter response of plant moisture stress as a function of soil moisture stress for two-year-old Douglas-fir seedlings preconditioned to 100 percent light their first and second growing seasons.	79
21	The fall and winter response of plant moisture stress as a function of soil moisture stress for two-year-old Douglas-fir seedlings preconditioned to 9 percent light their first growing season and full light the second.	80
22	The fall response of leaf conductance as a function of plant moisture stress for two-year-old Douglas-fir seedlings preconditioned to 100 percent and 9 percent light their first season and 100 percent light the second season.	81

LIST OF APPENDIX TABLES

<u>Table</u>		<u>Page</u>
A	Analysis of variance of summer and fall water relations of Douglas-fir seedlings.	119
B	Analysis of variance of fall water relations of low and high light-grown Douglas-fir seedlings.	120
C	Parameters of the allometric equation $\log Y = a + k \cdot \log X$ based on analysis of growth under different light preconditioning treatments.	121

LIST OF APPENDIX FIGURES

<u>Figure</u>		<u>Page</u>
A	Spectral distribution of light intensity at seedling level in the cuvette.	116
B	Calibration curve for LiCl Humidity Sensor, showing dates of calibration.	117
C	Soil moisture desorption curve for Jory soil.	118

EFFECTS OF LIGHT PRECONDITIONING ON THE SEASONAL WATER RELATIONS OF NURSERY GROWN DOUGLAS-FIR

INTRODUCTION

The growth of an organism observed at any one point in ontogeny has traditionally been considered as the result of two interacting forces, heredity and environment. Heredity determines the range of potential response of an individual to a complex of environmental factors, and environment determines where, within that range or ecologic amplitude, the response lies. The phenotypic response depends upon interaction of the two effects, but the precise nature of this interaction is difficult to determine. As Charles Darwin put forth in 1859,

When a variation is of the slightest use to a being, we cannot tell how much of it to attribute to the accumulative action of natural selection, and how much to the conditions of life. Thus, it is well known to furriers that animals of the same species have thicker and better fur the more severe the climate is under which they have lived; but who can tell how much of this difference may be due to the warmest-clad individuals having been favoured and preserved during many generations, and how much to the direct action of the severe climate? for it would appear that climate has some direct action on the hair of our domestic quadrupeds (p. 175).

Since Darwin's time we have progressed a great deal in our understanding of the hereditary basis for biological variation. In a

little over a century we have advanced to the point where we are able to map gene loci upon chromosomes and exert control over the genetic base of plant or animal populations through elaborate breeding programs. Similarly we have made strides in our understanding of environment, realizing to a greater degree how organisms respond to changes in factors such as light, temperature, moisture and nutrients. However, we are still neophytes in our ability to quantitatively and proportionately separate hereditary and environmental influences. How much of man's intelligence is hereditary and how much is environmentally induced? This current controversy illustrates something of the degree to which we are still absorbed and still perplexed by the problem which Darwin posed.

One of the difficulties involved here is that of having a clear concept of environment. The use of the term with reference to its interaction with an organism should have a meaning that is dynamic in time. That is, the effect of a given environment must exert influence over the growth of an organism not only through its current interaction, but subsequently as well. For example, in a plant, temperature affects rates of enzymatic reactions in photosynthesis which may lead to more or less carbohydrate production, and later, greater or fewer numbers of new cells. If the food is stored, a greater time may elapse before it is utilized. Regardless of stage of this process in time, the original influence is still active in regulating plant growth.

It does so now through control over the internal environment of the plant. In this sense any growth response may be viewed as at least the partial result of some prior effect of environment.

Any such influence must act over time. Equivalently, at any one point in time a complex of environments, past as well as current, must be operative. Photosynthesis may be limited by current rates of enzymatic reactions and also by the "reverse sink" effect. The former influence may be related to current temperature of the environment and the latter to effects of some previous conditions.

In forestry there are at least two areas where a clearer understanding of environment including as an integral component, preconditioning influences, would aid conduct and interpretation of research work. These are areas of provenance trial testing and nursery programs.

Where provenance trials have been established on the basis of transplanted material there has unquestionably been influence of preconditioning. To what extent this has affected analysis of supposed genotypic variation is unknown. On the basis of a dominant role of prior growing conditions, one would expect local seed sources to exhibit superior growth and foreign sources less so. As this is often the case, the question may be posed: Would source material originating from distant localities show more desirable phenotypic expression upon testing if it had been preconditioned to the environment of the

test site? Not all provenance testing is carried out with transplanted material, but even where seed has been used there is the effect of environment during seed formation of the mother plant. This may seem of little consequence, but Highkin (1958) has noted transmission of environmental stimuli through the pollen of pure line pea plants which affected subsequent growth.

In nursery operations involving open-rooted or container-grown seedlings cultural treatments are given planting stock during the first two years of growth which would have to be considered forms of preconditioning. As nursery treatments involving shading, fertilization, irrigation, or root system control become more sophisticated there is increasingly the possibility that in addition to their known positive effects upon seedling behavior, physiological processes are being influenced in unknown ways which may subsequently affect growth adversely. This would seem to be especially the case with rapidly expanding container seedling programs where conditions under which stock is grown differ markedly from the field environment into which the seedlings are eventually to be placed.

This study has been designed in consideration of the idea that preconditioning is an important component of environment and ought to be better understood as it influences the physiology and growth of Douglas-fir seedlings. In nursery programs in the Northwest, information on effects of preconditioning prior to planting of sites

susceptible to summer drought should be invaluable. Since light is an important component of a nursery environment it has been chosen as the basis for an investigation of preconditioning of water relations behavior in Douglas-fir seedlings.

LITERATURE REVIEW

Preconditioning influences in plants may be induced in a number of ways through varying factors of the environment such as light, moisture, temperature, or nutrients. In laboratory studies it is relatively easy to vary one factor at a time and observe some response in the material being studied. Under natural conditions, however, many factors vary simultaneously with appreciable interactions. For this reason many studies of preconditioning, especially those dealing with basic physiological processes, have been conducted in laboratory environments. Under these conditions processes of photosynthesis, respiration, or transpiration may be altered through acclimating plants to different growing conditions and then comparing them to other plants grown as a control.

Acclimation of Physiological Processes

Temperature Effects

In these types of studies, air temperature has been the factor most often varied to induce changes in rates of photosynthesis and/or respiration. When plants of Oxyria were grown at low temperatures and subsequently measured for net photosynthesis, the upper and lower compensation points as well as the point of optimum photosynthesis were shifted downwards to lower temperatures when compared to

warm-acclimated plants (Billings and Mooney, 1968). Dark respiration rates were increased by cold acclimation in this species (Billings et al., 1971) as well as in Abies and Acer (Bauer, 1969). In the latter case the greater the degree of freezing stress applied, the stronger was the effect on respiration rate. In certain woody desert perennials, the higher the acclimation temperature the greater was the increase in net photosynthesis at higher temperatures (Strain et al., 1966). Sorenson (1964) reports the same effect in Douglas-fir, the temperature optimum and upper temperature compensation point being shifted four to seven degrees Celsius higher for 36/21^oC (day/night) pretreatments as compared to 18/4^oC conditions.

Sorenson showed this effect to be transitory, however. When plants were raised for 45 days in each of two treatments then reversed for 10 days, rates of photosynthesis and dark respiration at 55 days were nearly the same as if the plants had spent the entire time under the latter conditions. Rook (1969) shows a similar rapid recovery in Pinus radiata. Indications are, however, that the rate of adjustment of plants to warm conditions after being preconditioned to cooler temperatures is rapid (Mooney and Harrison, 1970), more so in fact than when the opposite transition is made (Pharis et al., 1967).

In their work with Douglas-fir and ponderosa pine, Pharis et al. (1967) attribute this differential rate of adjustment to the action

of some enzyme whose production is temperature dependent and is used in the acclimation process. They speculate that the rate of production under cool conditions would be low and fall below the rate of destruction whereas during warming, the reverse would occur and increased net amounts of the enzyme would aid acclimation.

That the acclimation process is under genetic control has been shown by Billings et al. (1971). Alpine ecotypes of Oxyria digyna showed ideal homeostasis in net photosynthesis while arctic ecotypes showed only a lesser degree of plastic response. Thus, some plants may be more capable of metabolic acclimation to temperature than others.

Steward et al. (1971) demonstrated the importance of considering prior temperature, photoperiod, age, and stage of development of plants in laboratory studies of photosynthesis and photorespiration. In carrot, short day pretreatments resulted in plants more efficient in fixing CO₂ (rate of photosynthesis - rate of photorespiration) than did long day pretreatments.

Light Effects

Where conifers have been grown under high and low radiation levels, marked differences in their light saturation curves have been found. Both seedlings of Pinus taeda and Picea engelmannii exhibited higher net photosynthetic light saturation curves for plants grown

under shade than for sun plants (Bormann, 1958; Ronco, 1970). In Douglas-fir, Krueger and Ruth (1969) found steeper initial slopes in the light saturation curves of plants grown in 79 percent shade when compared to a 31 percent shade treatment. The maximum rates of net photosynthesis were unaffected by prior light treatments, however, and in Sitka spruce and western hemlock no differences due to light preconditioning appeared. Brix (1967), however, notes greater rates of light-saturated net photosynthesis in low light-conditioned Douglas-fir seedlings than in higher-light-grown plants. Greater photosynthetic efficiency of most shade-grown plants appears due to metabolic acclimation favoring growth under low light conditions.

Moisture Effects

The response of photosynthesis to moisture preconditioning is often related to functioning of stomata as well as inner leaf factors, variously termed mesophyll resistance. Transpiration is affected more by stomatal control, and as Hinckley et al. (1972) have shown, the latter was more of a limiting factor to recovery of net assimilation rate after a desiccation pretreatment in Robinia pseudoacacia than it was in two species of Abies. When both gymnosperms and the angiosperm were droughted to -18 bars over a seven-day period and then rewatered, recovery varied. Robinia increased in water potential by 10 to 13 bars within one hour whereas in Abies, the increase over

the same period was only 1 to 2.5 bars. Recovery of net assimilation rate paralleled decrease of stomatal resistance in Robinia, but lagged behind in Abies by as much as 24 hours. Thus, in Abies it appears that prior stress has a longer-lasting adverse effect upon mesophyll resistance to CO₂ uptake than upon stomatal resistance.

Other investigators have shown similar effects upon apparent stomatal behavior following a period of water stress. Jarvis and Jarvis (1963) report that spruce and pine seedlings which had been grown under low soil water potentials transpired less when measured under high soil moisture conditions than did plants which had been grown continuously in soil of high water potential. In Pinus radiata seedlings, transpiration rate was lower in plants severely stressed prior to transplanting than in well-watered plants. This difference was accentuated by watering both treatments adequately following transplanting. Later, however, the pattern reversed and the previously stressed plants transpired more than the others even up to three weeks after transplanting (Rook, 1973).

The after-effect of water stress on transpiration rate has been shown by Fischer (1970) and Fischer et al. (1970) to be due to depression of the ability of stomata to open in light. In leaf discs of bean plants, this appeared due to direct temporary damage to guard cell functioning.

Other investigators have noted a surge of growth in plants rewatered following a water stress period. In Pinus radiata, this added growth took the form of increased new root production and elongation within a period of 18 days after cessation of stress treatment. However, by 40 days the non-stressed plants had "caught up" in root growth, differences no longer being apparent (Rook, 1973). Miller (1965) stressed Pinus taeda seedlings to four levels of leaf water potential for 30 days, then observed growth as measured by needle elongation for 128 days thereafter as a function of prior level of stress treatment. Initially, the most stressed plants were behind non-stressed controls in terms of needle elongation, but by 128 days they had recovered and surged ahead of the controls. The greater the level of pre-treatment moisture stress, the greater was the post-treatment surge in needle elongation under well-watered conditions. Amer and Williams (1958) observed a similar temporary increase in growth rate due to a prior period of water stress in Pelargonium. They speculate that the large number of citations of this phenomena may indicate that it is an inherent property of drought-resistant plants. It remains to be explained, however, how the post-stress depression in transpiration rate apparently related to narrow stomatal apertures is compatible with a surge in growth rate at this time. It may be that stored carbohydrates play a central role in supplying respiratory substrate for rapid growth.

Several studies (Ruetz, 1958; Unterschuetz, 1970) including one done by this author have shown that seedlings of Douglas-fir grown outside in cold frames subsequently transpire more than do seedlings grown inside a growth chamber when measured under identical conditions of adequate moisture availability. This probably represents an acclimation to increased evaporative stress encountered in a natural environment.

These effects of prior growing conditions upon basic processes are useful in that they aid understanding of morphological variations induced in plants grown under different environments. Regardless of whether the immediate effect of the inductive period is observed or whether the cumulative effect upon growth and morphology is evident, physiological acclimation to varying environments has still occurred. The degree to which this acclimation is possible depends upon the inherent plasticity of the genotype.

Growth and Morphological Acclimation

Temperature Effects

Several authors report effects of air temperature upon current and subsequent growth of conifers. Hellmers (1962) found that regardless if the previous season's temperature was warm or cool, there was little effect upon needle growth of ponderosa pine. However, when previous seasons' growing temperatures had been warm,

current shoot growth was greater than when prior temperatures had been cool. This effect was only noted when current air temperatures were cool. Brix (1967) noted that an increase in air temperature from 13 to 18°C increased total growth, shoot growth, and the amount of leaf area produced per unit dry weight of Douglas-fir seedlings while net assimilation rates dropped. Temperatures above 18°C decreased shoot and increased root growth (Brix, 1971). Such an effect upon increased leaf area would further enhance the plant's future photosynthate-producing potential. Indeed, it is not only photosynthetic rate per se that affects a plant's future growth, but the allocation of photosynthate to shoot or root development. Because of this, a high rate of photosynthesis can result in smaller plants simply because the added dry matter is not utilized in needle growth (Sorenson, 1964).

Light Effects

The allocation of dry matter between shoot and root in conifers has been shown by an abundance of studies to be influenced by level of light intensity during growth. In Douglas-fir, the shoot-root ratio is higher in seedlings grown under shade than it is in ones grown under higher light conditions (Baker, 1945; Brix, 1967; Krueger and Ruth, 1969; Lavender et al., 1968; Lyr, 1964; Ruth, 1967). However, results such as these are suspect as Ledig et al. (1970) have pointed out since the shoot-root ratio decreases with increased growth in conifer seedlings

(Ledig and Perry, 1965; Ledig et al., 1970; Wareing, 1950). If high light-grown plants are larger than shade-grown plants, their lower shoot-root ratios may just be a consequence of greater total size. Ledig et al. (1970) point out the advantages of using an allometric form of analysis to examine shoot and root development over time as the plant increases in dry weight. This form of analysis is preferable to simple comparisons of shoot-root ratios. Bormann (1958) suggests that a better developed shoot system in shade-grown plants may be an environmentally-induced adaptation which enables such plants to photosynthesize more under low light conditions.

Other morphological and anatomical changes associated with low light-grown plants are increased internode length in Pinus taeda (Bormann, 1958; Ledig et al., 1970), and Picea abies (Gradetskias, 1969), increased angle between stem and fascicles of secondary needles in Pinus taeda (Bormann, 1958), decreased leaf thickness in Douglas-fir (Brix, 1967; Korstian, 1925; Tucker, 1966), in Picea engelmannii (Korstian, 1925), and in Pinus sylvestris (Zelawski et al., 1968), and a thinner cuticle in Douglas-fir (Korstian, 1925; Tucker, 1966), and in Picea engelmannii (Korstian, 1925). Also characteristic of shade-grown plants are increased ratio of leaf area to leaf dry weight in Douglas-fir (Brix, 1967; Krueger and Ruth, 1969), increased chlorophyll content in Picea abies (Gradetskias, 1969) and in Pinus sylvestris (Zelawski et al., 1968), and increased ratio of chlorophyll to carotenoids in Pinus sylvestris (Linder, 1971).

Moisture Effects

Several effects of moisture stress as a factor influencing growth have been demonstrated in conifers. Fritts et al. (1965) showed that in mature Pinus edulis, lack of available soil moisture during the growing season results in less total needle elongation on lateral branches. Cleary (1971) demonstrated the same phenomena in Pinus ponderosa. In Pinus sylvestris, poor environmental conditions one year in terms of atmospheric CO₂, moisture availability, and nutrient supply result in high amounts of lammas growth and prolepsis during a following year of improved conditions (Alden, T., 1971).

In oak and fruit tree seedlings, drought conditions during the second half of the growing season resulted in earlier dates of flushing the next spring than was the case for seedlings previously given sufficient moisture (Guzev, 1958). It is also of interest that J. N. Alden (1971) reports early bud-burst in Douglas-fir trees having high foliage potassium content. Potassium-deficient trees burst buds later.

All plants become adapted to carrying on optimum growth over discrete ranges of light, temperature, and moisture. Douglas-fir is no exception. A number of investigators have shown that seedlings of the species grow best in partial shade when other conditions are not limiting (Baker, 1945; Isaac, 1943; Korstian, 1925). Soil and air temperatures should be between 18 and 24^oC and photoperiod 18 hours

(Brix, 1971; Lavender and Overton, 1972). Neither author found any requirement for diurnal temperature fluctuations.

It has been well established that for conifers in western North America the principal climatic factor limiting growth is moisture. In fact, the science of dendrochronology is predicated upon the fact that variation in thickness of annual growth increments may be related to the limiting influences of climate, specifically moisture and temperature. Cool, wet conditions result in the formation of relatively wide tree rings whereas high temperatures and low moisture cause narrow growth rings to be formed (Fritts, 1966). However, it is recognized by dendrochronologists that not only is climate of the current growing season revealed in a tree's annual growth ring, but climate of the preceding years as well. This has been demonstrated by correlative statistical techniques for a number of species including Douglas-fir. A large current year's growth increment in this species was most strongly related to high precipitation and low temperatures of the previous fall period as shown by data from 120 different forest stands (Fritts, 1972).

MATERIALS AND METHODS

Plant and Soil Materials

Douglas-fir Pseudotsuga menziesii (Mirb.) Franco seedlings , of a North Santiam, Oregon seed source provided material for the study. The seed was from an area on the western slope of the Cascade Range at an elevation of approximately 3000 feet, centered just south of the northern boundary of Linn County in the Thomas Creek area.

For experimental purposes this plant material was grown in a soil of the Jory series, an acidic, leached clay with high surface organic matter. Mechanical analysis revealed a particle size distribution of 12 percent sand, 33 percent silt, and 55 percent clay. The soil was collected from a site near the MacDonald Forest nursery north of Corvallis, mixed well and sieved through 1/4 inch mesh screen to remove rocks and complex aggregates prior to filling of pots.

Techniques and Design of Experiment

A greenhouse and growth room as well as an adjacent outdoor cold-frame area were used for growing seedlings under various treatments. Most water relations data were collected on seedlings removed from this outdoor environment at different periods and

monitored indoors using laboratories of nearby facilities.

Douglas-fir seedlings of two general age groupings made up the experimental material. The first of these represented plants which had completed or were in various stages of their first season's shoot growth. These plants ranged in age from four to eight months at the time of measurement. Other plants were older, had completed a second season of shoot growth, and ranged in age from 20 to 23 months when their moisture relations were measured.

Both groups of seedlings were grown under the same varying levels of light intensity as preconditioning treatments during their first year of growth. These treatments were induced by shading and ranged from low light to a full sun environment. Seedlings of the younger age at time of measurement revealed characteristics of water relations behavior induced by the first season's growth under the different treatments, i. e., immediate effects of light preconditioning. Second-year seedlings upon measurement revealed information as to persistence of first-year preconditioning effects as well as information on effects of current conditions.

These latter plants, following completion of first-year shoot growth, were given a full light environment for the second growing season. This included plants of the same age group which had spent the first growing season under shaded conditions as well as some that had received full light for the first year. Full light the first season.

in this case constituted a control environment, and thus served as a basis for evaluating effects of preconditioning.

Stratification of seeds involved soaking for 24 hours in water, moistening with Captan 50W fungicidal solution, and storage at cool temperatures. After one week at room temperature seed began to germinate and young seedlings were placed in soil-filled pint plastic pots in a growth room. Seedlings had a modal germination date of April 25, 1971.

The seedlings remained in the growth room for two weeks under a light intensity of ca. 5 percent of full light. Photoperiod and thermoperiod were based on a 16 hour daylength with 23/18°C day-night temperatures. At two weeks of age the plants with first true leaves emerging were placed outside under three different levels of light intensity of approximately 9 percent, 44 percent, and 100 percent of full light.

The pots, base-perforated for drainage, were set down into soil so that the level of soil inside was the same as that of surroundings, and a weed shaving mulch spread over the surface to reduce evaporative loss. Soil moisture was maintained at field capacity by regular watering except just prior to measurement when plants were droughted to varying degrees to meet experimental requirements where soil moisture stress was an independent variable.

Two of the three light regimes were induced by layers of plastic and metal screening over the seedlings with "skirts" around the sides.

Values of light intensity were derived by comparison of diurnal light variation at seedling level with that above the screening measured with a Weston Illumination Meter (quartz filter) on August 8, 1971. The treatments were set up to represent a wide range of light intensity from low to full sun in order to elicit extremes of variation in seedling growth. With this design, variation between treatments would be expected to be far greater than any within treatment variability in terms of selected growth parameters. Seedlings remained under these treatments until August 24, 1971, when individually they began to be randomly selected for laboratory measurements. These measurements continued until completion of first-year studies on December 18.

During the following year, 1972, this portion of the study was repeated to gain additional information on growth of seedlings under the respective treatments. The date of germination was the same in 1972 as it was in 1971, and all other experimental procedures affecting aspects of seedling growth were the same for both years. In 1972 seedlings were sampled for dry weight of needles, stem, and root on two dates, May 12 and July 7, following germination and before the first laboratory measurements of August 11. Thereafter, periodic dry weight sampling occurred on August 13, September 22, November 3, and December 15 in addition to seedlings sampled at the time of laboratory investigation of water relations behavior. This periodic

sampling was based on a sample size of six plants randomly chosen from each of the three treatments.

Climatic data relating to changes in daily maximum and minimum air temperature over the study period were supplied by the U. S. Weather Bureau from their Hyslop Farm recording site north of Corvallis. These data were found to closely correspond to changes in air temperature observed at the Forest Research Laboratory and provided a means for explaining variation in certain aspects of seedling growth and physiology in their outdoor environment.

The second group of seedlings, i. e., those that were 20-23 months of age at time of laboratory measurement, had a slightly different post-germination year than did those just mentioned. These plants which had a germination date in mid-January, 1971, spent their first three months in a greenhouse and were transplanted to the pint-size pots in early April. On April 25, the time of outdoor treatment induction, they were taller and more etiolated than plants which had spent their first three months outdoors.

On September 15, 1971, these plants were transplanted to larger number 10 size metal cans. By this time all had completed shoot growth and set a terminal bud. These transplanted seedlings were then placed together under full light where they remained throughout the winter and next growing season until time of laboratory measurements between September 6 and October 27, 1972. Due to

time constraints the only treatments assessed for water relations behavior during this time were the 9 percent and 100 percent full light groups. As these older plants were few in number and destructive sampling would have been involved, no growth data were obtained prior to this period.

Water relations data were collected on plants using various laboratory instrumentation. A consistent procedure was followed in the case of each plant; measurements of transpiration, plant moisture stress, and soil moisture stress ultimately were made. Growth data in the form of total leaf area, needle, stem, and root dry weight were also gathered.

Results of these investigations have been expressed on a seasonal basis, and are compared herein on the basis of summer, fall, and winter periods. Such partitioning was done on the basis of changes in growth and physiological response of the plants rather than a strict "calendar-year" type delineation. The summer period was represented by seedlings in a state of active shoot growth and the fall period by seedlings that had ceased elongation growth and set a terminal bud. The transition from fall to winter was denoted by an obvious seedling response to low air temperatures as indicated by a simultaneous change in transpiration rate.

