Physiological Response of Loblolly Pine Seedlings to Moisture-Stress Conditioning and Their Subsequent Performance During Water Stress

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John R. Seiler

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APPROVED:

J. D. Johnson, Chairman

P. P. Feret

B. I. Chevone

M. G. Hale

J. A. Burger

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PHYSIOLOGICAL RESPONSE OF LOBLOLLY PINE SEEDLINGS TO MOISTURE-STRESS CONDITIONING AND THEIR SUBSEQUENT PERFORMANCE DURING WATER STRESS

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(ABSTRACT)

The effect of moisture stress conditioning on the drought tolerance and performance of three open-pollinated families of loblolly pine (Pinus taeda L.) during water stress were investigated. Seedlings were subjected to prolonged sublethal drought treatments which included a watered control, a moderate stress treatment (MWS, seedlings watered only when needle water potential reached -0.8 MPa) and a severe stress treatment (SWS, seedlings watered only when needle water potential reached -1.4 MPa). After the conditioning period, numerous physiological and morphological parameters were measured, and the performance of seedlings during water stress evaluated.

Significant decreases in needle osmotic potential occurred in moisture stress conditioned seedlings. As a result, turgor in conditioned seedlings was equal to or greater than control seedlings even at lower needle water potentials. Photosynthesis was decreased greatly with reduced needle water potential. However, the MWS and SWS seedlings maintained photosynthesis to water potentials 0.15 and 0.45 MPa lower than control seedlings, respectively. This reponse is likely the result of both osmotic adjustment, and an acclimation of the photosynthetic process resulting in less non-stomatal inhibition of photosynthesis at low needle water potentials. Initial needle conductance and transpiration, but not photosynthesis, were reduced greatly by the conditioning treatments, and resulted in improved water-use efficiency in conditioned seedlings. The response of stomata to changing vapor pressure deficit was increased through moisture stress conditioning. Root growth was affected more by moisture stress than shoot growth, causing a decrease in root/shoot ratio. Changes in root morphology as a result of conditioning are not likely to improve the drought tolerance of loblolly pine seedlings. SWS conditioning significantly improved the height growth increment and resulted in slightly greater shoot and root biomass of outplanted, containerized seedlings after the

first growing season, despite these seedlings being much smaller at the time of planting. Differences between seed sources did occur in the experiments, with a source from Texas generally showing the least response to moisture stress conditioning.

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DEDICATION

To my parents Robert H. and Dorothy M. Seiler,

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS v

DEDICATION vii

page

INTRODUCTION 1

LITERATURE REVIEW 4

Drought Tolerance 5
Osmotic Adjustment 6
Needle Conductance 11
Control of Water Loss 11
Response to Humidity 14
Root Growth 15
Effect of Water Stress 15
Importance in Drought Tolerance 17
Modification of Drought Resistance by Moisture Stress Conditioning 20
Effects of Water Deficits on Photosynthesis . . . 23

MATERIALS AND METHODS 26

Experiment One 26
Establishment 26
Data Collection 28
Experimental Design and Analysis 32
Experiment Two 34
Establishment 34
Data Collection 35
Experimental Design and Analysis 37
Experiment Three 38
Establishment 38
Experimental Design and Analysis 39
Experiment Four 40
Experimental Design and Analysis 41
Experiment Five 42

RESULTS 44

Experiment One 44

Growth Parameters 44
Chlorophyll Content 45
Osmotic Potential 50
Photosynthesis 52
Regression Analysis 52
Water stress effects 53
Seed source effect 55
Analysis of Variance 59
Water stress effects 60
Seed source effects 62
Needle Conductance 63
Regression Response 63
Water stress effects 65
Vapor pressure deficit effects 71
Seed source effects 73
Analysis of Variance 76
Water stress effects 77
Vapor pressure deficit effects 80
Seed source effects 82
Transpiration 85
Water-Use Efficiency 89
Experiment Two 89
Growth Parameters 89
Needle Water Potential Components 91
Photosynthesis 94
Regression Analysis 94
Analysis of Variance 97
Transpiration and Water-Use Efficiency . . . 100
Experiment Three 100
Water Potential Components 100
Water Stress Effects 100
Seed Source Effects 105
Chlorophyll Content 106
Growth Parameters 110
Seed Source Effects 112
Survival 118
Experiment Four 118
Osmotic Potential 118
Chlorophyll Content 121
Growth Parameters 122
Root Growth and Morphology Parameters 126
Experiment Five 129

DISCUSSION 134

Photosynthesis 134
Effects of Water Stress 134
Effect of Seed Source 141
Needle Conductance 144
Moisture Stress Conditioning Effects 144

Effect of Seed Source 152
Water Potential Components 153
Transpiration and Water-Use Efficiency 157
Seedling Growth and Morphology 159
Effects of Water Stress 159
Effect of Seed Source 162
Field Performance of Containerized Seedlings . . 163
Effect of Water Stress 163
Effect of Seed Source 165
Effect of Planting Site 166

SUMMARY AND CONCLUSIONS 168

LITERATURE CITED 173

Appendix page

A. LOBLOLLY PINE SEEDLING PHOTOSYNTHESIS DATA FOR EXPERIMENT ONE AND PREDICTED REGRESSION RESPONSES 184

B. LOBLOLLY PINE SEEDLING NEEDLE CONDUCTANCE DATA AND PREDICTED REGRESSION RESPONSES 188

C. LOBLOLLY PINE SEEDLING PHOTOSYNTHESIS DATA FOR EXPERIMENT TWO AND PREDICTED REGRESSION RESPONSES 196

VITA 199

LIST OF TABLES

Table		page
1.	Loblolly pine biomass as affected by 19 weeks of moisture stress conditioning	46
2.	Loblolly pine biomass as affected by seed source. .	47
3.	Loblolly pine chlorophyll content and a/b ratio as affected by 19 weeks of moisture stress conditioning.	48
4.	Loblolly pine chlorophyll content and a/b ratio as affected by seed source.	49
5.	Osmotic potential of needles stored frozen for eight weeks as influenced by moisture conditioning and seed source.	51
6.	Regression models, coefficients of determination and sample sizes for the three moisture stress treatments.	54
7.	Predicted loblolly pine photosynthesis as affected by water potential, moisture stress conditioning and seed source.	58
8.	Mean loblolly pine photosynthesis rate as affected by water potential and moisture stress conditioning.	61
9.	Loblolly pine seedling photosynthesis as affected by seed source.	64
10.	Regression models, coefficients of determination and sample sizes for the three moisture stress and VPD treatments.	66
11.	Predicted loblolly pine needle conductance as affected by water potential, moisture stress conditioning and VPD.	72

12. Predicted loblolly pine needle conductance as affected by moisture stress conditioning, seed source and VPD. 75

13. Mean loblolly pine needle conductance as affected by water potential and moisture stress conditioning. 78

14. Mean loblolly pine needle conductance as affected by water potential and VPD. 81

15. Mean loblolly pine needle conductance as affected by water potential and seed source. 84

16. Mean loblolly pine needle conductance as affected by seed source and 19 weeks of moisture stress conditioning. 86

17. Mean daily transpiration rate of loblolly pine seedlings as affected by seed source and moisture stress conditioning. 87

18. Mean water-use efficiency of loblolly pine seedlings as affected by 19 weeks of moisture stress conditioning. 90

19. Loblolly pine biomass as affected by 8 weeks of moisture stress conditioning. 92

20. Loblolly pine water potential components as influenced by 8 weeks of moisture stress conditioning. 93

21. Regression models, coefficients of determination and sample size for the two moisture stress treatments. 95

22. Loblolly pine photosynthesis rate as affected by water potential and 8 weeks of moisture stress conditioning. 99

23. Loblolly pine transpiration and water-use efficiency as affected by 8 weeks of moisture stress conditioning. 101

24. Needle water and osmotic potential in container grown loblolly pine as affected by length and severity of conditioning. 103

25. Needle water potential components in container grown loblolly seedlings as affected by moisture stress conditioning. 105

26. Container grown loblolly pine needle osmotic potential as affected by seed source and moisture stress conditioning. 107

27. Container grown loblolly pine chlorophyll content and a/b ratio as affected by moisture stress conditioning. 108

28. Container grown loblolly pine chlorophyll content and a/b ratio as affected by seed source. . . . 109

29. Container grown loblolly pine biomass before and after outplanting as affected by moisture stress conditioning. 111

30. Container grown loblolly pine biomass before and after outplanting as affected by seed source. . 113

31. Container grown loblolly pine biomass and height as affected by planting site. 115

32. Container grown loblolly pine growth increment after one year in the field as affected by moisture stress conditoning 116

33. Container grown loblolly pine growth increment after one year in the field as affected by seed source. 117

34. Container grown loblolly pine survival as affected by moisture stress conditioning, seed source and planting site. 119

35. Osmotic potential of needles stored frozen for eight weeks as affected by moisture stress conditioning and seed source. 120

36. Chamber grown loblolly pine chlorophyll content and a/b ratio as affected by 12 weeks of moisture stress conditioning. 123

37. Chamber grown loblolly pine chlorophyll content and a/b ratio as affected by seed source. 124

38. Chamber grown loblolly pine biomass as affected by 12 weeks of moisture stress conditioning. . . . 125

39. Chamber grown loblolly pine biomass as affected by seed source. 127

40. Loblolly pine root growth and morphological characteristics as affected by 12 weeks of moisture stress conditoning. 128

41. Change in loblolly pine root volume as affected by 12 weeks of moisture stress conditioning and seed source. 130

42. Containerized loblolly pine root growth the first 28 days following planting as affected by moisture stress conditioning 132

43. Containerized loblolly pine root growth the first 28 days following planting as affected by seed source. 133

LIST OF FIGURES

Figure page

1. Predicted rates of loblolly pine photosynthesis as affected by needle water potential and moisture stress conditioning. 56

2. Predicted needle conductance at ambient VPD (2.63 kPa) as affected by moisture stress conditioning and water potential. 68

3. Predicted needle conductance at low VPD (1.63 kPa) as affected by moisture stress conditioning and water potential. 69

4. Predicted needle conductance at high VPD (4.20 kPa) as affected by moisture stress conditioning and water potential. 70

5. Predicted loblolly pine photosynthesis as affected by needle water potential and 8 weeks of moisture stress conditioning 97

INTRODUCTION

Of all environmental factors water is the single most important factor affecting plant productivity, crop yields, species composition and distribution, and growth of forests (Chaney 1981, Kozlowski 1982, Kramer 1983a, Turner and Kramer 1981).

In the southeastern United States alone, over 1.3 million acres in 1982 were planted with tree species, with the overwhelming majority (97 percent) of this reforestaton completed using nursery-grown seedlings (USDA 1983). However, approximately 30 percent, or 350 million tree seedlings, do not survive their first year in the field. This high death rate is largely the result of poor planting stock and drought, and results in a large economic loss (Ventor 1981).

Newly planted tree seedlings are particularly sensitive to water stress because of their small, undeveloped root systems (Brix 1979), and often develop severe water deficits (Davies et al. 1973, Kozlowski 1975). In British Columbia, drought has been shown to be the greatest cause of mortality in the first five years after planting Douglas-fir

(Pseudotsuga menziesii (Mirb.) Franco) and western hemlock (Tsuga heterophylla (Raf.) Sarg.) (Arnott 1975), and poor survival of planted loblolly pine seedlings (Pinus taeda L.) has been attributed largely to drought (Stransky 1961).

Often times water stress is not severe enough to directly kill newly planted tree seedlings but does result in reductions in growth. Stransky and Wilson (1964) found that the terminal shoot elongation of potted loblolly pine and shortleaf pine (Pinus echinata L.) seedlings was inhibited by soil water potentials of only -0.20 MPa and was stopped completely as soil water potentials fell below -0.35 MPa. Likewise, Zahner (1962) found the height growth of four-year-old loblolly pine grown outdoors to be greatly affected by drought. Height growth of seedlings subjected to severe drought (no water for six months) was reduced by nearly 50 percent when compared with watered controls. Dry weight production of seedlings of Scots pine (Pinus sylvestris L.) was found to be sensitive to soil water potentials of only -0.05 MPa, with dry weight production being reduced by over 50 percent at soil water potentials of -0.15 MPa (Sands and Rutter 1959).

Since seedling survival and growth of are greatly affected by drought, improvement in drought tolerance could greatly increase their survival and growth. Numerous

factors are involved in determining the drought tolerance of higher plants including osmotic adjustment, stomatal conductance, and root growth. The role of stomatal conductance and root growth in drought tolerance has been well documented in many tree species. However, the role of osmotic adjustment in tree species is less clear.

Few studies have examined the possibilities of modifying factors responsible for drought tolerance by exposing seedlings to sublethal drought treatments. Drought tolerance could possibly be improved through such treatments and thus improve survival and growth of seedlings following outplanting.

With these considerations in mind, the objectives of this study were to :

1. evaluate the effects of moisture stress conditioning on needle conductance, photosynthesis, transpiration, and growth of loblolly pine;

2. examine the role of osmotic adjustment, needle conductance, and root growth in determining the response of loblolly pine to water stress;

3. determine the response of three open-pollinated families of loblolly pine to moisture deficits following moisture stress conditioning;

4. evaluate the effects of moisture stress conditioning on the survival and growth of containerized loblolly pine seedlings following outplanting in the field.

