AN ABSTRACT OF THE THESIS OF

Edward G. Newville for the Master of Science

in Forest Science presented on September 19, 1977

Title: ABSCISIC ACID LEVELS IN RELATION TO STOMATAL BEHAVIOR DURING DROUGHT AND RECOVERY IN CONTRASTING ECOTYPES OF DOUGLAS-FIR [*Pseudotsuga menziesii* (Mirb.) Franco]

Signature redacted for privacy.

Abstract approved: William K. Ferrell

Foliage of Douglas-fir seedlings [*Pseudotsuga menziesii* (Mirb.) Franco] from two contrasting environments was sampled during progressive stages of two consecutive cycles of induced drought for leaf resistance, plant water potential, and abscisic acid content (ABA). One group of seedlings was placed in a controlled environment room while another group was kept outside at a nursery under natural conditions.

During the first cycle of drought, the xeric and mesic ecotype seedlings maintained similar levels of transpiration. During a second cycle of drought, xeric ecotype seedlings from N.E. Washington, both in the growth room and nursery environments, delayed the initiation of stomatal closure until they reached greater levels of plant moisture stress than in the first drought cycle. There was

no apparent change in the stomatal behavior of the mesic ecotype seedlings during a second cycle of drought.

In both ecotypes, during the first drought cycle, a sharp increase in ABA content occurred simultaneous with the increase in leaf resistance within a narrow water potential range. However, after rewatering, high leaf resistances were not concurrent with high ABA content. In xeric ecotypes a threshold level of moisture stress again triggered an increase in ABA content, but leaf resistances remained low. The change in stomatal response induced by previous stress could not be explained by altered ABA levels. In mesic ecotypes in the growth room, ABA levels remained high after rewatering and slowly decreased with time, although recovery of pre-stress leaf resistance was mainly complete two days after rewatering. A rapid closure of stomata in these seedlings in response to a threshold level of moisture stress during a second drought cycle was not related to a corresponding change in ABA content.

Abscisic Acid Levels in Relation to Stomatal Behavior During Drought and Recovery in Contrasting Ecotypes of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco]

by

Edward G. Newville

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1978

APPROVED:

Signature redacted for privacy.

Professor of Forest Science

Signature redacted for privacy.

Head of the Department of Forest Science

Dean of the Graduate School

Date thesis is presented September 19, 1977

Typed by C. Joy Martin for Edward G. Newville

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSCISIC ACID LEVELS IN RELATION TO STOMATAL BEHAVIOR DURING DROUGHT AND RECOVERY IN CONTRASTING ECOTYPES OF DOUGLAS-FIR [*Pseudotsuga menziesii* (Mirb.) Franco]

I. INTRODUCTION

The range of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] extends over a wide geographical area of diverse climates. Numerous ecotypes have evolved in adaptive response to the various environmental conditions found across this spectrum of natural habitats. A significant environmental factor exerting an influence on selection pressure in both inland and coastal areas is the occurrence of a seasonal drought. However, in certain coastal areas of high precipitation and generally mild climate, the natural selection is based on other factors. Thus, the ability to withstand drought among ecotypes of Douglas-fir is highly variable.

Mortality of planted seedlings due to desiccation remains a serious obstacle to the successful reforestation of clear-cuts in many areas. In reforestation, efforts have been made to select genotypes with improved inherent physiological mechanisms to resist drought as well as to develop nursery practices that increase the drought resistant capabilities of planted stock. Also, drought resistant ecotypes from xeric habitats have been studied in an attempt to gain a general understanding of the manner by which they avoid desiccation and death.

Resistance to drought can be a function of either tolerance or avoidance of the moisture stress. The restriction of transpiration by the closure of stomata has been accepted as a significant drought avoidance mechanism in xeric ecotypes of Douglas-fir seedlings

(Unterscheutz et al. 1973). The physiological basis for this adaptation remains unclear.

Abscisic acid (ABA) is a naturally occurring plant growth hormone of Douglas-fir (Webber, 1974). In addition to a variety of physiological effects, ABA is thought to be involved in the regulation of stomatal aperture during moisture stress. Drought resistance in Douglas-fir seedlings could possibly be related to the control of stomatal aperture by ABA. The primary purpose of this investigation was to determine if differences in the stomatal behavior of xeric and mesic ecotypes of Douglas-fir during moisture stress and subsequent recovery were related to internal concentrations of ABA.

An inactive glucose ester of ABA has also been isolated from plants (Milborrow, 1974). Little is known concerning the physiological role (if any) of this "bound" form of ABA in Douglas-fir or any plant. High quantities of the ABA-conjugate exist in plants which have recovered from wilting and its accumulation has been suggested as a possible mechanism whereby the effects of preconditioning moisture stress influences subsequent stomatal behavior (Hiron and Wright, 1973).

The secondary purpose of this research was to test this possibility in xeric and mesic ecotypes of Douglas-fir by determining the change in levels of bound ABA during two consecutive cycles of induced moisture stress.

II. LITERATURE REVIEW

Response to local selection pressures results in the formation of physiological races or ecotypes through alteration of the gene pool. Douglas-fir occurs over a wide geographical area of diverse environment and would be expected to form numerous ecotypes.

The existence of drought resistant ecotypes of Douglas-fir has been demonstrated by Ferrell and Woodard (1966) and Pharis and Ferrell (1967). Resistance was found to be a combination of both tolerance and avoidance characteristics. Limited experiments by Zavitovski and Ferrell (1968) suggested that the superior drought resistance of inland sources of Douglas-fir was in part due to reduced transpiration when subject to moisture stress. Unterscheutz et al. (1974) made extensive investigations of stomatal behavior as a drought survival mechanism in Douglas-fir using sensitive equipment under controlled laboratory conditions. They compared the transpiration rates of seedlings from diverse geographical origins over a wide range of soil and plant water potentials. They also assessed the effect of pretreatment growing conditions on transpiration. They affirmed the results of prior investigation and concluded that restriction of transpiration illustrates an adaptive means by which seedlings conserve soil water and avoid eventual desiccation and death. Apparently, the stomata in seedlings of xeric and mesic origin differ in their response to moisture stress. This behavior was modified by previous stress.

Research with other species has shown similar differences between populations in their physiological response to drought that corresponds

to differences in the macro-climate of their respective natural habitats. Eickmeir, Adams, and Lester (1975) demonstrated that *Tsuga canadensis* seedlings from a southern area of Wisconsin were more efficient in minimizing water loss and maximizing CO_2 fixation under all conditions than their northern counterparts. After preconditioning in a warm regime at an osmotic stress of -4 bars, water use efficiency was also significantly improved in both groups. Preconditioning reduced transpiration by increasing stomatal resistance to water vapor loss.

Townsend and Roberts (1973), studying the effects of plant moisture stress on transpiration, obtained similar results with different seed sources of Red Maple (*Acer rubrum L.*). Ladiges (1974) also noted the variable response of transpiration among ecotypes of *Eucalyptus viminalis*. However, Ladiges found that the stomata of the more resistant xeric seedlings were less sensitive to moisture stress. These seedlings maintained significantly higher rates of transpiration under moisture stress than the seedlings from the mesic habitat.

The physiological means by which these different ecotypes affect adaptation of their stomatal behavior when under moisture stress has not been investigated.

The increase of endogenous ABA in plants subjected to moisture stress and the closure of stomata in response to exogenous ABA have been the subject of numerous investigations. Only representative reports from the literature will be cited here.

Mittleheuser and van Steveninck (1969) applied ABA to excised shoots of barley and wheat and found a reduction of transpiration caused by the closure of stomata. Applying very small amounts of (±) ABA (approximately 0.02 ug cm^{-2} of leaf) to leaves of *Xanthium pennsylvanicum*, Jones and Mansfield (1970) also induced stomatal closure. Response of the stomates was rapid. Kriedemann et al. (1972) detected the start of stomatal closure three minutes after application to the cut base of the leaf of corn.

Wright and Hiron (1969) showed that severe wilting can produce up to a 40-fold increase in levels of endogenous ABA. They postulated that ABA modulated stomatal closure in response to moisture stress. Since then, many other investigations have also shown that moisture stress causes a dramatic and rapid increase in endogenous ABA levels, Mizrahi et al. (1970), Beardsell and Cohen (1975), Loveys and Kriedemann (1973), Hiron and Wright (1973), Most (1971).

Zabadal (1974) reported that ABA began to increase in a narrow water potential range (-10 to -12 atmospheres) and suggested a critical water potential for ABA synthesis.

Though a definitive *in vivo* role for ABA in the modulation of stomatal aperture has not yet been proved (Hsiao, 1973), there are many suggestions of its direct or indirect involvement, Kriedemann et al. (1972), Zeevaart (1971), Cummins et al. (1971). This theory has been widely accepted. Recently, however, there have been reports that suggest that the stomatal aperture may not be determined solely by the internal ABA level. Under certain conditions the stomatal behavior and

ABA content of *Xanthium* leaves have been found not to be correlated with each other (Cummins, 1973, and Raschke and Zevaart, 1976). The suggestion that ABA is compartmentalized away from the site of action where it cannot act on the stomata has been made to account for these results (Cummins, 1973, and Raschke et al., 1976).

Though not discussed directly in the literature, it is possible that stomatal turgor may respond to environmental changes through several mechanisms of indirect action. Several "levels" of control may integrate to yield the final result. For instance, ABA and the plant hormone, kinetin, were found to have an antagonistic interaction affecting the stomatal aperture of barley by Cooper et al. (1972). Data published in a paper by Beardsell and Cohen (1975) also suggests the involvement of factors other than ABA. In that work, short term changes in water potential, stomatal resistance, and ABA content were determined in excised leaves of *Zea mays*. ABA levels rose sharply within 60-120 minutes after excision but stomatal closure always preceded the point of increase in ABA. When the excised stem was placed in water, the water potential and stomatal resistance recovered rapidly if the placement in water occurred before the increase in ABA content. After ABA content increased, placement in water immediately relieved plant moisture stress but both stomatal resistance and ABA content recovered slowly to normal levels. Thus, ABA did not appear to be involved in the initial closure of the stomata. The authors instead speculated that a redistribution of "compartmentalized" ABA accounted for the results. An implication of this data is that ABA may not be

involved in the short term changes associated with diurnal patterns of transpiration found under natural conditions. Limited data from sampling in the field by Ferrell and Newville (1976, unpublished data) using naturally established Douglas-fir seedlings are consistent with this hypothesis. Control of stomatal aperture by ABA may come into play once a certain level of moisture stress has been reached. In this case, levels of ABA may be more relevant to an understanding of drought avoidance and the stomatal response of the plant to a threshold level of moisture stress. Zabadal (1974) found that ABA began to increase at a critical water potential range. This was confirmed for Douglas-fir by Blake and Ferrell (1977). A sharp increase in the level of endogenous ABA occurred parallel with the increase in stomatal resistance after a critical water potential threshold had been attained.

Koshimuzu et al. (1968) showed that certain plant tissues contained, besides free ABA, a polar fraction identified as (+)-abscisyl-β-D-glucopyranoside. Stomatal closure occurs in response to applications of this compound only after hydrolysis of the glucose ester linkage (Jones and Mansfield, 1971). The compound is commonly referred to as bound ABA (Rudnicki and Pieniazek, 1971) though this is not intended to imply any type of binding to a macromolecule.

Bound ABA was found to be relatively stable in the plant (Milborrow, 1970) in amounts usually between 10 percent and 30 percent of the amount of ABA existing as a free acid (Milborrow, 1974). In one instance, its level exceeded by ten times the amount of free ABA (Goldschmidt et al., 1973). Milborrow (1970) found the ester to be

rapidly hydrolyzed by expressed sap from tomato shoots. He suggested that bound ABA and the enzymes involved in its hydrolysis to the free acid were separated into different cellular compartments.

Bound ABA appears to function in a storage capacity and presumedly is released to the physiologically active free acid under certain conditions. Hiron and Wright (1973) found high quantities of the ABA conjugate in plants that had recovered from wilting. They believed bound ABA to be involved in an adaptive response of plants to successive periods of moisture stress.

There are no published data concerning the occurrence of bound ABA in conifers. Preliminary investigations by Blake, Newville, and Ferrell (1975, unpublished data) indicated that free ABA was released by alkaline hydrolysis of the methanolic extract of foliage from Douglas-fir seedlings. This could be bound in a glucose ester or similar form.

III. MATERIALS AND METHODS

Seed Sources and Growing Conditions

In all experiments, seedlings [*Pseudotsuga menziesii* (Mirb.) Franco] of two contrasting ecotypes were used. The first experiment used seedlings from an area near Forks, Washington, and seedlings from the Bitterroot mountains near Missoula, Montana. The coastal climate of Forks on the Olympic Peninsula remains very humid for most of the year with 4400 mm annual precipitation. Seedlings were grown from a 1971 collection of seed taken from the 750 meter elevation level in the Soleduck area. By contrast, the Bitterroot mountains are relatively hot and dry with approximately 500 mm precipitation annually. Seedlings from this area were obtained from material remaining from a study by Bill Pope at OSU in 1974.

In the second set of experiments, seedlings from the St. Helens District in the Gifford-Pinchot National Forest and seedlings from an area near Spokane, Washington, were used. The former site in the central Washington Cascades has approximately 2500 mm annual precipitation, while the latter xeric site in Eastern Washington averages only 500 mm precipitation annually. Seed for the St. Helens population was collected in 1966 from the 1000 meter elevational level. Seed from the Spokane area was collected in 1974 from between elevations of 800 - 950 meters. The Bellingham nursery of the Washington State Department of Natural Resources donated the Spokane area seedlings in the summer of 1975. At this time the seedlings were one year old and planted in

tubes formed in a styrofoam block. These seedlings were replanted in #10 cans, 2-3 plants per can. Soil used for replanting was collected from the A-horizon of a forest site near Burnt Woods, Oregon. The soil was mixed well and sieved through a 1/2 inch mesh screen to remove rocks and aggregates prior to potting.

Seedlings from the other three geographical areas were planted individually in #10 cans and were 2-3 years old in 1975. All plants were grown outside under full sunlight at the FRL nursery and watered twice weekly during the dry season.

Experimental Design, Experiment #1

Seedlings from the Bitterroot and Forks area were moved into a growth room on January 15, 1976. The chamber environment was as follows: photoperiod, 16 hours; day temperature and humidity, 26°C and 62%; night temperature and humidity, 22°C and 92%; light intensity, 0.069 Langly.

All plants in the growth room were watered three times weekly for three weeks and at that time, watering was suspended. Sampling followed immediately on February 5, and continued until March 3, at which time the droughted plants were rewatered to follow their responses during recovery from moisture stress.