In general, Douglas-fir seedlings in their first season of growth maintained shoot activity into mid or late September when bud set

defined commencement of the "fall" period. In 1971, "winter" began around October 24, and in 1972, on September 27.

In addition to the main experiment already described, certain subsidiary studies were carried out. The purpose of these was to clarify certain aspects of Douglas-fir physiology already observed and to supply additional information on behavior under preconditioning treatments.

During the last four months of 1972, an experiment was devised to investigate a previously-observed change in transpiration rate with advent of sub-freezing air temperatures. A sample of four five-month-old seedlings which had ceased terminal growth were regularly monitored for transpiration rate under conditions of high moisture availability. These four plants from the 100 percent light treatment were brought into the laboratory the night preceding their day of measurement and the pots placed in one centimeter depth of water. Equilibration by capillary rise ensured a constant soil moisture content on each approximately biweekly sampling date. Changes in transpiration rate over time were taken to represent climatic influence upon seedling water relations.

Another experiment was carried out on plants growing under the three light regimes. Diurnal changes in plant moisture stress had been established by means of a pressure bomb (Waring and Cleary, 1967). To elucidate these changes, a diffusion porometer (Turner

et al., 1969; Turner and Parlange, 1970) was used to measure leaf resistance simultaneously with plant moisture stress on August 13, 1972.

Finally, critical growth information was gathered for the three treatments. Starting in late 1971, data on bud set and during the following spring, bud break, were collected for various-aged plants. This information was subsequently gathered for the 1972-73 bud set-bud break period as well and data presented on the basis of percentage of treatment population with dormant or active shoots. Lammas growth was monitored during 1972 for plants in their second season of growth which had been preconditioned the first.

Instrumentation

Transpiration rate of Douglas-fir seedlings was measured in a controlled environment apparatus similar to that of Bierhuizen and Slatyer (1964), and a modification of one described by Geppert (1968) and Ruetz (1968). Figure 1 presents the essentials of the apparatus with the symbolic representation explained on succeeding pages.

The light source was an incandescent lamp which, with the addition of several filters, provided a light intensity at seedling level in the cuvette of about 36 percent of full sunlight. The quality of this light was low in the infra-red and blue regions and high in the red with a peak intensity at 635 nm as measured by a recently calibrated

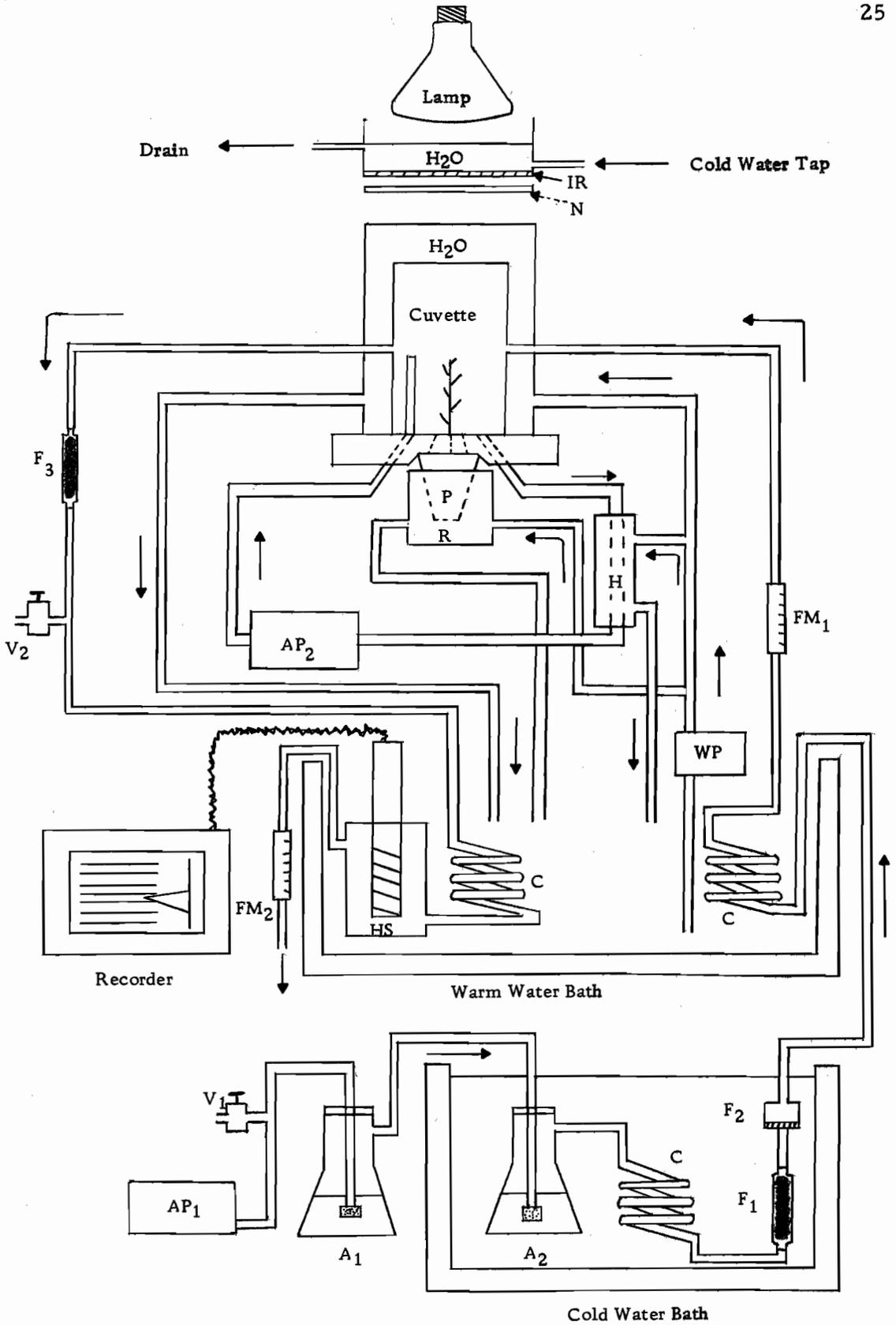


Figure 1. Controlled environment apparatus for measuring transpiration (description on following pages).

Figure 1 - Symbolic Representation

- Cold Water Bath - A Forma Scientific, Inc., refrigerated and heated bath and circulator maintained at $8.0 \pm 0.05^{\circ}\text{C}$.
- Warm Water Bath - Same as cold bath, but maintained at $19.1 \pm 0.05^{\circ}\text{C}$.
- Cuvette - A plexiglas chamber with a circulating water bath for control of air temperature. Top and base were separable and sealed by a rubber "O" ring.
- Lamp - A 1200 watt incandescent source arranged 2-1/2 inches above water bath gave a light intensity of $15,340 \text{ uw/cm}^2$ (about 35.5 percent of full light) between 400 and 700 nm at seedling level in the cuvette.
- IR - A glass plate infra-red filter placed in the bottom of the circulating water filter.
- N - A neutral density filter made of two thicknesses of cheesecloth.
- P - A black plastic pint container containing one seedling.
- AP₁ - A Neptune Dyna-Pump of diaphragm type for circulating air through the humidity control system.
- AP₂ - A Reciprotor piston pump controlled by a rheostat for circulating air through the wind simulation control system.
- A₁, A₂ - A flask containing distilled water and a fritted glass bubbler.
- V₁ - A pressure control valve used to adjust rate of air flow through the humidity control system.
- V₂ - A pressure control valve for maintaining a constant rate of air flow past the humidity sensor, HS.
- C - A coiled copper tubing heat exchanger.
- F₁ - A glass wool filter used to remove possible foreign particles or condensate from the air stream.
- F₂ - A sintered glass filter used as an extra safety precaution and having the same function as F₁.

Figure 1 - Symbolic Representation (continued)

- F_3 - A glass wool filter used to remove foreign particles from the air stream before it passed through the humidity sensor.
- FM_1 - A Manostat flowmeter used to monitor flow rate through the cuvette. Either Cat. No. 9144 or 36-541-22 was used depending on air flow requirements. No. 9144 measured flow rates between 0.2 and 2.0 liters/minute. No. 36-541-22 measured flow rates between 1.5 and 9.0 liters/minute.
- FM_2 - A flowmeter (No. G469) used to monitor flow rate through the humidity sensor. Its effective range was 0.4 to 2.6 liters/minute.
- HS - A Hygrodynamics, Inc. electric hygrometer sensing element used to measure relative humidity of the air leaving the cuvette.
- Recorder - A Taylor Transcope Electronic Recorder (Model 701JE2) used to record the response of the humidity sensing element.
- WP - A Gelber Corp. water pump used to supply warm bath water to three cooling systems: cuvette, soil, and wind loop heat exchanger.
- H - A heat exchanger in the wind simulation control system to maintain air in the loop at warm water bath temperature.
- R - A soil temperature control system consisting of a receptacle for a potted plant and a copper coil heat exchanger in a water bath.

Note: Not shown in Figure 1 are contact thermocouples in the cuvette for the purpose of measuring plant temperature, and a thermocouple inserted into the soil for the same purpose. These were connected to a 12-channel Leeds and Northrup recorder with a built-in temperature reference.

Isco spectroradiometer (Appendix Figure A). Thirty-six percent of full sunlight is greater than saturating light intensity for photosynthesis in Douglas-fir seedlings (Krueger and Ferrell, 1965).

Air entering the humidity control system was first saturated by bubbling through water at room temperature. Then it passed through a second bubbler in the cold bath. This removed moisture and ensured air of 100 percent relative humidity at 8.0°C . This air, after passing through a coil and two filters, was raised to warm water bath temperature by another coil and then passed through a flowmeter and entered the cuvette. The air was held at a constant flow rate of 0.66 liters/minute for small seedlings. Larger seedlings which transpired more required a higher flow rate and this varied depending on the plant. The alternate use of two flowmeters at FM_1 made this adjustment possible.

The base humidity of air entering the cuvette was 48.5 ± 0.4 percent at 19.1°C . This was stable and provided a reference point for evaluating a seedling's contribution to raising the humidity of the outgoing air. After leaving the cuvette, the moist air passed through a heat exchange coil at warm bath temperature and through the electric hygrometer sensing element. Measurement of humidity at this point was based on absorption or desorption of water vapor upon a LiCl wire-wound resistance core. The resistance of the unit changed as did the humidity of the passing air and was indicated on the chart

output of the Transcope Recorder. A calibration curve for recorder pen reading as a function of relative humidity of passing air appears in the Appendix, Figure B. Due to the importance of this relationship the curve was checked periodically while in use by weight changes of a desiccant, but no changes in calibration were observed. The curve has an accuracy of approximately ± 0.6 percent relative humidity. Also appearing in the Appendix is a detailed discussion of computation procedure in determining transpiration rate of a seedling.

The rate of air flow past the LiCl sensor was maintained at a constant 0.66 liters/minute by valve V_2 regardless of flow rate through the cuvette. It was determined that the LiCl element was sensitive to air pressure changes induced by varying the flow rate. Before the addition of V_2 to the system, an empirical correction factor was applied to pen readings to compensate for this effect. As air flow rate increased, output pen readings dropped slightly. This correction process was obviated by the addition of the regulator valve.

The apparatus, in addition to allowing for humidity control and measurement which was its prime function, also ensured that such measurements were made upon plants in an environment that was stable. Thus the temperature of the air in the cuvette was maintained constant by means of an external water jacket and continually circulating water from the warm bath at 19.1°C . However, due to sub-optimal efficiency in heat transfer through the cuvette wall, the inner air

temperature was several degrees above that of the water jacket. For plants measured during 1971 (young seedlings), mean needle temperature was $22.0 \pm 0.43^{\circ}\text{C}$ (1 SD), and for plants measured during 1972 (seedlings of the older age group), 21.1°C (range = 20.3°C to 21.8°C). Movement of the apparatus in mid-1972 from a small room with poor circulation to a larger one with better ventilation accounts for the difference in means.

Needle temperature was measured by means of three to five contact thermocouples spaced throughout the plant's foliage from top to base. The design for these was that of Fry (1965). In his studies the contact thermocouple which is fixed to the abaxial leaf surface by a spring gave temperature readings very close to an inserted thermocouple, the best method for measuring needle temperature of four tested. Gale et al. (1970) also found close agreement between a contact thermocouple similar to Fry's design and an infra-red thermometer in measurements of leaf temperature on Ficus.

In these experiments, mean needle temperature was considered a good estimate of air temperature in the foliage of the plant being tested. According to Fry (1965), the two are closely related under conditions similar to this experiment. The author's original intent was to make a comparison of the two parameters under varying levels of transpiration. However, the discovery was made that a vertical temperature gradient of several degrees Celsius existed in the cuvette.

This was due to absorption of radiant energy by the base of the cuvette, in particular the rubber plug holding the plant in place, and re-radiation upwards. Aspiration did not relieve the problem as the form of heat transfer was not convective. A high rate of air flow across the base of the cuvette was of some assistance, but the possibility for conductive transfer of heat away from this area by a water bath as was the case for the sides and top was lacking. Painting the rubber plug white was of some assistance, but did not relieve the problem. Thus to make the intended comparison would have necessitated placing shielded thermocouples in the plant's foliage which, along with the needle thermocouples, would have substantially interfered with the energy balance of the plant. Therefore, when cuvette temperatures are referred to herein the meaning is with reference to measured needle temperatures.

Another aspect of the stable cuvette environment was windspeed. This was kept constant at 50 cm/sec as measured by an Alnor hot-wire anemometer and was sufficient to minimize leaf boundary layer resistance on small Douglas-fir seedlings as determined by Unterschuetz (1970). Tests later proved the validity of this figure. Wind was supplied to the seedling by means of a vertical riser made of polyethylene tubing with a slit cut down one side. The air leaving the cuvette passed through a heat exchanger, H, at warm bath temperature.

Finally, for plants in pint-size containers soil temperature was controlled. This was accomplished by means of a small water reservoir kept at warm bath temperature by means of circulating bath water through a copper coil immersed in the reservoir. The center of this coil formed a receptacle for the plant. For 121 plants measured in 1971, soil temperature had a mean value of $21.4 \pm 0.76^{\circ}\text{C}$ (1 SD). For older plants in metal cans there was no control over soil temperature. Fifty-nine plants measured in 1972 had a mean temperature of 20.7°C (range = 18.2°C to 22.3°C). For each measurement a potted seedling was brought inside, placed in the apparatus and allowed to reach a point of stable transpiration rate. This usually required from one to three hours depending on the plant.

Immediately following measurement of transpiration rate, the plant was removed from the cuvette and plant moisture stress evaluated by means of the pressure bomb technique (Scholander et al., 1965). In this method the seedling was severed at soil level and the shoot placed in the bomb with cut end extruding. The air pressure in atmospheres necessary to force the sap back to the cut xylem surface is a measure of the negative tension in the xylem that existed prior to severance. This tension or moisture stress is physiologically meaningful as it corresponds numerically to xylem water potential, a fundamental parameter of water status in the plant.

Next, soil moisture stress was assessed by use of a desorption curve expressing soil matric potential in atmospheres measured by a pressure-membrane apparatus as a function of soil water content (Appendix Figure C). The latter was gravimetrically determined from a soil sample taken from the rooting zone of each potted plant in the study. Soil matric potential is a good measure of total soil moisture stress when osmotic potential is minimal as is likely the case with the Jory soil used here. Salter and Williams (1963) describe the pressure-membrane method and its use in relating soil moisture content to tension over a range from 0.33 to 15 atmospheres. This was the range used in this study, but with the additional extrapolation to 20 atmospheres. On a log-log graph showing a linear relationship, this was felt to be justified, but to extrapolate much beyond 20 atmospheres could lead to serious inaccuracy. Plants whose soil moisture stress was greater than 20 atmospheres were not included in the analysis.

Following evaluation of soil water status, total leaf surface area was determined. This formed the basis for expression of transpiration rates and was measured by means of an optical planimeter (Davis, 1966). In this technique, Douglas-fir needles were placed in the plane of an aperture through which a light beam passed. A solar cell received the light energy passing through the aperture

and converted it to electrical energy registering its output on a microammeter. The depression due to leaf surface area registers as a current drop on the meter.

This measuring procedure was followed on all the younger seedlings. On the older ones, however, leaf surface area was determined by a regression of surface area and dry weight. A sample of needles from the older study plants of each treatment was used to determine a regression of dry weight on surface area. The linear relationship obtained was then used to predict total leaf surface area from total leaf dry weight per plant. This method was necessitated due to the large amounts of foliage on the bigger seedlings which precluded direct measurement of their area.

The diffusion porometer used to measure leaf resistance does so on the basis of measuring the rate of change of humidity at a fixed temperature in a closed system due to water loss from needle tissue of known surface area. Boundary layer resistance is minimized by a small fan. Humidity is sensed by a LiCl element. The porometer used in this study was a commercially available unit--the Wren ventilated diffusion porometer. Operation procedure and calculations to arrive at leaf resistance are outlined by Turner and Parlange (1970).

Calculations and Methods of Analysis

Growth Analysis

Variations in seedling growth under the different light regimes should affect the plants' water relations. This is of critical importance where some plants may possess a better-developed root than shoot system or where the reverse holds true. Thus a form of analysis called allometry was used to consider variations in the relationship of shoot to root growth under the light regimes.

Allometry, a technique first devised by Huxley (1924) for use with animal systems, is the study of relative growth. By this method, any related pair of measurements may be compared, as branch length to diameter, fruit volume to weight changes, or in the case of this study, shoot dry weight to root dry weight. The allometric relationship between the latter two parameters expresses more than a simple shoot/root ratio as it takes into consideration changes in this ratio over time. As Ledig et al. (1970) have pointed out, the shoot/root ratio in conifers seems to decline with time over at least the first several years. When this decline is expressed on a logarithmic basis by the equation

$$\log (\text{shoot dry weight}) = a + k \cdot \log (\text{root dry weight}),$$

a linear relationship results. The slope of the allometric equation, k , represents the balance between shoot and root as the plant increases

in dry weight. In conifer seedlings where the rate of increase in size of root system is greater than the rate of increase in the shoot, i. e., where the shoot/root ratio declines over time as total dry weight increases, the allometric coefficient, k , is less than 1.0. If both shoot and root increased in dry weight at the same rate, k would be unity, and if the former surpassed the latter in rate of dry weight increase, k would be greater than 1.0.

By comparing parameters of the allometric equation developed for seedlings grown under the respective light regimes of this study, insight into the effects of light preconditioning upon the dynamic aspects of shoot-root balance was gained. In a like manner, equations expressing the balance between needle and root development and stem and root development under the different regimes provided additional insight.

A computer program based on a non-linear least squares curvfit of the form $Y = b_1 X^{b_2}$ was used to generate the appropriate allometric equations.

Water Relations Analysis

The method of analysis used in this portion of the study was similar to that used by Unterschuetz (1970) with several modifications. Briefly it involved developing an empirical model based on the relationship $PMS = f(SMS, L_c)$ where PMS is plant moisture stress,

SMS is soil moisture stress, and L_c is leaf conductance. Prior to fitting the empirical model all data were converted to base ten logarithms to provide a better fit. The representational form of this model is as follows;

$$Y_j = B_0 X_0 + B_1 X_1 + B_2 X_2 + B_3 X_1^2 + B_4 X_2^2 + B_5 X_1 X_2 + e$$

where

- Y_j = the measured plant moisture stress in atmospheres on seedlings of the j^{th} treatment-season combination
- B_0 = the effect common to all observations
- B_i = the effect of the i^{th} variable ($i = 1, 2, 3, 4, 5$)
- X_0 = 1
- X_1 = the measured soil moisture stress in atmospheres
- X_2 = the calculated leaf conductance in cm/sec based on measured transpiration rate
- X_1^2 = the square of soil moisture stress
- X_2^2 = the square of leaf conductance
- $X_1 X_2$ = the interaction between soil moisture stress and leaf conductance
- e = error

This model is useful as a response surface may be generated from it to portray graphically the effects of SMS and L_c upon PMS. However, where several treatment-season combinations are being compared, it is necessary to be able to test statistically for differences between treatments and seasons. It is important to know

whether differences between treatments, for example, are due to an effect of SMS upon PMS or an effect of L_c upon PMS or an interactive effect upon PMS. A means of testing for such effects involves combining terms from several individual treatment models into one large model.

As an example, in the case where the effects of two treatments in one season are being compared, as 9 percent light vs. 100 percent light for the fall period, two individual models each containing six terms as above are combined to form one full model with 12 terms. The model is then restrained in such a way that all the data from the two light regimes are lumped together to form a model with only six terms which does not differentiate between fall treatments. As a result, the residual sum of squares is increased in the restrained model over what it was in the full model. By comparing the degree of increase in residual sum of squares to an F-distribution with the appropriate degrees of freedom, one can ascertain whether or not accounting for treatment differences results in a significant difference in the full model.

Likewise, the full model (f) may be restrained term-by-term to test for differences in response of PMS between treatments due to SMS, L_c , or their interaction. In either case the restrained model (r) would have one term less than the full model, i. e., 11 terms, and the

increased residual sum of squares would be due to combining two terms.

Whether the full model is subject to overall treatment restraint, halving the number of terms, or term-by-term restraint successively resulting in one less term for each of the parameters in the model, the statistical computation is the same:

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

This computed F is then compared to the tabular F with degrees of freedom corresponding to d. f. r - d. f. f for levels of significance.

The computing procedure used enlisted the aid of a "canned" program known as *STEP or stepwise multiple regression. This program was used to generate "full models" for a number of treatment-season combinations. In general, these took the form of 2 x 1 factorials, the comparison being made of either two treatments for one season or one treatment over two seasons. The Appendix contains as an example analysis of variance tables for two such comparisons, viz., summer vs. fall over all treatments, and 9 percent light vs. 100 percent light for the fall period. No statistical analysis was applied to data comprising the winter period. These data represented a changing state in terms of the physiology of the seedlings, and not a static situation as seemed to be true in summer and fall.

Tri-variate response surfaces are shown for summer and fall models. Two-dimensional graphic portrayals of data appear for the remainder of the data, the reason being that certain important features of water relations behavior were not easily visualized on the response surfaces. This fact precluded their comprehensive use.

The two-dimensional graphed data are all represented by computer-fit lines or curves with the exception of summer and fall graphs of leaf conductance vs. plant moisture stress. These were analyzed statistically through the basic model, the data portrayed with tri-variate surfaces.

RESULTS

Climate

Monthly changes in air temperature during 1971 and 1972 are shown in Figure 2. Daily minima and maxima were averaged over the year for each month to show variation in temperature of the seedling's environment. Maximum and minimum temperatures rose to a high in July and August during the period of optimum growth, then declined over fall and winter. The largest daily temperature variation also occurred in July and August, the driest months of the year.

Nineteen-seventy-two was a warmer year than 1971. With the exception of April and December, every month had a higher maxima and minima in the second year, this being most apparent during June and July, the time of maximum shoot growth. In June, 1972, maximum and minimum air temperatures were 3.2 and 2.1^oC higher, respectively, than during the same month of the preceding year.