LITERATURE REVIEW

Water has a crucial role in almost all plant processes, ranging from photolysis of water in photosynthesis to hydrophobic bonding of macromolecules and the maintenance of form in nonwoody tissue. Therefore ,it is not surprising that almost any plant parameter can be changed by water stress, provided that the stress is strong enough and of sufficient duration (Hsiao 1973).

Since a shortage of water frequently limits plant growth and survival, selective pressure for adaptation to drought is often high (Pallardy 1981), resulting in a wide range of morphological and physiological adaptations to drought among plants.

As a result of strong selective pressures exerted by moisture stress, drought tolerance does vary among Pinus species. Heth and Kramer (1975), found loblolly pine to be less tolerant to drought than Monterey pine (Pinus radiata D. Don), but as tolerant to drought as shortleaf pine. Heth and Kramer (1975) further concluded that because of the long, dry summers Monterey pine is exposed to in its native habitat, is not surprizing that it exhibits greater drought

tolerance when compared to loblolly or shortleaf pine, which are rarely exposed to prolonged drought. Similarly, seed sources of loblolly pine from central Texas are known to survive better under drought than seedlings from east Texas. This improved drought tolerance is presumed to be the result of strong selection imposed by the harsh, arid central Texas environment (Bilan et al. 1977).

Drought Tolerance

Higher plants possess many physiological processes which allow them to tolerate drought to some extent (Hsiao et al. 1976). Briefly, drought tolerance involves dehydration postponement and deydration tolerance (Kramer 1980). Dehydration tolerance is the passive endurance of water stress and is relatively unimportant in tree species since most are intolerant of dehydration. Dehydration postponement involves morphological changes and physiological processes which minimize the water stress or reduce the internal impact (Hinckley et al. 1981). Unlike dehydration tolerance, dehydration postponement plays a major role in the drought tolerance of forest trees.

Dehydration postponement by trees represents an integrated response of numerous morphological and physiological adaptations which may be present in the

leaves, roots, and stems (Kozlowski 1982). These would include :

1. maintenance of the water status in transpiring organs by osmotic adjustment, water storage, and cell size reduction;

2. reduction of foliar water loss by closure of stomata, wax deposition on leaf surfaces, and leaf abscission;

3. maintenance of water absorption during periods of low soil moisture;

4. reduction of the energy load on leaves by production of smaller or fewer leaves, changes in leaf angle, and increases in convective cooling (Hinckley et al. 1981, Kozlowski 1982).

Osmotic Adjustment

Maintenance of partial or full turgor of plant cells under increasing water stress has been demonstrated for both herbaceous and woody plants (Kozlowski 1982). Maintenance of turgor can be achieved by lowering cell osmotic potential and by changes in elasticity of cell walls (Turner and Jones 1980). Since osmotic adjustment or lowering of cell vacuole osmotic potential can maintain positive turgor, osmotic adjustment has the capability of increasing drought tolerance while still maintaining cell enlargement, stomatal opening, and photosynthesis.

Osmotic adjustment may be either passive or active (Hinckley et al. 1981). Passive osmotic adjustment is due to an increase in the solute concentration of the cell

caused by dehydration of the tissue during stress. Active osmotic adjustment occurs when the osmotic potential becomes more negative at a given relative water content. These adjustments are the result of metabolism and transport of solutes and are not always easily separated from passive decreases in osmotic potential (Hinckley et al. 1980). Active osmotic adjustments confer a greater advantage for the plant during stress.

Herbaceous plants generally have a greater capacity for osmotic adjustment than woody plants (Hinckley et al. 1980). However, several studies have shown osmotic adjustment to be a significant factor in maintaining tree growth and water uptake during water stress. Osonubi and Davies (1978) subjected seedlings of English oak (Quercus robur L.) and silver birch (Betula verruccosa Ehrl.) to a number of consecutive drought periods. During this time leaf and root osmotic potential were sampled. Water stress had no significant effect on height, dry weight, or leaf area of oak seedlings. However, these parameters were significantly reduced by water stress in the birch seedlings. These difference were attributed to active osmotic adjustment in the oak seedlings.

Osmotic potential in oak roots grown in a drying soil was -1.0 MPa at a soil water potential of 0.0 MPa. Oak

seedlings were able to maintain a significant water potential gradient into the roots even when the soil water potential dropped to -1.5 MPa. However, in birch seedlings grown in a drying soil, root solute potential was only -0.6 MPa at a soil water potential of 0.0 MPa. Birch seedlings were unable to maintain a water potential gradient into the roots past -0.9 MPa. The authors suggested that solute accumulation in oak was responsible for the decrease in solute potential and the maintenance of growth as water potentials fell.

A follow up study conducted by Osonubi and Davies (1981) confirmed their earlier results. English oak seedlings subjected to a two-week drying cycle lowered their osmotic potential so that leaf turgor was maintained at around 1.0 MPa until after the ninth day. Osonubi and Davies (1981) concluded that a net accumulation of solutes must have occured for turgor to be maintained. In birch seedlings no solute regulation was detected and turgor declined rapidly when plants were not watered.

Western hemlock seedlings maintained at -1.0 MPa predawn shoot water potential for only two weeks exhibited considerable osmotic adjustment. Conditioned seedlings had an average osmotic potential of -2.0 MPa, whereas control plants showed an average osmotic potential of -1.67 MPa

(Kandiko et al. 1980). The authors concluded that this shift in osmotic potential allowed the seedlings to conserve water and survive at higher levels of stress.

Davies and Lasko (1978) examined the water potential components in irrigated and non-irrigated apple seedlings (Malus domestica Borkh.). Non-irrigated seedlings showed water potentials of 0.2 to 0.6 MPa lower than those of irrigated seedlings; however, turgor potentials remained comparable in both groups. They attributed this turgor maintenance to greater diurnal osmotic adjustment in the non-irrigated seedlings. The majority of the osmotic adjustment was believed to be due to concentration of solutes as water was lost from the leaf.

In a similar study by Davies and Lasko (1979), diurnal changes in osmotic potential were measured in a nine-year old apple tree. Osmotic potential was found to decrease by as much as 1.65 MPa during the day. However, only a 0.55 MPa decrease could be attributed to active increases in leaf metabolite levels with the remaining decrease in osmotic potential due to dehydration.

Nine year old apple trees (Pyrus malus L.) subjected to drought for three months adjusted osmotically (Jones and Higgs 1979). Osmotic potential in the drought-stressed trees, both at full and zero turgor, was 0.3 MPa lower than in the irrigated controls.

Changes in osmotic potential have also been found to occur in tree species over a growing season. Roberts and Knoerr (1977) examined several eastern forest tree species and found osmotic potential dropping in all species from summer to fall. Roberts (1978) found substantial seasonal osmotic adjustment in four tree species, reporting a drop in osmotic potential of as much as 1.6 MPa; whereas, apple trees showed only a 0.5 MPa drop in osmotic potential from July to September (Goode and Higgs 1973).

Other studies have suggested that large scale osmotic adjustment is not a typical drought avoidance mechanism in woody shrub species. Hinckley and others (1980) examined numerous drought hardy shrubs and only one species, Cornus sanguinea L., was found to have appreciable shifts in osmotic potential when water stressed. Similarly, Poole and Miller (1978) found no changes in osmotic potential in four evergreen shrub species from central Chile and aspen (Populus grandidentata Michx.), which is relatively intolerant to drought, showed no shift in its water content-potential curve when subjected to different irrigation regimes indicating no significant osmotic adjustment (Jarvis and Jarvis 1963).

In summary, it appears that osmotic adjustments do occur in many woody plant species. Nonami and Boyer (1982)

concluded that osmotic adjustments are likely to occur in all higher plants. However, there are few reports which show adjustments similar to those found in crop plants such as wheat and sorghum (Pallardy 1981). Further research is needed to fully evaluate the role of osmotic adjustment in tree species. The results of these studies suggest that the potential for osmotic adjustment varies widely among woody plants.

Needle Conductance

Control of Water Loss.

Stomata regulate the bulk of water loss from the leaves; therefore, stomatal adaptations within and among species that affect both the quantity and pattern of water loss are fairly common (Pallardy 1981). One such adaptation is differences in stomatal conductance to vapor transfer. Stomatal conductance has been shown to be an important factor regulating a species' ability to function under water stress. In eastern cottonwood (*Populus deltoides* Bart.), transpiration at various degrees of water stress was found to be controlled by stomatal conductance (Kelliher et al. 1980). Kelliher and Tauer (1980) found clones of eastern cottonwood originating from a wet site to have lower stomatal conductance than clones originating from a dry

site. As the plants were subjected to water stress, differences in stomatal conductance became even greater. Kelliher and Tauer concluded that this indicated greater drought resistance for the dry site clones since these clones were able to maintain higher stomatal conductance at all degrees of water stress.

In contrast, Douglas-fir seedlings grown from seed from a droughty areas have been shown to have lower transpiration rates than seedlings from wet areas (Ferrell and Woodard 1965, Unterscheutz et al. 1974, Zavitkovski and Ferrell 1968).

The capacity for early stomatal closure during a developing drought is also an important factor in drought tolerance (Kozlowski 1982). The field survival of a "high transpiration" clone of Monterey pine was only 50 percent compared to 100 percent for most clones. The differences in survival among clones was found to be due to the greater ability of successful clones to limit transpiration by decreased stomatal conductance (Bennett and Rook 1978).

Stomatal control in loblolly pine is also an important factor determining drought tolerance. Drought tolerant seedlings were found to transpire rapidly when water was available, but to conserve water under stress (van Buijtenen et al. 1976.). Douglas-fir seedlings from a xeric ecotype

and seedlings which had experienced prior moisture stress were found to decrease transpiration more rapidly than seedlings from a mesic environment or seedlings which have experienced no water stress (Unterscheutz et al. 1974).

Similarly, white spruce (Picea glauca (Moench) Voss), which were preconditioned through cold storage, showed decreased transpiration through stomatal closure when water was limiting, but did transpire rapidly when water was available. Freshly lifted seedlings, however, did not show this adaptive advantage (Blake 1983).

Federer and Gee (1976) found several species of northern hardwoods to efficiently control excess water loss by decreasing stomatal conductance during water stress. Similarly, Wuenscher and Kozlowski (1971) found stomatal conductance was lower in xerophytic northern hardwoods.

Numerous other researchers have demonstrated the control of water loss in plants through changes in stomatal conductance (Clemens and Jones 1978, Hinckley et al. 1978, Jarvis 1980, Osonubi and Davies 1980, Pereira and Kozlowski 1977, Tibbits and Bachelard 1981).

Response to Humidity.

The direct response of stomata to changes in atmospheric humidity is widespread and has been documented for numerous species (Losch and Tenhunen 1981). This response has obvious advantages for the conservation of water by providing a means by which plants might sense the environment in order to optimize water use (Cowen 1977). As atmospheric humidity decreases, plants respond by decreasing stomatal conductance.

Johnson and Ferrell (1983) examined this stomatal response in strawberry (Fragaria X ananassa Duch.), Engelmann spruce (Picea engelmanni Parry) and Douglas-fir. All three species showed high stomatal conductance at low vapor pressure deficit (high humidity) and low stomatal conductance at high vapor pressure deficit (low humidity).

Osonubi and Davies (1980) found that birch seedlings (Betula pendula Roth.) exhibited a similar stomatal response to vapor pressure deficit, which was enhanced when the plants were mildly stressed (-0.3 to -0.4 MPa pre-dawn leaf water potentials). They concluded that this enhanced response to vapor pressure deficit results in a significant increase in water use efficiency during mild water stress.

Johnson and Ferrell (1983) found that in Douglas-fir seedlings stomatal sensitivity to atmospheric humidity

increased with initial increases in water stress. However, once needle water potential fell below -2.0 MPa, stomatal response to humidity decreased, until the stomates became insensitive at approximately -4.0 MPa.

Schulze and Kuppers (1979) investigated the effects of long-term (more than one day) changes in leaf water potential on the stomatal response to humidity using hazel (Corylus avellana L.). At all levels of leaf water potential, they found stomata responded in a very similar manner to humidity, but the response lessened with increasing plant water deficits. In contrast Osonubi and Davies (1980) and Johnson and Ferrell (1983) found that the stomatal response to humidity was initially enhanced by water stress.

Root Growth

Effect of Water Stress.

Water stress generally brings about a cessation of root growth. However, root growth is often times less affected by water stress than shoot growth, resulting in an increase in root/shoot ratio (Kramer 1983a). This increase in root/shoot ratio observed under water stress is generally believed to be due to greater water stresses developing in the shoot (Kramer 1983a).

Several reports have actually observed an overall increase in root growth due to mild water stress. Sharp and Davies (1979) found that mildly stressed maize seedlings had an absolute increase in root growth. This effect was attributed to more effective osmotic adjustment in the roots or that water stress inhibited shoot growth before limiting photosynthesis, causing an overall increase in photosynthate available to the roots (Kramer 1983a, Sharp and Davies 1979).

English oak under water stress has been found to change root morphology from a few short, thick roots to a profusion of long thin roots (Osonubi and Davies 1978). It was concluded that this change in root structure was an obvious advantage for absorption of water from a drying soil. Similarly, Clemens and Jones (1978) attributed improved drought tolerance in Eucalyptus robusta Sm. following drought conditioning to changes in root size or structure.