In the sampling, pre-dawn moisture stress was estimated (just prior to the lights being turned on) by the measurement of xylem sap potential (Waring and Cleary, 1967) using a pressure bomb (Scholander et al.,

1965). Duplicate measurements were made for each plant using the terminal shoots. Total leaf resistance was determined four to five hours later using a modified version (Blake and Ferrell, 1977) of a tubular diffusion porometer described by Moreshet and Yocum in 1972. Values on five plants agreed well (R^2 = 0.97) with those made at the same time with a null balance porometer described by Beardsell et al. (1972). Needles were also collected for the analysis of ABA at this time. The procedure for the sampling, extraction, and determination of the levels of ABA will be described elsewhere as in this first experiment all samples were destroyed by accident. No data exist for ABA levels in this first experiment.

Experiment #2

In the second set of experiments, seedlings from the Spokane and St. Helens area were moved into the growth room on July 14, 1976. Conditions in the growth room were identical to those of the previous experiment. A duplicate group of seedlings remained at the nursery under full sunlight. Using this material, a parallel experiment was run in order to contrast the results obtained from the growth room to those obtained under more natural conditions. Frequent watering of all plants continued as before for two weeks until July 27. Water was withheld after this time.

In the growth chamber, pre-dawn moisture stress was determined at the end of the dark period as before. Total leaf resistance was measured directly on intact foliage using an aspirated porometer described by Turner and Parlange (1970). Surface area measurements used in the

calculation of leaf resistance were made on a Licor portable surface area meter (Lambda Instruments Corporation). The value of the one-sided planer surface was conferted to total surface area by multiplication by a factor of 2.36 (Gholz et al., 1976). Leaf resistance for each seedling was measured at two different sites on the plant. Sampling was restricted to the current year's growth.

The determination of leaf resistance was timed to approximately coincide with the period of maximum moisture stress. This coincidence was estimated to occur toward the end of the light cycle, so sampling was done 14 hours after the lights turned on. The endogenous levels of free and bound ABA were also estimated from needles collected at this time. Severe moisture stress in these plants was allowed to build up until August 8, when all plants were rewatered. Saturation of the soil was maintained for two days, then watering was again discontinued. Change in the levels of ABA and leaf resistance in relation to pre-dawn moisture stress was again followed during the recovery period and subsequent secondary drought cycle.

Seedlings outside at the nursery were in an area that could be covered by a clear sheet of plastic to exclude rainfall. Watering was discontinued on July 27 and care was taken after that time to cover the seedlings in the event of an approaching storm. Pre-dawn moisture stress was determined prior to sunrise. Determination of leaf resistance and collection of needles for ABA analysis was done between noon and 1:00 pm PST, the approximate time of maximum moisture stress. Sampling methods were identical to those described previously. In

order to sample under environmental conditions as uniform as possible, sampling was done on relatively cloudless days. After reaching high levels of moisture stress, the seedlings were rewatered on August 16. Again, sampling was conducted after a two-day recovery period, and then, while the plants were subjected to a second drought cycle.

Analysis of Abscisic Acid

ABA was extracted and analyzed using a procedure described by Zabadal (1974) with a few modifications. Duplicate samples of approximately 150 needles each were removed from the stem and adjusted to equal weight on a torsion beam balance. One sample (for ABA analysis) was immediately covered with methanol in a small vial and stored at 0°C. The other sample was dried in an oven overnight at 60°C then weighed to determine needle dry-weight. Final concentrations of ABA in the leaf were expressed on a dry-weight basis. Again, all foliar samples were restricted to the current year's growth.

The extraction of ABA was carried out under minimal lighting to avoid isomerization of the hormone. The needles in methanol were ground in a Sorvall Omni-mixer and the residue filtered and rinsed with methanol until the filtrate was clear. Five-hundred ul of a 1 ng/ul concentration of a manufactured trans (±) ABA dissolved in methanol was added to the needle extract as an internal standard. Twenty-five mls of H_2O, adjusted to ph 8.3 with sodium bicarbonate, was also added. The methanol was then removed from the solution in a vacuum evaporator. The resulting water solution was partitioned against 50 mls of methylene

chloride ($MeCl_2$) three times. The $McCl_2$ fraction was discarded each time. The aqueous phase was taken to ph 2.5 with HCl and partitioned against $MeCl_2$ (3X-50 mls), this time retaining the $MeCl_2$ fraction. The aqueous phase contains the ABA-glucoside conjugate (bound ABA) and was retained for later analysis. The $MeCl_2$ fraction was partitioned three times against 50 mls of water taken to ph 10 with sodium hydroxide. The aqueous phase was retained and taken to ph 3.0 with HCl and partitioned against $MeCl_2$ (3X-50 mls). The aqueous phase was discarded and the $MeCl_2$ fraction taken to dryness in a vacuum evaporator. The residue was taken up in 1-2 mls of $MeCl_2$ and transferred to a small test tube and again taken to dryness under a stream of nitrogen. This residue was esterfied using diazomethane, dried and taken up in ethyl acetate. The ethyl acetate solution was put directly into a gas chromatograph.

The ABA-glucoside was isolated and quantified from the previously mentioned aqueous phase by first adjusting the ph to 10.5-11.0 and heating for one hour in a water bath at 65°C. The solution was then cooled, adjusted to ph 3.0 and 500 ng of trans (±) ABA added as an internal standard. It was then partitioned against $MeCl_2$, following the analysis from the second partitioning step onward.

Diazomethane for the methylation of ABA to the ester form was generated just prior to use from a manufactured precursor, trade name Diazald, using a specially designed apparatus available from the manufacturer. The diazomethane was added to the ABA residue in each small test tube. The reaction goes to 100 percent completion and is

virtually instantaneous. The solution was dried and the residue taken up in ethyl acetate as previously mentioned.

The gas-liquid chromatography was done using a 250 ft. stainless-steel open tubular column and a ^{63}Ni electron capture detector. The column was coated twice with a two percent solution of SP-2340 (Supelco, Inc.) dissolved in chloroform. The column temperature was 190°C, injection port temperature 205°C, detector temperature 280°C. The helium carrier gas flow rate was 15 cc min^{-1}. The purge gas, a 90:10 mixture of argon-methane, flow rate was 55 cc min^{-1}. Pulse interval was maintained at 15. Pure cis-ABA and pure trans-ABA standards were injected at the beginning of each run using approximately 1 ul of 5 ng ul^{-1} standards. Pure trans-ABA was produced for standardization purposes by irradiating commercially available pure cis (±) ABA (ICN Pharmaceuticals, Inc., Lot #8594-A) with ultraviolet light. Cis and trans isomers were then separated on silica gel thin layer plates with benzene : ethylacetate : acetic acid (15 : 3 : 1 v/v). The trans isomer migrates before the cis isomer of ABA. The bands were separated, redissolved in ethylacetate, and filtered to remove the silica coating. The concentration was checked by gas-liquid chromatography and the volume adjusted to give the appropriate final concentration. The identity of cis and trans ABA peaks in the samples was confirmed by comparison of retention times relative to pure standards on several columns coated with SP-2340 as well as EGSS-X (Applied Science Laboratories, State College, PA 16801) and relative changes in the area of the cis and trans peaks after ultraviolet radiation of the sample. Abscisic acid has been positively identified in Douglas-fir by Webber

(1974) using mass spectrometry, optical rotatory dispersion, and gas-liquid chromatography. Blake and Ferrell (1975, unpublished data) also confirmed with mass spectrometry the identity of a cis-ABA peak isolated during the gas-liquid chromatography of Douglas-fir needle extracts using an open tubular column coated with EGSS-X.

In the calculation of the final endogenous levels of free or bound ABA in the sample, the area under the cis and trans peaks was measured with a digitizer (Hewlett-Packard) and the following formula applied:

$$\text{ABA content, ng (g dry wt.)}^{-1} = \frac{\frac{\text{area of cis peak}}{\text{area of trans peak}} \times \text{amount of internal standard, 500 ng}}{\text{dry weight of sample, grams}}$$

The absolute recovery of ABA during the extraction was determined to be approximately 65 percent. A sample chromatogram of methylated plant extract appears in Figure 1.

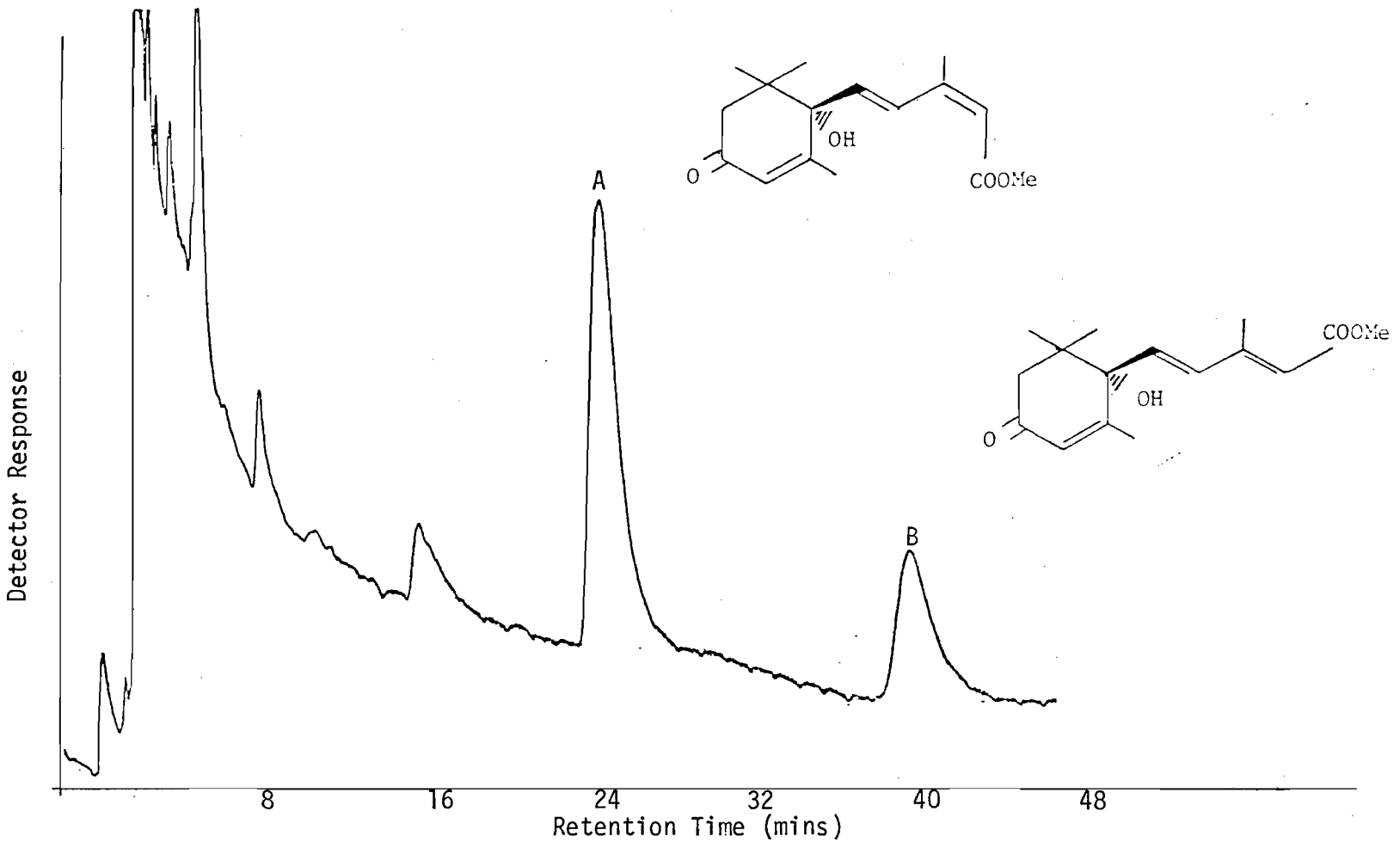

Figure 1. Gas-liquid chromatography analysis of methylated plant extract (hydrolized glucoside-ABA fraction). Peak "A" is 2-cis methyl abscisate, peak "B" is 2-trans methyl abscisate. Chromatography conditions as noted in text.

IV. RESULTS

Growth Room Study - Winter, 1975/1976

The data concerning changes in levels of ABA is missing from the first experiment. Because of this loss, the drought study was repeated the following summer. However, analysis of the data collected on leaf resistance and plant moisture stress (PMS) alone yielded some unexpected results. In Figure 2, for both seed sources, pre-dawm PMS is plotted against the leaf resistance measured 4-5 hours after the beginning of the light cycle. The response of the Bitterroot seedlings appears to be curvilinear and a hand-drawn curve approximates this relationship. No curve is drawn for the highly variable response of the Forks area seedlings.

Contrary to expectations, the xeric Bitterroot seedlings appeared to have lower leaf resistances at moderate stress levels than the Forks group. A definite, though highly variable, reaction of leaf resistance to a build-up of stress of about -12 bars occurred within the Forks group. The Bitterroot group began to exhibit higher leaf resistances only at greater levels of stress, about -14 or -15 bars. The response was more uniform with a less sudden increase in leaf resistance. These results did not confirm the preconception that the more xeric species would more sharply control water vapor loss under stressed conditions.

All seedlings were rewatered after they reached levels of pre-dawn PMS in excell of -35 bars. Results of samples taken four and six

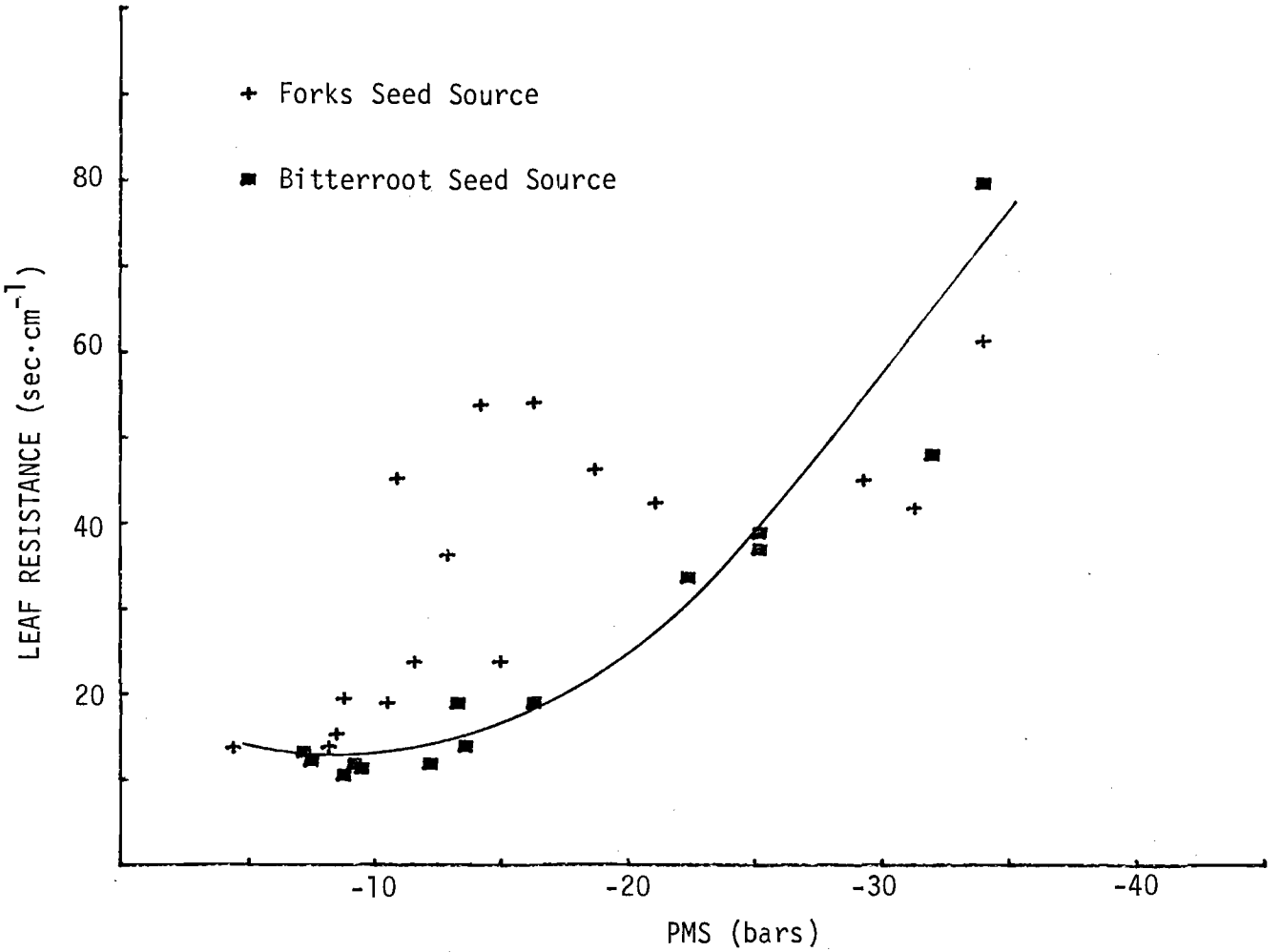

Figure 2. Pre-dawn moisture stress (PMS) and approximate minimum diurnal leaf resistance in growth room for Bitterroot and Forks area seedlings.

days after recovery are given in Table 1.