Seedling Growth

Gross Morphology

Growth of seedlings under the light treatments differed between the two years (Table 1). Based on a sample of 12 plants of ca. seven months age more dry matter was accumulated in 1971 than in 1972,

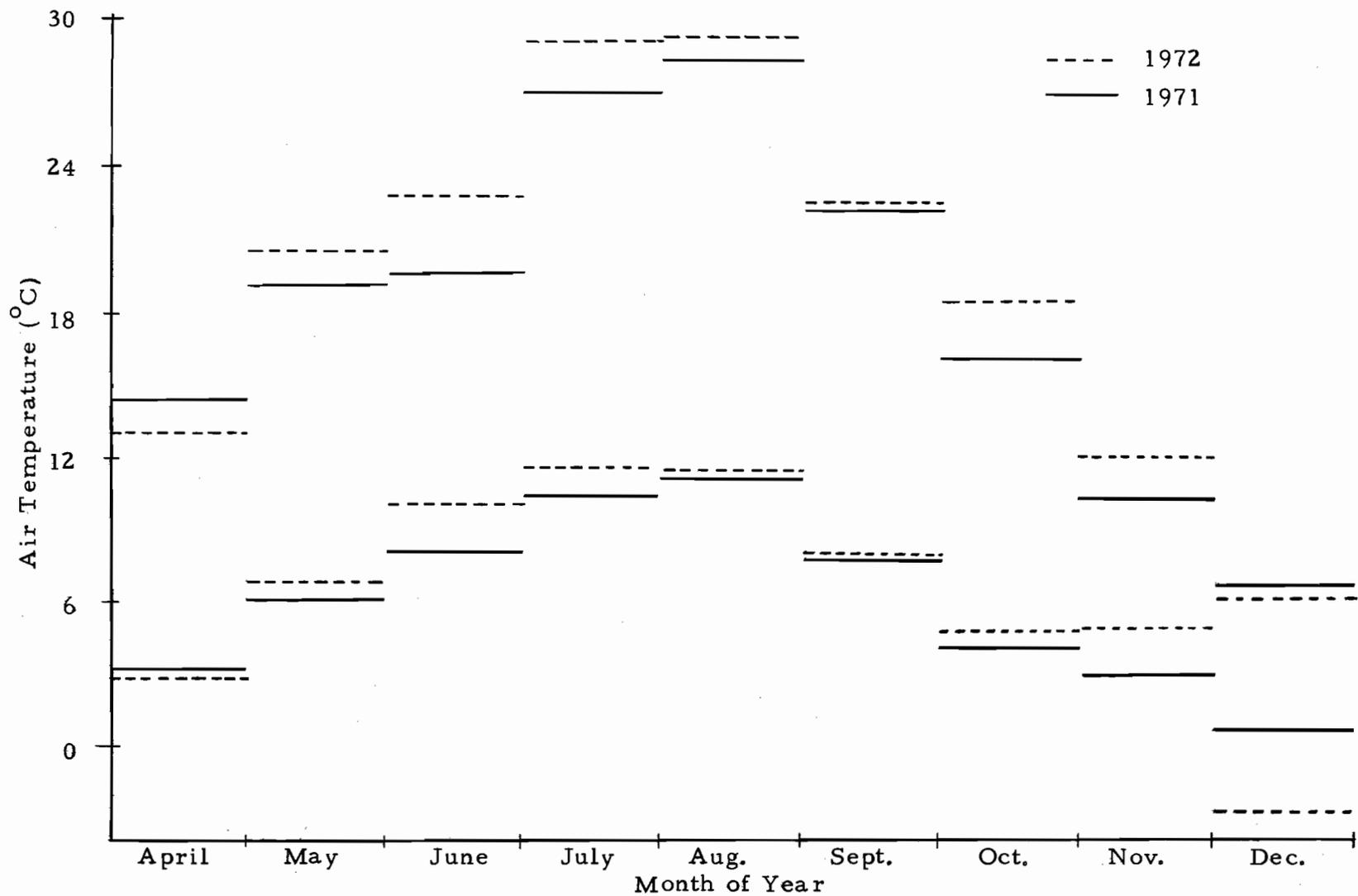


Figure 2. Mean minimum and maximum monthly air temperature at Hyslop Farm, Corvallis, Oregon for the period April to December of 1971 and 1972.

Table 1. Effect of light treatment on seedling dry weight in milligrams measured at seven months for 1971 and 1972 (leaf surface area/leaf dry weight ratio in cm^2/g is also shown).

Year	Light Preconditioning					
	9 percent		44 percent		100 percent	
	Seedling Dry Weight	Leaf Surface Area/Dry Weight	Seedling Dry Weight	Leaf Surface Area/Dry Weight	Seedling Dry Weight	Leaf Surface Area/Dry Weight
1971	154	145	467	109	355	82
1972	147	154	255	112	314	93
t-Test of yearly means	NS	NS	**	NS	NS	**

** Significant at 1% level

NS - No significant differences

regardless of treatment. In 1971, seedling dry weight was greatest in plants of the 44 percent light treatment, high in plants grown under full light, and lowest for those under 9 percent light. In 1972, the latter still grew least, but the 44 and 100 percent light plants were reversed from their order in 1971, i. e., the 100 percent light plants exhibited greatest dry weight at seven months followed by the 44 percent light-treated plants. The only statistically significant difference between mean dry weights for the two years was found for the 44 percent light treatment.

The variation in gross dry matter production seemed also to be reflected in the ratio: leaf surface area/leaf dry weight. This ratio, determined for 20 to 40 plants of each treatment that had ceased shoot growth, was larger in 1972 than in 1971 for each treatment. A regular increase in the ratio occurred as the amount of light decreased. For seedlings given full light the difference in means between the two years was significant at the 1 percent level.

Allometric Analysis

A basic consideration in the application of allometric growth analysis to plants is the change in the ratio of parameters compared over time. In this study the ratio of shoot dry weight to root dry weight of Douglas-fir seedlings generally declined with increasing age regardless of growing conditions. This decline is shown for 100

percent and 9 percent light-grown plants in 1972 in Figure 3, each point being based on a mean of six plants measured during that year.

The computed allometric coefficients for preconditioning treatments and years appear in Table 2, each being based on a sample size of between 43 and 50 plants. Each coefficient is diagnostic of treatment effect upon the rates of shoot and root development. A comparison of the value of the coefficients with their graphic portrayal in Figure 3 clarifies their meaning. In 1972, the growth of 9 percent light-grown seedlings was characterized by an allometric coefficient of 0.483 whereas that of 100 percent light-grown plants in the same year was 0.709. A coefficient below 1.0 indicates that as the seedling grows larger, more dry matter is being allocated to root than to shoot development, the trend being more pronounced for seedlings with smaller coefficients. This was the case for the low light treatment with $k = 0.483$, and as the change in shoot/root ratio with total dry weight in Figure 3 indicates, the ratio declined more rapidly for seedlings of this treatment than it did for seedlings of the high light treatment. Also, it is apparent that the 9 percent light-grown plants never reached the large size that the 100 percent light-grown plants did in 1972 as was indicated in Table 1.

In 1972, the allometric coefficients increased with level of light intensity, but in 1971 the relationship was reversed with the 9 percent light-grown plants having the largest coefficient, 1.014. This indicates

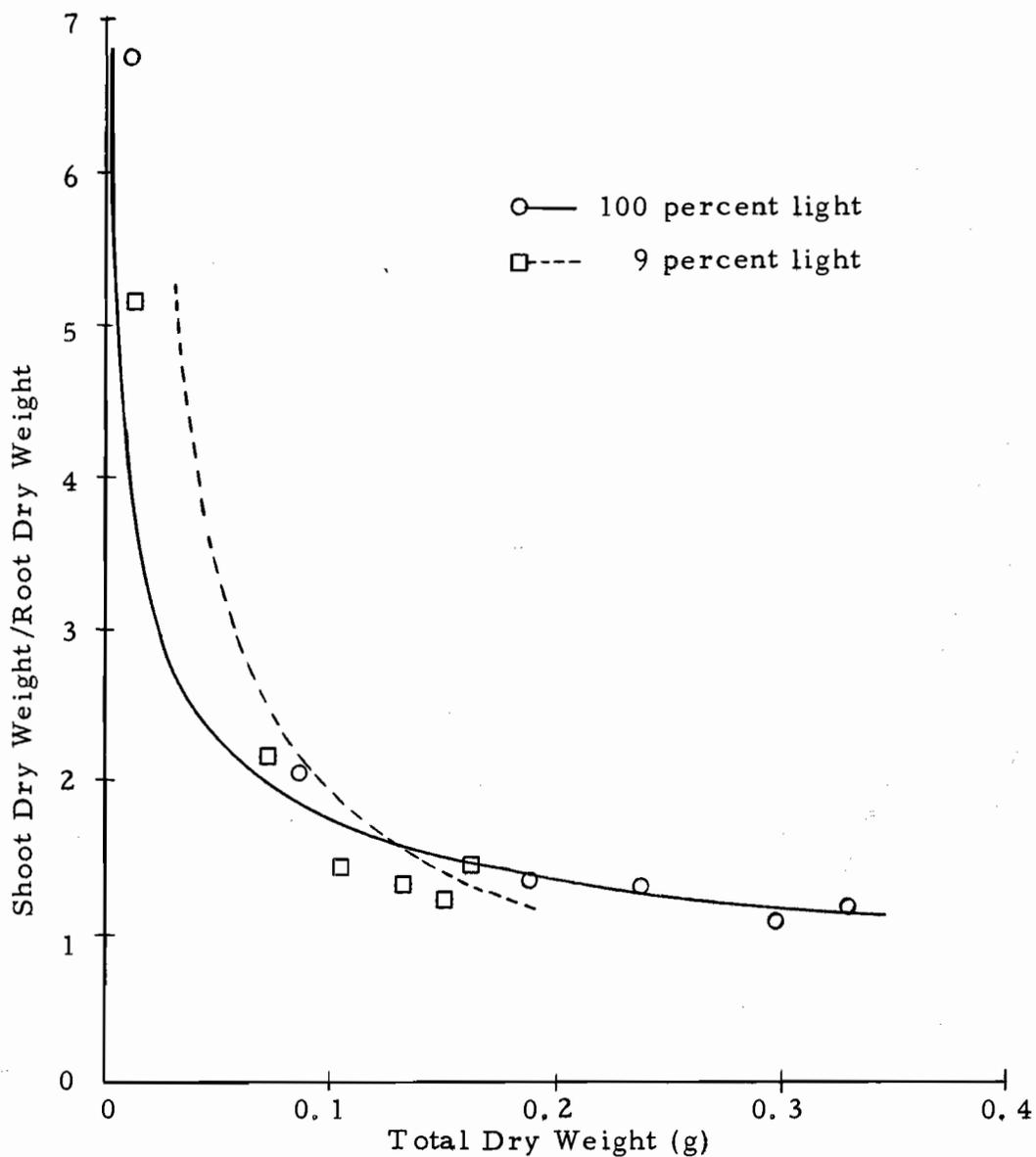


Figure 3. Decline in shoot/root ratio with size of plant for 100 percent and 9 percent light-grown seedlings. Each point is the mean of six plants.

Table 2. Variation in slope, k , of the allometric equation, $\log Y = a + k \cdot \log X$ with level of light preconditioning and year for different combinations of organs in Douglas-fir. Shoot, needle, and stem dry weights are represented as a function of root dry weight.

Comparison		Light Preconditioning			F - Tests of Slopes	
		100 percent	44 percent	9 percent	Comparison	Significance
Y	X					
<u>1971</u>						
<u>Shoot</u>	vs. <u>Root</u>	<u>0.703</u>	<u>0.978</u>	<u>1.014</u>	100% vs. 44%	**
Needle	vs. Root	0.702	0.915	1.064	100% vs. 44%	*
					100% vs. 9%	NS
Stem	vs. Root	0.853	1.146	0.916		
<u>1972</u>						
<u>Shoot</u>	vs. <u>Root</u>	<u>0.709</u>	<u>0.585</u>	<u>0.483</u>	100% vs. 44%	*
					100% vs. 9%	NS
Needle	vs. Root	0.586	0.556	0.444	100% vs. 44%	NS
					vs. 9%	
Stem	vs. Root	1.011	0.648	0.607	100% vs. 44%	**

* Significant at 5 percent level

** Significant at 1 percent level

that for these seedlings the shoot/root ratio was increasing slightly over time as the seedling grew larger. The complete allometric equations for all treatments and combinations of organs for both years appear in Appendix Table C.

Whether some of these differences were statistically significant or not was only partially determined. In a test of significance of different allometric coefficients, one of the assumptions is that the sample variances are homogeneous (Snedecor and Cochran, 1967). This assumption was tested at the 5 percent level for the various treatment-year interactions and in some cases was found invalid. In those cases where the assumption held, F-tests of the slopes were performed and the results shown in Table 2.

The contributions of needle and stem growth to total shoot development are also shown in Table 2. In 1971, an increase in light from 44 to 100 percent resulted in greater root growth at the expense of needle growth (significant) and stem growth (untested). In 1972, the same transition increased shoot growth rate due to more allocation of dry matter to stem than to needles. Although not significant, the change from 9 to 44 percent light appeared to increase rate of needle growth more than stem growth.

The non-homogeneity of treatment variances seemed related to size of the seedling. Without exception, the variance about the individual regressions was greater where total seedling dry weight as

recorded in Table 1 was greater. Thus it seems that some of the statistical conclusions where differences were not significant may be unreliable and due to differences in sample variance. In the case of the comparison for 1972 of shoot dry weight vs. root dry weight for 100, 44, and 9 percent light treatments, the 100 vs. 44 percent comparison showed significant differences, but the 100 vs. 9 percent comparison revealed none, a conclusion at odds with ranking of means. In this case, the 100 and 9 percent treatments had homogeneous variances at the 0.95 probability level, but not at the 0.93 level. The F-test of slopes was justified on the basis of testing at the conventional 5 percent significance level, but a strong possibility exists that non-homogeneity of variances influenced the F-test, resulting in a type II statistical error. In other words, the slopes of 100 and 9 percent treatments may in reality have been different, but were judged the same out of unequal variances. Thus trends in the data may be more important than statistical conclusions based on "homogeneous" variances.

Phenology

Seedlings of the older age group exhibited phenological variation during their second growing season which was related to first-year preconditioning. Those plants which were given 9 percent light the first year broke terminal buds earliest the following spring

(Figure 4) followed by 44 percent light-grown ones. This difference in timing of emergence of new growth during 1972 was observed on seedlings that had been under full light since the previous fall. An interesting observation was also made just prior to that time.

On July 28, 1971, 80 percent of the seedlings under the 9 percent light treatment had ceased shoot growth and set a terminal bud. For the median light treatment observed at the same time this figure was 57 percent and for the full light-grown, 43 percent. Thus a direct correlation was apparent, i. e., those seedlings that had terminated shoot growth earliest the previous summer broke bud earliest the next spring. These seedlings also suffered more spring frost damage as indicated in Figure 4.

When the same plants, still under full light conditions but now somewhat decreased in numbers from sampling, were again monitored for timing of bud break the next spring, i. e., 1973, no pronounced differences were apparent (Figure 5). As bud break occurred later for these plants in 1973 than it had in 1972 no frost damage as judged by dieback of new needles was evident in any of the treatments.

After initial observation of a correlation in time between fall bud set and spring, 1972 bud break, the same relationship was noted again on other seedlings. During late 1972, percent bud set was assessed at three different times for first-year seedlings under two treatments, 9 and 44 percent light. Again the low light-treated plants

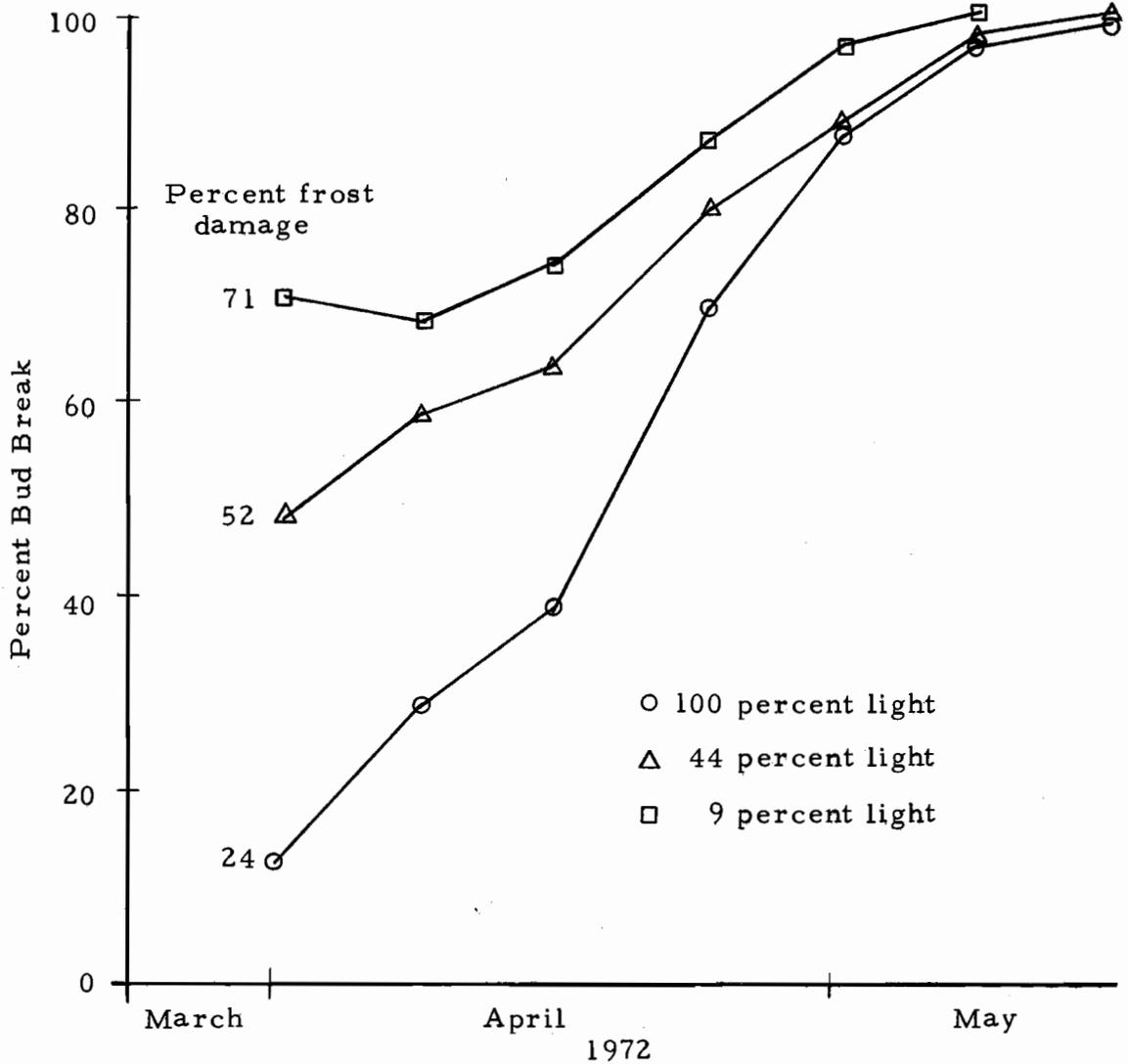


Figure 4. Timing of 1972 bud break on seedlings preconditioned to varying light regimes during 1971 showing amount of 1972 frost damage. Percentages are based upon proportion of total plants having green foliage visible through terminal bud scales.

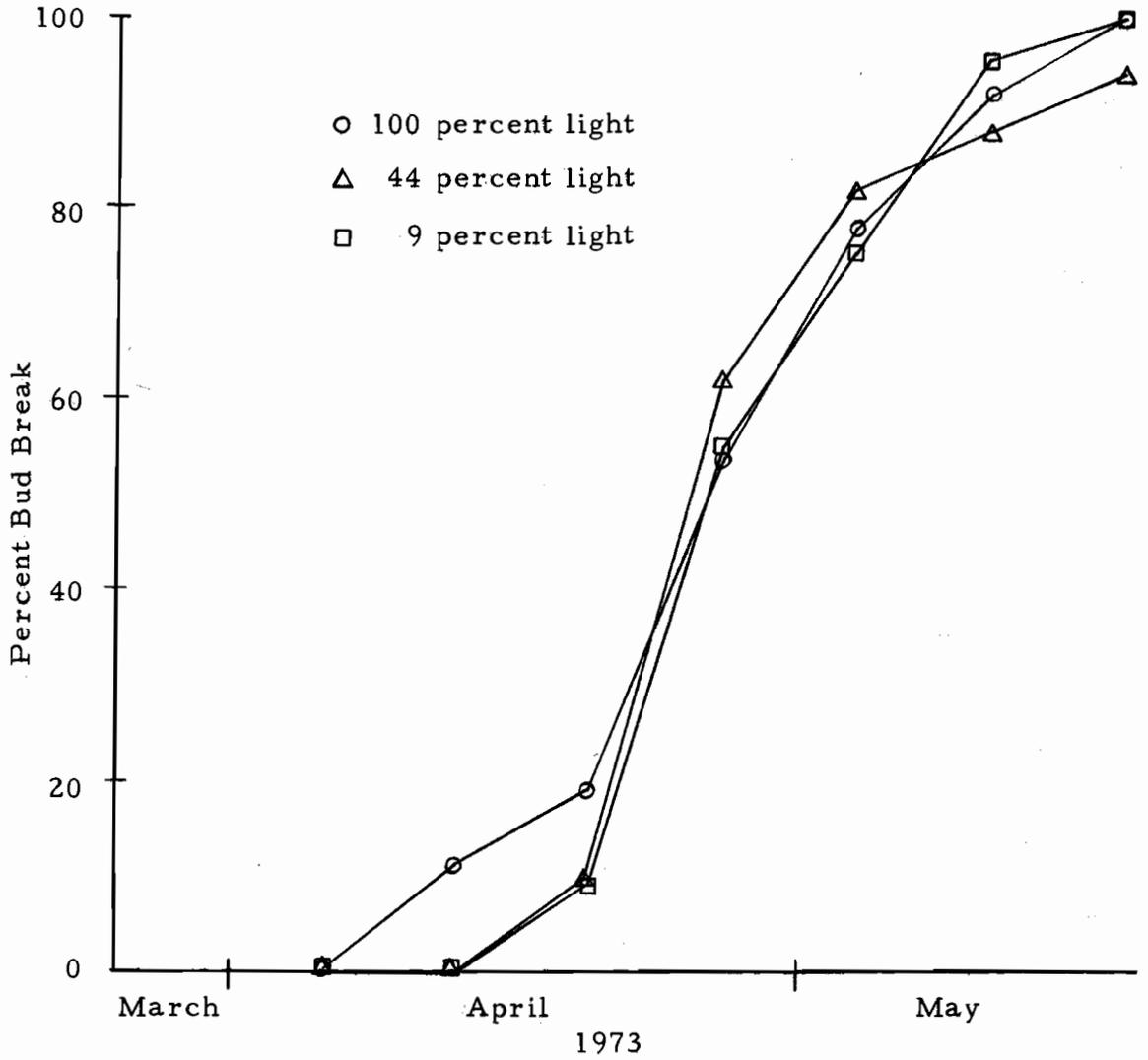


Figure 5. Timing of 1973 bud break on seedlings preconditioned to varying light regimes during 1971.

set bud before the other group. The next spring, bud break on the former preceded that of the latter by about one week.

Seedlings of the treatments shown in Figure 4 also exhibited varying degrees of lammas growth during the 1972 season. Of those grown under 9 percent light the first season, 45 percent had second-flushed by July 1. For the 44 and 100 percent light treatments, these figures were 49 and 24 percent, respectively. Later assessment revealed little change. Thus the previously shaded seedlings were about twice as likely to second-flush as those given a full light pre-treatment.

Seedling Water Relations

First-year Behavior

Water relations of Douglas-fir seedlings were found to vary with light treatment and season of the year. In the case of first-year seedlings all water relations data for fall and winter periods were collected on plants grown during 1971. Summer data, however, consisted of a combination of data collected during 1971 and 1972 (Table 3). As seedling growth differed markedly between the two years, it was necessary to ascertain whether or not summer water relations behavior also varied with year.

The multiple regression model based on the relation, $PMS = f(SMS, L_C)$, was constructed for those summer treatment-year

Table 3. Sample sizes for water relations analysis. All data on first-year seedlings were collected during 1971 except where otherwise indicated.