Root wrenching (undercutting the roots with a dull, wedge-shaped blade) has been shown to improve subsequent root growth in Monterey pine, resulting in improved seedling survival following outplanting (Rook 1969). The resulting change in root morphology is believed to be largely due to mild water stress which develops immediately after wrenching. Monterey pine seedlings, upon recovery from the

initial water stress, respond with a large increase in root growth and a redistribution of photosynthate to the roots (Rook 1971, Stupendick and Shepherd 1980).

Other reports have shown root growth in pines to be more affected by water stress than shoot growth. Loblolly pine and white pine (Pinus strobus L.) root growth when subjected to three 7-day drying cycles (minimum soil water potentials of approximately -1.6 MPa) was drastically reduced (Kaufmann 1967). Root growth, was successively reduced with each drying cycle, with only 6 percent of the total growth occurring in the third cycle. Needle growth however, showed a constant, uniform decrease during all drying cycles. Kaufmann (1967) concluded that this root growth response was largely due to root maturation and subsequent quiescense in response to stress. A similar phenomena has been reported for Aleppo pine (Pinus halepensis Mill.), where the roots of this species became suberized and quiescent at soil water potentials below - 0.7 MPa (Leshem 1970).

Importance in Drought Tolerance.

Although root growth is often inhibited by water stress, overall root morphology plays a major role in the drought tolerance of many tree species. A recent review by Pallardy (1981) cited many examples of the importance of root growth and root morphology in increasing soil water availability to

the plant. Survival under drought of Douglas-fir seedlings originating from a xeric source was greater than that of seedlings originating from a mesic source. This difference in survival was found to be correlated with the capacity of roots to penetrate below 30 cm in the soil. Similarly, a relationship between survival under drought in sugar maple (Acer saccharum Marsh.) and root morphology has been shown. Seedlings from a drought tolerant ecotype possessed a root system composed of a large primary root and a dense mass of fine roots. Drought susceptible seedlings had a diffuse, shallow root system and lacked a prominent primary root. Relationships between rooting patterns and relative drought tolerance in Caribbean pine (Pinus caribaea Morelet), northern white ceder (Thuja occidentalis L.), and Japanese larch (Larix leptolepis (Zieb. and Zucc.) Gord.) were also reviewed by Pallardy (1981)

Periera and Kozlowski (1976) attributed the greater drought tolerance of Eucalyptus camaldulensis Dehn. over Eucalyptus globulus Labill. to the ability of the former to produce a deep extensive root system. Lopushinsky (1973) concluded that when lodgepole pine (Pinus contorta Dougl.) is absent on dry sites it is due to poor initial root elongation.

The rooting habit of Ponderosa pine (Pinus ponderosa Dougl.) and Douglas-fir has also been shown to be a major factor affecting their success in various habitats (Jackson and Spomer 1979) and drought tolerance of some families of loblolly pine in Texas has been attributed to extensive root growth (van Buijtenen et al. 1976).

Cannell et al. (1978) found that loblolly pine seedlings from northern coastal families of North Carolina produced greater root masses and lengths in proportion to their shoot weights and grew faster under mild water stress. Further, northern coastal family seedlings, grown under water stress, had root/shoot ratios highly correlated with volumes of 8-year old trees of the same families grown on well-drained sites. Cannell et al. (1978) suggests that trees exhibiting better growth on well-drained sites may be doing so because of their ability to avoid drought through the production of larger, more extensive root systems. In contrast, a study by Feret (1982) showed root-shoot relationships of ponderosa pine seedlings grown under varying moisture stress to have little or no correlation with either field performance or adaptation to mesic or xeric sites.

Modification of Drought Resistance by Moisture Stress Conditioning

Tree seedlings grown in the nursery bed or containers rarely encounter substantial water stress until they are outplanted. As a result, seedlings often develop severe water stress resulting in decreased survival and growth. Often times, seedlings are "hardened" by exposure to sublethal drought treatments. This hardening process may result in two effects : 1) the plant may become more tolerant to low water potentials; or 2) they may avoid moisture stress by water conservation and maintenance of water absorption at low soil water potentials (Clemens and Jones 1978).

Early work by Ashton (1956) with sugar cane (*Saccharum officiarum*) suggested a modification of drought resistance in response to moisture stress conditioning. Ashton (1956) found that the reduction in photosynthesis in response to increasing water stress became less and less with succesive drought cycles and that pre-stressed plants recovered more quickly from water stress.

Clemens and Jones (1978) examined the effects of water stress conditioning on the subsequent water use of *Acacia* and *Eucalyptus*. Control plants that had received water daily used significantly more water than plants that had

been repeatedly subjected to water stress. In a more detailed study (Clemens and Jones 1978) on water stress conditioning of Eucalyptus robusta (swamp mahogany), control plants were found to use 46 percent more water than conditioned plants. Additionally, water stress conditioning had little effect on plant height and leaf area.

Drought resistance in English oak was also improved through water stress conditioning. Osonubi and Davies (1978) subjected seedlings of English oak and silver birch to a number of consecutive periods where water was withheld from the soil for six days and then watered on the seventh day. A control group was watered every day. Water stress conditioning reduced the growth of birch seedlings but had little effect on the oak seedlings. Oak seedlings subjected to water stress conditioning produced long thin roots and accumulated solutes in the roots and leaves. This resulted in the maintenance of turgor and high leaf conductance in the oak seedlings as the soil dried.

Similarly, pre-stressing seedlings of Banksia serrata L.F. resulted in morphological and physiological changes which made the seedlings more resistant to drought. These changes included slower growth rates, lower stomatal conductance, and lower osmotic potential (Tibbits and Bachelard 1981).

Cotton (Gossypium hirsutum L. Tamcot SP37) plants subjected to several stress cycles in which water was withheld from the soil until midday leaf water potentials reached approximately -2.0 MPa exhibited considerable acclimation to low leaf water potentials as compared to control plants. Conditioned plants maintained photosynthesis and stomatal conductance to lower water potentials and exhibited considerable osmotic adjustment (Ackerson and Hebert 1981).

Brown et al. (1976) found preconditioning in cotton plants to alter the stomatal response to leaf water potential. Cotton plants were preconditioned by subjecting them to 8 water stress cycles, where plants were not watered until visibly wilted. Preconditioning resulted in the stomates on the lower surface of the leaves to remain open to water potentials approximately 1.4 MPa lower than in control plants and resistance values were lower in conditioned plants at all levels of leaf water potential. Osmotic adjustment of the guard cells of 0.7 MPa was given as a partial explanation for this shift (Brown et al. 1976).

Recent studies using sunflower (Helianthus annus L.) examined the acclimation of photosynthesis to low soil water potentials (Matthews and Boyer 1982, 1983, 1984). Plants were acclimated to several cycles of low water potential or

to a constant, moderately-low water potential. When acclimated plants were returned to a high soil water potential there was no difference in light or carbon-dioxide response of photosynthesis when compared to control plants. At low soil water potentials, acclimated plants showed less inhibition of light-saturated photosynthesis as compared to non-acclimated plants. Additionally, chloroplast activity as measured *in situ* and *in vitro* with isolated chloroplasts was less inhibited at low soil water potentials in acclimated than in non-acclimated plants. Matthews and Boyer (1982, 1983, 1984) also concluded that inhibition of photosynthesis was controlled more by chloroplast activity than by stomatal closure and that acclimation was greater at the chloroplast level than at the stomatal level.

Effects of Water Deficits on Photosynthesis

Water deficits can decrease photosynthesis either by increasing resistance to diffusion of carbon dioxide or by affecting the photosynthetic and respiratory mechanism (Boyer 1976, Hinckley et al. 1981). As water stress develops photosynthesis is reduced initially by stomatal closure and then as the leaves become severely dehydrated the photosynthetic process is eventually inhibited (Luukkanen cited in Kozlowski 1982).

Changes in stomatal conductance during drought have a major impact on photosynthesis. Boyer (1976) cites numerous investigators who have shown a strong correlation between stomatal conductance and photosynthesis inhibition during drought. Generally, as leaf water potential decreases, both net photosynthesis and stomatal conductance decrease.

Brix (1962) found a strong correlation between reductions in transpiration and photosynthesis with increasing water stress in both loblolly pine and tomato seedlings, indicating that photosynthesis is limited by a decrease in conductance to carbon dioxide uptake. Loblolly pine seedling photosynthesis was found to drop rapidly below a needle diffusive pressure deficit of 4 atmospheres (roughly equivalent to a water potential of −0.4 MPa) and reached zero by a diffusive pressure deficit of 11 atmospheres.

The decrease in conductance commonly associated with decreases in photosynthesis has been found to be associated with increased resistance to diffusion of carbon dioxide at both the stomatal and mesophyll level (Bunce 1977, Kozlowski 1982, Wuenscher and Kozlowski 1970).

Although stomatal conductance and photosynthesis are often highly correlated, increased resistance to carbon dioxide diffusion is not the only factor controlling photosynthesis during water deficits (Boyer 1976).

Chlorophyll synthesis can be highly sensitive to low leaf water potential (Bourque and Naylor 1971, Virgin 1965). In this way, water deficits may reduce photosynthesis by inhibiting chlorophyll formation.

Chloroplast activity is also affected by low leaf water potentials. Several studies have found good correlations between chloroplast activity of sunflower and photosynthetic activity over a wide range of leaf water potentials (Boyer and Bowen 1970, Boyer 1971, Matthews and Boyer 1982, 1983, 1984).

Additionally, recent work with isolated mesophyll cells of Phaseolus and Lycopersion showed decreased photosynthetic activity with only moderate decreases in osmotic potential of the incubation media (-1.08 to -1.59 MPa). The results suggest that during a reduction in water potential, non-stomatal inhibition of photosynthesis may occur simultaneously with stomatal closure (Mawson and Coleman 1983). Berkowitz and Gibbs (1983) found the photosynthetic capacity of isolated spinach chloroplast to be inhibited (64 to 74 percent inhibition) when stressed with 0.67 molar sorbitol (-2.0 MPa) in the reaction media. Further, Berkowitz and Gibbs (1983) presented strong evidence supporting stromal acidification as a mediating mechanism for the inhibition of photosynthesis at low osmotic potentials.

MATERIALS AND METHODS

Experiment One

Establishment

Three geographic sources of loblolly pine were used in the experiment. One open-pollinated family per source was chosen in order to minimize the variance within sources. Two families (female parents clone R-525 and clone R-523) were obtained from the Virginia Division of Forestry at the New Kent Forestry Center seed orchard. Clone R-523 originated from the Virginia Coastal Plain, while clone R-525 originated from the Virginia Piedmont. The third family, originating from east Texas (female parent clone GR1-8), was obtained from the Texas Forest Service and was selected because of its known superior drought tolerance.

The seeds from each source were stratified by soaking them in water for 24 hours, followed by surface sterilization with 7 percent hydrogen peroxide for 15 minutes. The seeds were then rinsed three times, drained, and stored at 2°C for two months.

Following stratification, seeds from each source were germinated and grown in a greenhouse in Bay Leach, Super

Cells (160cc) containing a 3:1 (v:v) ratio of sterilized nursery soil and peat. The nursery soil had a loamy-sand texture and was obtained at the New Kent Forestry Center. Seedlings were grown for an initial four month period in which they were kept well watered and fertilized weekly with 20-20-20 water soluble fertilizer (Peters General Purpose, Fogelsville, PA), supplemented monthly with micro-nutrients (Peters Soluble Trace Element Mix, Fogelsville, PA). Seedlings were grown under ambient light conditions, supplemented with fluorescent lights to maintain a 16-hour photoperiod. Temperatures ranged from 30° during the day to 18°C during the night.

Following the initial growth period, seedlings were transferred into one liter (11 cm diameter) plastic pots containing the same 3:1 soil mix. Seedlings were watered regularly and fertilized weekly for a one month acclimation period, followed by 16 to 22 weeks of moisture stress conditioning depending on which block of seedlings were being measured in the experiment.

The water stress treatments were as follows :

1. no water stress; seedlings were watered to keep pre-dawn needle water potential above −0.5 MPa,

2. moderate water stress (MWS); seedlings were watered only when pre-dawn needle water potential fell below −0.8 MPa,

3. severe water stress (SWS); seedlings were watered only when pre-dawn needle water potential fell below −1.4 MPa.

Needle water potentials used to determine the water stress treatment were measured on a single fascicle of needles using the pressure chamber technique (Scholander et al. 1965). The SWS treatment was chosen such that seedlings were often wilted, but no mortality resulted. The MWS treatment was chosen to represent a level of stress approximately midway between the controls and SWS seedlings.

Following water stress conditioning, all treatments were watered to approximately equal soil water potential. Drought stress was then imposed on the seedlings by allowing the soil to dry with no further watering.

Data Collection

Needle osmotic potential was measured for each treatment using thermocouple psychrometers (Wescor, Logan, UT). Prior to the drought stress cycles, needles from each treatment were collected, sealed in 10 ml plastic test tubes and stored frozen (-5°C) until used for the osmotic potential measurements.

For the osmotic potential measurements the frozen needles were placed into small aluminum chambers (2.2 cm O.D. X 7.5 cm long, 0.4 cm I.D. X 5.3 cm long). The psychrometers were then sealed into the tops of the chambers. To insure that all cell membranes were disrupted the chambers

containing the needle samples were frozen in liquid nitrogen. The chambers and psychrometers were then submerged in a 30°C water bath for 12 hours, after which a water potential measurement was taken. This measurement was assumed to equal the osmotic potential of the sample (Brown 1972).