Table 1. LEAF RESISTANCE AND PRE-DAWN PMS LEVELS AFTER REWATERING FORKS AND BITTERROOT AREA SEEDLINGS.

Days After Rewatering	Forks Seed Source		Bitterroot Seed Source	
	PMS (bars)	L_r (cm/sec)	PMS (bars)	L_r (cm/sec)
4	-6.5	21.0	-5.8	5.1
4	-7.5	20.9	-8.2	6.8
6	-6.8	21.6	-8.2	6.0
6	-7.8	51.7	-9.1	11.1

This data were to be the beginning of sampling throughout a second complete drought cycle. A growth room malfunction terminated the study prematurely.

Examination of Table 1 shows that transpiration of Bitterroot seedlings recovered within four days to normal pre-stress levels. In contrast, the Forks seed source maintained high leaf resistances for at least six days due to the after-effects of moisture stress. How differences in levels of ABA may have accounted for these differences in stomatal behavior unfortunately is unknown.

Growth Room Study - Summer, 1976

Effects of Moisture Stress on Leaf Resistance

The absolute levels of leaf resistances in the following data cannot be quantitatively compared to the data from the previous experiment for several reasons. First, two porometers of different design

were used in the experiments. Though both have been calibrated correctly, the Turner model appears substantially more reliable especially at resistances greater than 50 $sec \cdot cm^{-2}$. Secondly, the timing of sampling in the second growth room study was changed to coincide more with the maximum stress period of the daily cycle. This was done in order to better detect any changes in ABA levels induced by moisture stress. Even in a growth room of constant temperature, humidity and light intensity, Douglas-fir seedlings go through a diurnal fluctuation of leaf resistance and PMS. Sampling 4-5 hours after the lights are turned on roughly follows the period when leaf resistances should be at a minimum. Sampling toward the end of the light cycle coincides with a time of maximum PMS and usually maximum leaf resistance. In the growth room, some measurements were made of the diurnal change of leaf resistance and PMS for both seed sources. Examples are presented in Figures 3 and 4. Lastly, reservations should be made in comparing the results of the two experiments not only because different xeric and mesic seed sources are involved, but also because of the seasonal timing of the experiments. The first study, begun in January, utilized seedlings that had developed dormancy outside. Bud break did not occur during the experiment. In the summer study of 1976 all plants had set a terminal bud just prior to the start of the study.

Figure 5 shows the response of leaf resistance to increasing PMS in the dry site seedlings from the Spokane area. Results of two consecutive drying cycles are plotted together for comparison. The fitted curves were derived according to a non-linear least squares curve

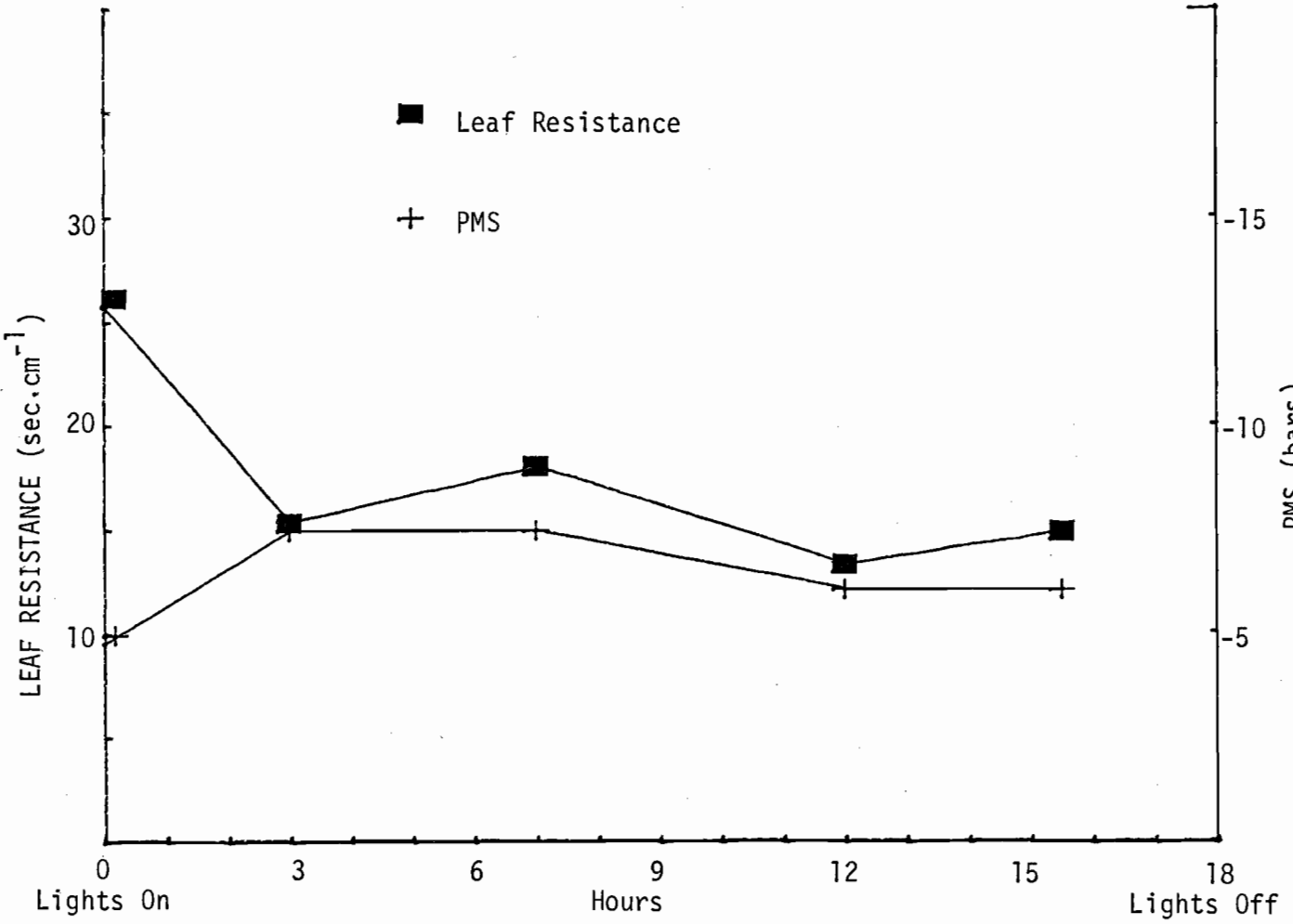

Figure 3. Variation of leaf resistance and plant moisture stress (PMS) during light cycle in growth room for Spokane area seedlings.

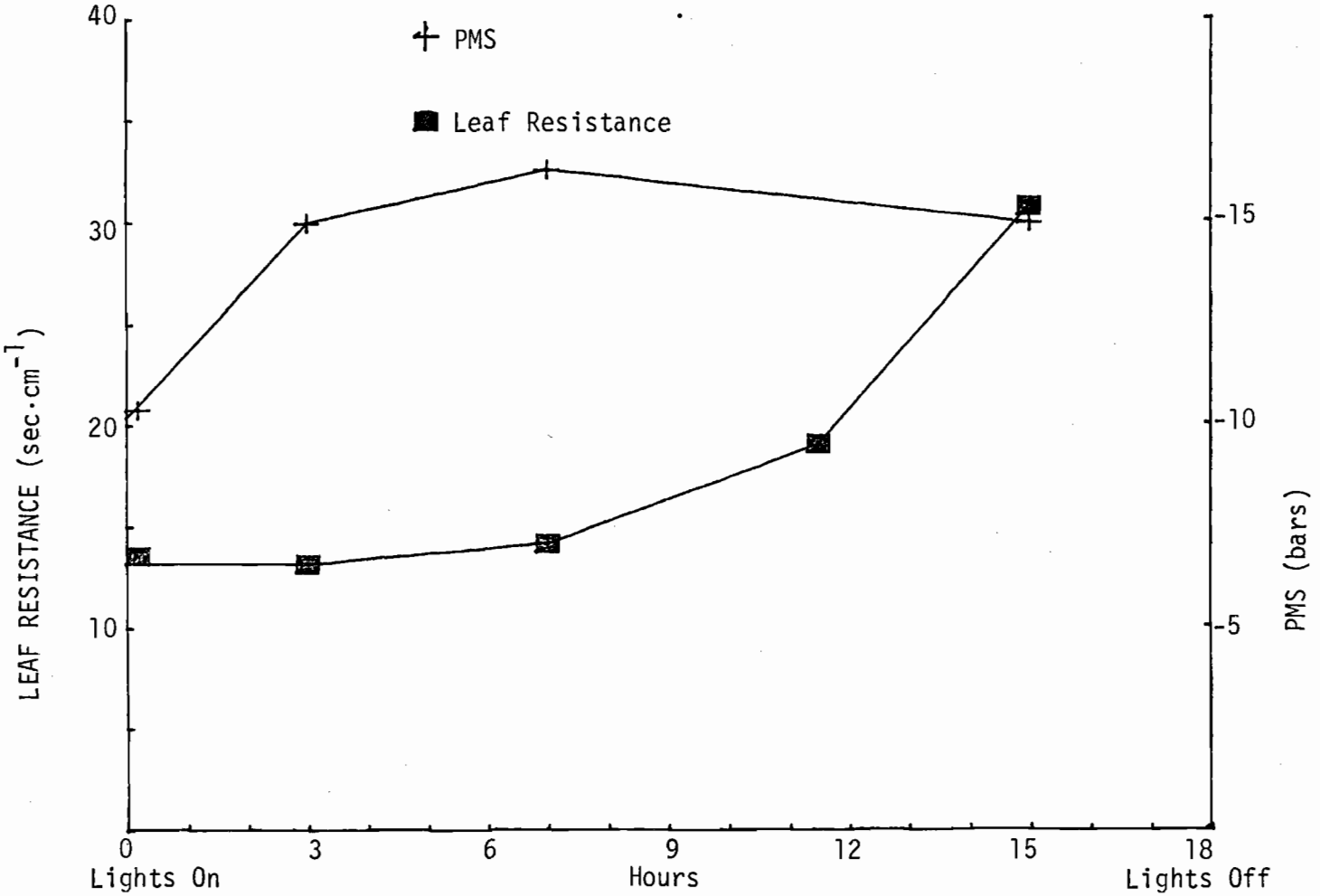

Figure 4. Variation of leaf resistance and plant moisture stress (PMS) during light cycle in growth room for rewatered Spokane area seedlings.

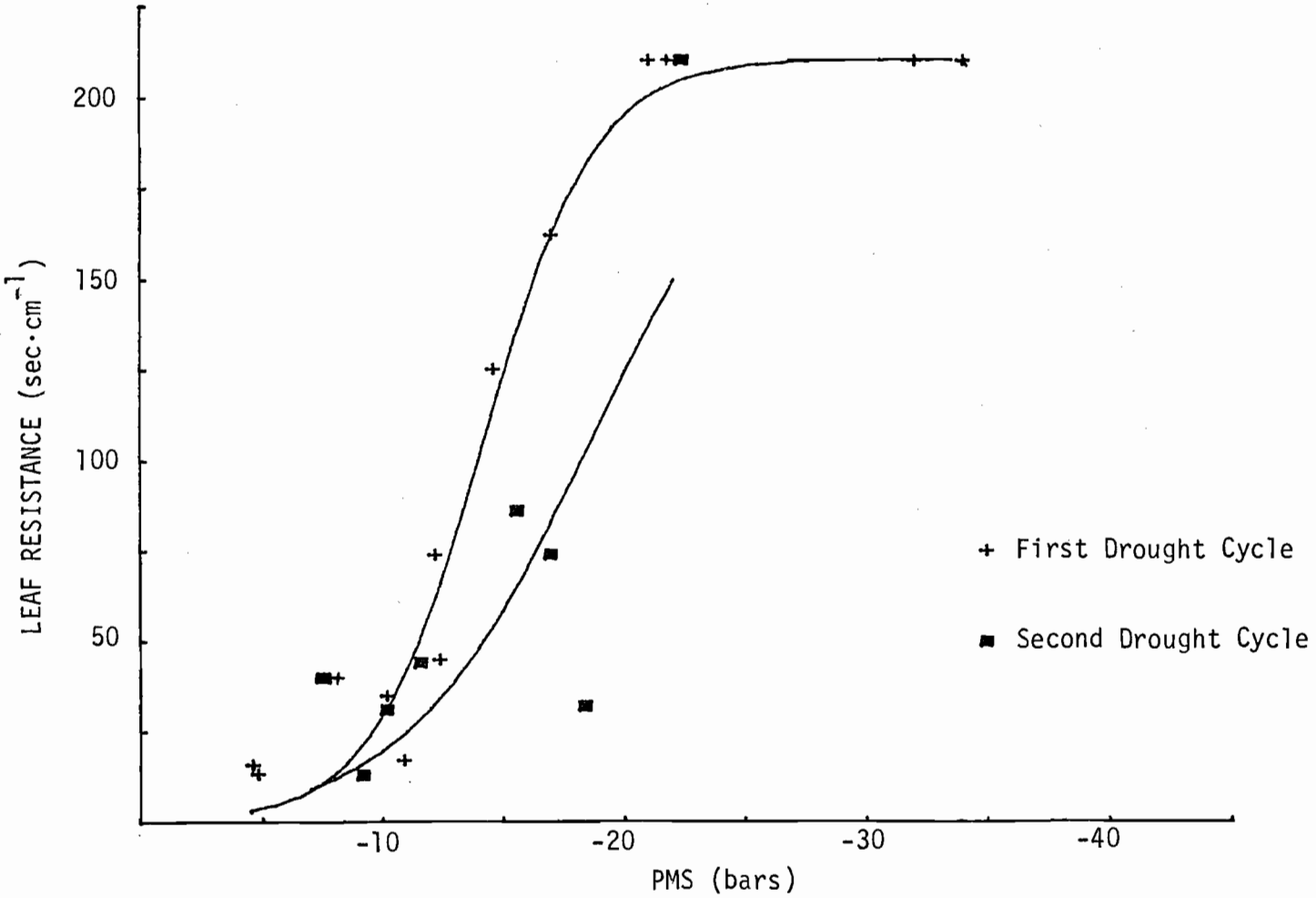

Figure 5. Leaf resistance and pre-dawn PMS in growth room during two consecutive drying cycles of Spokane area seedlings.

fitting program. The logistic function: $$y = \frac{B_1}{1 + B_2 e^{B_3 x}}$$

was chosen as the model to fit the data. An upper limit of 210 cm sec^{-1} was placed on leaf resistance values.