Season	Light Treatment		
	100%	44%	9%
	<u>First-year Seedlings</u>		
Summer	1971 - 11	1971 - 5	1971 - 4
	1972 - 8	1972 - 8	1972 - 8
Fall	12	18	18
Winter	16	15	17
	<u>Second-year Seedlings</u>		
Summer	-	-	-
Fall	13	-	12
Winter	12	-	5

comparisons which were based on a sample size greater than six, the number of parameters in the model. Appropriate comparisons revealed no differences between 1971 and 1972-grown plants under 100 percent light, but differences at the 5 percent level for a comparison of combined data from 9 and 44 percent light treatments compared between years. The treatments could not be tested individually for variation due to year as sample sizes for the 44 percent treatments of five and four, respectively, were less than the number of parameters in the regression model.

Additionally, the summer data were examined for variation due to treatment. A comparison of all 1972 data revealed no differences between treatments. As full light-grown plants did not significantly differ in their water balance between years, these combined data were compared to combined data for 9 percent light-grown plants. Again, no significant differences were present.

With no significant variation due to treatment in the summer data and only limited evidence for variation due to year, combining all the data to give a sample size of 44 representative of a summer response seemed justified. The resulting response surface of the equation $PMS = f(SMS, L_c)$ is shown in Figure 6.

On this surface the data points lie roughly along a diagonal between "corners" representing low PMS, low SMS, high L_c , and high PMS, high SMS, low L_c . For the seedlings upon which the model

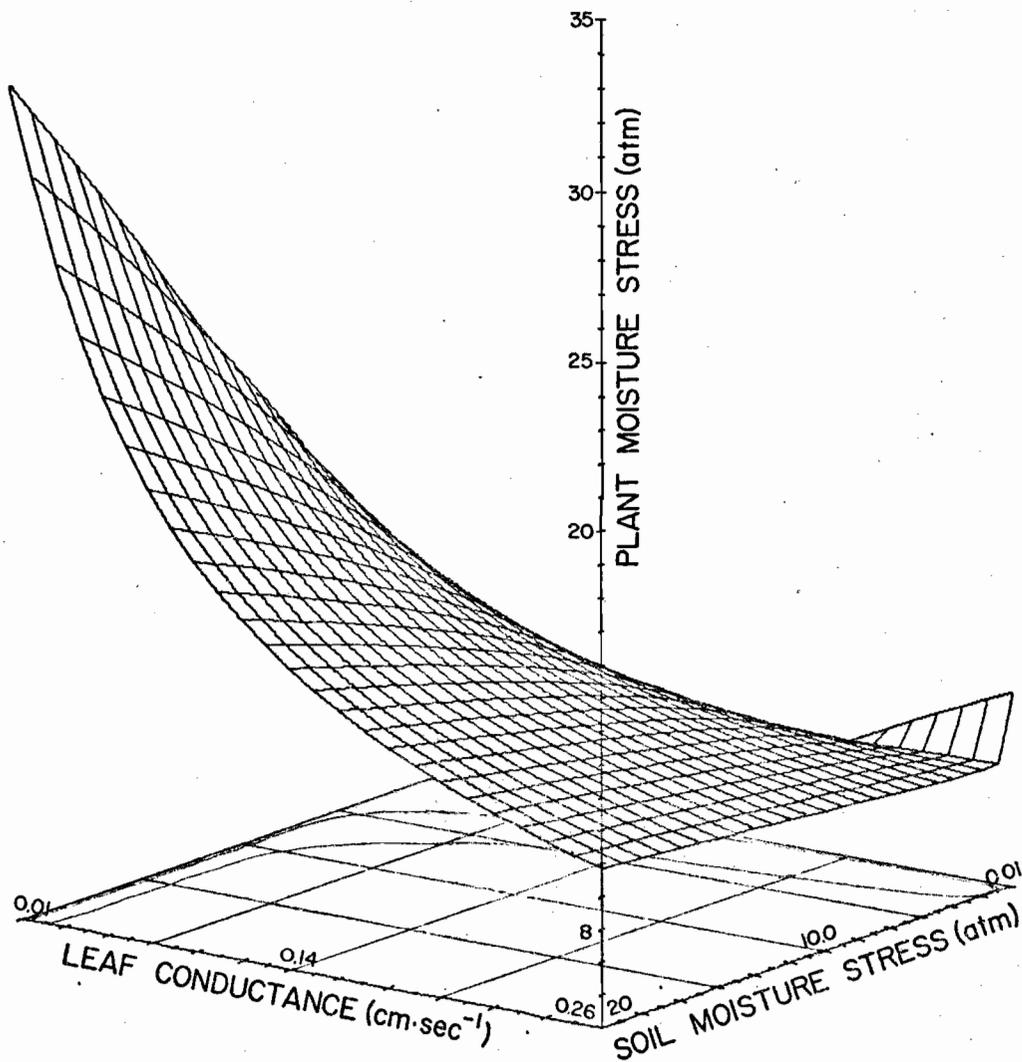


Figure 6. Trivariate response surface representing summer behavior of Douglas-fir seedlings based on the relationship, $PMS = f(SMS, L_c)$. The equation for the surface is: $\log PMS = 0.838 - 0.259 \log SMS - 0.291 \log L_c + 0.018 (\log SMS)^2 - 0.217 (\log L_c)^2 - 0.418 (\log SMS \times \log L_c)$. The projection of the data field is shown on the horizontal plane of 5 atmospheres PMS.

is based this would reflect the behavior during soil moisture depletion. That is, as soil moisture stress increases, the moisture stress in the plant also rises and leaf conductance falls due to stomatal closure.

For seedlings in a state of active summer shoot growth, drying of the soil results initially in a slow response in PMS to around 10 atmospheres and later a more rapid change as soil stresses rise to 20 atmospheres. This is apparent from differences in slope of the surface at high and low PMS. Leaf conductance declines rapidly beginning at around 10 atmospheres PMS; later the decline is much slower as PMS approaches 30 atmospheres. The "tail" at low PMS, SMS and high L_c is probably not an artifact as three data points lie in this region of the surface. It may represent increased PMS due to restricted water uptake below 0.3 atmospheres SMS.

During the fall after shoot growth has ceased and terminal buds formed, a different response was observed (Figure 7). Most notable are the higher levels of moisture stress in seedlings at medium and high values of leaf conductance. During soil drying, PMS initially rises rapidly from around 12 atmospheres then tapers off more at higher stresses as leaf conductances decline markedly. For any level of SMS, PMS responds to an abrupt change in L_c . Leaf conductance declined more gradually in the summer. Figure 8 further simplifies interpretation of the two surfaces.

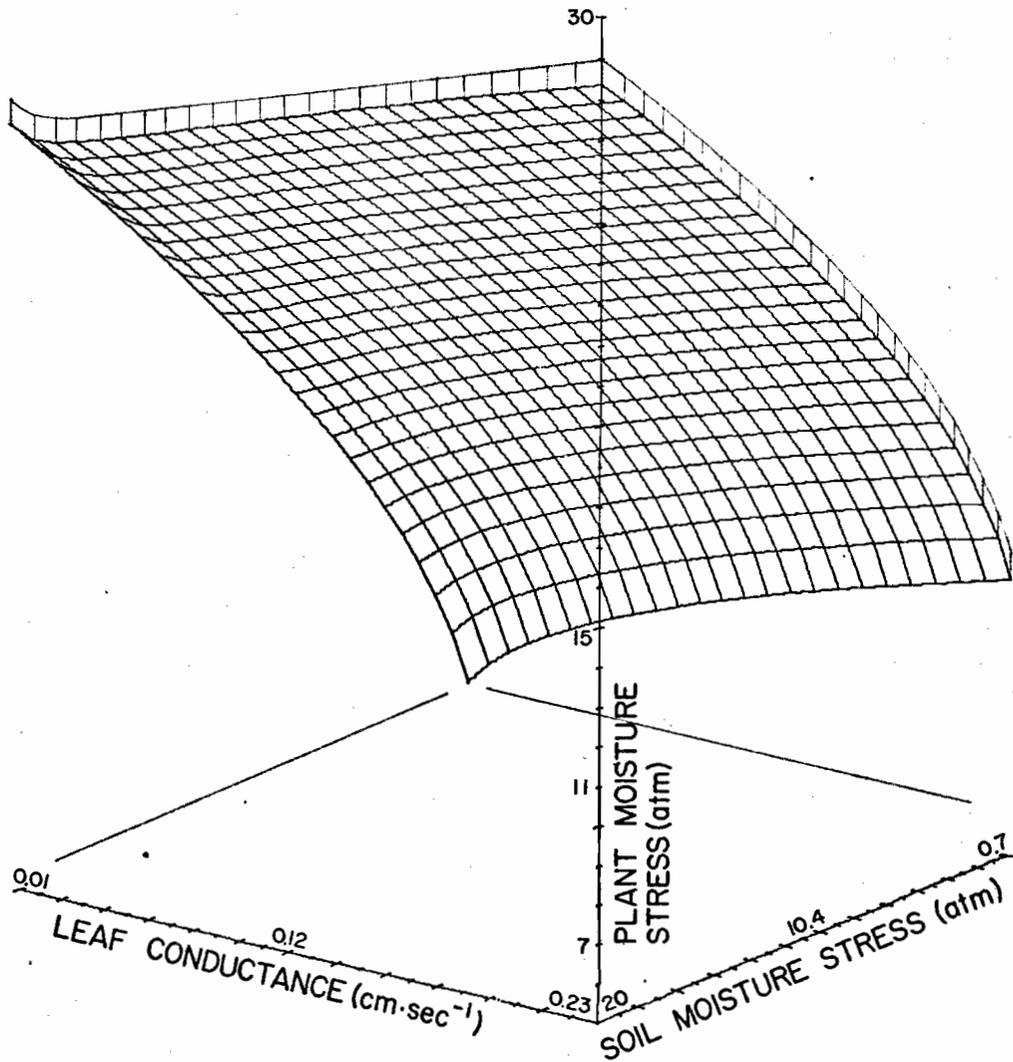


Figure 7. Trivariate response surface representing fall behavior of Douglas-fir seedlings based on the relationship, $PMS = f(SMS, L_c)$. The equation for the surface is: $\log PMS = 1.290 + 0.054 \log SMS + 0.253 \log L_c + 0.087 (\log SMS)^2 + 0.036 (\log L_c)^2 - 0.110 (\log SMS \times \log L_c)$. (It may be noted that this surface has depth. This is in fact not the case; extra lines have been drawn to give this effect for purposes of visual clarity.)

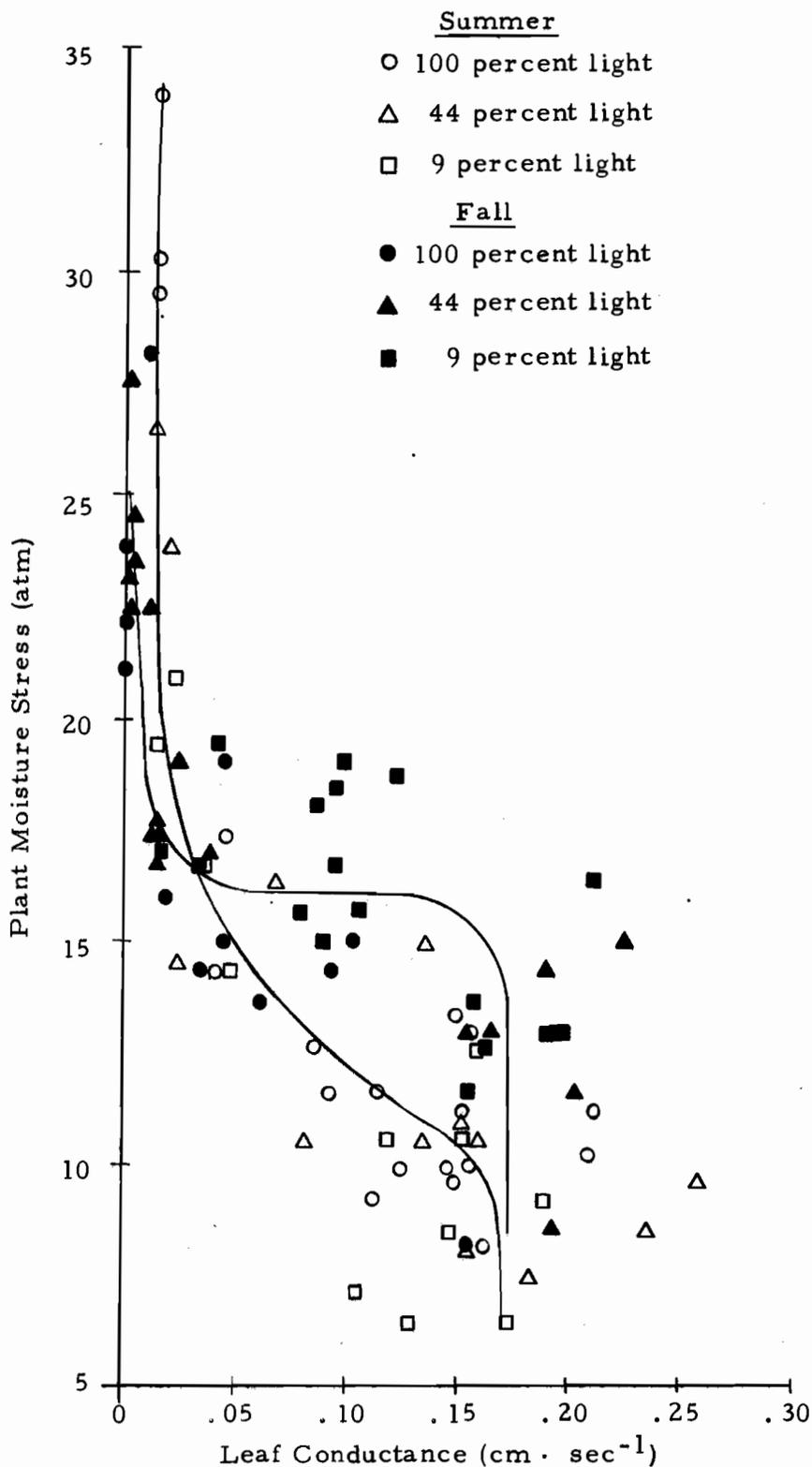


Figure 8. Leaf conductance as a function of plant moisture stress of Douglas-fir seedlings grown under three light treatments measured during fall and summer.

During the summer, plant moisture stress increases gradually between 10 and 15 atmospheres while during the fall this decline is rapid until about 16-17 atmospheres. The changes are presumably due to differential stomatal control. Then, at low leaf conductances where the curves cross, and beyond, plant moisture stress continues to increase at a faster rate for summer than for fall seedlings. On the response surfaces at low leaf conductances and high SMS, it is evident that PMS in the summer at any value of L_c below about $0.03 \text{ cm} \cdot \text{sec}^{-1}$ is higher than during the fall. The surface "tails off" at a higher PMS (ca. 30 atm) in the summer than the fall (ca. 25 atm) reflecting a slower decline in L_c during the time of active shoot growth.

Figure 7 is based on combining all fall treatments together to give a total sample size of 48 seedlings. This was not justified on the basis of there being no differences between treatments as significant differences were found (Appendix Table B), but rather from the point of view that similar seasonal variations were reflected in each treatment, this being most obvious for the low light-grown plants. By combining the data, a more complete model was obtained than if any one treatment in itself had been used. The sample size of 48 so obtained was close to 44 for the summer model based on combined treatments and years.

Highly significant differences at the 0.99 level of probability were found between summer and fall models (Appendix Table A). This

due to the following factors, each statistically significant: 1) inequality of intercepts, i. e., $B_{o_{summer}} \neq B_{o_{fall}}$, 2) differences in effect of SMS upon PMS, and 3) a different SMS \times L_x interactive effect upon PMS.

Additional insight into summer behavior was gained through diurnal sampling of plant moisture stress on well-watered seedlings with active shoot growth under treatment. Figure 9 reveals the nature of changes observed on July 29, 1972, a hot, clear summer day in which air temperatures reached a high of 36°C. PMS recorded as a mean of two seedlings rose from low pre-dawn levels to a peak in the morning which varied between treatments, then declined throughout the remainder of the day. The highest stressed seedlings were those of the 100 percent light treatment. These reached a maximum PMS of 14.1 atmospheres measured at 8:10 AM. Throughout the mid-portion of the day stresses in all seedlings remained below 11 atmospheres.

In order to clarify the basis for such changes in daytime PMS, leaf conductance measured with a diffusion porometer was monitored simultaneously with PMS on August 13, 1972. Figures 10 and 11 indicate that the maximum early morning PMS coincided in time with a strong increase in leaf conductance. This would indicate that probably opening of stomata in the morning accompanied by transpiratory loss allowed PMS to rise to a high level. Closure of stomata then

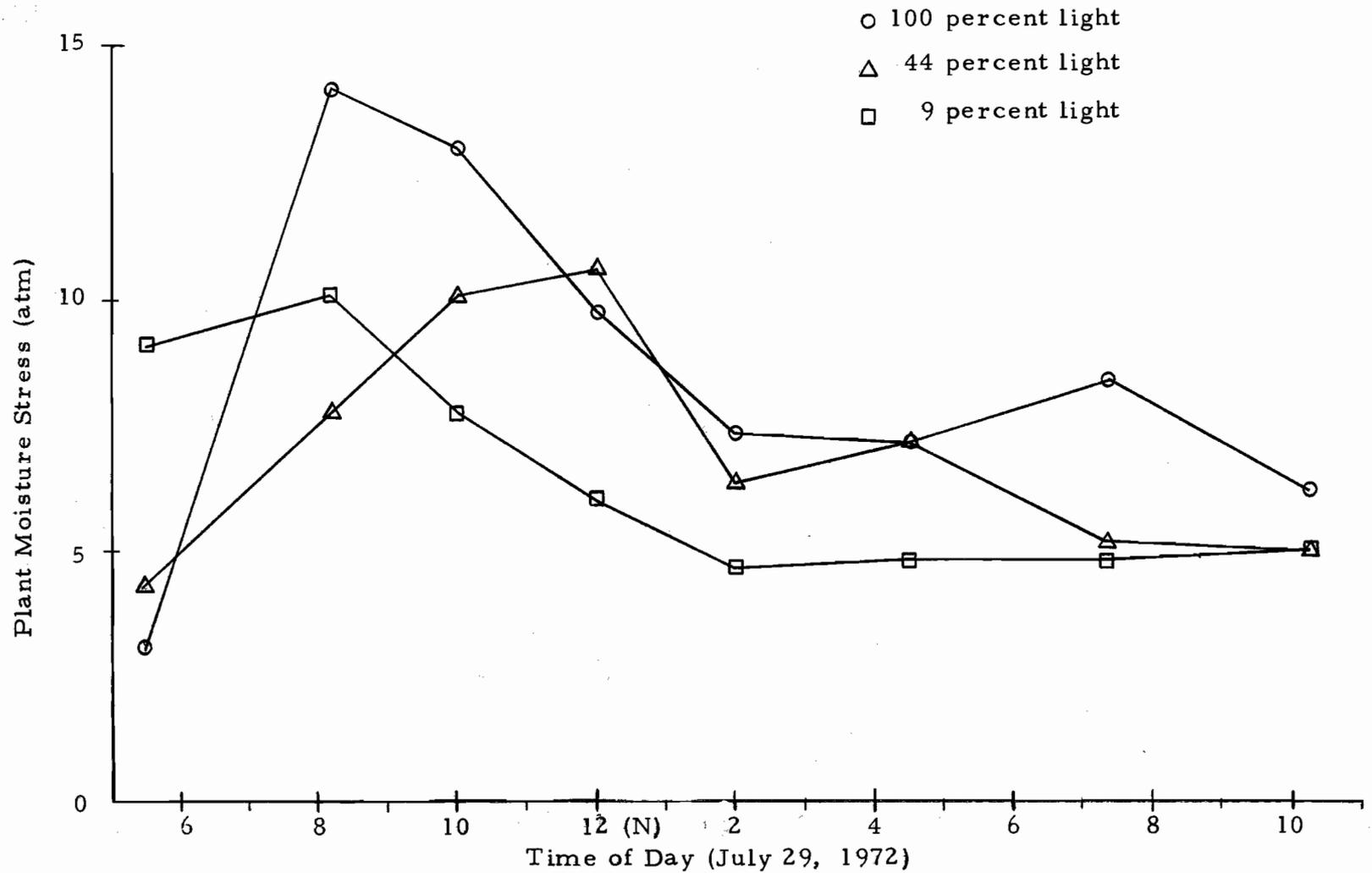


Figure 9. Variation in plant moisture stress with time of day on July 29, 1972 for Douglas-fir seedlings growing under three light regimes.

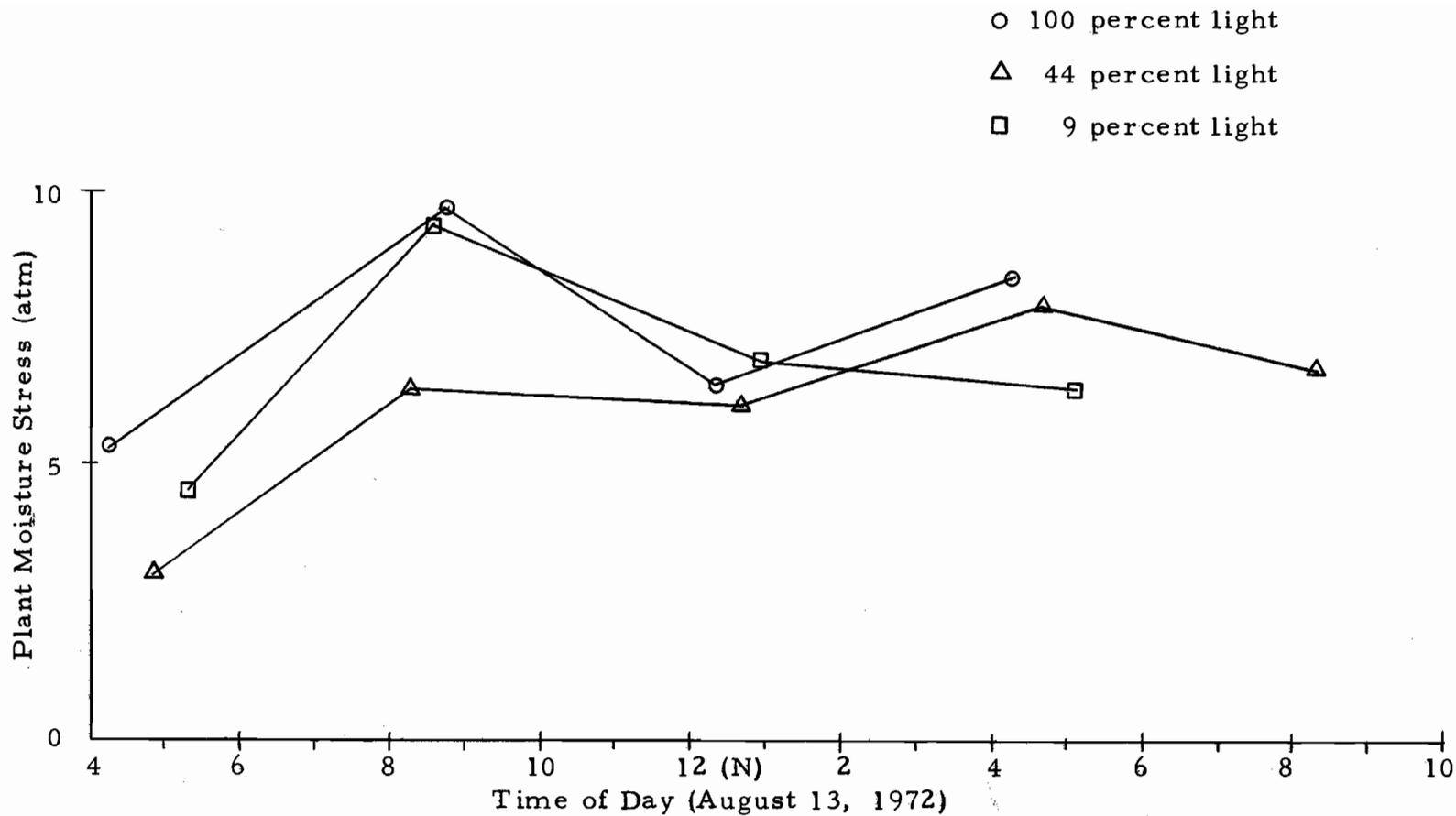


Figure 10. Variation in plant moisture stress with time of day on August 13, 1972 for Douglas-fir seedlings growing under three light regimes.