Additionally, at this time chlorophyll content was determined on a sample of all treatments. Chlorophyll content was measured spectrophotometrically following grinding and extraction with an 80 percent acetone solution (Kirk 1968).

Needle conductance was measured on all seedlings every other day during the drought cycle using a steady state porometer (Interface Instruments, Corvallis, Oregon). Measurements were taken on a set of sample needles at three cuvette humidities (ambient humidity (approximately 50 percent), 15 percent relative humidity, and 70 percent relative humidity). Humidity in the cuvette was decreased to 15 percent or increased to 70 percent by bubbling air through a saturated solution of lithium chloride or distilled water, respectively. Conductance was measured first at ambient humidity and then at the two treatment humidities (15 and 70 percent relative humidity).

Between measurement periods plants were kept in a large walk in growth chamber maintained at 55 percent relative humidity and a 16-hour photoperiod (150 uMoles/m^2 s of PAR) with 23°C days and 17°C nights. For measurements of needle conductance plants were placed under a high intensity metal arc lamp (1100 uMoles/m^2 s (PAR) at the plant level and 650 uMoles/m^2 s (PAR) inside the cuvette) and allowed to acclimate for one hour after which needle conductance was measured.

Following completion of the experiment, leaf area of the sample needles used to measure conductance was determined using a technique described by Johnson (1984), which relates total needle length and volume to surface area. Stomatal conductance values were then computed on the basis of total leaf surface area.

Additionally, using the same seedlings, estimates of transpiration were obtained through daily pot weighing. Pots were mulched with one inch of perlite to minimize evaporation. Daily mean rates of transpiration were estimated by dividing grams of water lost by the time between two successive pot weighings and expressed as grams of water lost per gram dry weight leaf tissue per hour.

Rates of net photosynthesis were measured for each plant with an infrared carbon dioxide analyzer in an open system

(Anarad model 600, Santa Barbara, CA). Rates were measured every other day during the drought stress cycle and expressed as milligrams of CO2 assimilated per gram dry weight leaf tissue per hour.

Between measurement periods the plants were kept in a growth chamber maintained at 60 percent relative humidity and a 16-hour photoperiod (330 uMoles/m^2 s of PAR) with a 23°C day and a 17°C night. Seedlings were again mulched with one inch of perlite to minimize evaporative water loss.

For photosynthesis measurements, seedlings were placed in large continuously stirred tank reactors supplied with charcoal filtered ambient air (Heck et al. 1978) and allowed to acclimate for one hour. Light was supplied with high pressure sodium vapor lamps which produced 400 uMoles/m^2 s (PAR) at the leaf surface. Following the acclimation period one liter glass cuvettes were placed over the seedlings for measurement of carbon dioxide depletion rate. Temperature within the cuvettes averaged 29 $\pm$ 1.8 °C.

Needle water potential was determined with a pressure chamber on a single fascicle of needles following every photosynthesis and needle conductance measurement.

The drought cycle was continued until photosynthesis, conductance and transpiration were reduced to near zero for all treatments. Following the drought cycle, seedlings were

destructively sampled for root, shoot and needle dry weight. Additionally, water-use efficiency was calculated by utilizing the photosynthesis and transpiration data and was expressed as milligrams of CO2 fixed per gram of water lost.

Experimental Design and Analysis

The study was a factorial experiment with a completely randomized block design using three blocks. Due to the length of time necessary for both the needle conductance and photosynthesis measurements only one block of seedlings was measured at a time. Block one was measured first following 16 weeks of conditioning, block two was measured after 19 weeks of conditioning, and block three was measured last following 22 weeks of conditioning. One seedling was measured for each water stress/seed source/block combination.

A separate set of seedlings, subjected to identical treatment conditions, was measured for the needle conductance and photosynthesis measurements. Needle osmotic potential and chlorophyll content were measured on a pooled sample of needles collected from both the needle conductance and photosynthesis seedlings following 16 weeks of conditioning.

Regression analysis was used to analyze the response of photosynthesis and needle conductance to needle water potential. For photosynthesis, the water stress treatment significantly influenced the response to water potential. Therefore, to simplify the analysis, separate regression models were produced for each water stress treatment.

Stomatal conductance was significantly influenced by both the water stress treatment as well as by the three relative humidity treatments used during measuring. Vapor pressure deficits were calculated from the humidity and air temperature used for the measurement and then grouped into three classes; namely, high vapor pressure deficit (4.20 $\pm$ 0.25 KPa), moderate vapor pressure deficit (2.35 $\pm$ 0.28 KPa) and low vapor pressure deficit (1.63 $\pm$ 0.17 KPa). Nine separate regression models were then produced for each water stress/vapor pressure deficit combination.

Additionally, to separate significant differences in the photosynthesis and conductance response to water potential as influenced by moisture stress conditioning, seed source and vapor pressure deficit, an analysis of variance was used. Water potential values were grouped into 8 discrete levels (less than 1.0, 1.00 to 1.19, 1.20 to 1.39, 1.40 to 1.69, 1.70 to 1.99, 2.00 to 2.29, 2.30 to 2.59 and greater than 2.60 (-MPa)) and an analysis then performed using water

potential as a class variable with eight discrete levels. This permitted any significant changes in photosynthesis or conductance due to water potential class, moisture stress conditioning, seed source or interactions among these factors to be separated using Duncan's multiple range test. In order to meet the assumption of homogeneity of variance, a log transformation of photosynthesis and conductance was used.

Analysis of variance followed by Duncan's multiple range test was also used to separate treatment differences due to water stress conditioning or seed source for the following variables : osmotic potential, chlorophyll content, root/shoot ratio (g/g), and shoot and root dry weight, as well as transpiration rate and water-use efficiency for any given day during the drought cycle.

Experiment Two

Establishment

To verify the results obtained from the photosynthesis measurements in experiment one a second series of measurements were obtained. Year old seedlings of a single open-pollinated family (female parent 48-1425) were obtained from the Virginia Division of Forestry Center. Seeds were obtained from Continental Forest Industries and originated

from the Virginia Coastal Plain. The seedlings were operationally grown at the New Kent Forestry Center, handlifted and stored at 2°C until used in the experiment.

The seedlings were planted in two liter (14 cm diameter) plastic pots containing the same 3:1 nursery soil mix as used in experiment one. Seedlings were grown for an initial four week period where they were kept well watered and fertilized as in experiment one, followed by eight weeks of water stress conditioning where one-half of the seedlings were subjected to the SWS treatment used in experiment one. The remaining trees were watered regularly and served as controls. Following the water stress conditioning period all trees were well watered and drought imposed by allowing the soil to dry with no further watering.

Data Collection

Water potential and osmotic potential were sampled for both treatments using thermocouple psychrometers. Freshly collected needles were placed inside aluminum chambers as described in experiment one. Psychrometers were then sealed into the chambers and allowed to equilibrate for 12 hours in a water bath at 30°C after which a water potential measurement was taken. The chambers containing the needle samples and psychrometers were then frozen in liquid

nitrogen to rupture all plant membranes (Brown 1972). The chambers were placed back into the water bath and following a second 12 hour equilibration period osmotic potential was measured. Turgor potential was estimated as the difference between the osmotic potential measurement and the water potential measurement.

Rates of net photosynthesis were measured every other day on four seedlings of each treatment in the system described in experiment one. However, only the latest flush of growth produced during the conditioning period was placed under the cuvette for measurement of CO2 depletion rate. Immediately following every photosynthesis measurement, needle water potential was determined on a single fascicle of needles with a pressure chamber. Between measurement periods, plants were kept in a greenhouse under ambient light conditions, supplemented with a high intensity metal arc lamp to maintain a 16-hour photoperiod.

Estimates of daily mean transpiration rates were measured on a separate set of seedlings as described in experiment one. Four plants of each treatment were maintained in a growth chamber with a 16-hour photoperiod (150 uMoles/m^2 s of PAR) with 23°C days and 17°C nights and were weighed every day for estimates of water loss.

Following completion of the photosynthesis and transpiration measurements seedlings were harvested and shoot and root dry weight determined. Water-use efficiency was also calculated as in experiment one.

Experimental Design and Analysis

A completely randomized design was utilized in the experiment. Four seedlings per treatment were sampled for both the photosynthesis and transpiration measurements, while nine trees per treatment were sampled for the osmotic potential measurements.

Regession analysis was used to analyze the response of photosynthesis to decreasing water potential. In a similar manner as experiment one, an analysis of variance was performed by grouping water potential into discrete classes to separate differences in photosynthesis due to water potential, moisture stress conditioning or the interaction of these two factors. A Students' T-test was utilized to determine differences due to water stress conditioning for osmotic potential, transpiration rate and water-use efficiency.

Experiment Three

Establishment

To evaluate the effect of conditioning on the performance of loblolly pine seedlings following outplanting a field study was conducted in which seed from the same sources as experiment one were germinated and grown in Ray Leach, Super Cells (160 cc) containing the same nursery soil mix as previously described. The seedlings were grown for an initial 18 week period under natural lighting, supplemented with fluorescent lights to maintain a 16-hour photoperiod. During this initial growth period, seedlings were watered regularly and fertilized weekly as in experiment one. Following this initial period, seedlings were exposed to 14 weeks of water stress conditioning similar to previous experiments. Water stress treatments were monitored on a sample of seedlings from each treatment population.

At monthly intervals during the water stress treatments, water potential and osmotic potential were sampled on all treatments with thermocouple psychrometers as described in experiment two.

At the end of the conditioning treatments, seedlings were outplanted with planting bars on two sites located at the Reynolds Homestead Research Center (Patrick Co., VA). One site was located in an alluvial bottomland, while the other

was on a drier upland site. As part of a larger project (Fox 1984), both sites were prepared by shearing all residual material using a KG-blade mounted on the front of a Caterpillar D7-G tractor. The areas were then raked and the residual material piled into windrows, followed by disking using a Rome, 16 disk offset harrow.

Prior to outplanting and at the end of the first growing season, height was measured on all seedlings. Additionally, shoot and root dry weight was measured on a subsample of seedlings after the water stress treatments and after the first growing season in the field. Seven seedlings were sampled prior to outplanting for each water stress/ seed source/block combination and three were sampled after the first growing season for each water stress/seed source/site/block combination. Chlorophyll content was determined after the water stress treatments on a pooled sample of needles for each water stress/seed source treatment combination.

Experimental Design and Analysis

A nested factorial design with three blocks was used in the experiment. Seedlings were planted in 24 tree plots for every water stress/seed source combination. Analysis of variance followed by Duncan's multiple range test was

utilized to separate treatment differences due to water stress conditioning, seed source and site for the following variables : height growth, survival, shoot dry weight, root dry weight, root/shoot ratio (g/g), chlorophyll content, osmotic potential and turgor potential.

Experiment Four

To determine how root growth and morphology were affected by water stress conditioning an experiment was designed in which seeds from the same sources as experiment one were germinated and grown in a growth chamber for an initial four month period in one liter (11 cm diameter) plastic pots containing the same soil mix as previous experiments. The seedlings were grown under a 16-hour photoperiod (150 uM/m^2 s PAR) with 23°C days and 17°C nights. During this initial growth period seedlings were fertilized as in experiment one.

Following four months of growth, seedlings were transplanted into two liter (14 cm diameter) plastic pots containing the same soil mix. During transplanting, total root length was estimated on all seedlings using a modified line transect method (Tennant 1975). Seedling root system were photographed quickly on a 1X1 cm grid for later determination of length. Care was taken to keep the

seedlings moist at all times. Additionally, total root volume was measured using a water displacement technique described by Burdett (1979).

The seedlings were then grown in a growth chamber under the same ambient conditions for an initial four week acclimation period, after which plants were exposed to 12 weeks of moisture stress conditioning as in previous experiments. Following the conditioning treatments, seedlings were destructively sampled, and root volume, root length, root dry weight and shoot weight determined. Chlorophyll content and needle osmotic potential were measured as described in experiment one.

Experimental Design and Analysis

A nested-factorial design, with four randomized complete blocks were used in the experiment. Due to the length of time thought necessary for all measurements, the blocks were split into two time groups. All seedlings underwent 12 weeks of moisture stress treatments. However, the moisture stress treatments for seedlings in blocks 3 and 4 were begun three weeks later than seedlings in blocks 1 and 2. One seedling was sampled for each moisture stress/seed source/block combination.

Analysis of variance followed by Duncan's multiple range test was used to separate treatment differences due to water stress conditioning and seed source for the following variables : osmotic potential, chlorophyll content, root length, root volume, root dry weight and shoot weight.

Experiment Five

This experiment was conducted to evaluate initial root growth of containerized loblolly pine seedlings during water stress. Seedlings were propagated and conditioned as in experiment three were transplanted into 20 liter (30 cm) plastic pots containing a mixture of alluvium and A1 horizon of a Calvin silt loam (Typic Hapludult, mixed thermic) obtained from a forested site in Montgomery County, Virginia. After transplanting the seedlings were watered to approximately field capacity and drought imposed by withholding all further watering. Following 14 days without water, needle water potential was measured with a pressure chamber and plants rewatered. Drought was again imposed by withholding water and seedlings destructively harvested 14 days later. The pots were carefully broken apart and the seedlings removed. All new roots growing beyond the original root plug were removed and their dry weight obtained. Additionally, shoot and original root weight were obtained.