Figure 6 shows the same parameters plotted for the mesic seedlings in the growth room study.

The general response of leaf resistance to increasing PMS for both groups of seedlings corresponds to the results obtained by Blake and Ferrell (1977). Leaf resistance increased sharply within a critical water potential range. However, the critical level of PMS that triggers the increase in leaf resistance appears to vary between ecotypes and in the xeric seedlings after previous exposure to severe stress. The stomata of the xeric seedlings remained open until the plant reached greater levels of stress. After previous exposure to drought, the stomata of this group appear even less sensitive to severe stress.

Limited data were collected on the stomatal behavior of the mesic seedlings during the second drought cycle. Available sample points are plotted but a separate curve is not shown. Stomatal resistance of these seedlings was comparable to levels recorded during the first drought cycles. Any change in stomatal behavior was not evident.

In both mesic and xeric seedlings, leaf resistances two days after rewatering were comparable to pre-stress levels.

Effects of Moisture Stress on ABA Levels

Figure 7 represents the relationship between pre-dawn PMS and free ABA level in the Spokane group. All curves relating ABA

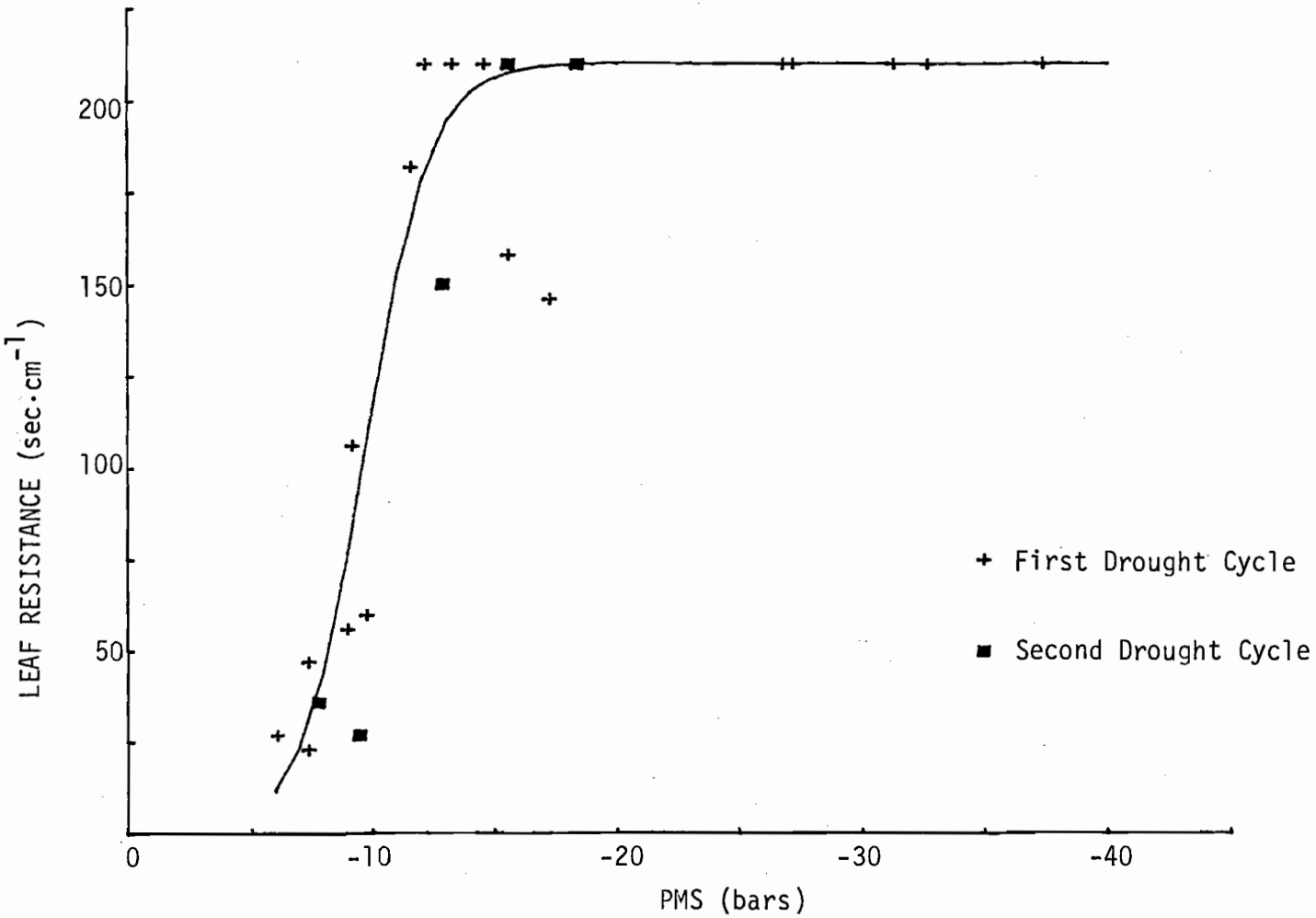

Figure 6. Leaf resistance and pre-dawn PMS in growth room during two consecutive drying cycles of St. Helens area seedlings.

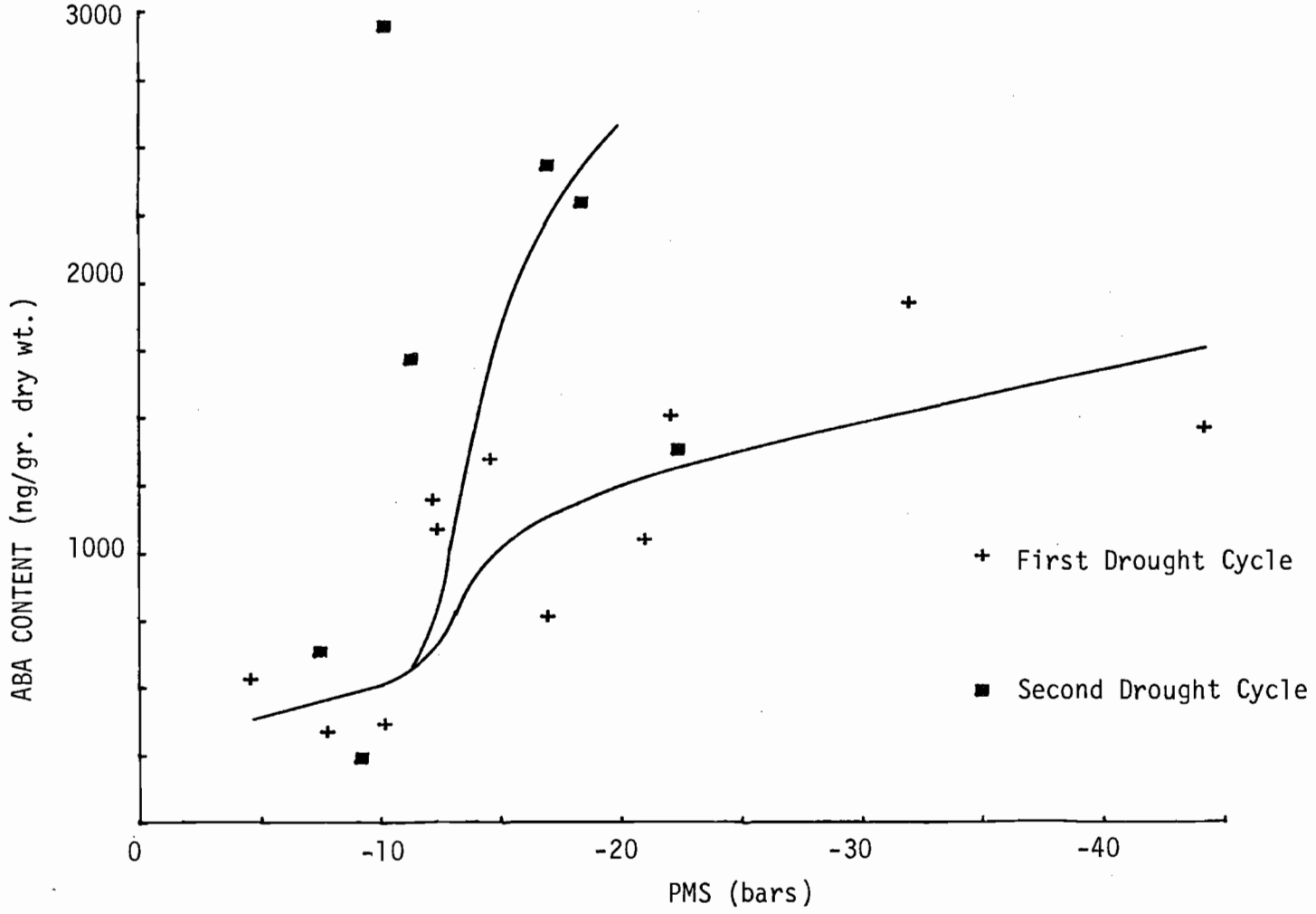

Figure 7. Needle ABA content and pre-dawn PMS in growth room during two consecutive drying cycles of Spokane area seedlings.

content to PMS are hand-drawn. During the first drought, ABA content tripled at stresses in excess of -10 bars. This roughly corresponds to the critical sap potential level for the increase in leaf resistance. This association has been described in Douglas-fir by Blake and Ferrell (1977) as well as in other species, Zabadal (1974), Hemphill and Tukey (1975).

However, this relationship appears to break down in these seedlings during the second drought cycle. Both ABA and leaf resistance levels returned to low pre-stress levels within two days after rewatering. But, while an identical critical level of stress triggers an enormous increase in ABA content, no increase in leaf resistance is evident until the plants attain higher levels of stress. In this case, the two events do not appear to be synchronous.

Table 2 lists data collected during the second drought cycle. Comparing values of ABA content and leaf resistance shows that in several plants, whole leaf ABA content is not related to stomatal functioning. ABA levels equal or exceed those correlated with stomatal closure in the first drought cycle. Yet, little or no change in leaf resistance is apparent.

Table 2. PRE-DAWN PMS, LEAF RESISTANCE, AND ABA CONTENT IN SPOKANE SEEDLINGS DURING A SECOND EXPOSURE TO DROUGHT.

PMS (bars)	Leaf Resistance (cm/sec)	ABA (ng/gr. dry wt.)
-9.2	13	245
-7.5	40	640
-10.2	31	3020
-11.6	44	1720
-17.0	74	2435
-18.4	32	2300
-22.4	210	1385

Figure 8 shows the relationship between xylem sap potential and free ABA level in the St. Helens group of seedlings. At the start of the first drought cycle, ABA levels were already quite high and increasing rapidly. This coincides with an apparently lower critical level of PMS needed to induce stomatal closure in this group. The increase in ABA approximately parallels the increase of leaf resistance for these plants during the first drought cycle.

Two days after rewatering, ABA remained at an elevated level and then decreased slowly with time. As mentioned, leaf resistances had returned to pre-stress levels and appeared to be functioning normally. These results are summarized in Table 3. Again, in several cases, stomatal aperture seems unaffected by high ABA content.

Table 3. PRE-DAWN PMS, LEAF RESISTANCE, AND ABA CONTENT IN ST. HELENS AREA SEEDLINGS DURING SECOND EXPOSURE TO DROUGHT.

PMS (bars)	Leaf Resistance (cm/sec)	ABA (ng/gr. dry wt.)
-7.8	36	2220
-9.5	27	2530
-12.5	150	1200
-15.6	210	1370
-16.3	-	1400
-18.4	-	1130
-20.4	-	830

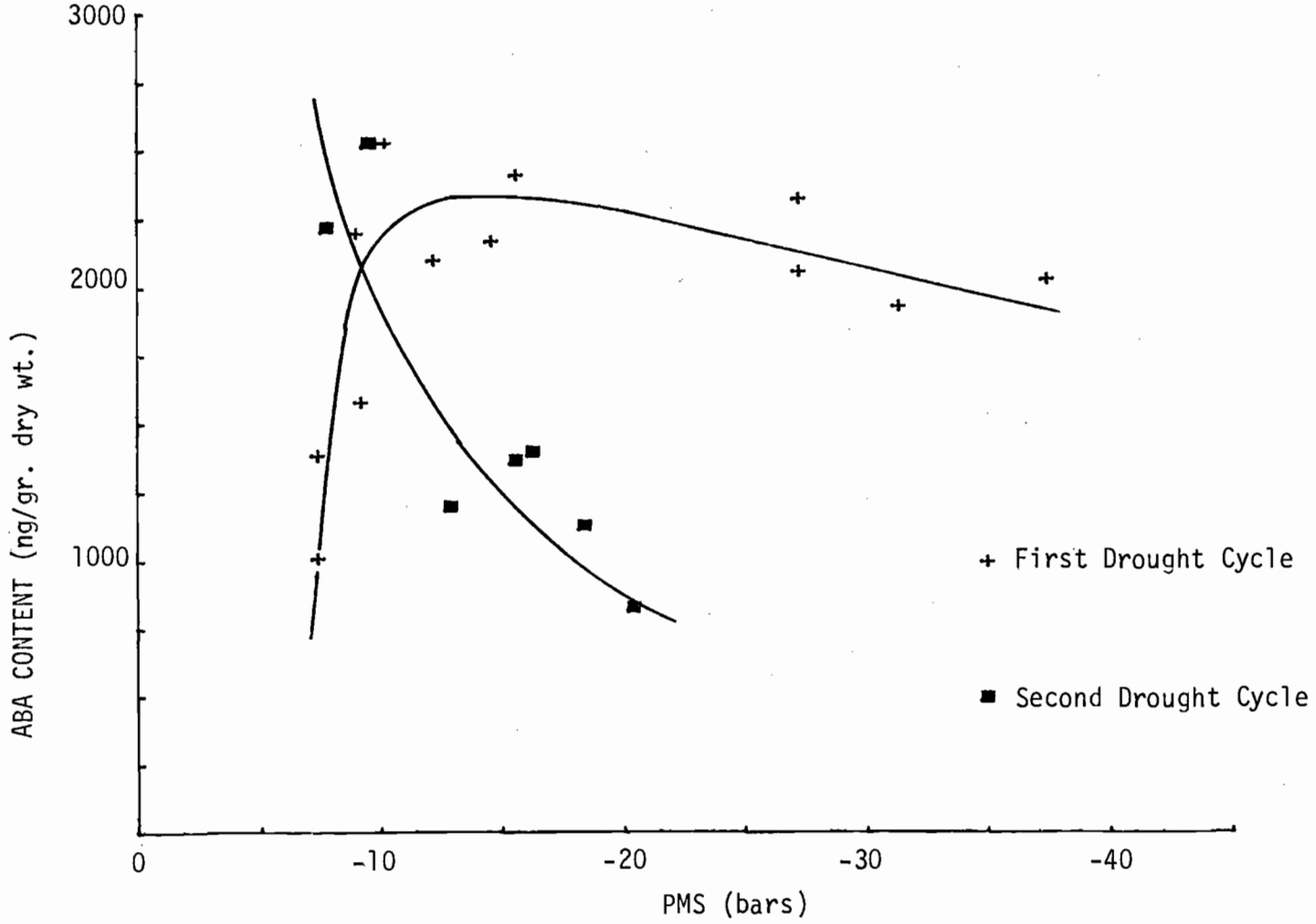

Figure 8. Needle ABA content and pre-dawn PMS in growth room during two consecutive drying cycles of St. Helens area seedlings.