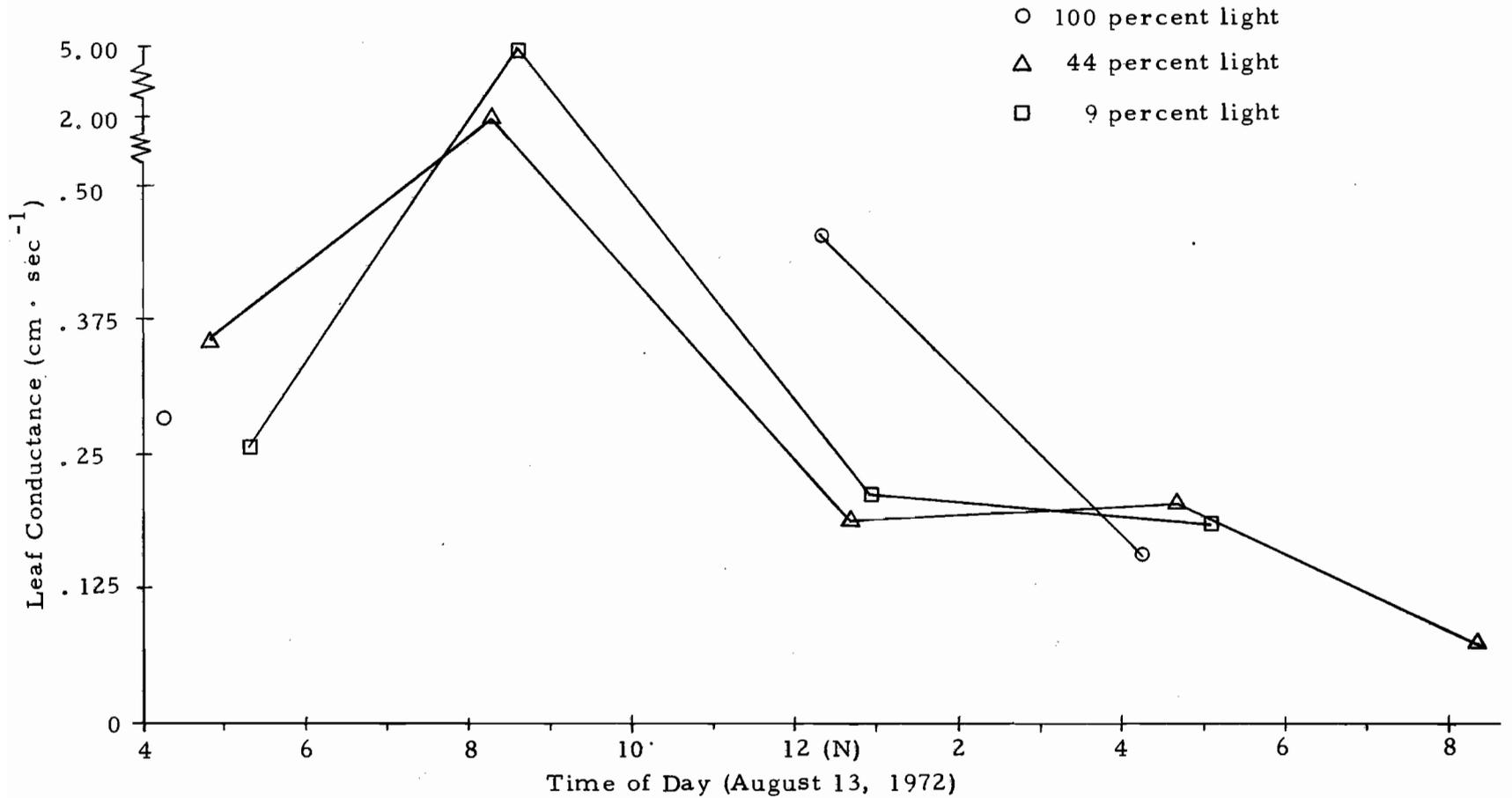


Figure 11. Variation in leaf conductance with time of day on August 13, 1972 for Douglas-fir seedlings growing under three light regimes.

lowered the stress in the plant. August 13 was cooler than July 29 with a maximum temperature of 25°C , but even so a morning peak in PMS was observed. This occurred at about 10 atmospheres. Diurnal monitoring of PMS on two other summer days indicated that mid-day maxima were usually around 10 atmospheres, but not higher than 12.

The morning leaf conductances above $0.25 \text{ cm} \cdot \text{sec}^{-1}$ are somewhat in doubt. The first seedling sampled from the full light treatment was accidentally measured before it was realized that the plant was slightly moist from dew. Thus this treatment was not sampled again until noon when the moisture had evaporated. It is likely that some small water droplets from this first plant remained in the porometer for several hours and affected subsequent readings, making them slightly higher than true values. Leaf conductances for Douglas-fir above $0.25 \text{ cm} \cdot \text{sec}^{-1}$ are suspect for this reason and additionally for the reason that leaf conductances measured in the laboratory were rarely above $0.25 \text{ cm} \cdot \text{sec}^{-1}$. However, the upward trend between first and second sampling periods is likely real as later measurements would be less affected by an initial source of error of this type.

These findings compare well with the response surface for summer behavior (Figures 6 and 8). Leaf conductance of plants measured in the laboratory declined at around 10 atmospheres PMS. "Field data" indicates that stomatal closure as interpreted from diurnal

leaf conductance changes prevented stresses in the seedling from increasing beyond 10 to 12 atmospheres except under exceptionally high evaporative stress conditions as those prevailing on July 29, 1972, when stresses reached 14 atmospheres. Thus the inferred stomatal response to increasing plant moisture stress in the summer may be assumed to be similar under laboratory and "field" conditions.

A breakdown of fall water relations behavior by treatment appears in Figures 12 and 13. Shown are the data for treatments found to be significantly different by testing of the regression model, viz., 100 and 9 percent light treatments (Appendix Table B). The 44 percent light treatment was not significantly different from either of the other two treatments.

Figure 12 shows the relation between soil and plant moisture stress. The seedlings grown under low light were at higher plant moisture stresses than those grown under full light conditions. The two treatments differed in this respect with a probability greater than 0.95.

Similarly, for any level of leaf conductance, the low light-grown plants were under greater stress than those grown under high light (Figure 13). This pattern in the water balance characteristic of seedlings in the fall was radically altered as air temperatures dropped in September and October with the onset of winter.

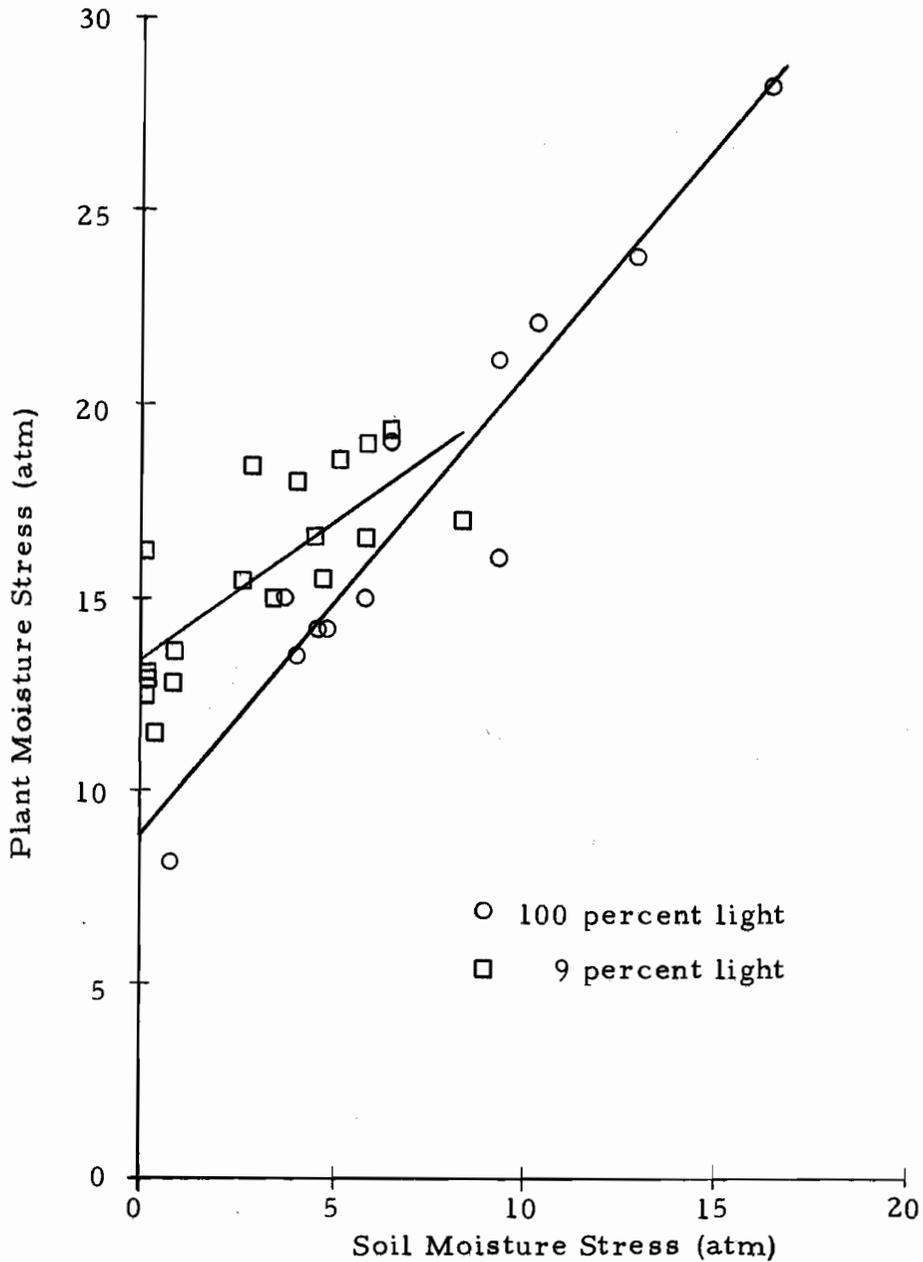


Figure 12. The fall response in plant moisture stress as a function of soil moisture stress for six-month-old Douglas-fir seedlings grown under 100 percent and 9 percent light.

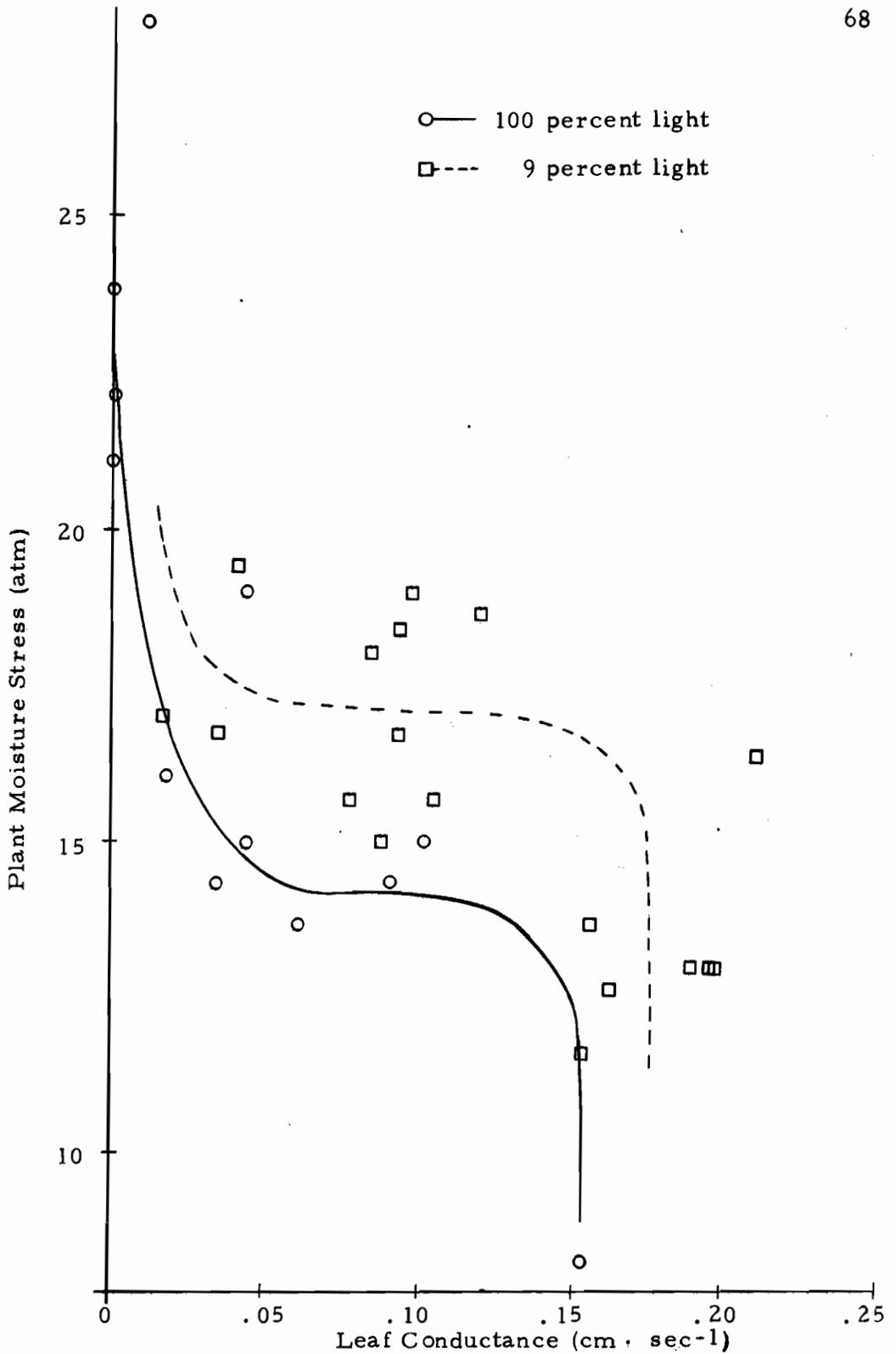


Figure 13. The fall response in leaf conductance as a function of plant moisture stress for six-month-old Douglas-fir seedlings grown under 100 percent and 9 percent light.

Figure 14 shows how transpiration rate at 22°C of a sample of four well-watered seedlings from the 100 percent light treatment was affected by low outdoor air temperature. As temperature fell, transpiration rate declined over a period from September 4 to December 21, 1972. This decline was most pronounced before October 30 and after November 22. In the interim time, minimum temperatures were somewhat constant around 5°C and there was little change in transpiration rate. The pronounced drop in transpiration on September 28 was probably related to the previous night's frost. This was the first occurrence of sub-freezing temperatures in 1972 and was reflected as well in transpiration rates of seedlings from other light treatments. Because of this apparently causal relationship of first sub-freezing temperatures, September 27 was taken to represent the end of the fall period and beginning of winter.

Figures 15 and 16 further portray changes in the water balance due to low temperature. In the former leaf conductance is shown to have dropped noticeably at levels of PMS where during the fall it had been high. For 9 percent light-grown plants, leaf conductances below 15 atmospheres PMS in the winter were mostly below 0.05 cm/sec. In the fall, they were above 0.15 cm/sec. About a five-fold drop in leaf conductance had taken place after October 24. Seedlings from other treatments showed a similar response.

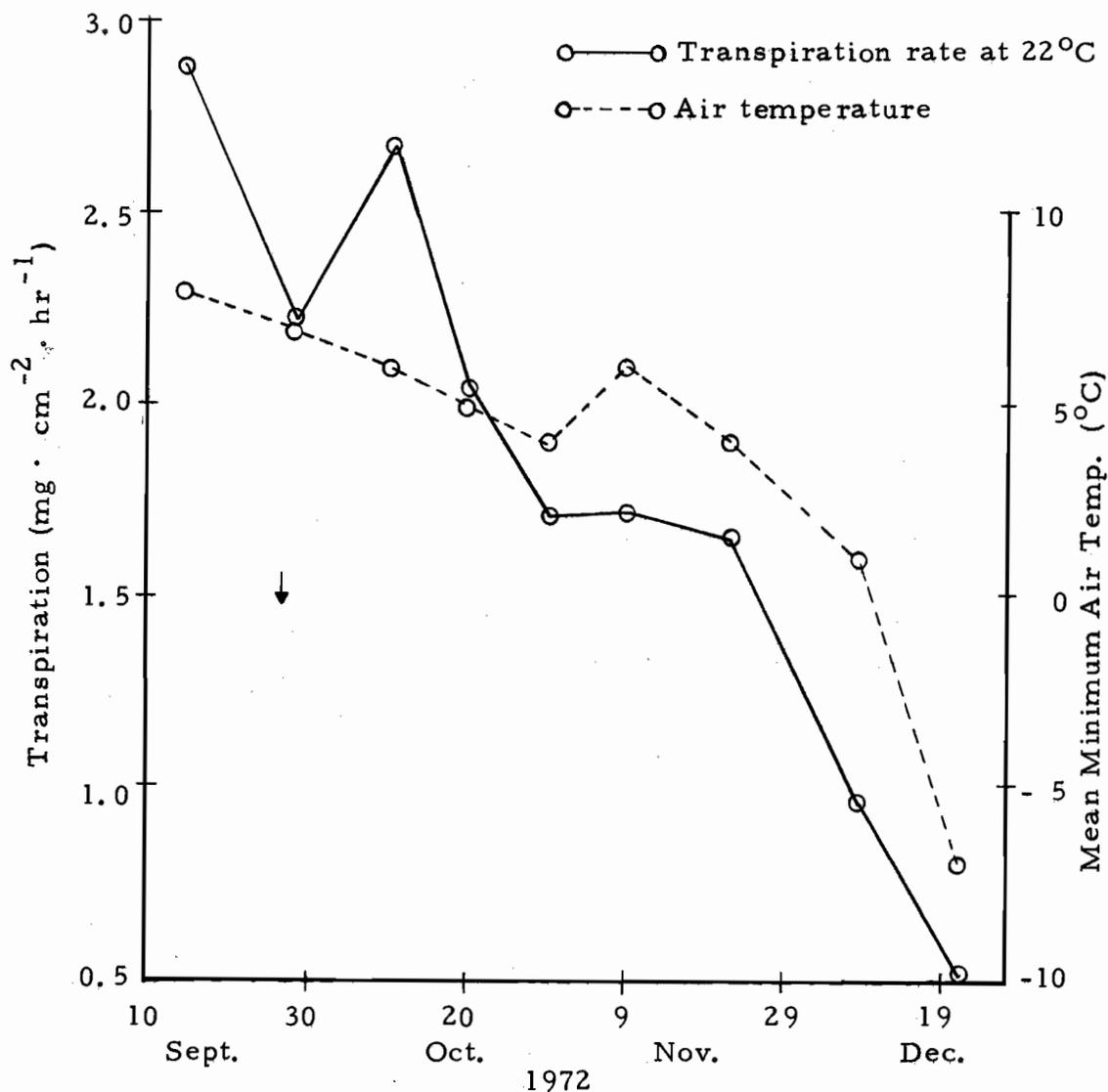


Figure 14. The decline in transpiration rate of Douglas-fir seedlings with low air temperature during the last four months of 1972. Air temperature is shown as the mean of daily minima over the preceding period. The arrow denotes the first frost of the year.

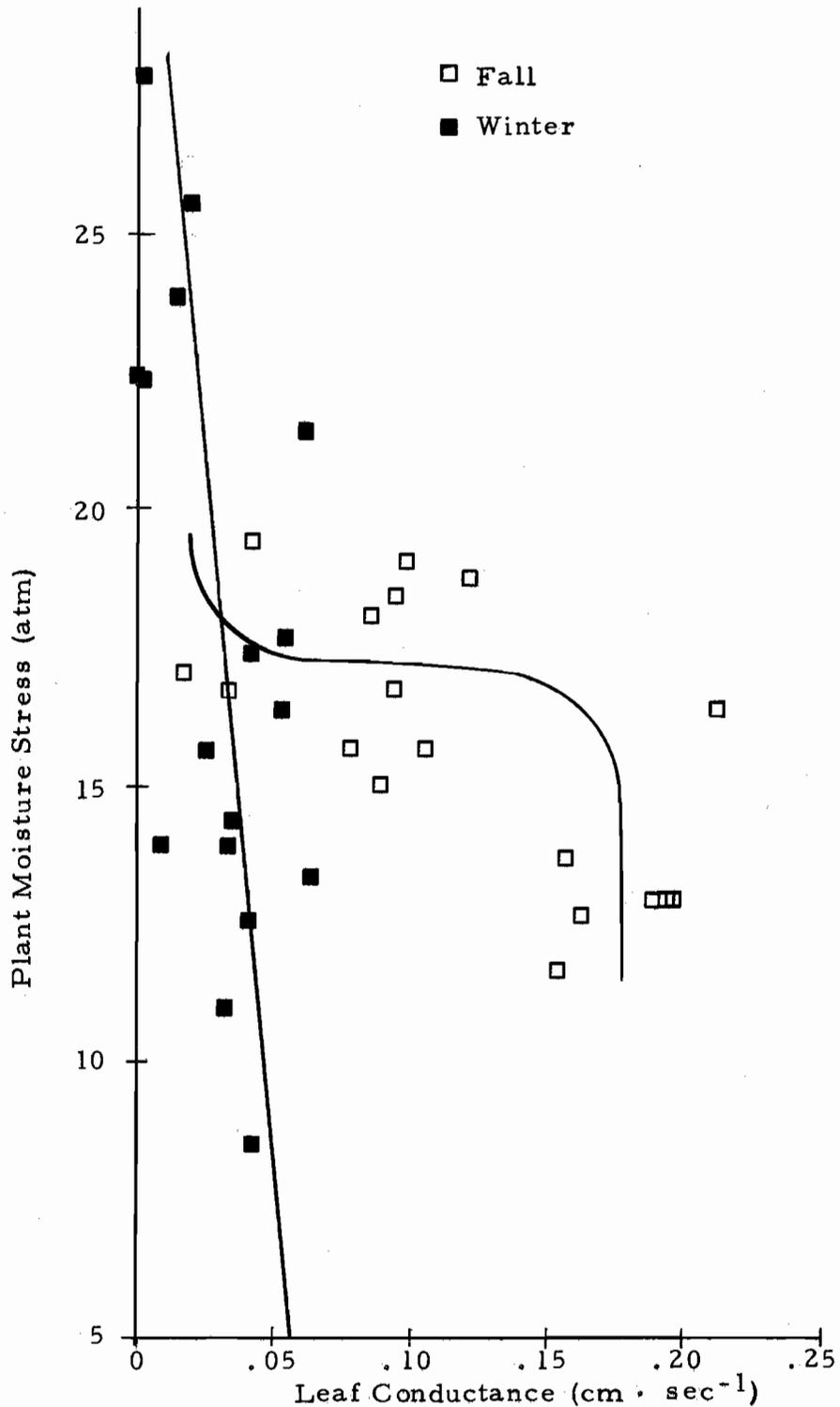


Figure 15. Fall and winter response of leaf conductance as a function of plant moisture stress for 9 percent light-grown Douglas-fir seedlings. The fall seedlings were ca. six months old and the winter, ca. eight months old.