The study was a factorial experiment with a completely randomized block design with three blocks. One seedling was measured for each moisture stress/seed source/block combination. Since needle water potential had such a strong influence on new root growth, an analysis of covariance was used, with needle water potential as a covariate. Least-square means (LSM) were calculated and treatment differences for new root weight and new root/old root ratio were separated using probability values for the hypothesis Ho : LSM(i) = LSM(j), where i and j equal the various levels of moisture stress and seed sources.

RESULTS

Experiment One

Growth Parameters

Seedling growth parameters as affected by moisture stress conditioning are shown in table one. Shoot and root dry weight, and root/shoot ratio were all significantly affected by the moisture stress treatments . Average shoot dry weight was decreased 63 percent as a result of the severe water stress (SWS) treatment, decreasing from 7.89 g in control plants to 2.90 g in the SWS plants. The moderate water stress (MWS) treatment resulted in a 35 percent decrease in shoot weight with an average shoot weight of 5.09 g.

Root dry weight exhibited a greater decrease in response to the moisture stress treatments, ranging from 3.68 g in the control plants to 1.99 g and 1.07 g (46 and 71 percent decrease) in the MWS and SWS seedlings, respectively.

The greater reduction in root growth, in response to the moisture stress treatments, resulted in a significant decrease in root/shoot ratios (Table 1). The average root/shoot ratio of 0.47 in control plants was significantly

higher than either of the preconditioned treatments averaged 0.40 in the MWS treatment and 0.38 in the SWS treatment.

The effect of seed source on growth was less pronounced (Table 2). Shoot dry weight did not differ significantly among seed sources; however, root dry weight was significantly lower in the Texas seed source, averaging 1.94 g compared to 2.40 g in both the Coastal Plain and Piedmont sources. As a result of this decreased root growth, the Texas seed source also had a significantly lower root/shoot ratio of 0.33 compared to 0.45 and 0.48 in the Coastal Plain and Piedmont sources, respectively, which did not differ significantly.

Chlorophyll Content

Amounts of chlorophyll a, chlorophyll b and total chlorophyll expressed as mg per cm^2 of needle surface area did not differ significantly as a result of the moisture stress treatments (Table 3). However, the chlorophyll a/b ratio was significantly lower in the SWS trees, averaging 2.23 compared to 2.43 $mg \cdot cm^{-2}$ in the controls. Chlorophyll content as affected by seed source is shown in Table 4. Seed source, however, had no significant affect on any chlorophyll parameters.

Table 1. Loblolly pine biomass as affected by 19 weeks of moisture stress conditioning.[1]

Treatment	shoot weight		root weight		root/shoot ratio	
	(g)		(g)			
Control	7.89	A	3.68	A	0.47	A
Moderate Stress	5.09	B	1.99	B	0.40	B
Severe stress	2.90	C	1.07	C	0.38	B

[1]Means followed by the same letter within a column do not differ significantly (alpha = 0.05); means are averaged over all seed sources.

Table 2. Loblolly pine biomass as affected by seed source.[1]

Seed Source	shoot weight		root weight		root/shoot ratio	
	(g)		(g)			
Coastal Plain	5.25	A	2.40	A	0.45	A
Piedmont	4.86	A	2.40	A	0.48	A
Texas	5.77	A	1.94	B	0.33	B

[1]Means followed by the same letter within a column do not differ significantly (alpha = 0.05); means are averaged over all moisture stress treatments.

Table 3. Loblolly pine chlorophyll content and a/b ratio as affected by 19 weeks of moisture stress conditioning.[1]

Treament	Chlorophyll a	Chlorophyll b	Total Chlorophyll	a/b Ratio
	(mg/cm² leaf area)			
Control	33.4	13.8	47.0	2.43 A
Moderate stress	34.2	14.9	48.9	2.32 A B
Severe stress	31.7	14.3	46.0	2.23 B

[1]A/B ratios followed by the same letter do not differ significantly (alpha = 0.05); no other means within columns differed significantly; means are averaged over all seed sources.

Table 4. Loblolly pine chlorophyll content and a/b ratio as affected by seed source.[1]

Seed source	Chlorophyll a	Chlorophyll b	Total chlorophyll	a/b Ratio
	---------- (mg/cm² leaf area) ----------			
Coastal Plain	33.3	14.6	47.8	2.29
Piedmont	33.0	14.0	47.0	2.39
Texas	32.9	14.3	47.2	2.32

[1]No means within columns differed significantly; means are averaged over all moisture stress treatments.

Osmotic Potential

The osmotic potential of needles stored frozen for two months as influenced by seed source and water stress is presented in Table 5 . A significant interaction between seed source and water stress treatment occurred; therefore, mean osmotic potential values for all seed source/water stress combinations (nine means) were compared together. In general, the SWS treatment resulted in the lowest osmotic potential; however, this trend was dependent on seed source. In the Coastal Plain source, control trees actually had the lowest osmotic potential of -3.27 MPa. This value, however, did not differ significantly from the SWS treatment which averaged -2.96 MPa.

Both the Piedmont and Texas seed sources showed more expected results. In the Piedmont source, osmotic potentials did not differ significantly between the controls and the MWS treatment, averaging -2.40 and -2.27 MPa, respectively. The SWS treatment in the Piedmont source resulted in a significantly lower osmotic potential of -3.09 MPa. The Texas seed source exhibited osmotic potentials ranging from -2.36 to -2.81 MPa in the control and SWS trees, respectively. However, these values were not significantly different at the alpha = 0.05 level.

Table 5. Osmotic potential of loblolly pine needles stored frozen eight weeks as influenced by moisture stress conditioning and seed source.[1]

Treatment	Seed Source: Coastal Plain	Piedmont	Texas
	(MPa)		
Control	-3.27 A	-2.40 C D	-2.36 C D
Moderate stress	-2.42 C D	-2.27 D	-2.73 B C D
Severe stress	-2.96 A B	-3.09 A B	-2.81 A B C

[1]Means followed by the same letter do not differ significantly (alpha = 0.05); means can be compared in all directions.

Photosynthesis

Regression Analysis.

The three moisture stress treatments were found to have a significant influence on the response of photosynthesis to water potential. This conclusion was based on an examination of the data as well as on statistical procedures. When the data from the three treatments were modeled together, using moisture stress treatments as a variable in the regression equation, moisture stress and moisture stress interactions were found to be highly significant parameters. Additionally, when the log of photosynthesis was regressed on water potential squared (a good single predictor for all treatments) moisture stress conditioning resulted in significantly different intercepts when compared to controls. Therefore, in order to simplify the regression models, the three moisture stress treatments were analyzed separately.

Equations were developed by first screening initial variables using stepwise and backward elimination procedures. Variables selected with these procedures were then utilized to build the final regression models. The final models chosen were selected based on coefficients of determination, Cp statistics and residual analysis. All models contain only variables with slope estimates

significantly different than zero, and the models all showed good residual plots.

Regression equations developed to illustrate the response of photosynthesis to water potential for the three treatments are presented in Table 6 . Coefficients of determination ranged from 0.92 in the controls to 0.70 in the MWS treatment. All three equations contain water potential and various polynomial combinations of water potential. Seed source also had a significant effect in all three models, generally as an interaction with water potential, but also as a main effect in the MWS model.

Water stress effects. The predicted response of photosynthesis to decreasing water potential, averaged for the three seed sources, is shown in figure one. Predicted values are only presented in the range of water potentials where sufficient data points occurred for all three treatments. The predicted responses for the three treatments, along with their corresponding data points, are presented in appendix a (Figures a1 through a3).

The most striking difference as a result of the moisture stress conditioning is a shift in the response surface to the right, so that higher rates of photosynthesis occur at lower needle water potentials in both the MWS and SWS treatments (Figure 1). For example, the rate of

Table 6. Regression models, coefficients of determination and sample sizes for the three moisture stress treatments.[1]

Treatment	Model	R^2	n
Control	$Ln(Ps) = 13.6 - 12.1(WP) + 11.1Ln(WP) + 0.32(WP^3) - 0.02(SD*WP^3)$	0.92	32
Moderate stress	$Ln(Ps) = 4.0 - 1.9(WP) - 4.4(SD) + 8.5(SD*WP) - 5.2(SD*WP^2) - 1.0(SD*WP^3)$	0.70	56
Severe stress	$Ln(Ps) = - 3.3 + 9.9(WP) - 5.9(WP^2) + 1.0(WP^3) - 0.02(SD*WP^3)$	0.81	79

[1]Ps = photosynthesis, WP = water potential, SD = seed source (1, 2 or 3 for Coastal Plain, Piedmont and Texas, respectively).

photosynthesis which occurs at -1.4 MPa in the control plants does not occur until approximately -1.55 MPa and -1.80 MPa in the MWS and SWS trees, respectively.

A similar trend was observed in the data (Figures a1 through a3). Although a large amount of variation existed, it is evident that few control plants showed positive photosynthesis below approximately -2.0 MPa (Appendix a, Figure a1). However, in the SWS treatment a large number of trees showed significant photosynthesis below -2.0 MPa, with some photosynthesis occurring below -2.5 MPa (Appendix a, Figure a3).

Another apparent effect of conditioning on photosynthesis is that maximum predicted photosynthesis rates are higher than control plants (Figure 1). In both the MWS and SWS trees maximum rates are approximately 6.0 mg $CO2 \cdot g^{-1} \cdot hr^{-1}$; whereas, in control plants the maximum rate is approximately 5.2 mg $CO2 \cdot g^{-1} \cdot hr^{-1}$. Additionally, maximum rates occurred at lower needle water potentials in both conditioning treatments, occurring at approximately -1.1 MPa compared to -0.9 MPa for the control plants.

Seed source effect. The fact that seed source and water potential interact, implies that the effect of seed source varies with changing needle water potential. On the average, the Coastal Plain seed source exhibited the highest

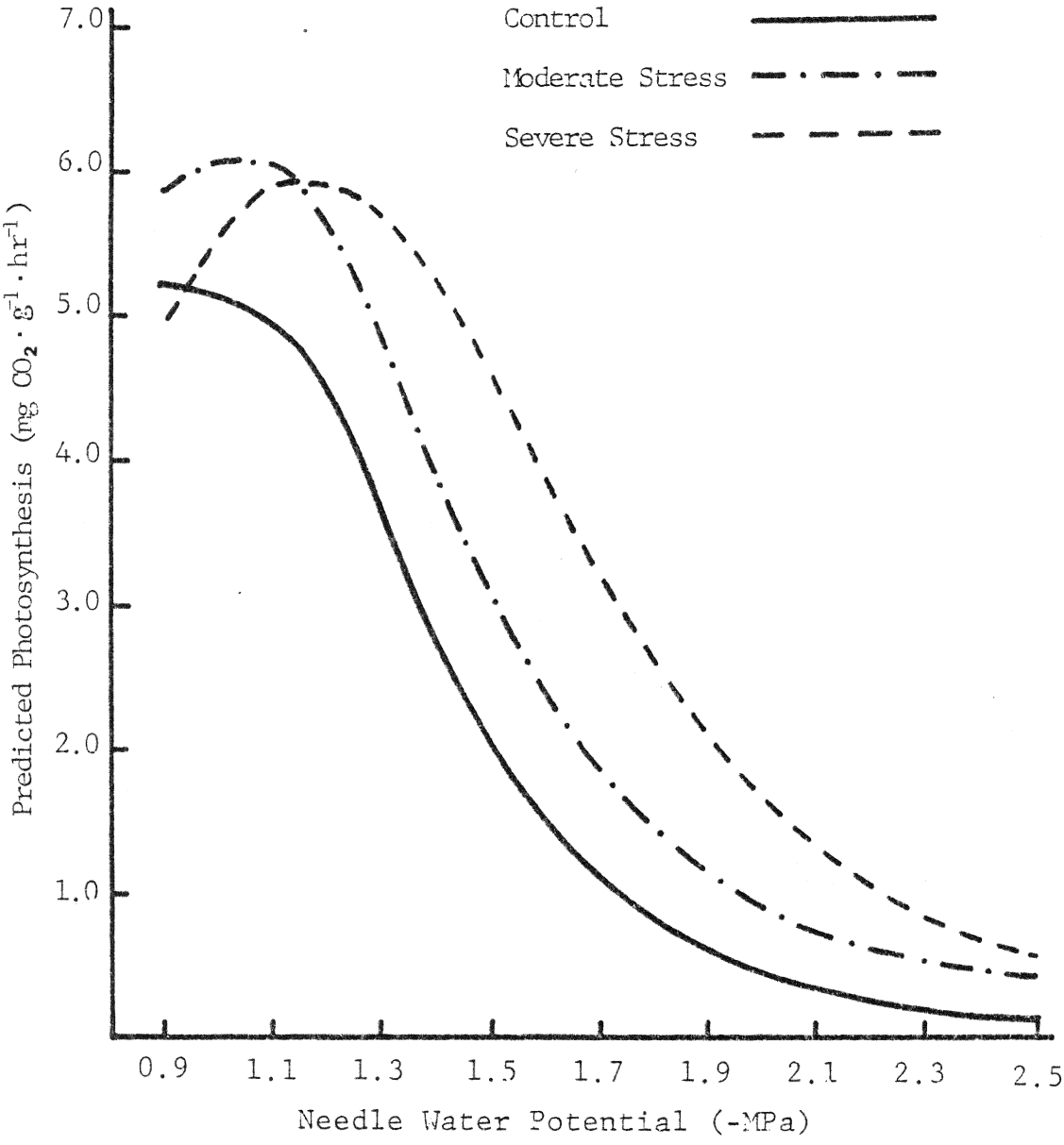


Figure 1. Predicted loblolly pine photosynthesis as affected by needle water potential and 19 weeks of moisture stress conditioning.

rates of photosynthesis at all needle water potentials, followed by the Piedmont source and the Texas source which exhibited the lowest photosynthesis (Table 7).