Nursery Drought Study - Summer, 1976

Effects of Moisture Stress on Leaf Resistance

Figure 9 shows the response of leaf resistance to increasing PMS in Spokane seedlings kept outside at the nursery. The data recorded during two consecutive drought cycles are plotted together. The logistic response model was used to fit curves to the data points.

Figure 10 shows the same parameters plotted for the mesic St. Helens seedlings kept outside. No curve appears on the graph due to the high variability of the data.

PMS values in these and all figures and tables are pre-dawn measurements of xylem sap water potential. PMS measured just prior to sunrise allows for recovery of plant water potential and approximates a period of equilibrium with the soil water potential. Soil water potential, rather than plant water potential during the day, has been found to be more closely related to stomatal resistance during the day (Tan and Black, 1976). Running (1976), sampling Douglas-fir, reached the same conclusion concerning the relationship between pre-dawn PMS and the maximum leaf conductance measured during the day.

Initial examination of all data from the experiments described here, in both the growth room and at the nursery, indicated that the relationship between leaf resistance and the xylem sap potential during the day was poorly defined. As the drought cycle progressed, pre-dawn PMS rose steadily, while mid-day levels of PMS increased to high levels, decreased, then increased slowly once more. Changes in leaf resistance,

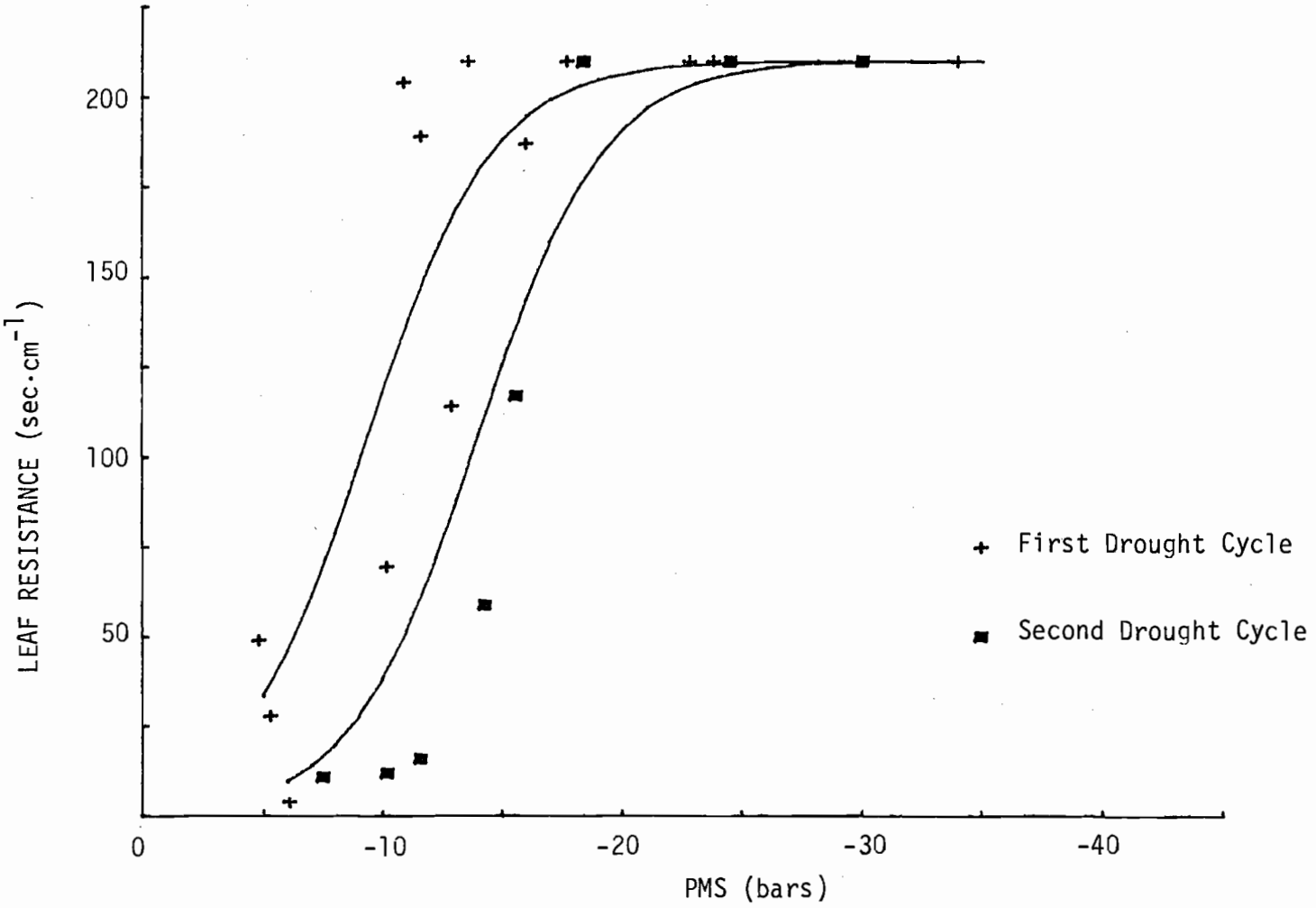

Figure 9. Leaf resistance and pre-dawn PMS outside at the nursery during two consecutive drying cycles of Spokane area seedlings.

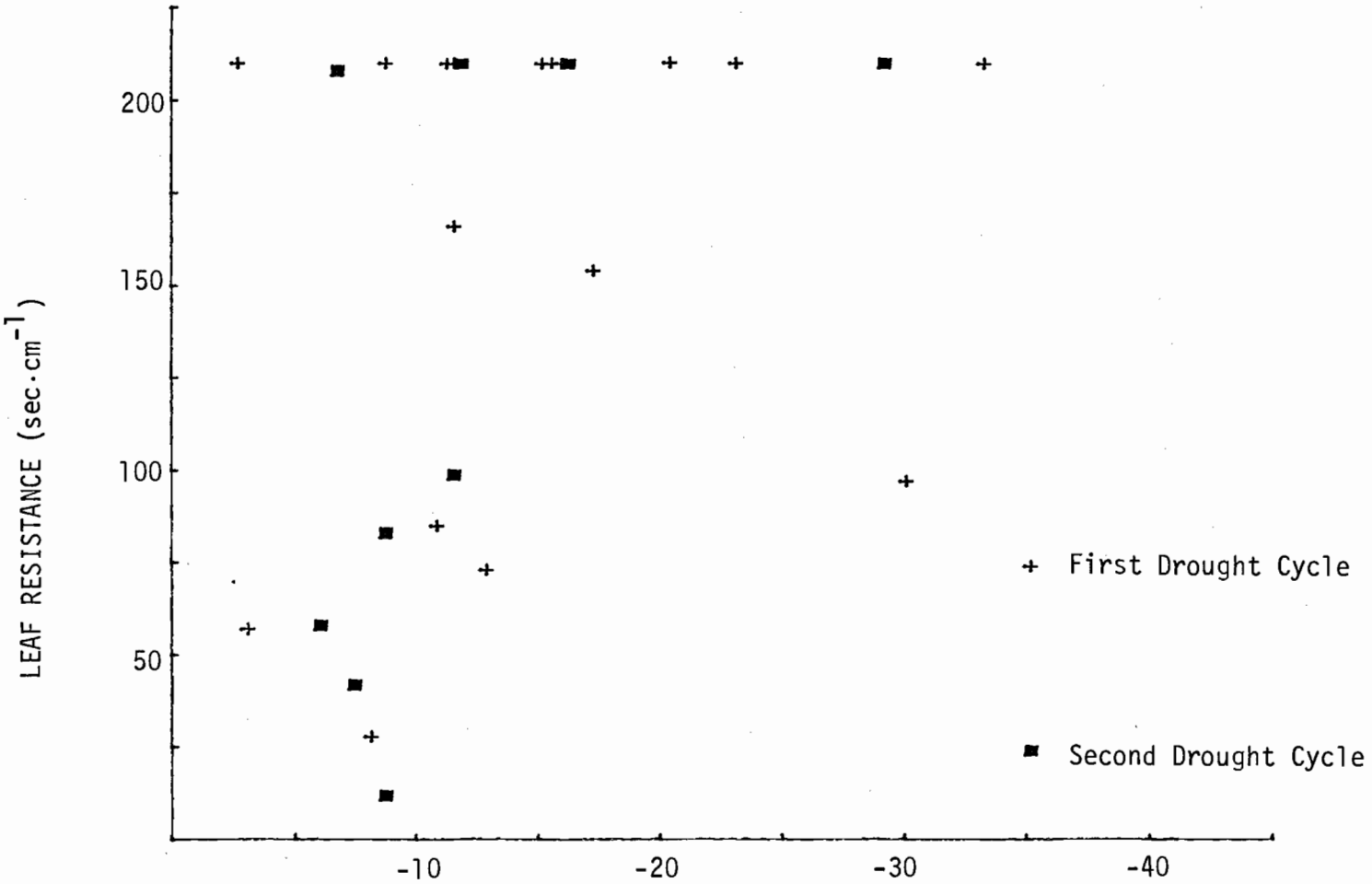

Figure 10. Leaf resistance and pre-dawn PMS outside at the nursery during two consecutive drying cycles of St. Helens area seedlings.

as well as levels of ABA, appeared more responsive to threshold levels of pre-dawn PMS, ultimately the soil water supply.

However, correlation between pre-dawn PMS and mid-day leaf resistance grows more tenuous when the experiment is moved out of the growth room and into the natural environment. Plants respond to factors affecting water demand (temperature, humidity, and radiation), as well as those affecting the water supply (soil water potential, soil and root hydraulic conductivity). Tan and Black (1976) found that in natural stands of Douglas-fir, stomatal resistance was well correlated with soil water potential and the vapor pressure deficit (VPD) during the day. PMS during the day, a result of the interaction of water demand and supply factors, modified by plant behavior (stomatal resistance), was by itself less indicative of leaf resistance.

In the growth room, water demand is held constant. Outside, it is changing and daily fluctuations in leaf resistance and PMS become much greater. Two examples of the typical diurnal changes measured during the drought study are presented in Figures 11 and 12.

Since samples in the drought study were taken on a number of different days, variable atmospheric conditions at the nursery likely affected the measurements of stomatal resistance. The method of sampling, while not eliminating this defect, should have held its effect to a minimum. All measurements were made on relatively clear, warm days and though VPD at the times of measurement were undoubtedly different, they were not variable in the extreme. Examination of weather data collected at the nursery indicates the VPD to have been between 25 to 35 mb during most times of leaf resistance measurement.

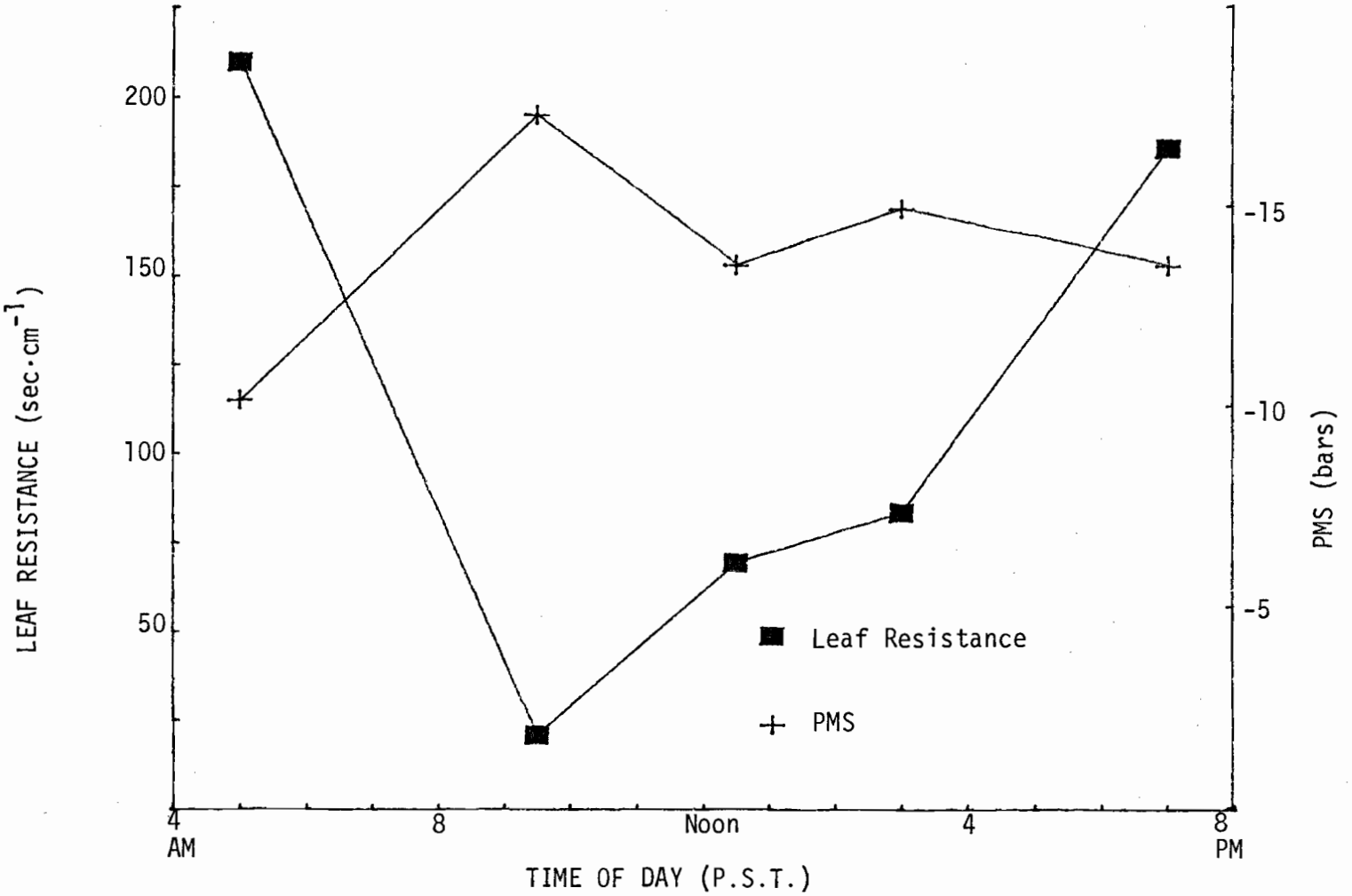

Figure 11. Diurnal change in leaf resistance and PMS in Spokane area seedlings outside at the nursery. August 27, 1976.