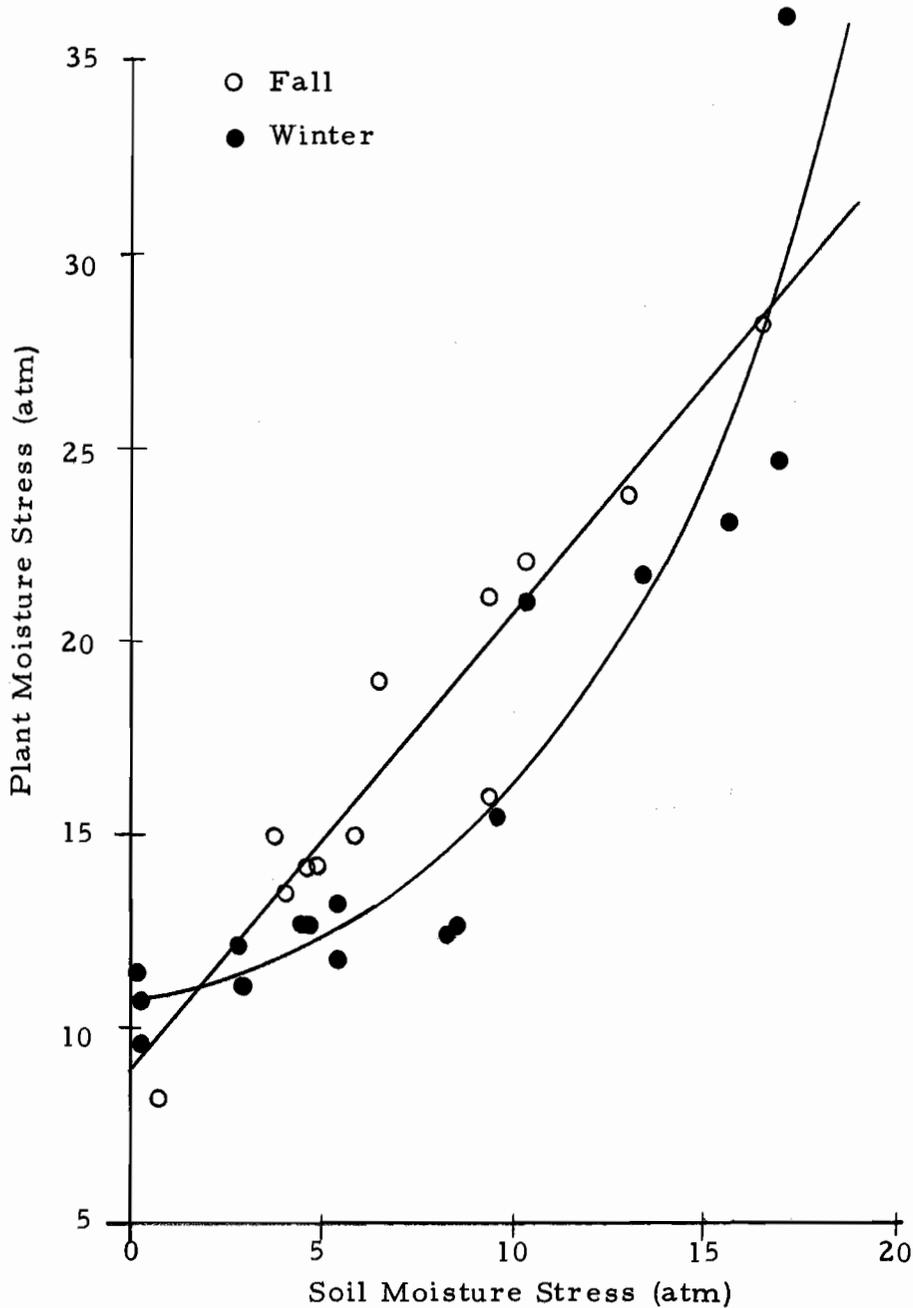


Figure 16. Fall and winter response of plant moisture stress as a function of soil moisture stress for 100 percent light-grown Douglas-fir seedlings. The fall seedlings were ca. six months old and the winter, ca. eight months old.

This difference in water loss was also reflected in plant moisture stress (Figure 16). Apparently related to increased water economy is a tendency for stresses in the seedlings in winter to be lower than in the fall over a wide range of soil moisture. This is shown for full light-grown plants, but was also true for the median treatment. A high amount of variation in the data for low light-grown plants obscured differences.

At low soil moisture stresses of one or two atmospheres, an anomaly was observed. Seedlings growing in soil at field capacity tended to be at a slightly higher PMS in the winter than in the fall. This was true even though leaf conductance was less indicating more closed stomata. At high SMS the same pattern was again present giving the winter relationship an exponential shape compared to the linear fall pattern. Although this difference at low soil stress in 100 percent light-grown plants may not be significant, the same trend also characterized the other two light treatments.

Since transpiration rates fell gradually over time after the first frost, the plants measured subsequently were in somewhat of a state of flux, but certain general interpretations of treatment variation were allowable. Figure 17 shows a comparison of 9 and 100 percent light treatments where the seedlings of the former are obviously under greater plant moisture stress than the latter over a wide range of soil moisture. The 44 percent light-treated plants lay intermediate.

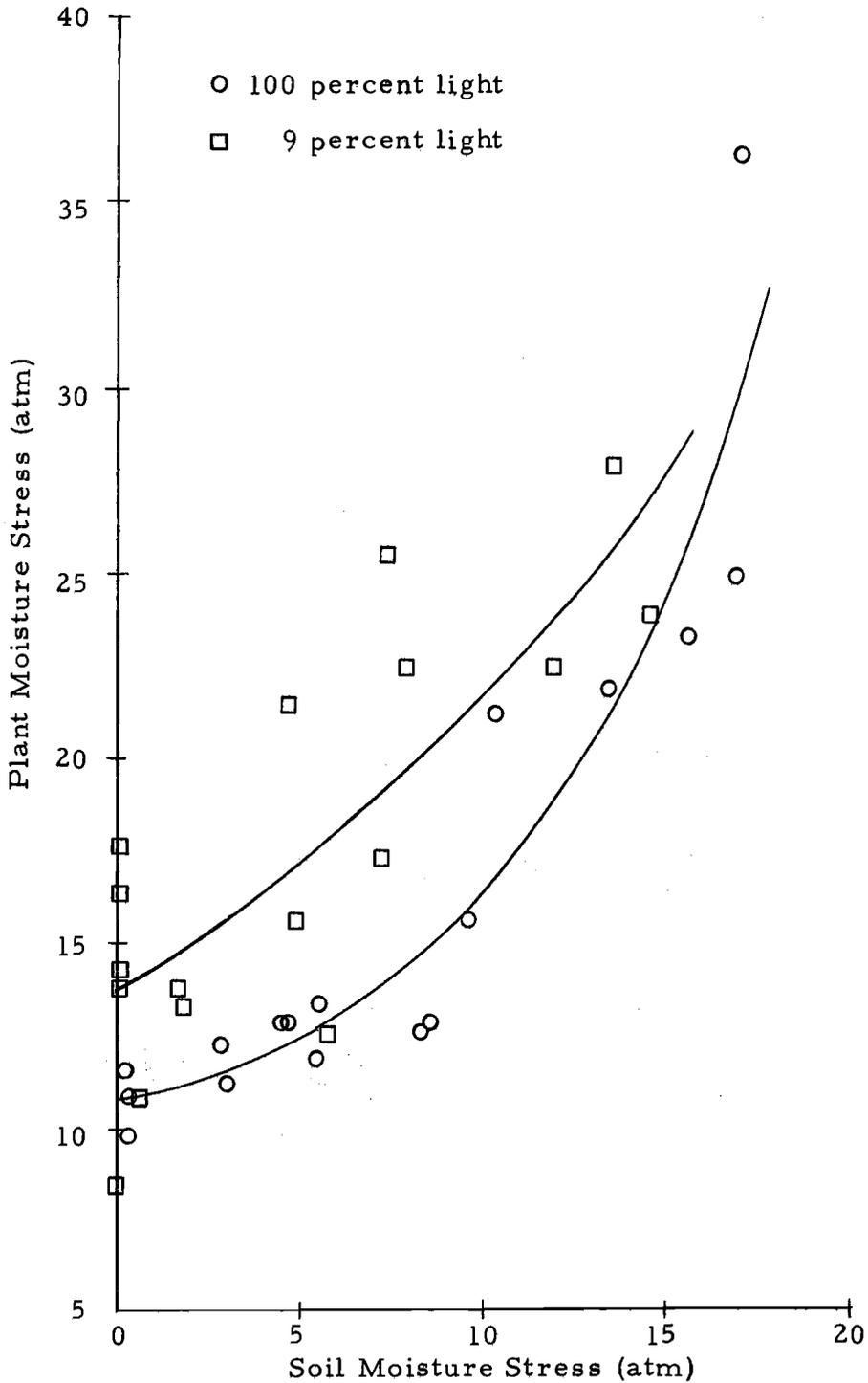


Figure 17. Winter response of plant moisture stress as a function of soil moisture stress for 100 percent and 9 percent light-grown Douglas-fir seedlings at an age of eight months.

Figure 18 shows this treatment along with some data collected during late February, 1972, just prior to bud break (Figure 4).

Again the exponential relationship between PMS and SMS is evident. It has been seen (Figure 16) that in the winter, PMS appears to be greater for seedlings in soil near field capacity than it is in the fall. Here, the collection of data points represented by seven seedlings indicates that in spring the reverse change may occur. That is, the high winter PMS at low SMS seems to drop to a lower level more similar to that of the previous fall. This group of seven seedlings had a mean leaf conductance of ca. $0.06 \text{ cm} \cdot \text{sec}^{-1}$, slightly higher than winter levels, but still not as high as off-recorded summer and fall conductances.

The winter pattern in leaf conductance with changes in plant moisture stress for two treatments appears in Figure 19. Data from the 44 percent light treatment again were intermediate. The seedlings grown under high light seemed to maintain lower plant moisture stress than those grown under low light at small leaf conductances.

Second-year Behavior

Seedlings which were preconditioned their first year and grown under a control regime for the second year exhibited differences which appeared related to their first-year light treatment. These seedlings upon assessment of fall and winter behavior showed a response similar

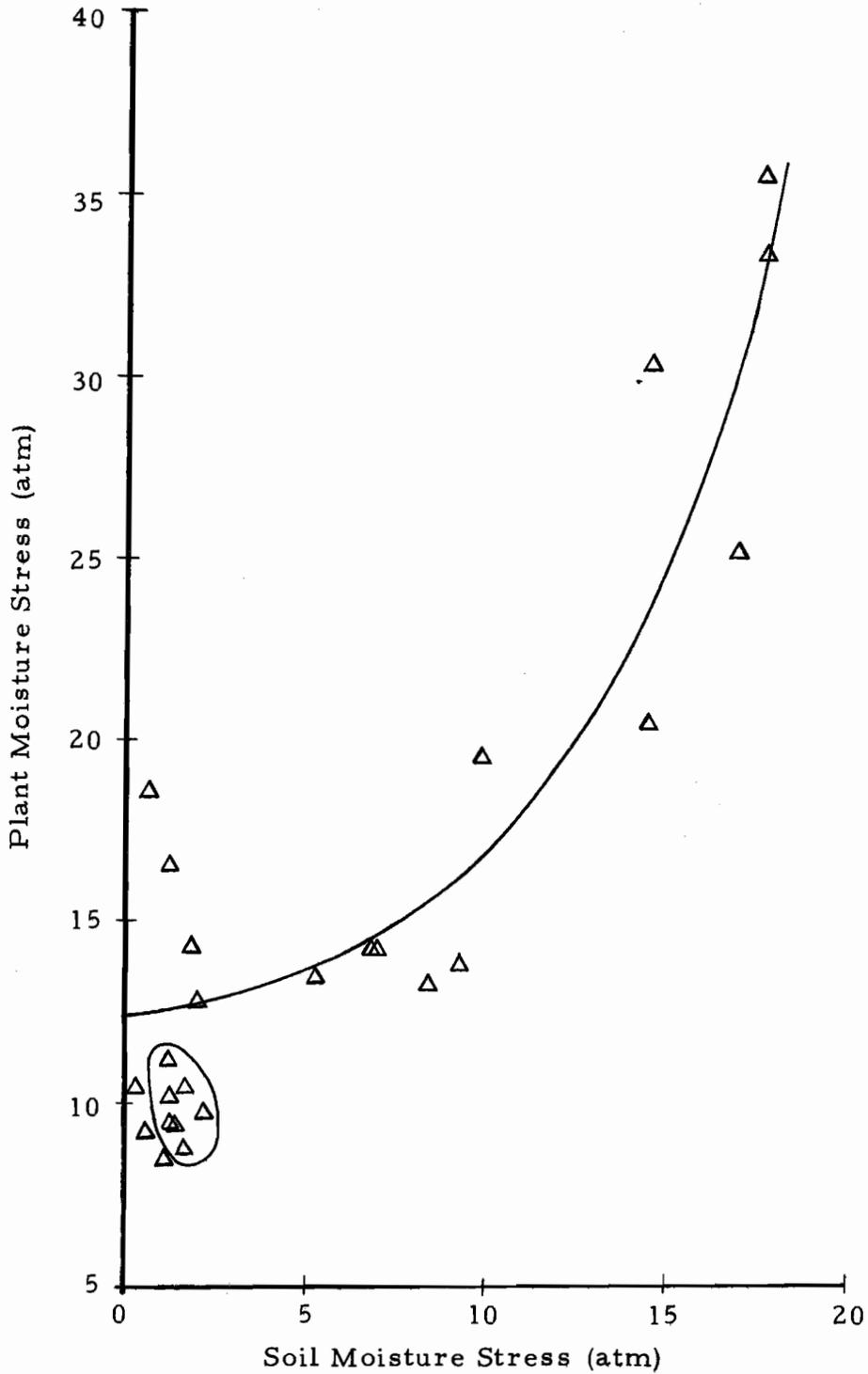


Figure 18. Winter response of plant moisture stress as a function of soil moisture stress for 44 percent light-grown Douglas-fir seedlings. Also shown is the spring response of seven seedlings.

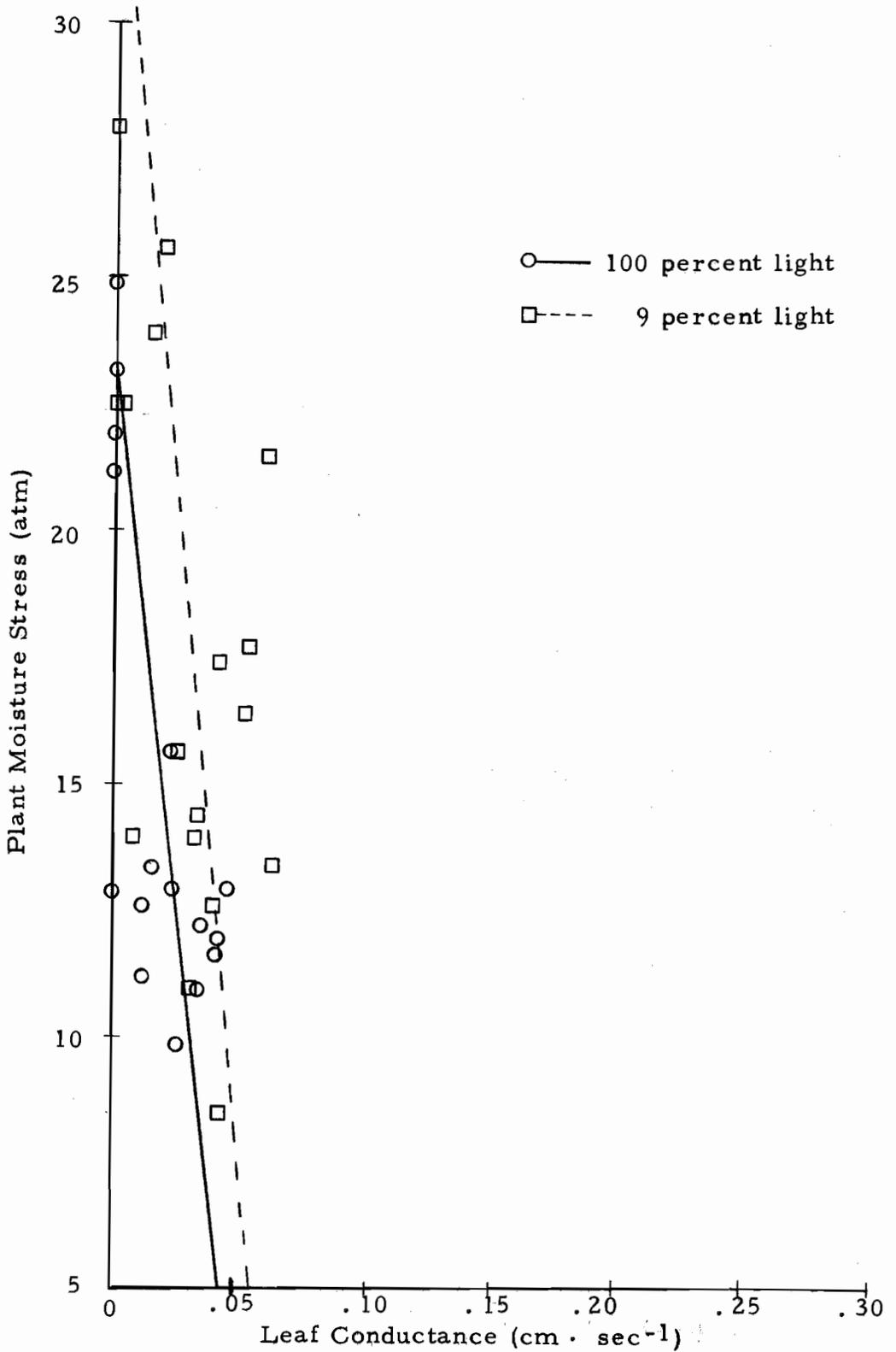


Figure 19. The winter response of leaf conductance as a function of plant moisture stress for 100 percent and 9 percent light-grown Douglas-fir seedlings at an age of eight months.

to that of first-year seedlings (Figures 20 and 21). Both high and low light-preconditioned plants showed the characteristic exponential trend in PMS with increasing SMS during winter. However, the higher plant stress in winter than fall at low SMS which appeared in younger seedlings was absent in these older plants. Also, reversal of the fall and winter trends under dry soil conditions occurred at a lower soil moisture stress in the second-year seedlings than it had in the first.

A comparison of the high with low light-preconditioned seedlings indicated that over a wide range of soil moisture stress the latter were under greater plant moisture stress than the former. A test of the overall model relating PMS to SMS and L_c revealed no significant differences between the two treatments. However, this may have been related to extreme variability in measurement of parameters on these older seedlings.

Examination of the relation between L_c and PMS (Figure 22) showed no likely differences except at moisture stresses below 20 atmospheres where the low light-preconditioned seedlings seemed to have lower leaf conductances than the other. The decline in leaf conductance in these older plants seemed to occur at a slightly higher plant moisture stress than it did in the younger seedlings.

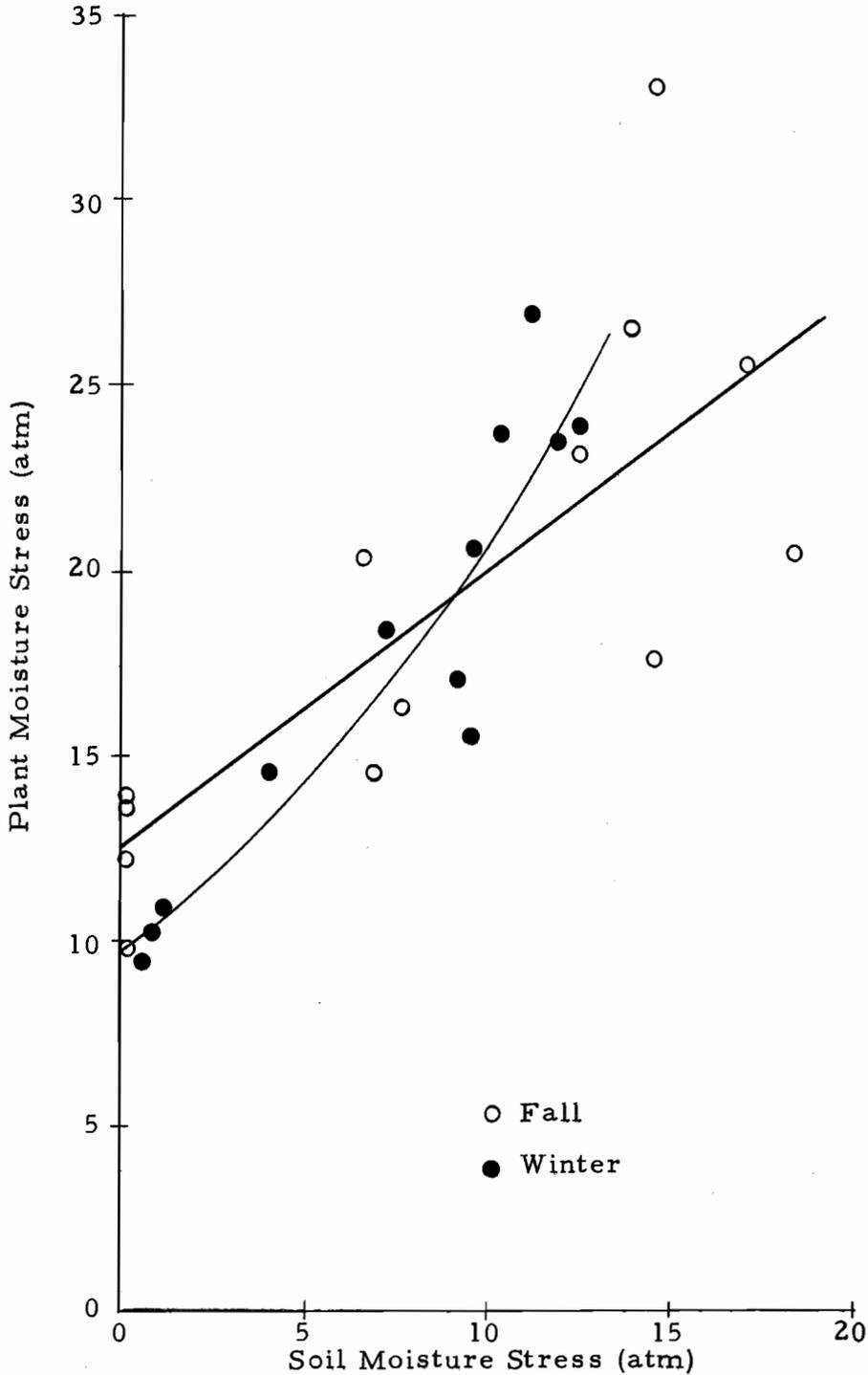


Figure 20. The fall and winter response of plant moisture stress as a function of soil moisture stress for two-year-old Douglas-fir seedlings preconditioned to 100 percent light their first and second growing seasons. The fall seedlings were ca. 20 months old and the winter, ca. 23 months old.