Differences in predicted photosynthesis due to seed source in SWS trees generally increases with decreasing needle water potential, with a maximum difference between the Coastal Plain and Texas source at a needle water potential of -1.7 MPa (3.65 versus 2.94 mg $CO_2 \cdot g^{-1} \cdot hr^{-1}$, in the Coastal Plain and Texas source respectively, a difference of 0.71). As needle water potential decreases further differences in photosynthesis among seed source diminishes.

The effect of seed source is more complex with MWS trees. Under watered conditions the Coastal Plain seedlings show much higher predicted photosynthesis rates (Table 7). However, at needle water potentials of -1.3 MPa, the Texas source shows the highest predicted rates. As needle water potential decreases further, the Coastal Plain source again shows the highest rates. The control response consistently shows the Coastal Plain source with the highest rates. However, differences were never very large, with a maximum difference between the Coastal Plain and Texas source occurring at -1.3 MPa (3.74 versus 3.39 mg $CO_2 \cdot g^{-1} \cdot hr^{-1}$, a difference of 0.35) (Table 7).

Table 7. Predicted loblolly pine photosynthesis as affected by needle water potential, moisture stress conditioning and seed source.

	Seed Source											
	Coastal Plain				Piedmont				Texas			
	Water Potential				Water Potential				Water potential			
	------ (-MPa) -------				------ (-MPa) -------				------ (-MPa) -------			
Treatment	0.9	1.3	1.7	2.1	0.9	1.3	1.7	2.1	0.9	1.3	1.7	2.1
	---------- (mg CO2/g hr) ----------											
Control	5.28	3.74	1.27	0.37	5.19	3.56	1.14	0.30	5.11	3.39	1.02	0.25
Moderate stress	7.53	4.69	1.97	0.82	5.72	4.82	1.85	0.70	4.34	4.96	1.73	0.60
Severe stress	4.97	6.03	3.65	1.60	4.89	5.74	3.28	1.30	4.81	5.47	2.94	1.06
Severe minus Control	-0.31	2.29	2.38	1.2	-0.30	2.18	2.14	1.00	-0.30	2.08	1.92	0.81

The acclimation of photosynthesis to low needle water potentials also differed among seed sources. This is apparent by observing the difference between control and SWS seedlings at the various levels of needle water potential (Table 7). Under watered conditions (needle water potentials of -0.9 MPa), control plants showed the highest predicted rates of photosynthesis and the difference between control and SWS seedlings does not differ among seed sources. However, at lower needle water potential values, SWS plants showed the highest rates of photosynthesis and the degree of acclimation (difference between control and SWS seedlings) is greatest for the Coastal Plain source, followed by the Piedmont source and the Texas source with the smallest difference. For example, at -1.7 MPa needle water potential, the Coastal Plain source shows a difference of 2.38 between the control and the SWS treatment, while the Texas source shows a difference of only 1.92 (Table 7).

Analysis of Variance.

When the photosynthesis data were analyzed using analysis of variance techniques, seed source, moisture-stress conditioning and water potential all significantly influenced photosynthesis. However, only seed source affected photosynthesis independently of the other factors. A significant two-way interaction occurred between water

potential and moisture stress conditioning, and therefore these factors were not examined independently.

Water stress effects. The effect of moisture conditioning on the response of photosynthesis to decreasing needle water potential (moisture stress conditioning X water potential interaction) is shown in Table 8. Photosynthesis does not differ significantly among moisture stress treatments or water potentials through the first four water potential classes (up to -1.40 to -1.69 MPa). However, in the water potential class -1.70 to -1.99, both conditioning treatments exhibit significantly higher photosynthesis than control plants. In this range of water potential, control plant photosynthesis was near zero, averaging 0.41 mg $CO_2 \cdot g^{-1} \cdot hr^{-1}$. compared to the MWS treatment which averaged 1.56 mg $CO_2 \cdot g^{-1} \cdot hr^{-1}$. The SWS treatment resulted in significantly higher photosynthesis, than either the control or MWS plants, averaging 3.27 mg $CO_2 \cdot g^{-1} \cdot hr^{-1}$.

Based on regression results, it is likely that differences would have occurred between control seedlings and conditioned seedlings in the water potential class -1.40 to -1.69; however, no control data points fell in this region. Control seedling water potentials fell quickly below -1.40 MPa and despite efforts to obtain a broad range of data points, no needle water potentials were ever

Table 8. Mean loblolly pine photosynthesis as affected by water potential and moisture stress conditioning.[1]

Water Potential Class	Treatment		
	Control	Moderate stress	Severe stress
(-MPa)	(mg CO2/g•hr)		
< 1.0	4.59 A B	6.12 A	4.78 A B
1.0 - 1.19	5.41 A B	5.56 A	6.07 A
1.2 - 1.39	5.60 A	4.98 A B	5.71 A
1.40 - 1.69	--[2]	5.47 A B	4.99 A B
1.70 - 1.99	0.41 D E F	1.56 C	3.27 B
2.00 - 2.29	- 0.06 E F	0.49 D E	1.68 C
2.30 - 2.59	0.08 E F	0.39 D E F	0.95 C D
> 2.60	- 0.11 F	- 0.04 F	0.17 D E F

[1]Means followed by the same letter do not differ significantly (alpha = 0.05); comparisons can be made in all directions; means are averaged over all seed sources.
[2]No data occurred at this level for the control treatment.

recorded in the region between -1.40 and -1.69 MPa in control seedlings.

As needle water potentials fell further, SWS seedlings maintained significantly higher photosynthesis than control plants, or MWS plants, averaging 1.68 mg $CO_2 \cdot g^{-1} \cdot hr^{-1}$ in the range of water potentials between -2.00 and -2.29 MPa.

In this same range of water potentials (-2.00 to -2.29 MPa), control plants showed a negative photosynthesis rate (-0.06 mg $CO_2 \cdot g^{-1} \cdot hr^{-1}$), indicating the plants were releasing more CO_2 by respiration than they were fixing by photosynthesis. SWS plants, in the range of water potentials measured, never showed a negative photosynthesis rate and continued significant photosynthesis (0.95 mg $CO_2 \cdot g^{-1} \cdot hr^{-1}$) down to water potentials between -2.30 and -2.59 MPa and exhibited a positive rate (0.17 mg $CO_2 \cdot g^{-1} \cdot hr^{-1}$) below water potentials of -2.60 MPa.

Seed source effects. The effect of seed source on photosynthesis is shown in Table 9. Averaged over all treatments and water potentials both the Coastal Plain and Piedmont sources exhibited significantly higher photosynthesis (alpha=0.1) than the Texas source, both averaging 1.23 mg $CO_2 \cdot g^{-1} \cdot hr^{-1}$, compared to 1.11 in the Texas source. It is somewhat surprising that seed source and water potential did not exhibit a significant

interaction since in the regression analysis this was a significant factor in all models. This could however, be due to variation occurring within water potential classes, which may have inflated the error term.

Needle Conductance

Regression Response.

As with photosynthesis, moisture stress conditioning significantly influence the response of needle conductance to water potential. When the data from the three treatments were modeled together, moisture stress conditioning and moisture stress conditioning interactions were found to strongly contribute to the model. Additionally, when the treatments were modeled separately slope estimates for various parameters were found to differ significantly among the moisture stress conditioning treatment.

In addition to moisture stress conditioning, vapor pressure deficits also significantly influenced needle conductance. In the MWS and SWS treatments, when the log of needle conductance was regressed on the log of water potential, vapor pressure deficit was found to significantly influence the intercept. Additionally, vapor pressure deficit and vapor pressure deficit interactions were significant parameters in both the MWS and SWS seedling

Table 9. Loblolly pine seedling photosynthesis as affected by seed source.[1]

Seed Source	Photosynthesis Rate
	(mg CO_2/g•hr)
Coastal Plain	1.23 A
Piedmont	1.23 A
Texas	1.11 B

[1]Means followed by the same letter do not differ significantly (alpha = 0.05); means are averaged over all moisture stress treatments and all water potentials.

models. Vapor pressure deficit was found to have no significant affect on the control seedlings.

Considering these results, seven separate models were then produced, one for each vapor pressure deficit class for both the MWS and SWS treatment and one model for control plants. Models were produced using the same criteria as explained for the photosynthesis data.

Regression equations developed for the various treatment combinations are shown in Table 10 . All models contain only variables with significant slope estimates and all showed good residual plots. The coefficients of determination ranged from a low of 0.28 for the SWS trees under low vapor pressure deficit to a high of 0.81 for control plants. The low coefficient of determination of 0.28 is partially due to the nearly flat response of conductance to needle water potential for this treatment and not necessarily to a poor fit. Water potential and various polynomial or log transformations of water potential explained the greatest amount of variation in the models. However, various interactions of seed source and water potential terms also significantly contributed to all models.

Water stress effects. Figures 2 through 4 show the predicted response of needle conductance to water potential

Table 10. Regression models, coefficients of determination and sample sizes for the three moisture stress and VPD treatments.[1]

Treatment	VPD	Model	R^2	n
	(kPa)			
Control	--	$Ln(Gn) = 28.6 - 8.8(SD) - 36.5(WP) + 25.8\ Ln(WP) + 6.7(WP^2) - 0.47(WP^3) + 10.1(SD*WP) - 7.9((SD*Ln(WP)) - 1.8(SD*WP^2) + 0.13(SD*WP^3)$	0.81	154
Moderate stress	4.20	$Ln(Gn) = -2.81 - 2.57\ Ln(WP) + 0.280(SD*WP^2)$	0.70	74
	2.35	$Ln(Gn) = -2.66 - 2.39\ Ln(WP) + 0.154(SD*WP^2)$	0.56	81
	1.63	$Ln(Gn) = -3.15 - 1.97\ Ln(WP) + 0.175(SD*WP)$	0.42	48
Severe stress	4.20	$Ln(Gn) = -2.69 - 1.93\ Ln(WP) + 0.0017(SD*WP^3)$	0.66	93
	2.35	$Ln(Gn) = -2.40 + 2.93(SD) - 0.52(WP^2) + 0.07(WP^3) - 3.43(SD*WP) + 3.86((SD*Ln(WP)) + 0.347(SD*WP^2)$	0.74	101
	1.63	$Ln(Gn) = -2.99 - 0.030(SD*WP^3)$	0.28	70

1. VPD = vapor pressure deficit, Gn = needle conductance, WP = water potential, SD = seed source (1, 2 or 3 for the Coastal Plain, Piedmont or Texas source, respectively), vapor pressure deficit did not significantly influence the model in control plants.

at the three vapor pressure deficits averaged for the three seed sources. Predicted values are only presented in a range of water potentials where sufficient data occurred for all treatments. The predicted lines along with their corresponding data are presented in appendix b (Figures b1 through b7).

The most striking response to moisture stress conditioning is that initial needle conductance values (under well watered conditions) are greatly reduced compared to control plants (Figures 2, 3 and 4). For example under ambient vapor pressure deficits (2.35 kPa) at needle water potentials of -1.1 MPa, conditioned seedlings exhibited conductance values of 0.068 and 0.047 $cm \cdot s^{-1}$ for the MWS and SWS treatments, respectively, compared to 0.095 $cm \cdot s^{-1}$ for control plants. This response is especially significant considering that initial photosynthesis rates did not differ among the three moisture stress treatments (Table 8, Figure 1).