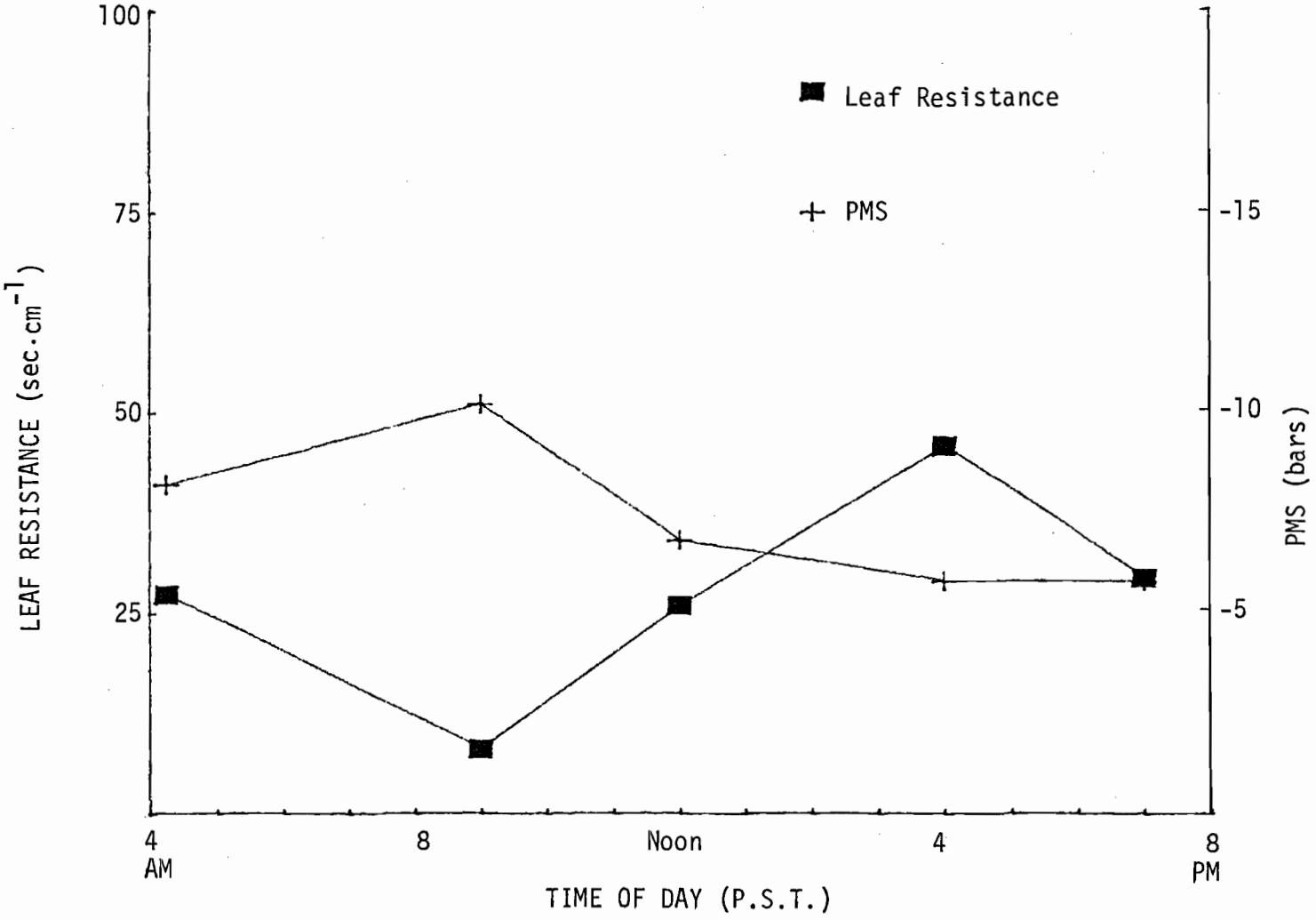

Figure 12. Diurnal change in leaf resistance and PMS in St. Helens area seedlings outside at the nursery. August 27, 1976.

VPD in the growth room remained constant at 11.5 mb during the light cycle. Higher VPD results in higher leaf resistance at corresponding soil water potentials (Tan and Black, 1976).

Figure 9 shows the response of leaf resistance in the xeric Spokane seedlings outside to be similar to the growth chamber seedlings in all respects but one. Leaf resistance values at any particular PMS level are somewhat greater outside. This could be a direct result of the greater outside VPD. Significantly, the response of the xeric seedlings to a secondary drought is identical in nature to those in the growth chamber. That is, less stomatal sensitivity to moisture stress after previous exposure.

The relationship shown in Figure 10 between pre-dawn PMS and leaf resistance in the mesic seedlings is not as well defined as in the xeric group. Several instances of high leaf resistance following low pre-dawn stress occur. Variation in the VPD may account for the variability of the data, especially if the stomatal aperture of the mesic seedlings was more responsive to high VPD than the stomatal aperture of the xeric seedlings.

No substantial differences in the stomatal behavior of the two groups of seedlings are apparent with increasing moisture stress in the nursery study. However, leaf resistance in the mesic group, unlike the xeric, does not appear to change during a second drying cycle.

Effects of Moisture Stress on ABA Levels

Figure 13 shows the relationship between needle ABA content of the Spokane seedlings outside and increasing PMS during two consecutive periods of drought. During the first drought cycle, ABA levels rose

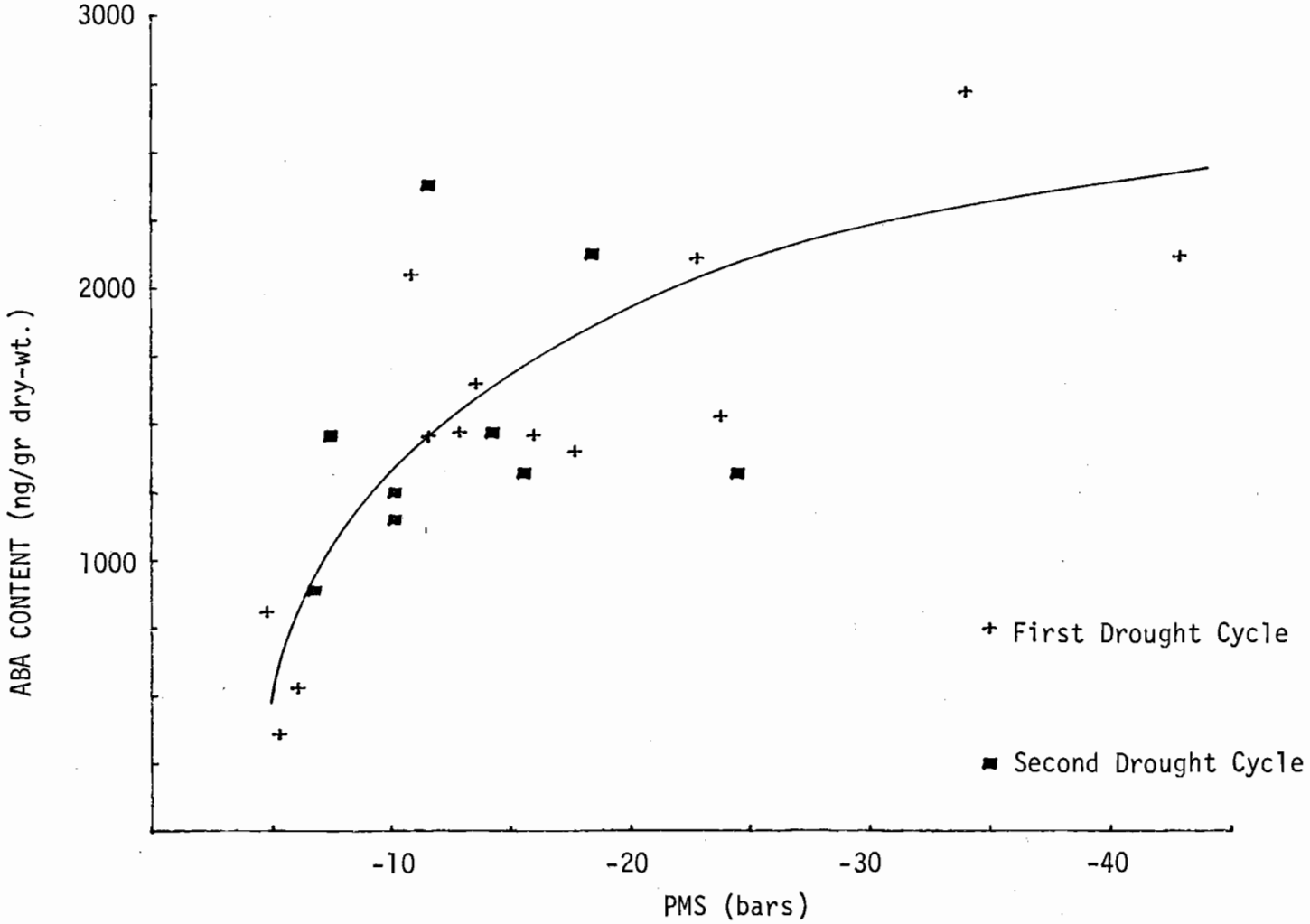

Figure 13. Needle ABA content and pre-dawn PMS during two consecutive drying cycles of Spokane area seedlings outside at the nursery.

simultaneously with the increase in leaf resistance. These changes occurred at slightly lower water potentials than before in the growth room. A threshold level of stress was not detected in this case as no samples were collected between -5.3 and -10.9 bars, the point of ABA and leaf resistance increase.

ABA levels increased six fold between these points while leaf resistance rose from 28 $sec \cdot cm^{-1}$ to 205 $sec \cdot cm^{-1}$ at midday. ABA would seem to be the agent modulating the stomatal response to water stress. However, again, this relationship between increased leaf resistance and ABA level does not extend to a second drought cycle. After rewatering, leaf resistances had returned quickly to pre-stress levels and were unaffected by high endogenous ABA content.

Table 4 lists part of the data collected during the second drought cycle. ABA levels in these seedlings did not recover completely two days after rewatering though a drop of about 30 percent did occur. These high residual levels of ABA mask any subsequent change in needle ABA content induced by moisture stress during a second cycle of drought. ABA content remained high and bore no relation to stomatal behavior.

Table 4. LEAF RESISTANCE, PRE-DAWN PMS, AND ABA CONTENT IN SPOKANE SEEDLINGS AT THE NURSERY DURING A SECOND EXPOSURE TO DROUGHT

PMS (bars)	Leaf Resistance ($sec \cdot cm^{-1}$)	ABA (ng/gr dry-wt.)
-6.8	--	890
-7.5	11	1460
-10.2	12	1150
-10.2	17	1250
-11.6	16	2380
-14.3	59	1470
-15.6	117	1320
-18.6	210	2125
-24.5	210	1340

Figure 14 shows the needle ABA content in the St. Helens seedlings outside in relation to increasing pre-dawn PMS. Any direct relationship between those parameters, or either one in conjunction with leaf resistance, appears obscurred, insubstantial.

Changes of Bound ABA Content Induced by Moisture Stress

Tandem measurements of bound-ABA and free-ABA were made throughout all of the previous drought studies. The levels of bound-ABA are recorded in the appendices together with all data collected during the experiments.

Change in bound-ABA content was not found to be correlated with any other parameter. It exceeded levels of free-ABA two to three times in some cases, represented as low as 20 percent in others. In general, its level was close to free-ABA levels.

Though free-ABA may be released from a bound form, the absolute level of bound-ABA measured here did not reflect either the potential for an increase in free-ABA or the physiological state of a seedling with respect to moisture stress. The measurement could be in error.

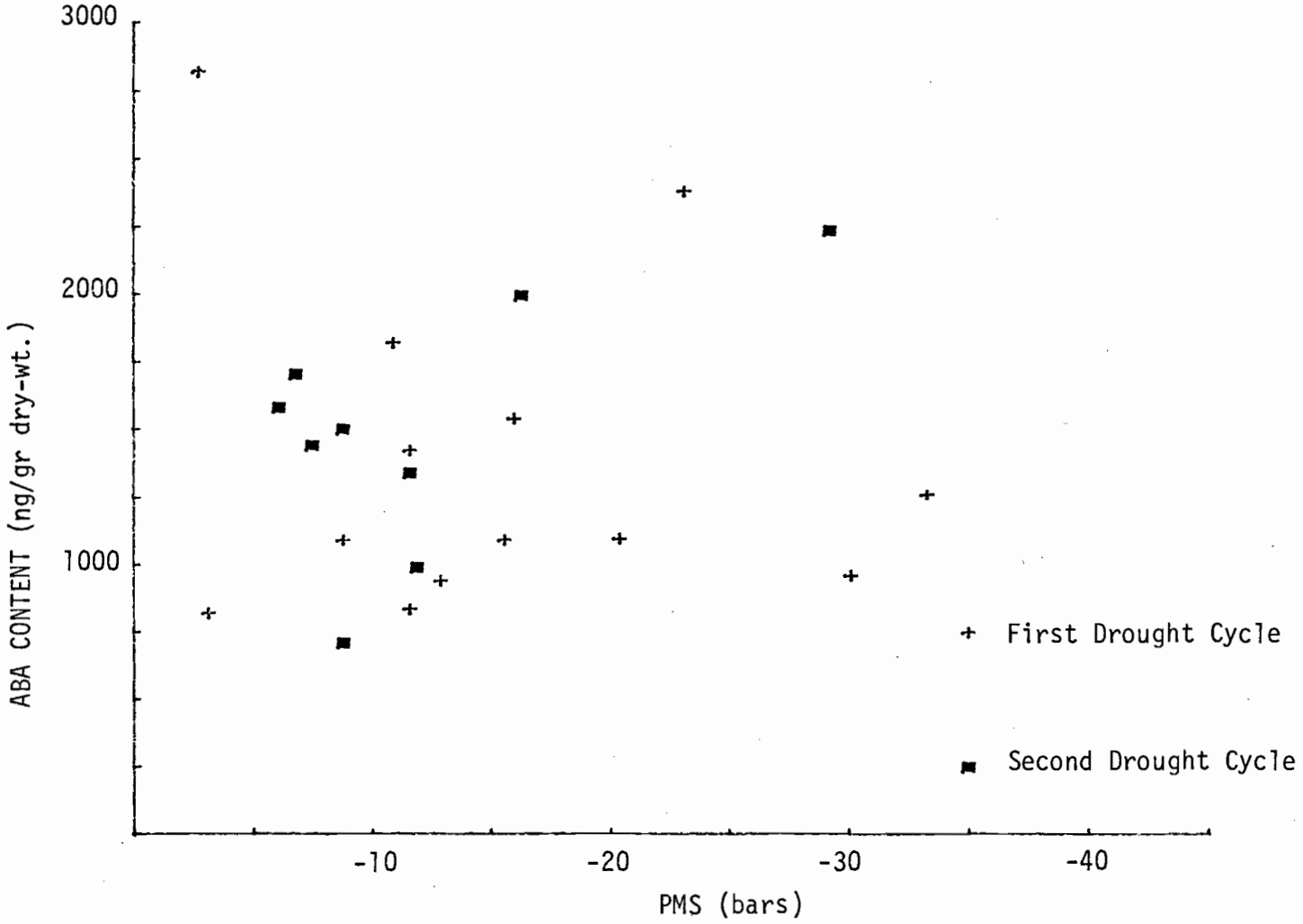

Figure 14. Needle ABA content and pre-dawn PMS during two consecutive drying cycles of St. Helens area seedlings outside at the nursery.