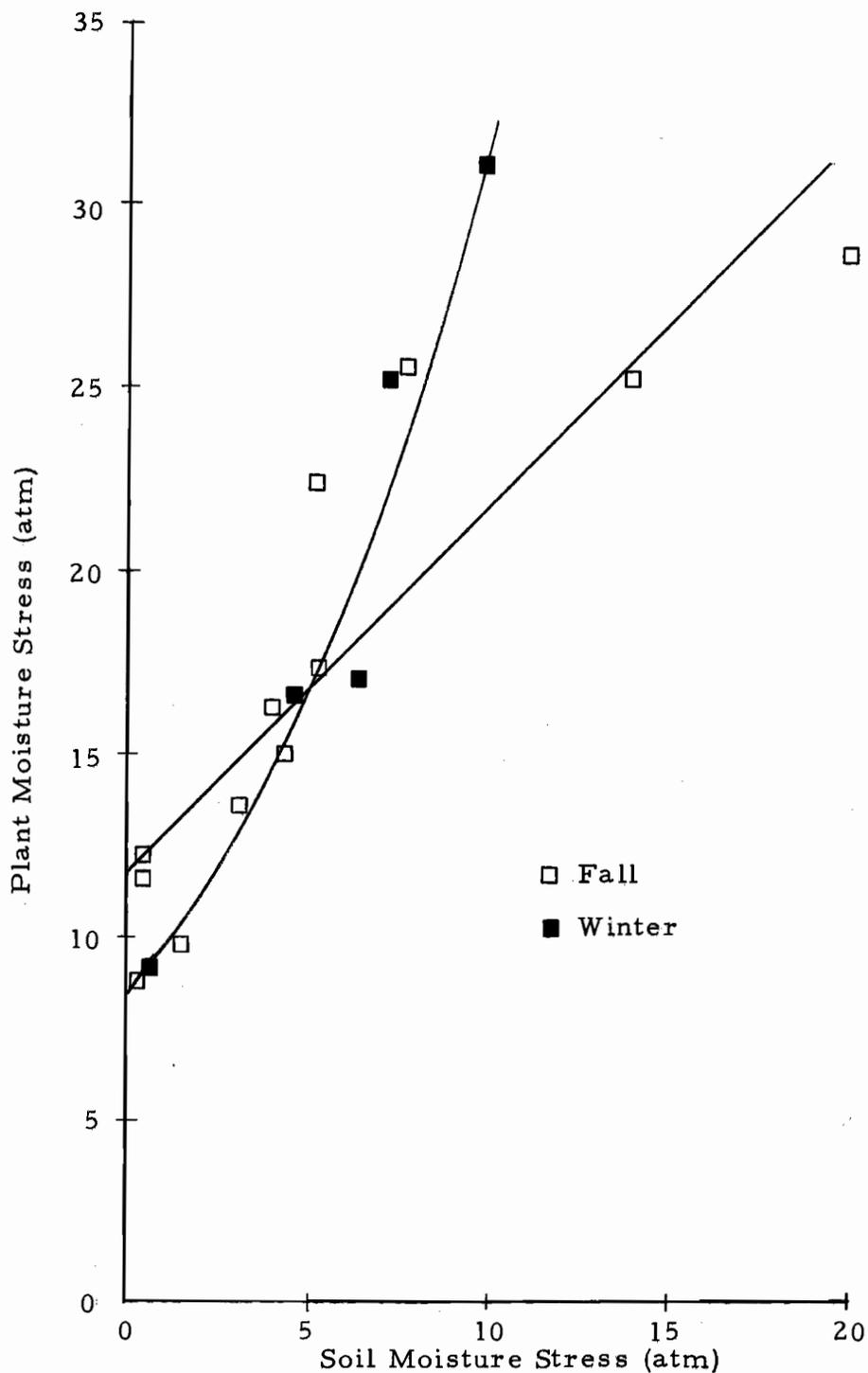


Figure 21. The fall and winter response of plant moisture stress as a function of soil moisture stress for two-year-old Douglas-fir seedlings preconditioned to 9 percent light their first growing season and full light the second. The fall seedlings were ca. 20-months old and the winter, ca. 23-months old.

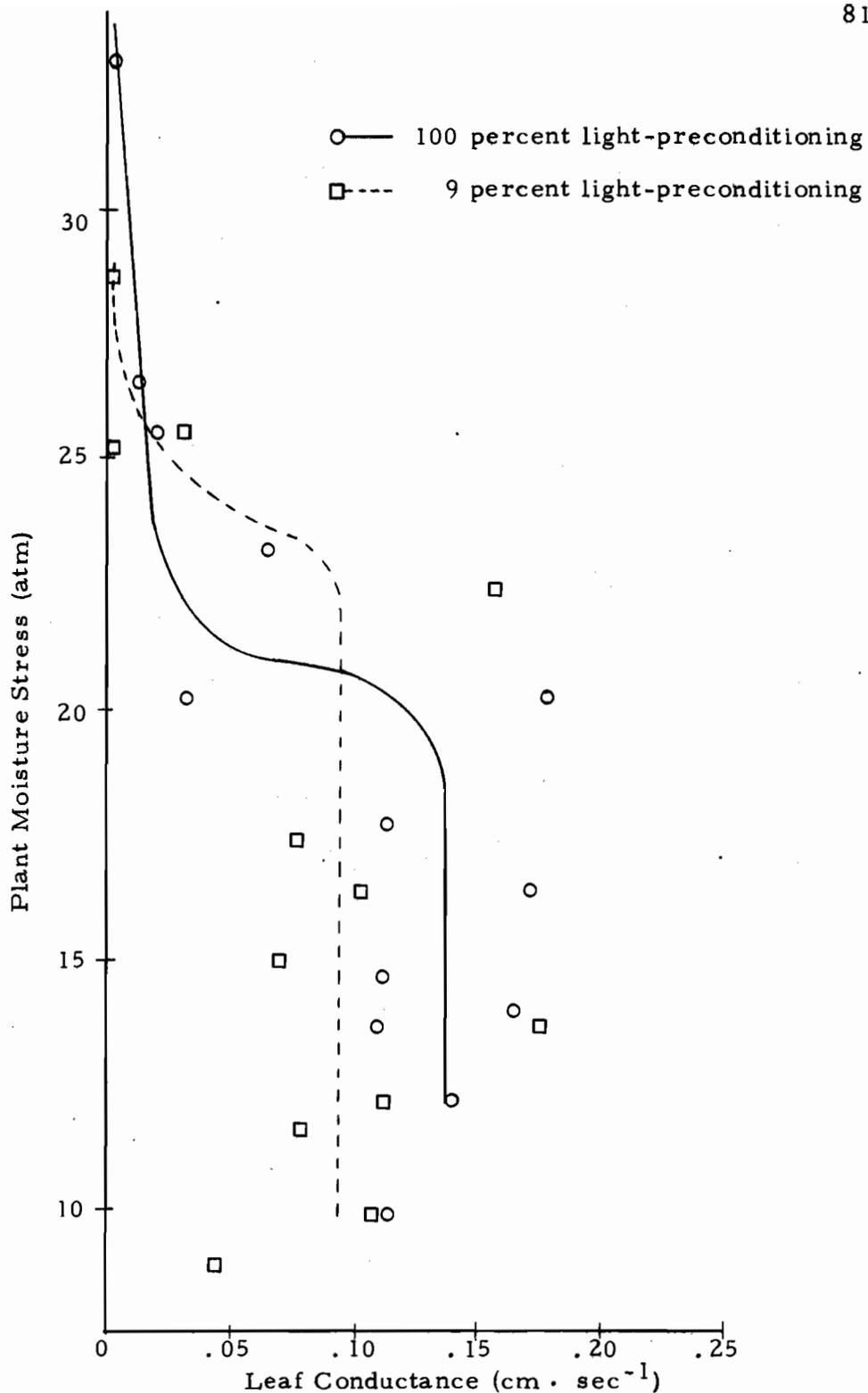


Figure 22. The fall response of leaf conductance as a function of plant moisture stress for two-year-old Douglas-fir seedlings pre-conditioned to 100 percent and 9 percent light their first season and 100 percent light the second season.

DISCUSSION

Douglas-fir seedlings growing in a nursery environment are subjected to the vagaries of changing climate. An experimenter working under such conditions has limited control over important variables affecting plant growth. Some variables may be controlled more than others, as light intensity and soil moisture in this study, but others such as temperature, photoperiod, and diurnal, seasonal and yearly changes in all factors often vary independently in an experiment of this sort. This adds variability to data and may complicate interpretations. An advantage, however, accrues in the added knowledge that is gained concerning behavior of seedlings as part of a natural system. This allows for more direct application of research results obviating extrapolation between growth chamber and field.

Growth Analysis

Seedlings grown in this study during 1971 and 1972 showed marked differences in proportionate distribution of dry matter between root and shoot systems. The increase in shoot development under low light conditions in 1971 was consistent with the findings of many investigators based on simple shoot-root ratios. The fact that these ratios increase under shading was confirmed here based on allometric analysis of 1971 growth.

In 1972, however, the effect of low light was to increase to a greater degree the amount of dry matter allocation to seedlings' root systems. This unexpected finding led to an investigation into possible sources of variation between the two years. As cultural treatments, germination dates, soil, and plant material had all been held constant both years, these were ruled out as accountable factors.

There were in addition to changes in allometric growth between the two years, differences in total dry weight. As seedlings grown in 1971 were larger for every treatment than those grown in 1972 and other conditions had been constant, it was felt that climatic factors could have been involved. Indeed this seems likely as 1971 was a cooler year than the following one. The summer of 1972 was especially warm with high temperatures occurring over the period of most rapid seedling growth. As these temperatures were often close to and above 24°C , the upper limit of the optimum growth range in different seed sources of Douglas-fir (Lavender and Overton, 1972), it is possible that seedling size was limited in this year by high temperatures. Less net photosynthesis from higher respiration rates in 1972 leading to smaller dry weight increase in plants seemed a plausible explanation for the dry weight differences.

Not only were seedlings larger in 1971 than 1972, but optimum growth occurred under the median treatment, 44 percent light. This agrees with the findings of Baker (1945), and others that Douglas-fir

seedlings grow best under moderate shade. In 1972, the full sun-grown plants grew best. Thus it would appear that the climatic conditions during 1971 were more optimum in terms of the growth requirements of the species than was the case in 1972.

Assuming that temperature primarily accounted for these yearly differences, then the variation in yearly trends of allometric coefficients might be explained on the basis of a pronounced light-temperature interaction. The effects of temperature seemed to have the most marked effect upon the low light-grown plants in terms of their dry matter allocation. The difference between allometric coefficients, 1.014 and 0.483 is extreme. In 44 percent light-grown plants the difference between years was less, and in 100 percent, nil. In terms of total growth, that light treatment generally recognized as being most favorable for growth of Douglas-fir seedlings was the one greatest affected by the temperature differences, viz., 44 percent light. In 1972, total dry weight at seven months was almost half of what it had been in 1971. Growth under the low and high light treatments was only slightly less in 1972 than 1971.

In other words, during 1972 high temperatures apparently resulted in a greater rate of dry matter allocation to roots than to shoots. The effect seemed to override any tendency of low light conditions to favor shoot development and was most pronounced in the 9 percent light-grown plants, diminishing in importance as the light

intensity under which seedlings were grown increased. Brix (1967) has shown for young Douglas-fir seedlings grown under a range of low light intensities that as growing temperature increased from 13 to 18°C, the amount of dry matter in stem and branches increased and that in roots decreased. In a later study (Brix, 1971), he showed that at 10 percent of full sun, growing temperatures above 18°C result in decreased shoot and increased root growth. Thus in his work and in this study the effects of high temperatures decreased allocation of dry matter to the shoot system of growing seedlings.

High temperatures as well as full light conditions appear in this study to have had a similar stimulatory effect upon root growth. This may have adaptive significance in that such plants would be at an advantage in terms of their ability to compete for moisture under hot, potentially desiccating conditions.

Although hotter growing conditions affected the distribution of dry matter to shoot and root, other morphological variations were less affected. In both years the low light-grown plants were markedly etiolated in appearance with longer internodes and less mutual needle shading. Also, the changes in the ratio of leaf surface area to leaf dry weight were the same for both years, the ratio increasing with decreasing light intensity. Other investigators have noted the same (Brix, 1967; Krueger and Ruth, 1969). The fact that the ratios were slightly higher in 1972 than in 1971 may be a consequence of greater

dry weight of plants in 1971 leading to thicker and more dense needles. Or, it may be due to increases in leaf area during the warmer year. Brix (1967) notes greater ratios of leaf area to dry weight with increase in temperature between 13 and 24 °C which he attributes not to an increase in area of individual leaves, but to an increase in leaf number per unit weight.

In 1972 an increase in light from 9 percent to 44 percent resulted in a larger increase in the amount of dry matter allocation to needles than to stem. As light increased to 100 percent, further shoot development was largely in terms of allocation to stem growth. This would be expected if a small seedling growing under adverse conditions of 9 percent light and high temperatures allocated surplus food produced due to increased light to needle growth rather than stem growth. This would sharply increase the plant's photosynthate-producing potential. After a certain optimum rate of needle development had been reached, extra food produced would be devoted to stem growth. This trend is supported by the 1972 data.

In 1971, however, it is less easy to make similar judgments due to the irregular transition in seedling size with increasing light, greater plant size, and the reverse trend in allometric growth. However, the transition from 9 percent to 44 percent light appeared to favor increased stem growth more than needle growth. In the entire study the highest rates of stem growth relative to root growth were

found for the treatment exhibiting greatest total dry weight. A coefficient of 1.146 characterized plants grown under the 44 percent light treatment during 1971.

Ledig et al. (1970) report a re-analysis of shoot-root ratios in data of Steinbrenner and Rediske (1964) for growth chamber-grown Douglas-fir seedlings. They show that those grown under high light (1600 ft-c) and moisture had an allometric coefficient for the regression of shoot on root of 0.749, but that this was around 0.3 for plants grown under 400 ft-c of light. Comparing these coefficients to those of the 9 percent light-grown plants in this study indicates that the proportionate distribution of photosynthate between shoot and root in Douglas-fir is extremely variable, but generally less than 1.0.

Ledig et al. (1970) also report that in Pinus taeda seedlings the relative growth of shoot to root is constant at around 0.7 over a wide range of light and moisture conditions. As changes in the rates of shoot and root development indicate phenotypic adjustments to different growing conditions it may be that some species of conifers are better able to make these adjustments than others. This may be a feature of growth under genetic control, different species possessing greater or lesser plasticity with regard to dry matter allocations.

Phenology

A correlation in time between bud-set and bud-break has been

observed for a range of light-treated Douglas-fir seedlings. Low light-grown plants set buds earlier in the fall and burst buds earlier the following spring than did full light-grown plants. This correlation between prior growing conditions and spring bud burst is rare, but not unprecedented in the literature. Guzev (1958) observed that seedlings of oak and fruit trees grown under droughty conditions flushed earlier the following spring than ones previously given adequate moisture. Griffin (1974) has noted a similar response in Douglas-fir. He grew seedlings of 212 families under three moisture regimes. Seedlings given medium drought set bud sooner than ones grown under high moisture or heavy drought. The following spring the medium- and heavily-droughted treatments broke bud sooner than those given adequate moisture the prior year.

J. N. Alden (1971) in his study of freezing resistance in Douglas-fir noted that 11-15-year-old trees which developed frost resistance earliest in fall broke buds earliest the next spring. Those trees having late bud break were also the last to deharden. This information combined with the results from this study suggests that seedlings which set bud early may develop frost resistance sooner. Campbell and Sorensen (1973) have confirmed this relationship for different provenances of Douglas-fir. In spring these same plants may deharden sooner and break bud earlier than plants which had set bud and hardened later the previous fall. Caution should be exercised,

however, in equating bud set and bud break with changes in hardiness. As van den Driessche (1969) has demonstrated, two-year-old Douglas-fir seedlings may form terminal buds well before any major change in hardiness occurs.

Alden additionally demonstrated a correlation between potassium content of current year's foliage and bud break. Trees with normal amounts of potassium broke bud earlier than did potassium-deficient trees. He suggests that ready availability of potassium enhances early development and loss of freezing resistance. As this element appears strongly tied in with stomatal functioning in a number of plants (Willmer and Pallas, 1973), its role in bud break and freezing resistance in Douglas-fir may be mediated through control over water relations by stomata. Potassium deficiency in plants is known to reduce stomatal openings and consequently photosynthesis and transpiration.

The variation in timing of bud break of seedlings in this study may also be related to the fact that low light-grown plants are more efficient photosynthesizers than higher light-grown plants. Little (1970) has shown that in needles of one-year-old Abies balsamea most of the often-observed springtime starch build-up just prior to bud break is derived from current photosynthesis rather than stored carbohydrate. Later, they demonstrated a positive correlation between increase in starch content and rates of net photosynthesis

(Little and Loach, 1973). Shiroya et al. (1966) demonstrated that spring flushing may be delayed by four to five weeks in Pinus strobus seedlings due to shading the plants prior to bud break. These findings suggest that timing of spring bud break may be dependent upon the ability of seedlings to carry on high photosynthetic rates at this time whether related to high light levels or temperature. Seedlings previously grown under low light would likely produce photosynthate more rapidly when subsequently placed under higher light conditions because of decreased mutual needle shading, higher light-saturated photosynthetic rates, and other factors related to low-light preconditioning. This could lead to earlier bud break in these seedlings than in ones preconditioned to high light with less efficient photosynthetic systems.

In addition to affecting timing of bud break, low light-preconditioning resulted in increased lammass growth. This observation is similar to that noted by T. Alden (1971) in Pinus sylvestris. He found that adverse environmental conditions one year in terms of CO₂, water, and nutrient supply to seedlings resulted in a large number of plants with summer shoots the following year. He postulated that under poor conditions low levels of carbohydrate reserves were built up, insufficient to maintain growth throughout a second growing season under improved conditions. The seedlings thus set bud early. Later, after current photosynthesis had again produced a

reserve food store, a second flush occurred. A similar process may have occurred in the case of low light-grown plants in this study.

Water Relations

Summer and Fall Behavior

The water balance of Douglas-fir seedlings differs markedly between summer and fall. Seedlings in a state of active shoot growth appear to close their stomata more gradually with increasing plant moisture stress than do those that have set bud, judged by changes in slope of declining leaf conductance. This closure also occurs sooner in the summer with respect to plant stress than it does in the fall. As a result, trivariate response surfaces for the two seasons based on the model, $PMS = f(SMS, L_c)$, exhibit marked differences in response to soil drought.

In the summer, plant moisture stress increases slower and from an initially lower plant stress than in the fall. More open stomata during the latter period allow for more rapid desiccation of the plant and higher rates of rising plant stress. However, under very severe soil drought, plant moisture stress of fall seedlings is lower than in the summer, apparently due to greater cuticular water loss of young needle tissue at the latter time.

During the period of active needle and stem elongation it is to the plant's advantage to maintain a high leaf water potential, and so a

high turgor pressure. This ensures that metabolic processes associated with photosynthesis, respiration, and growth and differentiation of new tissues will proceed at efficient rates not being limited by moisture stress conditions. After meristematic activity subsides and elongation and differentiation of the new shoot has largely ceased, the requirement of high water potential for growth functions may not be as critical. Thus the apparent differences in stomatal functioning may be adaptive in that early closure of stomata in the summer ensures a high water potential in the plant, favoring growth processes. At the same time such closure is gradual enough that the plant can still maintain a certain amount of CO_2 exchange with the ambient air, critical in the case of four-month-old seedlings with little stored food. Partial closure of stomata may occur thus conserving moisture, but not completely eliminating CO_2 uptake.

Variations in plant moisture stress between summer and fall were related to an effect of soil moisture stress and the interaction between soil stress and leaf conductance. Changes in leaf conductance alone did not significantly affect differences in plant moisture stress between the two seasons. This would imply that leaf conductance is changing in response to stress in the soil more than it is to stress in the plant, and that this relationship is different in the fall than in the summer. Examination of Figures 6 and 7 indicates that this interaction is more pronounced in the summer than the fall. In

summer, plant moisture stress-leaf conductance interaction is different at low and high levels of soil stress. In fall, the change in plant stress with decreasing leaf conductance is similar at low and high levels of soil moisture. Unterschuetz et al. (1973) report a soil stress-transpiration interaction in greenhouse and nursery-grown Douglas-fir seedlings of different ecotypes which is similar to that reported here.

It may be that during active shoot growth a "message" is relayed to leaves from roots in response to soil drought which results in closure of stomata. Translocation of a growth inhibitor such as abscissic acid or its precursor from sites of synthesis in the root to leaves might be responsible for this kind of effect. Indeed there is evidence that ABA causes stomatal closure (Mittelheuser and Van Steveninck, 1969) and that such closure may occur in response to root stress (Mizrahi et al., 1970). However, the exact role of ABA and other growth regulators in stress physiology is presently unclear.

In the fall, the lower the light that plants were grown under, the greater was the plant moisture stress for any given level of leaf conductance. Also, the low light-grown plants were under greater plant moisture stress than were the full light-grown plants at equivalent soil moisture. This was likely a consequence of less root and greater shoot development in the former. Water uptake could easily lag behind water loss raising the stress in plants with an inadequate

root system. On the other hand, the fact that low light-grown plants close their stomata at a higher plant stress could be due to other causes.

Plants grown under shade may not normally be subjected to the higher stresses of a sun-grown plant and thus adjust their stomatal functioning accordingly. This could occur through some anatomical change affecting guard cell functioning or through the osmotic relations involved. If potassium ion flux indeed accounts for increased guard cell turgor and stomatal opening (Humble and Hsaio, 1970), then foliage of low and high light-grown plants may vary with respect to potassium relations. In the winter, 9 percent light-grown plants seemed to be at a higher leaf conductance than 100 percent light-grown plants for any level of plant moisture stress, implying more open stomata in the former. This difference in stomatal behavior observed in both fall and winter along with the variation in timing of bud break could be explained on the basis of differing foliage potassium relations, stomata of low light-grown plants perhaps being influenced to a differing degree from those grown under full light.

Winter Behavior

In this study, low nighttime temperatures in late fall brought about decreased transpiration rate. This was especially noticeable at the time of first freezing or sub-freezing temperatures. Other

investigators have shown a strong correlation between hardiness changes and low temperatures especially first frosts. Van den Driessche (1969a, b) reports this in Douglas-fir, and Parker (1955) in Pinus ponderosa and Abies grandis. Simultaneously with increased hardiness in spruce and pine, transpiration rates have declined (Christersson, 1972), and in Pinus nigra the period of maximum hardiness coincided with the period of lowest transpiration (Parker, 1963). Parker (1961, 1963) noted that low transpiration rates were not related to lowered leaf water content in a number of coniferous species, and Timmis (1973) showed that hardy Douglas-fir tissue was at a higher moisture content than non-hardy tissue over a wide range of needle osmotic stress.

Christersson (1972) speculates that since transpiration rates of unhardened spruce and pine seedlings in darkness were similar to rates of hardened seedlings in light, a stomatal closure may have occurred in the hardened seedlings. In Xanthium, Drake and Raschke (1973) note that chilling of greenhouse-grown plants caused a reduction in stomatal conductance. Mature, semi-arid site Pinus ponderosa have wide open stomata all winter when air temperatures are above freezing. Below freezing they close completely (Drew et al., 1972). The winter stomatal response of mature trees may be different than in seedlings.

This study supports the contention that decreased leaf conductance in winter is not related to changes in water potential of leaf tissue, but to a direct effect of stomatal closure. At low plant moisture stresses of 10 to 15 atmospheres leaf conductance may be 20 percent of what it was in the fall even though soil moisture is near field capacity.

Michael (1966) noted a high resistance to desiccation in detached twigs of Douglas-fir and other conifers in the winter which she attributed to stomatal closure. Thus the hardening process in Douglas-fir seedlings confers drought resistance as well as frost resistance. Indeed, the effects of drought and frost upon leaf tissue are very similar in that as in drought, protoplasm is dehydrated by movement of water out of cells to sites of intercellular ice formation. Siminovitch and Briggs (1953) found a linear relation between resistance of Robinia bark cells to desiccation and resistance to freezing.

As a result of closure of stomata due to low temperatures in seedlings of this study, plant moisture stress was less. This is apparent over a wide range of soil moisture stress in comparison to the fall trend. The exponential nature of the change in plant moisture stress with drying soil in the winter was observed in all treatments for first and second-year seedlings. It is not clear why this winter pattern is exponential rather than linear as in fall, but Timmis (1973) noted a very similar relationship in hardy Douglas-fir needles as

compared to non-hardy which appeared related to changes in osmotic potential. Figure 16 shows that the deviation between fall and winter relationships is greatest between about 15 and 25 atmospheres of plant moisture stress. It may be that this is a critical stress in the plant with regard to susceptibility to frost damage. During the fall, stomatal closure normally occurs at around 15-20 atmospheres. This point may be critical to avoidance of frost damage just as during the fall it is a critical point with regard to drought. At higher stresses winter changes seem not to result in decreased plant stress and this was most apparent in the older seedlings (Figures 20 and 21).