Predicted needle conductance in conditioned trees also tended to drop less quickly in response to decreasing water potentials. This is particularly true for the SWS trees, which under low needle water potentials exhibited higher predicted needle conductance than either controls or MWS trees (Figures 2, 3 and 4). This effect is most striking

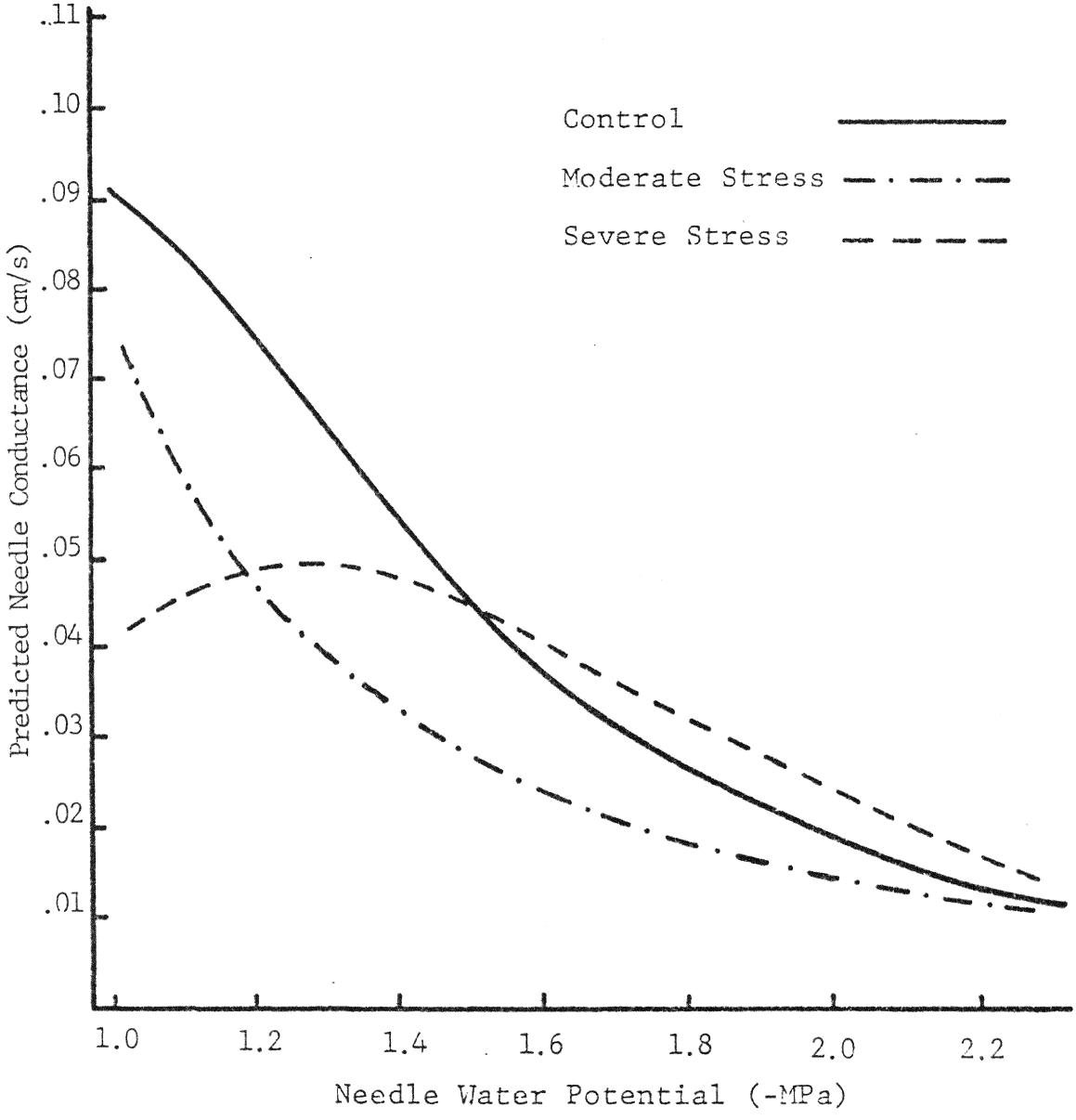


Figure 2. Predicted loblolly pine needle conductance at ambient vapor pressure deficit (2.63 kPa) as affected by moisture stress conditioning and water potential.

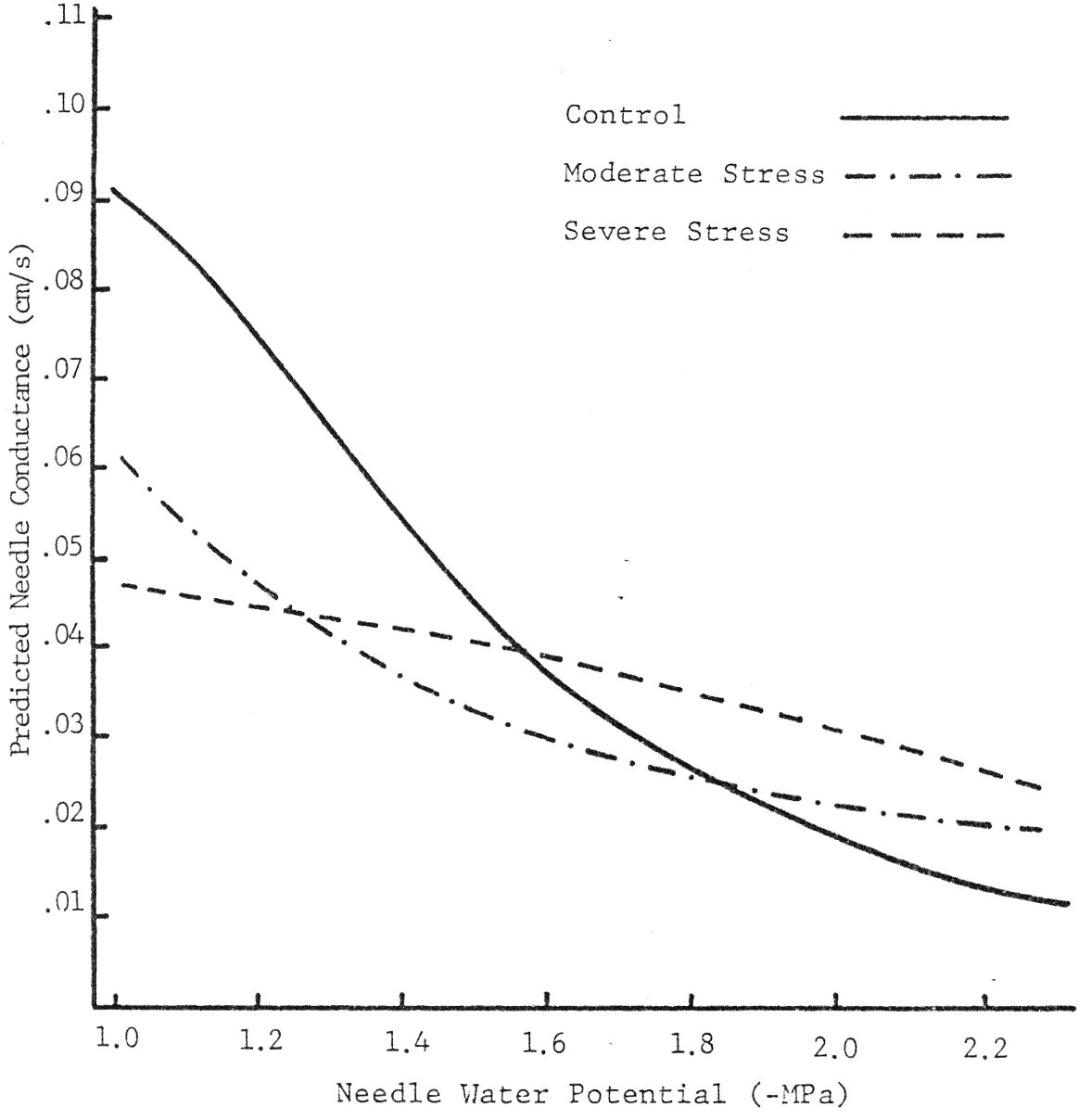


Figure 3. Predicted loblolly pine needle conductance at low vapor pressure deficit (1.63 kPa) as affected by moisture stress conditioning and water potential.

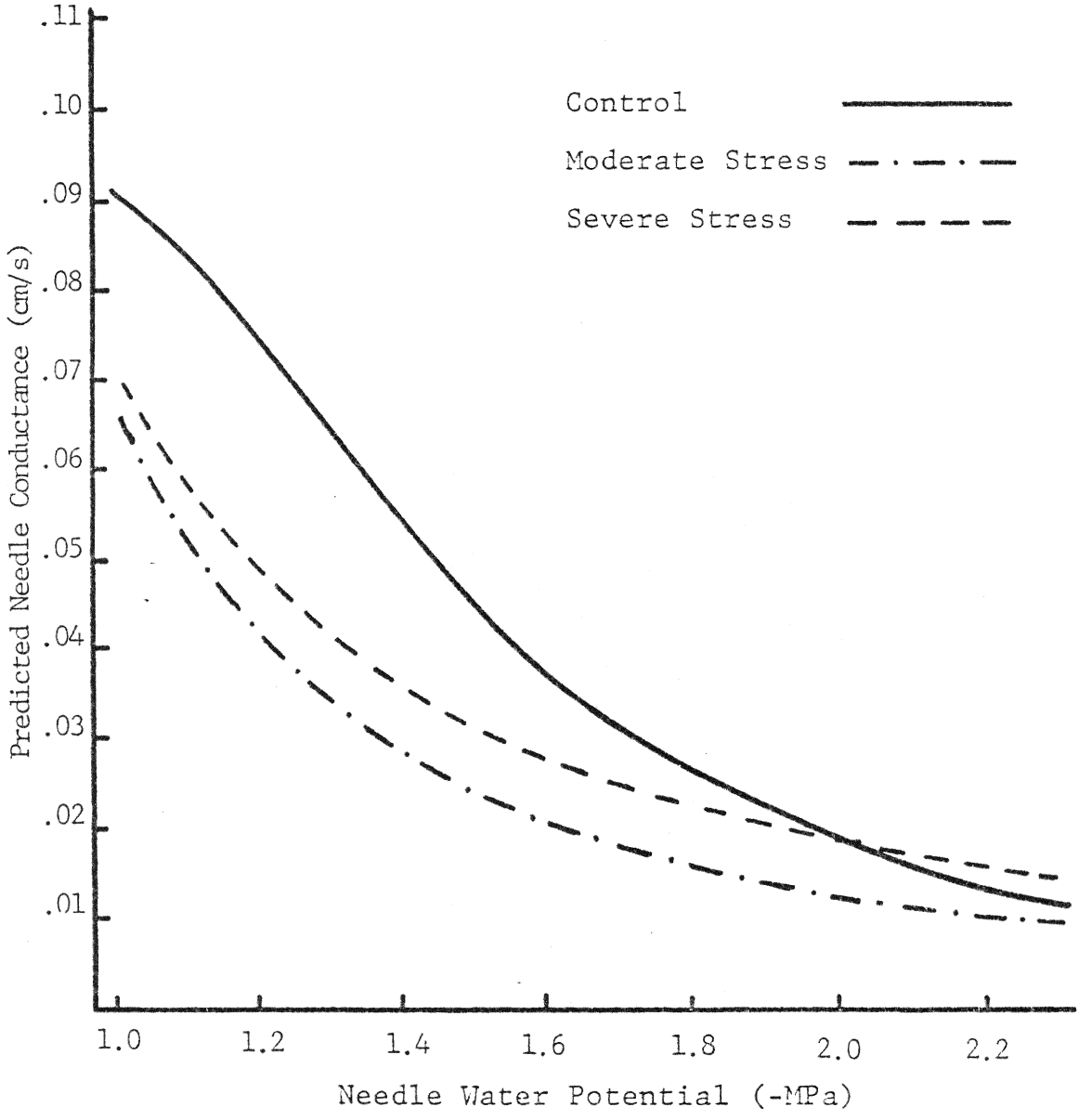


Figure 4. Predicted loblolly pine needle conductance at high vapor pressure deficit (4.20 kPa) as affected by moisture stress conditioning and water potential.

under low vapor pressure deficits, where SWS trees show a very gradual drop in needle conductance and as a result, exhibit higher conductance values than control plants below needle water potentials of -1.5 MPa (Figure 4).

Vapor pressure deficit effects. The effect of vapor pressure deficit on predicted needle conductance is shown in Table 11 . Predicted needle conductance in control plants was not significantly influenced by vapor pressure deficits but values are presented for comparison. At a water potential of -1.1 MPa, vapor pressure deficit had little affect on MWS plants, ranging from 0.051 $cm \cdot s^{-1}$ at a vapor pressure deficit of 4.20 kPa, to 0.058 $cm \cdot s^{-1}$ at a vapor pressure deficit of 2.35 kPa. Unexpectedly, needle conductance in SWS seedlings at water potentials of -1.1 MPa, was lowest at a vapor pressure deficit of 1.63 kPa, averaging 0.046 $cm \cdot s^{-1}$, compared to 0.057 $cm \cdot s^{-1}$ at a vapor pressure deficit of 4.20 kPa. However, at lower needle water potentials, a definite pattern was evident in both conditioning treatments. As needle water potential decreased conductance became much greater at low vapor pressure deficits.

This response was very striking in the SWS trees, where at needle water potentials of -1.9 MPa, needle conductance was 0.014 units higher (0.034 versus 0.020) at a vapor

Table 11. Predicted loblolly pine needle conductance as affected by water potential, moisture stress conditioning and VPD.[1]

Water Potential	Water Stress Treatment						
	Moderate			Severe			Control
	VPD			VPD			
	(kPa)			(kPa)			
	1.63	2.35	4.20	1.63	2.35	4.20	
(-MPa)	(cm/s)						
1.1	0.053	0.058	0.051	0.046	0.047	0.057	0.085
1.3	0.041	0.039	0.034	0.044	0.050	0.041	0.065
1.5	0.033	0.029	0.024	0.041	0.045	0.031	0.045
1.7	0.028	0.022	0.018	0.038	0.037	0.025	0.031
1.9	0.024	0.017	0.014	0.034	0.029	0.020	0.022
2.1	0.022	0.014	0.012	0.029	0.021	0.017	0.016
2.3	0.019	0.011	0.010	0.025	0.015	0.014	0.012
2.5	0.018	0.010	0.008	0.021	0.011	0.012	0.010

[1] VPD = vapor pressure deficit; control seedlings were not significantly influenced by vapor pressure deficit.

pressure of 1.63 kPa compared to a vapor pressure deficit of 4.20 kPa. Moderate stress trees below water potentials of -1.3 MPa, generally exhibited needle conductance values 0.01 units higher under low compared to high vapor pressure deficits.

This response can also be seen by comparing figures 2, 3 and 4, and was again most striking in the SWS plants where conductance drops rapidly in response to water potential at high vapor pressure deficits (Figure 3), but shows a near linear, gradual drop under low vapor pressure deficits (Figure 4).