Millborrow and Mallaby (1975) report that in alkaline methanol the glucosyl ester of ABA transesterfies to form methyl abscisate. The extraction procedure used here, while still ideal for the quantification of the amount of the free acid of ABA, is apparently unsuitable for the glucosyl conjugate. Methyl abscisate would be removed during the extraction and levels of the glucosyl conjugate underestimated.

V. DISCUSSION AND CONCLUSIONS

The objective of this study was to relate endogenous hormone levels to differences in stomatal behavior in ecotypes of Douglas-fir seedlings during drought and recovery. However, the results, particularly during a second drought cycle, conflicted with certain expectations held at the onset of the study. The conclusion reached by Unterscheutz et al. (1974), that xeric ecotype seedlings decrease transpiration rates more in relation to increasing moisture stress than do mesic ecotypes was not confirmed.

In this study, xeric seedlings did not exhibit greater sensitivity to water vapor loss. The absence of stomatal sensitivity in xeric ecotypes during drought was accentuated after a previous exposure to severe drought when xeric seedlings delayed stomatal closure until greater levels of stress. Unterscheutz et al. interpreted their results to indicate that pre-conditioning moisture stress has the opposite effect, "Seedlings which have experienced prior soil moisture stress decrease transpiration more in response to low plant water potentials than do plants which have experienced no soil moisture stress." This conclusion was based on the observation that plants preconditioned in a cold frame exhibited lower transpiration rates in comparision to plants grown inside in a growth room (when measured under conditions of both low soil and plant water potential). Presumedly the outside grown seedlings preconditioned to higher levels of evaporative demand under the full sunlight. They were kept well watered so the possibility of actual severe soil stress

preconditioning is precluded. However, their data indicates that seedlings preconditioned outside had far greater transpiration rates under conditions of low to moderate stress. This was true for both ecotypes.

This type of behavior after moisture stress preconditioning is similar to that noted by Thomas et al. (1976) in sorghum (*sorghum vulgare*) under field conditions. McCree (1974) and Brown et al. (1976) have also shown that stomatal response to decreasing soil moisture potential of chamber grown sorghum and cotton plants is altered by previous moisture stress. In all cases, the initiation of stomatal closure in plants preconditioned to stress occurred at higher levels of stress than in plants which were not preconditioned. This parallels the results of preconditioning found here with xeric ecotypes and indicates a possible increased physiological tolerance to drought. This adjustment in the stomatal response has been attributed to a reduction in the osmotic potential of leaves from preconditioned plants (Thomas et al., 1976) although this has not been verified.

What might be the physiological or ecological value of this change in stomatal behavior for any of these plants? Restriction of transpiration also diminishes CO_2 exchange and ultimately photosynthesis. Increased drought tolerance after preconditioning would provide enhanced capacity for growth under moderate moisture stress by extending the range in which photosynthesis may occur. The competitive ability of the plant would be increased and its potential geographic range extended. During severe drought, growth and photosynthesis become irrelevant. Under these circumstances, preconditioned

plants, in these and the other studies cited, minimize water loss as well as, or better than, plants with no prior exposure to water stress.

Presently, there are no clear indications whether this type of change of stomatal behavior after preconditioning is more characteristic of xeric species or ecotypes than mesic ones. Stomatal behavior of mesic Douglas-fir seedlings was not altered in this study by preconditioning moisture stress, however, only a limited number of observations were made.

There is evidence to suggest that other physiological processes in species adapted to xeric habitats are less affected by water stress than those in species found in mesic environments. Inhibition of protein synthesis, irreversible changes in membrane permeability, and disruption of polyribosomes have all been found to be minor in certain drought tolerant species as compared to drought sensitive species (Dhindsa and Cewley, 1977; Bewley, 1973; Brandle et al., 1973; Brandle et al., 1977).

It is interesting to note that sorghum has long been considered one of the most drought hardy crop plants. Sanchez-Diaz and Kramer (1971) found that in comparison to corn, sorghum, when under water stress, began to close stomata later and at lower water potentials. The water saturation deficit of stressed corn was much greater at all levels of water potential than that of sorghum. Jarvis and Jarvis (1963a) also found that the more drought resistant species Spruce (*Pices abies*) and Pine (*Pinus sylvestris*) reach lower relative turgidities before stomatal closure than do the more drought sensitive Birch (*Betula verrocosa*) and Aspen (*Populus tremula*). Spruce

maintained transpiration over a wide range of water content (Jarvis and Jarvis, 1963b).

Some of the differences between the results obtained here and those of Unterscheutz et al. could be attributed to the different age of the seedlings used in the experiments. All plants used in this study were two to three years old; those used by Underscheutz et al. were in the first year of growth. Stomatal behavior of certain ecotypes of Douglas-fir could change after the first year of growth. Limited experiments by Drew (1974) found transpiration in two year old seedlings to be less sensitive to plant moisture stress than during the first year of growth.

Transpirational control may be particularly important in the early establishment of a seedling before appreciable penetration of the soil by the root system. Alternately, the tolerance of the seedling to desiccation may change with age and/or prior exposure to drought. Enhanced drought tolerance would make the seedling less reliant on drought avoidance mechanisms. It should be emphasized that drought avoidance, resulting in the conservation of water by reducing transpiration, will do the plant little good if the soil moisture is depleted by the competing vegetation.

Also, xeric ecotypes differ in the exact form of drought resistance that has evolved. Ferrell and Woodard (1966) assessed levels of both drought tolerance and avoidance in first year xeric and mesic ecotypes of Douglas-fir. Xeric ecotypes from N.E. Washington had enhanced drought avoidance mechanisms; those from Arizona had greater tolerance levels. Yet, tolerance in the Washington group and avoidance

in the Arizona group were no greater than those found in the mesic seedlings from Vancouver Island. Thus, the characteristics of stomatal behavior under moisture stress may not be uniform for all xeric ecotypes but instead reflect the influence of particular local conditions.

There have been several recent investigations into the role ABA might play in the adaptation of plants from contrasting environments to drought (Dorffling et al., 1977; Milborrow and Robinson, 1973; Larque-Saavedra and Wain, 1977). These reports emphasize the high levels of ABA induced by water stress in drought tolerant varieties (Larque-Saavedra and Wain, 1977) and the slow decrease of ABA parallel with the delayed recovery of stomatal functioning in mesophytic species (Dorffling et al., 1977). The latter type of behavior may represent a safety mechanism against drought enabling plants to recover cell turgidity rapidly. These studies, as well as most of the research relating changes in ABA content to water relations have noted the effects of a rapid dehydration induced by leaf excision, hot (35°C), dry air from blowers, or both. The data reported here in this study were gathered under conditions similar to the slow progressive effects of natural drought. Besides restricting the introduction of unknown variables, this procedure also allows precise determination of the point of increase in leaf resistance and ABA content with increasing plant moisture stress.

In this study, during an initial cycle of drought, critical levels of xylem sap potential existed in both ecotypes that apparently stimulated a simultaneous increase in ABA content and leaf resistance. This suggests that increased endogenous ABA results in the closure of stomata at a critical level of stress and represents the physiological

basis for a drought survival mechanism in Douglas-fir. However, after rewatering and recovery, and during a second cycle of drought, high ABA content was not associated with increased leaf resistances. Recovery of pre-stress leaf resistances in both groups was mainly complete two days after rewatering, but ABA levels remained high in mesic seedlings. Also, during a second drought of the xeric seedlings, a threshold level of moisture stress again triggered a sharp increase in ABA content although leaf resistances were unchanged. In neither ecotype is stomatal behavior after preconditioning moisture stress always directly related to endogenous ABA content.

The delayed closure of stomata in xeric seedlings after preconditioning moisture stress is believed to represent an increased physiological tolerance to drought. The change in stomatal behavior of this ecotype is entirely independent of whole leaf ABA content. This indicates that after preconditioning moisture stress, the rapid closure of stomata in xeric ecotypes of Douglas-fir in response to a threshold level of PMS may not be the direct result of endogenous ABA.

Studies on the short term changes in ABA content and stomatal aperture induced by rapid dehydration indicates that an increase of ABA is not a necessary prerequisite for the induction of stomatal closure (Beardsell and Cohen, 1975; Dorffling et al., 1977). It seems likely that ABA is not involved in the short term changes of stomatal aperture associated with diurnal patterns of transpiration under natural conditions. However, considerable evidence indicates that ABA is responsible for the stomatal response of plants to a threshold level of stress (Zabadal, 1974; Blake and Ferrell, 1977) and that raised ABA

levels, once induced by water stress, are responsible for the occurrence and duration of the after-effect of wilting on stomatal openings (Dorffling et al., 1977). This relationship apparently does not extend to xeric and mesic ecotypes of Douglas-fir after preconditioning moisture stress.

Two hypotheses are suggested in the literature in explanation of instances similar to those found in this study where stomatal behavior was not related to whole leaf ABA content. If one or both are eventually shown to be accurate, the findings here would then not conflict with either the idea that ABA is the regulator of stomatal aperture under all circumstances or that ABA is responsible for rapid stomatal closure at a threshold level of plant moisture stress. The two hypotheses are:

a) The sensitivity of guard cells to ABA changes after either prolonged moisture stress or high ABA levels (Kriedemann et al., 1972).

b) ABA may be removed from the site of action into storage sites where it cannot act on the stomata (Cummins, 1972; Raschke, 1975), and that stomatal closure can result from the "redistribution" of this "compartmentalized ABA" (Beardsell and Cohen, 1975).

Most of the published literature relating changes in ABA content to water relations involves species from mesic environments. Lancaster et al. (1977) recently found that exogenous ABA was largely ineffective in inducing the stomatal closure of yellow lupin (*Lupinus luteus*), a strongly drought tolerant plant. Still, rapid dehydration of yellow

lupin, induced either by leaf excision or by uprooting plants, caused approximately a ten-fold increase in endogenous ABA. The authors suggested that though ABA levels increase after water stress, ABA is not the regulator of stomatal aperture in yellow lupin.

Drought resistance is a general characteristic of Douglas-fir that is particularly well developed in xeric ecotypes of the species. The stomatal physiology of drought resistant plants (yellow lupin, Douglas-fir) could be distinct from mesophytic plants in that either ABA is not involved in rapid stomatal closure at a threshold level of PMS, or the relationship is not indicated in all circumstances by measurements of whole leaf ABA content. ABA is a plant growth hormone with numerous effects besides the postulated regulation of stomatal aperture. Its role in drought resistance, or in the response of the plant to moisture stress, could also involve one or more of these other functions. The inhibition of growth or the effects of ABA on protein and RNA synthesis are examples. The data presented here makes a direct cause and effect relationship between ABA content and stomatal behavior during drought of Douglas-fir seedlings appear questionable under certain circumstances, and suggests that increased ABA levels induced by water stress are not directly related to the drought resistant characteristics of particular families, varieties, or ecotypes.

BIBLIOGRAPHY

Beardsell, M.F. & Cohen, D. 1975. Relationships between leaf water status, abscisic acid levels, and stomatal resistance in Maize and Sorghum. Plant Physiology. 56:207-212

Beardsell, M.F., Jarvis, P.G., & Davidson, B. 1972. A null-balance diffusion porometer suitable for use with leaves of many shapes. J. Appl. Ecol. 9:677-690

Bewley, J.D. 1973. Desiccation and protein synthesis in the moss *Tortula ruralis*. Can. J. Bot. 51:203-206

Blake, J. & Ferrell, W.K. 1977. The association between soil and xylem water potential, leaf resistance, and abscisic acid content in droughted seedlings of Douglas-fir (*Pseudotsuga menziesii*). Physiol. Plant. 39:106-109

Brandle, J.R., Hinckley, T.M., & Brown, G.N. 1977. The effects of dehydration-rehydration cycles on protein synthesis of black locust seedlings. Physiol. Plant. 40:1-5

Brandle, J.R., Schnare, P.D., Hinckley, T.M., & Brown, G.N. 1973. Changes in polysomes of black locust seedlings during dehydration-rehydration cycles. Physiol. Plant. 29:406-409

Brown, K.W., Jordan, W.R., & Thomas, J.C. 1976. Water stress induced alteration in the stomatal response to leaf water potential. Physiol. Plant. 37:1-5

Cooper, M.J., Digby, J., & Cooper, P.J. 1972. Effects of plant hormones on the stomata of barley: a study of the interaction between abscisic acid and kinetin. Planta. 105(1):43-49

Cummins, W.R. 1973. The metabolism of abscisic acid in relation to its reversible action on stomata in leaves of *Hordeum vulgare* L. Planta 114(2):159-167

Cummins, W.R., Kende, H. & Raschke, K. 1971. Specificity and reversibility of the rapid stomatal response to ABA. Planta 99:347-351

Davies, W.J., Kozlowskii, T.T. 1975. Effects of applied abscisic acid and plant water stress on transpiration of woody angiosperms. Forest Science. 21(2):191-195

Dhindsa, R.S. & Bewley, J.D. 1977. Water stress and protein synthesis. V. Protein synthesis, protein stability, and membrane permeability in a drought-sensitive and a drought-tolerant moss. Plant Physiol. 59:295-300

Dorffling, K., Streich, J., Kruse, W., & Muxfeldt, B. 1977. Abscisic acid and the after-effect of water stress on stomatal opening potential. Z. Pflanzenphysiol. Bd. S. 43-56

Drew, A.L. 1974. Effects of light preconditioning on the seasonal water relations of nursery-grown Douglas-fir. Ph.D. thesis. Oregon State University, Corvallis, OR.