Older plants having already passed through one winter period may be more resistant to frost damage than are first-year seedlings. Thus they may not maintain lower plant moisture stresses in winter as compared to fall over the wide range that characterizes young seedlings. Indeed, Figure 22 indicates that during the fall stomatal closure seems to occur at a higher plant moisture stress (above 20 atmospheres) than it did in younger seedlings indicating less susceptibility to drought damage. Insofar as drought resistance and frost resistance are similar physiologically, these older plants may not be as susceptible to freezing injury.

J. N. Alden (1971) reports that in both normal and potassium-deficient Douglas-fir trees the potassium content declined over winter increasing again in spring. Kartelev (1968) also reports lower

potassium content of foliage of Pinus sylvestris during the winter. As potassium is involved in the osmotic relations of guard cells it may be that stomatal closure in conifers during the winter is related to lower potassium levels in needles at this time. Christersson (1972) speculates that abscissic acid may regulate transpiration rate in conifers during the winter. Numerous studies have shown that ABA causes stomatal closure and that stomatal movements are related to influx and efflux of potassium ions of guard cells. Thus it may be that ABA closes stomata in the winter through regulation of available potassium ion in foliage. If the low light-grown plants in this study had higher potassium content of their foliage than full light-grown plants then stomata might close in the winter in response to potassium decline, but not to the same degree as the latter and leaf conductance would vary accordingly. Such changes in leaf conductance were observed in this study. Differences in potassium content of light-treated seedlings may explain such treatment variation, but on the other hand, variation in shoot and root system development may be responsible. The data from these experiments support the latter viewpoint, but do not exclude the possibility of the former.

The data in Figure 16 indicate that in spring plant moisture stress of seedlings in soil near field capacity may be less than during the previous winter. This may be associated with dehardening of seedlings at this time or there may be no difference in response of

plant stress to soil stress between winter and spring. There is a large amount of variability in the winter data.

There is some evidence the water relations influences due to first-year growth under low light conditions may still be present a year later under full light. This is understandable if the basis for these differences is morphological as shown in this study. A small root system developed one year in response to shaded conditions would likely still be somewhat reduced the second year in comparison to seedlings given full light for two growing seasons. Pawsey (1972) has shown that height growth differences in Pinus radiata for one and one-half years are still present and enhanced six years later. Thus a seedling's environment early in its ontogeny is of critical importance to later growth.

Practical Implications

With reference to nursery programs involving Douglas-fir stock, the results of this study suggest that seedlings grown under low or medium light conditions have limited ability to avoid internal drought. They are in fact less drought resistant and more subject to spring frost damage than seedlings grown under full sunlight. This is critical when the seedlings are to be planted on harsh south-facing slopes. Early closure of stomata and a well-developed root system

as well as later bud break favor survival in such a situation. Low light-grown seedlings do not possess these features.

When temperature is not limiting, total dry weight of seedlings may be optimized under slight shade, and any shading would probably enhance shoot growth, desirable from the standpoint of productivity. However, survival of such seedlings is in jeopardy on many planting sites in the Northwest due to their inability to avoid internal desiccation. A low light cultural treatment may be beneficial if seedlings are to be planted on more mesic sites. why?

It is of interest, however, that with a growing season characterized by high temperatures total growth may be reduced and shading as a cultural treatment may result in better root development at the expense of shoot growth. Experimenters should be aware of this apparent light-temperature interaction as it affects distribution of dry matter in Douglas-fir seedlings.

The Canadian Forestry Service (Mathews, 1971) recommends that container-grown Douglas-fir seedlings of coastal and interior seed sources be given 30 percent shade for the first four months of growth prior to planting at an age of six months. As container-grown seedlings have a restricted root distribution, shading under otherwise favorable growing conditions may cause further reductions in root development.

Some nurseries in the Northwest provide shaded conditions for certain species of open-rooted stock. Here again this practice might be to the eventual detriment of the seedlings through reduced drought resistance even though a larger seedling may result.

Long ago, Korstian (1925) recommended that a plant be subjected to light conditions in the nursery approximating those of the site to be planted. It is clear from this study that Douglas-fir seedlings make phenotypic adjustments to their environment. If suddenly transferred from one set of nursery conditions to another the adjustment required may be so severe that the seedling eventually succumbs.

SUMMARY AND CONCLUSIONS

Changes in growth and water relations of Douglas-fir seedlings were induced through control of the light regime in a nursery environment. These were measured and related to certain limiting climatic effects and where possible, causal influences of seedling growth upon water relations were defined.

Allometric analysis of first-year seedling growth indicates that reducing light intensity over a range from 100 percent to 9 percent of full light decreases the rate of root development and increases that of the shoot. When conditions less favorable prevail, as when temperatures over the growing season are high, total seedling dry weight is restricted and increased dry matter produced is allocated to root rather than shoot development. Under these conditions, a reduction in light intensity increases the rate of root development and decreases that of the shoot, the opposite of what may be observed under more favorable conditions. The effect of this apparent temperature-light interaction upon allometric growth is most pronounced in the case of seedlings grown under low light. Under favorable growing conditions, first-year Douglas-fir seedlings exhibit maximum dry weight increase under moderate shade.

Seedlings given low light their first growing season set bud sooner in fall and break bud earlier the following spring than do those

plants previously grown under full light the first year. This can result in increased spring frost damage. The first-year preconditioning influence upon timing of spring bud break during the second growing season does not extend into a third year. Those seedlings grown under low light their first growing season have about twice as much lammas growth the following summer under full light conditions as plants grown continuously under high light.

Water relations behavior during the time of summer shoot growth is characterized by an initially low rate of increase in plant moisture stress at low soil stress, the increases being greater as the soil dries and leaf conductance declines. This is presumably related to gradual and early closure of stomata. In the fall after shoot growth has ceased, such closure occurs at a higher plant moisture stress and is more abrupt. At this time, rate of increase in plant stress is initially high under low soil stress, but is less at high levels of plant moisture stress. The net result is that during the fall, moisture stress of seedlings is higher over a wide range of soil moisture than it is in the summer. These seasonal differences are interpreted as an adaption of the stomatal apparatus to maintaining a high leaf water potential necessary in summer for growth processes. Cuticular water loss is greater in the summer during active shoot growth than during the fall.

During the fall and winter, first-year seedlings grown under low light are less able to maintain low plant moisture stress under drought than those grown under high light intensity. These low light-grown plants have a poorly-developed root system with a larger top, whereas the full light-grown plants exhibit the opposite tendency, having a morphology more favorable to uptake and retention of moisture. Seedlings' growth under varying light regimes appears to affect water relations, the high light-grown plants possessing features favoring drought avoidance and the low light-grown, features which make them more susceptible to drought damage.

Concomitantly with the first sub-freezing temperatures of winter, leaf conductance falls. The decline continues, correlating well with the decrease in minimum daily temperatures at this time. This appears related to some direct effect upon closure of stomata rather than to closure mediated through osmotic effects of increased plant moisture stress. This change was observed in all plants in winter regardless of prior growing conditions. As a result, moisture stress in seedlings is lower than during summer or fall over a wide range of equivalent soil moisture stress.

The effects of first-year preconditioning to low light intensity upon water relations may still be present a year later following transferral from low to a full light environment. Although not statistically conclusive, the results of this study support this contention.

Douglas-fir seedlings make pronounced phenotypic adjustments to growing conditions in the nursery. These changes affect dry matter distribution between root and shoot, and water relations. As a result, seedlings preconditioned to low light intensity have limited ability to avoid desiccation, are more susceptible to spring frost damage, and less likely to become established following planting on many sites in the Northwest.

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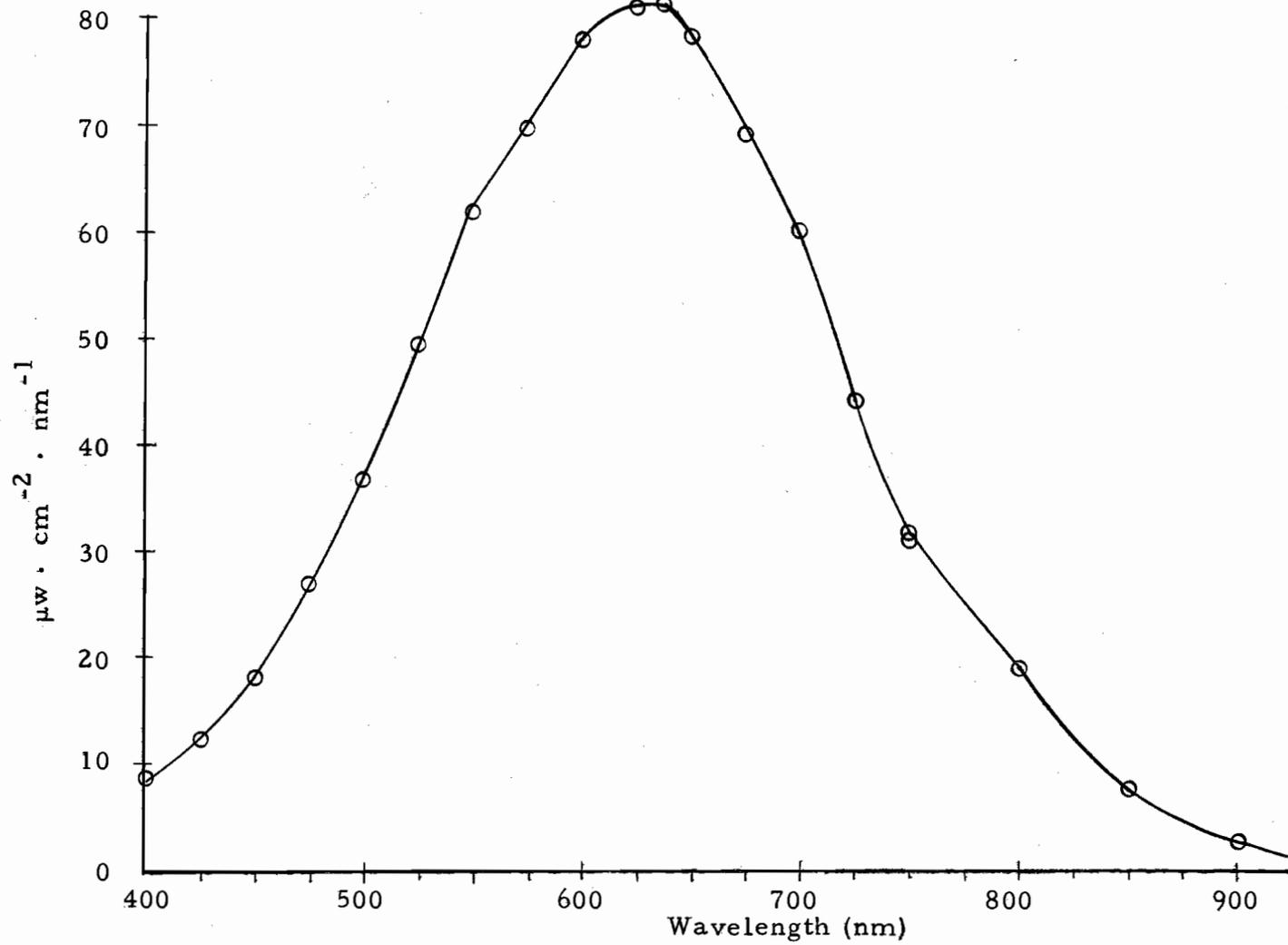
APPENDIX

Computation of Transpiration Rates and Associated Parameters

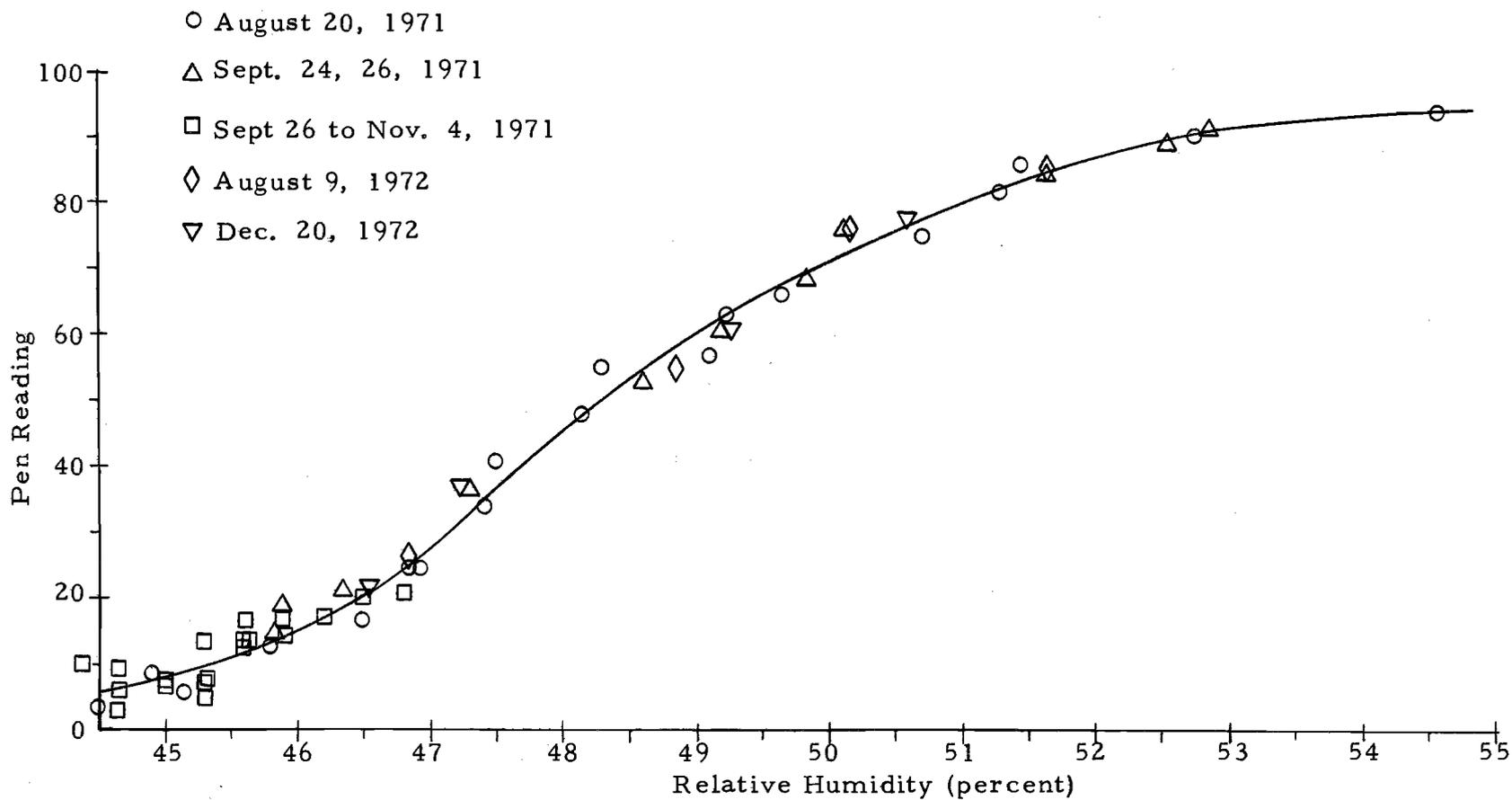
As a stable humidity control system had been developed, it was possible to rely on this as a baseline reference for each plant being measured without continual monitoring of humidity of incoming cuvette air. Occasional checking verified this assumption. By means of the relative humidity calibration curve for the LiCl sensor in use, recorder pen reading was converted to relative humidity and in turn to absolute humidity by multiplying by the saturation vapor pressure at 19.1°C , sensor temperature. This value in milligrams/liter was then corrected for the reference humidity, i. e., humidity of incoming air, by subtraction. Then, by multiplying this figure by air flow rate through the cuvette in liters/minute and by a 60 minutes/hour conversion factor, and dividing by total leaf area (based on upper and lower leaf surfaces), transpiration rate in $\text{mg H}_2\text{O}/\text{cm}^2/\text{hr}$ was obtained.

Due to the presence of the seedlings in the cuvette, vapor pressure gradients were smaller for seedlings that were heavily transpiring and larger for ones losing less moisture. Transpiration rates were not corrected for this source of error. However, the variation in vapor pressure gradients was not large, ranging from 9 to 12 mg/liter for most plants.

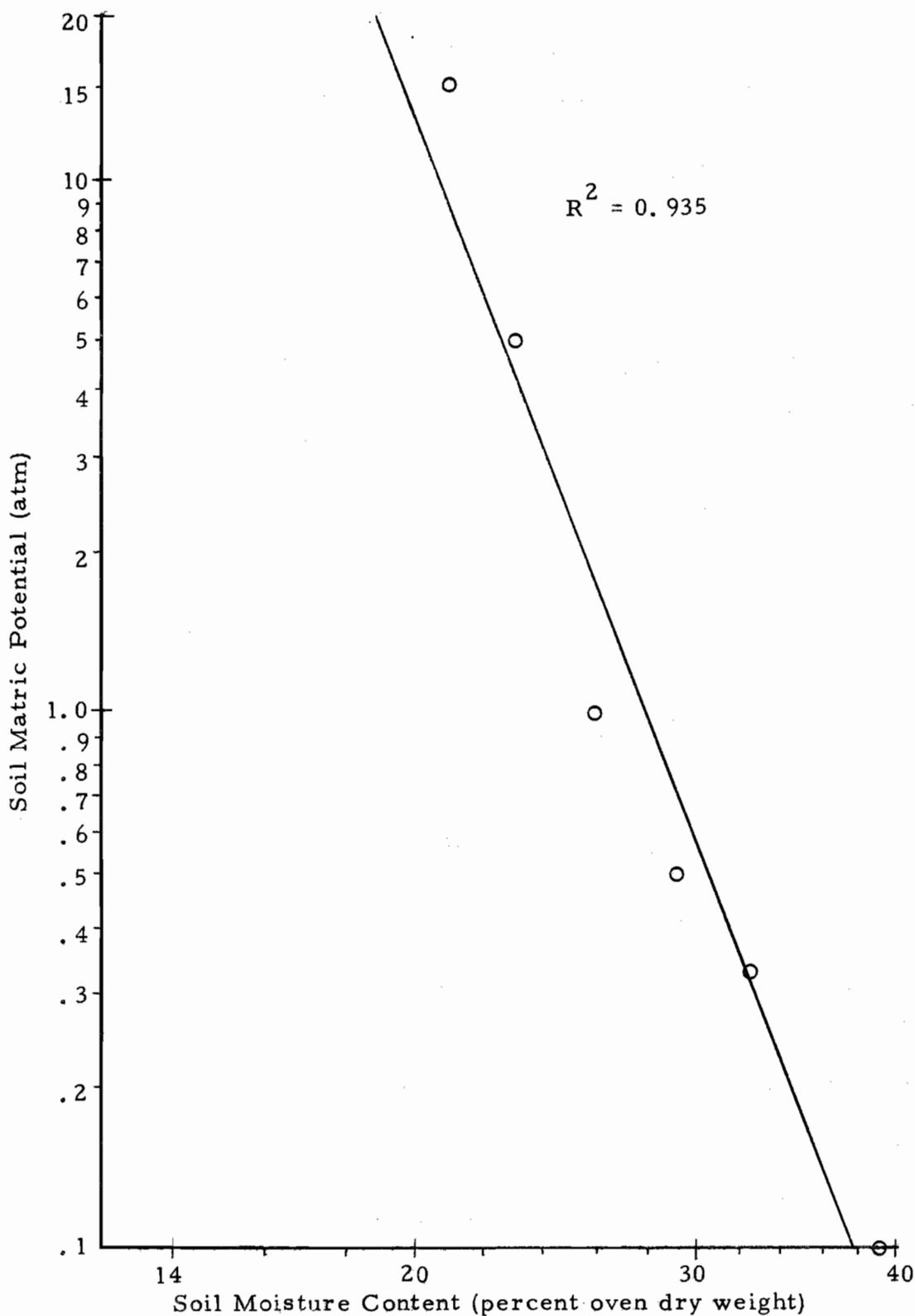
The calculated vapor pressure gradient was divided by transpiration rate for each plant to give leaf resistance in sec/cm. This figure was then inverted to give the reciprocal of resistance, i. e., leaf conductance. The latter was used in subsequent analyses rather than leaf resistance as it was felt to be easier to interpret in the case where tri-variate response surfaces were generated from data, and it eliminated the necessity of working with large resistances. As stomata close, leaf resistance approaches infinity, but leaf conductance approaches zero. The latter figures were easier to manipulate in data analysis. Also, this author is of the opinion that conceptually, leaf conductance is more clearly related to the parameter being measured, viz., transpiration, than is leaf resistance, even though the latter seems to be a more popular term.



Appendix Figure A. Spectral distribution of light intensity at seedling level in the cuvette.



Appendix Figure B. Calibration curve for LiCl Humidity Sensor, showing dates of calibration.



Appendix Figure C. Soil moisture desorption curve for Jory soil.

Appendix Table A. Analysis of variance of summer and fall water relations of Douglas-fir seedlings.

Comparison: Summer - combining treatments
(9, 44, 100 percent light) and years
(1971, 1972)

vs.

Fall - combining treatments (9, 44,
100 percent light) for 1971

Source of Variation	df	SS	MS	F
Season	6	0.20880	0.03480	9.30**
Fit of Model	6	124.72495	20.78749	
Full Model (SSR) ^a	12	124.93375	10.41115	
Residual (SSE)	80	0.29906	0.00374	
Total (SST)	92	125.23281	1.36123	

Breakdown of Seasonal Variation

Equality of Intercepts	1	0.02030	0.02030	5.43*
SMS	1	0.01664	0.01664	4.45*
L_c	1	0.01207	0.01207	3.23 NS
SMS^2	1	0.01075	0.01075	2.88 NS
L_c^2	1	0.00886	0.00886	2.37 NS
$SMS \times L_c$	1	0.01492	0.01492	3.99*
	6			

** Significant at 1 percent level

* Significant at 5 percent level

NS - Not significant

^a $R^2 = 0.998$

Appendix Table B. Analysis of variance of fall water relations of low and high light-grown Douglas-fir seedlings.

Comparison: 9 percent vs. 100 percent light
(fall, 1971)

Source of Variation	df	SS	MS	F
Preconditioning	6	0.05507	0.00918	5.28**
Fit of Model	6	43.72961	7.28827	
Full Model (SSR) ^a	12	43.78468	3.64872	
Residual (SSE)	18	0.03129	0.00174	
Total (SST)	30	43.81597	1.46053	

Breakdown of Variation Due to Preconditioning

Equality of Intercepts	1	0.00014	0.00014	0.08 NS
SMS	1	0.00792	0.00792	4.56*
L _c	1	0.00317	0.00317	1.83 NS
SMS ²	1	0.00101	0.00101	0.58 NS
L _c ²	1	0.00530	0.00530	3.05 NS
SMS x L _c	1	0.00394	0.00394	2.27 NS
	6			

** Significant at 1 percent level

* Significant at 5 percent level

NS - Not significant

^aR² = 0.999

Appendix Table C. Parameters of the allometric equation $\log Y = a + k \cdot \log X$ based on analysis of growth under different light preconditioning treatments. Comparisons between different organs of Douglas-fir seedlings are shown for 1971 and 1972 growth.

Comparison		Light Preconditioning					
		100 percent		44 percent		9 percent	
Y	X	a	k	a	k	a	k
<u>1971</u>							
Shoot	vs Root	0.860	0.703	1.690	0.978	1.495	1.014
Needle	vs Root	0.648	0.702	1.102	0.915	1.147	1.064
Stem	vs Root	0.311	0.853	0.617	1.146	0.379	0.916
<u>1972</u>							
Shoot	vs Root	0.657	0.709	0.469	0.585	0.330	0.483
Needle	vs Root	0.343	0.586	0.312	0.556	0.211	0.444
Stem	vs Root	0.394	1.011	0.157	0.648	0.133	0.607