Eickmeier, W., Adams, M.S., & Lester, D. 1975. Two physiological races of *Tsuga canadensis* in Wisconsin. Can. J. Bot. 53(9): 490-951.

Ferrell, W.K. & Woodard, E.S. 1966. Effects of seed origin on drought resistance of Douglas-fir [*Pseudotsuga menziesii*, (Mirb.) Franco]. Ecology. 47(3):499-503

Gholz, H.L., Franklin, K., & Waring, R.H. 1976. Leaf area differences associated with old-growth forest communities in the western Oregon Cascades. Can. J. For. Res. 6:49-57

Goldschmidt, E.E., Goren, R., Even-Chan, Z., & Bittner, S. 1973. Increase of free and bound abscisic acid during natural and ethylene induced senescense of citrus fruit peel. Plant Physiol. 51:879-882

Hsiao, T.C. 1973. Plant responses to water stress. Annual Review of Plant Physiology, Annual Reviews Inc., Palo Alto, CA. pp. 527

Hemphill, D.D. & Tukey, H.B. 1975. Effect of intermittent mist on abscisic acid levels in *Evonymus alatus*: leaching vs. moisture stress. Hort. Science 10:369-370

Hiron, R.W.P. & Wright, S.T.C. 1973. The role of endogenous abscisic acid in the response of plants to stress. J. of Exp. Bot. 24: 769-781

Jarvis, P.G. & Jarvis, M.S. 1963a. The water relations of tree seedlings. IV. Some aspects of the tissue water relations and drought resistance. Physiol. Plant. 16:501-516

Jarvis, P.G. & Jarvis, M.S. 1963b. The water relations of tree seedlings. II. Transpiration in relation to soil water potential. Physiol. Plant. 16:236-253

Jones, R.J. & Mansfield, T.A. 1970. Suppression of stomatal opening in leaves treated with abscisic acid. J. Exp. Bot. 21:714-719

Jones, R.J. & Mansfield, T.A. 1971. Antitranspirant activity of methyl and phenyl esters of abscisic acid. Nature 231:331-332

Koshimizu, K., Inui, M., Fukui, H., & Mitsui, T. 1968. Isolation of (+)-abscisyl-β-D-glucopyranoside from immature fruit of *Lupinus luteus*. Agr. Biol. Chem. 32:789-791

Kriedemann, P.E., Loweys, B.R., Fuller, G.L., & Leopold, A.C. 1972. Abscisic acid and stomatal regulation. Plant Physiol. 49(5): 842-847

Ladiges, P.Y. 1974. Variation in drought tolerance in *Eucalyptus viminalis*. Aust. J. Bot. 22(3):489-500

Lancaster, J.E., Mann, J.D., & Porter, N.G. 1977. Ineffectiveness of abscisic acid in stomatal closure of yellow lupin, *Lupinus luteus* var. Weiko III. J. Exp. Bot. 28:184-191

Larque-Saavedra, A. & Wain, R.L. 1974. Abscisic acid levels in relation to drought tolerance in varieties of *Zea mays*. L. Nature. 251:716-717

Loveys, B.R. & Kriedemann, P.E. 1973. Rapid changes in abscisic acid-like inhibitors following alterations in vine leaf water potential. Physiol. Plant. 28:476-479

McCree, K.J. 1974. Changes in the stomatal response characteristics of grain sorghum produced by water stress during growth. Crop Science 14:273-278

Milborrow, B.V. 1970. The metabolism of abscisic acid. J. Exp. Bot. 21:17-29

Milborrow, B.V. 1974. The chemistry and physiology of abscisic acid. Annual Review of Plant Physiology, Annual Reviews Inc. Palo Alto, CA. pp. 275-276

Milborrow, B.V. & Mallaby, R. 1975. Occurrence of methyl (+) abscisate as an artifact of extraction. J. Exp. Bot. 26:741-748

Milborrow, B.V. & Robinson, D.R. 1973. Factors affecting the biosynthesis of abscisic acid. J. Exp. Bot. 24:537-548

Mittleheser, C.J. & van Steveninck, R.F.M. 1969. Stomatal closure and inhibition of transpiration induced by (R.S.)-abscisic acid. Nature 221:281-282

Mizrahi, Y., Blumenfeld, A., & Richmond, A.E. 1970. Abscisic acid and transpiration in leaves in relation to osmotic root stress. Plant Physiol. 46:169-171

Morshet, S. & Yocum, C.S. 1972. A condensation type porometer for field use. Plant Physiol. 49:944-949

Most, B.H. 1971. Abscisic acid in immature apical tissue of sugar cane and in leaves of plants subjected to drought. Planta 101: 67-75

Pharis, R.P. & Ferrell, W.K. 1966. Differences in drought resistance between coastal and inland sources of Douglas-fir. Can. J. Bot. 44:1651-1659

Raschke, K. 1975. Stomatal action. Annual Review of Plant Physiology 26:309-340

Raschke, K., Pierce, M., & Popiela, C.C. 1976. Abscisic acid content and stomatal sensitivity to CO_2 in leaves of *Xanthium strumarium* L. after pretreatments in warm and cold growth chambers. Plant Physiol. 57:115-121

Raschke, K. & Zeevaart, J.A.D. 1976. Abscisic acid content, transpiration, and stomatal conductance as related to leaf age in plants of *Xanthium strumarium* L. Plant Physiol. 58:169-174

Rudnicki, R. & Pieniazek, J. 1971. Free and bound abscisic acid in developing and ripe strawberries. Bull Acad. Polon. Sci. 19: 421-423

Running S.W. 1976. Environmental control of leaf water conductance in conifers. Can. J. of For. Res. 6:104-112

Sanchez-Diaz, M.F. & Kramer, P.J. 1971. Behavior of corn and sorghum under water stress and during recovery. Plant Physiol. 48: 613-616

Scholander, P.F., Hammel, H.T., Bradstreet, E.D., & Hemmingsen, E.A. 1965. Sap pressure in vascular plants. Science 148:339-346

Tan, C.S. & Black, T.A. 1976. Factors affecting the canopy resistance of a Douglas-fir forest. Boundary-Layer Meteorology 10:475-488

Thomas, J.C., Brown, K.W., & Jordan, W.R. 1976. Stomatal response to leaf water potential as affected by preconditioning water stress in the field. Agronomy J. 68:706-708

Townsend, A.M. & Roberts, B.R. 1973. Effect of moisture stress on Red Maple seedlings from different seed sources. Can. J. Bot. 51(10):1989-1995

Turner, N.C. & Parlange, J.Y. 1970. Analysis of operation and calibration of a ventilated diffusion porometer. Plant Physiol. 46:175-177

Unterscheutz, P.F., Ruetz, W., Geppert, R., & Ferrell, W.K. 1974. The effect of age, predonditioning, and water stress on transpiration rates of Douglas-fir (*Pseudotsuga menziesii*) seedlings of several different ecotypes. Physiol. Plant. 32:214-221

Waring, R.H. & Cleary, D.B. 1967. Plant moisture stress: evaluation by pressure bomb. Science 155:1248-1254

Weber, J.E. 1974. The occurrence of abscisic acid in the dormant shoots of Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco]. Ph.D. thesis. Oregon State University, Corvallis, OR.

Wright, S.T.C. & Hiron, R.W.D. 1969. (±)Abscisic acid, the growth inhibitor induced in detached leaves by a period of wilting. Nature 224:719-720

Zabadal, T.J. 1974. A water potential threshold for the increase of abscisic acid in leaves. Plant Physiol. 53:125-127

Zavitkovski, J. & Ferrell, W.K. 1968. Effect fo drought upon rates of photosynthesis, respiration, and transpiration of seedlings of two ecotypes of Douglas-fir. Bot. Gaz. 129(4):346-350

Zeevart, J.A.D. 1971. (±)Abscisic acid content of spinach in relation to photoperiod and water stress. Plant Physiol. 48:86-90

APPENDICES

GROWTH ROOM DROUGHT STUDY

Bitterroot Seed Source

predawn PMS	day PMS	day L_r
-7.5	-12.3	12.2
-7.2	-10.2	13.2
-8.8	-11.1	10.5
-9.2	-12.2	11.8
-9.5	-20.7	11.3
-12.2	-15.8	11.8
-13.6	-11.9	13.9
-13.3	-19.0	19.0
-16.3	-17.1	19.0
-22.4	-29.3	33.7
-25.2	-29.9	37.0
-25.2	-31.6	38.9
-32.0	-36.4	48.0
-34.0	-38.8	79.7

Rewatered

			# of days after rewatering
-5.8	-10.5	5.1	4
-8.2	-10.0	6.8	4
-8.2	-	6.0	6
-9.2	-	11.1	6

Forks Seed Source

predawn PMS	day PMS	day L_r
-4.4	-12.9	13.7
-8.2	-13.4	13.8
-8.5	-13.6	15.3
-8.8	-16.0	19.5
-10.5	-16.5	19.0
-10.9	-18.7	45.2
-11.6	-17.7	23.8
-12.9	-17.7	36.3
-14.2	-19.0	53.8
-15.0	-21.1	23.9
-16.3	-22.1	54.0
-18.7	-21.4	46.2
-21.1	-22.1	42.4
-29.3	-34.2	45.1
-31.3	-27.2	41.8
-34.0	-35.0	61.3

Rewatered

			# of days after rewatering
-6.5	-12.2	21.0	4
-7.5	-12.8	20.9	4
-6.8	-	21.6	6
-7.8	-	57.7	6

GROWTH ROOM DROUGHT STUDY

Spokane Seed Source

First Drought Cycle					
predawn PMS	day PMS	predawn L_r	day L_r	free ABA	bound ABA
-4.6	-10.7	20	16	535	1440
-7.8	-11.7	15	40	340	120
-10.2	-12.1	19	35	370	-
-12.4	-15.5	19	45	1090	915
-12.2	-18.7	90	74	1200	1970
-14.6	-16.3	74	125	1350	900
-17.0	-15.8	66	162	770	260
-21.0	-25.3	210	210	1055	520
-32.0	-33.3	107	210	1930	1560
-44.2	-46.7	174	97	1465	2140

Second Drought Cycle						
predawn PMS	day PMS	predawn L_r	day L_r	free ABA	bound ABA	days after rewater
-9.2	-8.2	25	13	245	2225	2
-7.5	-12.9	67	40	640	1540	2
-10.2	-15.0	13	31	3020	3600	9
-11.6	-17.7	107	44	1720	2350	9
-17.0	-23.1	96	74	2435	2570	9
-18.4	-19.0	68	32	2300	2500	9
-22.2	-27.9	210	210	1385	1420	13

St. Helens Seed Source

First Drought Cycle					
predawn PMS	day PMS	predawn L_r	day L_r	free ABA	bound ABA
-7.4	-8.2	6.6	23	1385	2230
-7.4	-9.6	10.8	47	1010	560
-9.0	-12.5	-	56	2200	850
-9.2	-11.1	-	106	1580	1160
-9.8	-13.0	49	60	2530	1660
-12.2	-18.0	67	210	2100	-
-13.3	-18.5	210	210	-	440
-14.6	-19.4	55	210	2170	1820
-15.6	-22.1	30	158	2410	3470
-17.3	-19.7	210	146	-	1210
-27.2	-33.3	210	210	2060	3160
-27.2	-32.3	210	210	2325	-
-31.3	-34.7	210	210	1935	620
-37.4	-31.6	210	210	2030	1890

Second Drought Cycle						
predawn PMS	day PMS	predawn L_r	day L_r	free ABA	bound ABA	days after rewater
-7.8	-8.0	36	36	2220	920	2
-9.5	-8.2	54	27	2530	1950	2
-12.9	-20.4	205	-	1200	590	13
-15.6	-18.4	210	-	1370	764	13
-16.3	-19.7	-	-	1400	608	13
-18.4	-18.7	210	210	1130	1810	13
-20.4	-22.2	-	-	830	2090	13

NURSERY DROUGHT STUDY

Spokane Seed Source

First Drought Cycle						Second Drought Cycle						
predawn PMS	day PMS	predawn L_r	day L_r	free ABA	bound ABA	predawn PMS	day PMS	predawn L_r	day L_r	free ABA	bound ABA	days after rewater
-6.1	-8.5	7.4	3.9	530	-	-6.8	-8.5	86	-	890	895	2
-4.8	-8.2	93	49	810	1640	-7.5	-8.0	15	11	1460	1760	2
-5.3	-8.7	125	28	360	755	-10.2	-8.8	17	12	1150	1830	10
-10.9	-16.0	-	204	2050	1960	-10.2	-18.4	101	17	1250	1900	14
-11.6	-15.6	-	189	1455	180	-11.6	-11.3	17	16	2380	2390	8
-12.9	-17.0	210	114	1470	1970	-14.3	-11.2	42	59	1470	1390	10
-13.6	-14.9	210	210	1650	1355	-15.6	-15.6	210	117	1320	-	19
-16.0	-17.0	-	187	1460	1760	-18.4	-18.3	187	210	2125	-	14
-17.7	-15.3	-	210	1400	1530	-24.5	-23.1	210	210	1340	-	19
-22.8	-24.0	-	210	2110	2955							
-23.8	-19.0	-	210	1530	1125							
-34.0	-34.0	210	210	2725	2820							
-42.9	-44.2	210	-	2120	2440							

St. Helens Seed Source

First Drought Cycle						Second Drought Cycle						
predawn PMS	day PMS	predawn L_r	day L_r	free ABA	bound ABA	predawn PMS	day PMS	predawn L_r	day L_r	free ABA	bound ABA	days after rewater
-2.7	-8.8	210	210	2820	133	-6.1	-5.7	54	58	1580	4990	2
-3.1	-9.7	193	57	820	-	-6.8	-8.2	150	207	1705	1210	2
-8.8	-11.9	-	210	1090	-	-8.8	-	20	12	1500	2790	8
-10.9	-12.1	210	85	1820	2440	-8.8	-9.9	210	83	710	2010	10
-11.6	-12.6	210	210	835	570	-7.5	-8.2	25	42	1440	1200	10
-12.9	-15.1	210	73	940	280	-11.6	-13.8	182	99	1340	2130	14
-11.6	-16.7	161	166	1420	890	-11.9	-15.0	210	210	990	1630	19
-15.6	-17.0	-	210	1090	1240	-16.3	-18.0	210	210	1995	1845	14
-16.0	-17.7	-	210	1540	1310	-29.2	-24.5	210	210	2235	1695	19
-20.4	-22.3	-	210	1095	920							
-23.1	-26.5	-	210	2380	1900							
-33.3	-29.3	-	210	1260	1980							
-30.1	-28.3	-	97	960	1720							