Also of interest in Table 11, was that conditioned trees under high vapor pressure deficits (4.20 kPa) generally never exhibited higher conductance values than control trees. Only under low vapor pressure deficits and low water potentials, did conditioning result in much greater predicted conductance values compared to control plants. For example, at a water potential of -2.1 MPa, control plants exhibited a needle conductance of 0.016, compared to SWS trees which exhibited a needle conductance of 0.029 $cm \cdot s^{-1}$ at low vapor pressure deficits, but only 0.017 $cm \cdot s^{-1}$ under high vapor pressure deficits.

Seed source effects. Seed source had a significant influence in all needle conductance prediction equations.

However, the magnitude of the effect was dependent on moisture stress conditioning. Table 12 shows predicted needle conductance values as influenced by seed source, moisture stress conditioning and water potential.

Control plants exhibited the greatest difference due to seed source. Under high needle water potentials (-1.1 MPa), predicted conductance values were over twice as large in the Coastal Plain compared to the Texas seed source, 0.124 versus 0.052 $cm \cdot s^{-1}$, respectively. The Piedmont source fell midway between the two, with a predicted value of 0.080 $cm \cdot s^{-1}$ (Table 12). This large difference between seed sources in control plants diminished as needle water potential decreased. At needle water potentials of -2.3 MPa predicted conductance values were 0.013, 0.012 and 0.011 $cm \cdot s^{-1}$ for the Coastal Plain, Piedmont and Texas sources, respectively.

With both conditioning treatments, seed source had less of an effect on predicted conductance values. At most levels of needle water potential and vapor pressure deficit, the Texas seed source had the greatest predicted conductance values for both moisture stress conditioning treatments (Table 12). However, differences were very slight, with the exception of predicted values at low vapor pressure deficits (1.63 kPa) and MWS conditioning, where the Texas source

Table 12. Predicted loblolly pine needle conductance as affected by moisture stress conditioning, seed source and VPD.[1]

		Seed Source											
		Coastal Plain				Piedmont				Texas			
		Water Potential				Water Potential				Water Potential			
		-------- (-MPa) --------				-------- (-MPa) --------				-------- (-MPa) --------			
Treatment	VPD	1.1	1.5	1.9	2.3	1.1	1.5	1.9	2.3	1.1	1.5	1.9	2.3
	(kPa)	---------- (cm/s) ----------											
Control		0.124	0.066	0.028	0.013	0.080	0.043	0.021	0.012	0.052	0.027	0.016	0.011
Moderate stress	2.35	0.057	0.028	0.016	0.010	0.057	0.029	0.017	0.010	0.059	0.030	0.018	0.012
	4.20	0.049	0.023	0.013	0.008	0.051	0.024	0.014	0.010	0.052	0.026	0.016	0.011
	1.63	0.043	0.025	0.017	0.012	0.052	0.033	0.023	0.019	0.063	0.042	0.033	0.028
Severe stress	2.35	0.050	0.040	0.025	0.014	0.047	0.045	0.029	0.015	0.044	0.051	0.033	0.017
	4.20	0.057	0.031	0.020	0.014	0.057	0.031	0.020	0.014	0.057	0.032	0.020	0.015
	1.63	0.048	0.045	0.041	0.035	0.046	0.041	0.033	0.024	0.045	0.037	0.027	0.017

1. VPD = vapor pressure deficit; control seedlings were not significantly affected by vapor pressure deficit.

showed much higher values than either the Coastal Plain or Piedmont sources. In fact, under MWS conditioning and low vapor pressure deficit, predicted conductance values were higher than any other level of conditoning or vapor pressure deficit for the Texas source.

An exception to the Texas source exhibiting the highest conductance values in conditioned trees occurred with the SWS seedlings subjected to low vapor pressure deficits (1.63 kPa). At these treatment levels, Coastal Plain seedlings had the highest conductance values, followed by the Piedmont seed source and the Texas seed source with the lowest conductance. This trend is the result of predicted conductance values increasing greatly under low vapor pressure deficits in both the Coastal Plain and Piedmont seed sources subjected to SWS conditioning; whereas the Texas seed source exhibited no response and in some cases a slight decrease (Table 12).

Analysis of Variance.

Seed source, water potential and vapor pressure deficit were significant as main effects when needle conductance was analyzed with analysis of variance techniques. However, these factors occurred in various significant two-way interactions and therefore were not examined independently. Two-way interactions which were significant in the analysis

were : moisture stress conditioning X water potential, vapor pressure deficit X water potential, seed source X moisture stress conditioning, and seed source X water potential. No three-way interactions were significant in the analysis.

Water stress effects. Moisture stress conditioning had a significant influence on the response of needle conductance to decreasing needle water potential (moisture stress conditioning X water potential interaction). Through the first four water potential classes (down to -1.40 to -1.69 MPa) control plant needle conductance (averaged over all seed sources and vapor pressure deficits) did not differ significantly, ranging from a high of 0.110 $cm \cdot s^{-1}$ at water potentials between -1.0 and -1.19 MPa, to a low of 0.087 $cm \cdot s^{-1}$ between water potentials of -1.20 and -1.39 MPa (Table 13). However, at these same levels of needle water potential, control plant conductance was significantly higher than either conditioning treatment. Needle conductance values were approximately 40 to 55 percent lower than control plants for the MWS and SWS trees, respectively. Needle conductance values, however, did not differ significantly between MWS and SWS trees.

Below water potentials of -1.70 MPa, both control seedlings and MWS seedlings exhibited a large decrease in

Table 13. Mean loblolly pine needle conductance as affected by water potential and moisture stress conditioning[1]

Water Potential Class	Treatment		
	Control	Moderate Stress	Severe stress
(-MPa)	(cm/s)		
< 1.0	0.097 A B	0.055 C D	--
1.00 - 1.19	0.110 A	0.068 B C	0.046 C D
1.20 - 1.39	0.087 A B	0.056 C D	0.046 C D
1.40 - 1.69	0.103 A B	0.054 C D	0.048 C D
1.70 - 1.99	0.040 E F	0.021 G H	0.040 D E
2.00 - 2.29	0.013 H I J	0.014 H I	0.024 F G
2.30 - 2.59	0.013 H I J	0.012 I J K	0.018 G H
2.60 - 2.89	0.007 K L	0.009 K L	0.009 J K L
> 2.90	0.008 K L	0.009 J K L	0.006 L

[1]Means followed by the same letter do not differ significantly (alpha = 0.05); comparisons can be made in all directions; means are averaged over all seed sources and vapor pressure deficits.

needle conductance, averaging 0.040 and 0.021 $cm \cdot s^{-1}$, respectively, at water potentials between -1.70 and -1.99 MPa (Table 13). At this level of water potential, conductance in MWS seedlings was significantly lower than control conductance. SWS seedlings, however, showed only a slight drop in needle conductance averaging 0.040, at water potentials between -1.70 and -1.99 MPa. This level of needle conductance is equal to control seedlings at the same needle water potentials and was not significantly different than MWS or SWS seedlings initial values.

As needle water potential decreased further, control seedlings exhibited a significant 68 percent decrease in needle conductance averaging 0.013 $cm \cdot s^{-1}$ between water potentials of -2.00 and -2.29 MPa. At this level of water stress, MWS seedling needle conductance fell only 33 percent and did not differ significantly compared to controls, averaging 0.014 $cm \cdot s^{-1}$. SWS seedlings had an average needle conductance value of 0.024 $cm \cdot s^{-1}$, which was significantly higher than either of the other two treatments.

At needle water potentials between -2.30 and -2.59 MPa, needle conductance fell only slightly in all three treatments, ranging from 0.018 $cm \cdot s^{-1}$ in the SWS trees, to 0.013 and 0.012 $cm \cdot s^{-1}$ in the control and MWS trees, respectively. Conductance in SWS trees was not

significantly higher than the control, but was when compared to MWS plants. Below water potentials of -2.60 MPa, no significant differences in conductance occurred due to moisture stress conditioning.

Vapor pressure deficit effects. The effect of vapor pressure deficit on the response of needle conductance to water potential (vapor pressure deficit X water potential interaction) is shown in Table 14 . As expected, needle conductance decreased dramatically in response to increasing water potential. However, under low vapor pressure deficit (1.63 kPa) this decrease was more gradual, resulting in higher conductance values at lower needle water potentials under this vapor pressure deficit treatment. For example, at water potentials between -1.77 and -1.99 MPa both the low (1.63 kPa) and the ambient (2.35 kPa) vapor pressure deficits exhibited significantly greater needle conductance values than at high vapor pressure deficit (4.20 kPa), averaging 0.039 and 0.036 cm• s^{-1}, compared to 0.023 cm• s^{-1}, respectively.

In the next lower water potential class (-2.00 to -2.29 MPa), needle conductance at low vapor pressure deficit was greater than conductance values at either ambient (2.35 kPa) or high (4.20) vapor pressure deficits. This was the result of conductance falling only 31 percent from 0.039 (at water

Table 14. Mean loblolly pine needle conductance as affected by water potential and VPD.[1]

Water Potential Class	Vapor Pressure Deficit (kPa) 1.63	2.35	4.20
(-MPa)	(cm/s)		
< 1.0	0.099 A	0.090 A B	0.074 A B C
1.00 - 1.19	0.080 A B C D	0.081 A B C	0.073 A B C D
1.20 - 1.39	0.051 C D E	0.055 B C D E	0.044 E
1.40 - 1.69	0.054 C D E	0.059 B C D E	0.048 D E
1.70 - 1.99	0.039 F	0.036 F	0.023 G H I
2.00 - 2.29	0.027 F G H	0.018 H I J K	0.017 H I J K
2.30 - 2.59	0.019 H I J	0.015 I J K	0.013 J K L
2.60 - 2.89	0.026 F G	0.007 M	0.008 L M
> 2.90	0.016 K L	0.007 M	0.008 M

[1]VPD = vapor pressure deficit; means followed by the same letter do not differ significantly (alpha = 0.05); comparisons can be made in all directions; means are averaged over all moisture stress treatments and seed sources.

potentials between -1.70 and -1.99 MPa) to 0.027 $cm \cdot s^{-1}$ (at water potentials between -2.00 and -2.29 MPa) under low vapor pressure deficit, compared to a 50 percent decrease from 0.036 to 0.018 $cm \cdot s^{-1}$ under ambient vapor pressure deficit. Conductance values under high vapor pressure deficit, having fallen dramatically at previous needle water potentials (e.g. -1.70 to -1.99 MPa), decreased only slightly further to 0.017 $cm \cdot s^{-1}$ at water potentials between -2.00 to -2.29 MPa.

With further decreases in needle water potential, conductance values under low vapor pressure deficits continued to be significantly higher than either of the other vapor pressure deficit treatments.

Of additional interest in Table 14, is that the high vapor pressure deficit class always had the lowest conductance values. Although this was not always statistically significant, this trend was consistent within all water potential classes above -2.59 MPa.

Seed source effects. A significant water potential X seed source interaction also occurred in the analysis and this response is presented in Table 15 . In general, all seed sources showed large decreases in needle conductance with decreasing water potential; however, the responses were slightly different for the three seed sources. Conductance

values were very similar among seed sources through the first four water potential classes (down to water potentials between -1.40 and -1.69 MPa) and did not differ significantly. However, initial values were lowest in the Texas source, which agreed with regression results shown in Table 12 .

The Texas source exhibited the least reduction through the first four water potential classes, and at water potentials between -1.40 and -1.69 MPa, had an average conductance value of 0.061 $cm \cdot s^{-1}$, which did not differ significantly from its original value of 0.079 $cm \cdot s^{-1}$.

At water potentials between -1.70 and -1.99, the Piedmont source exhibited significantly lower conductance than either of the other two sources, averaging 0.025 $cm \cdot s^{-1}$ compared to 0.039 and 0.038 $cm \cdot s^{-1}$ for the Texas and Coastal Plain sources, respectively. Below water potentials of -2.00 MPa no significant differences in conductance occurred as a result of seed source.

The effect of seed source and moisture stress conditioning (averaged over all water potentials and vapor pressure deficits) on needle conductance is shown in Table 16 . Control seedling needle conductance was significantly affected by seed source. The Texas source had significantly lower conductance averaging 0.044 $cm \cdot s^{-1}$, compared to 0.060

Table 15. Mean loblolly pine needle conductance as affected by water potential and seed source.[1]

Water Potential Class	Seed Source: Coastal Plain	Piedmont	Texas
(-MPa)	(cm/s)		
< 1.00	0.083 A	0.096 A	0.079 A B
1.00 - 1.19	0.083 A B	0.077 A B	0.071 A B C
1.20 - 1.39	0.042 D E	0.056 B C D E	0.045 C D E
1.40 - 1.69	0.048 D E	0.058 B C D	0.061 A B C D
1.70 - 1.99	0.038 F G	0.025 G H	0.039 E F
2.00 - 2.29	0.016 H I	0.023 G H	0.020 H I
2.30 - 2.59	0.013 I J	0.016 H I	0.016 H I
2.60 - 2.89	0.010 J K	0.009 J K	0.007 K
> 2.90	0.008 K	0.007 K	0.008 K

[1]Means followed by the same letter do not differ significantly (alpha = 0.05); comparisons can be in all directions; means are averaged over all moisture stress treatments and vapor pressure deficits.

$cm \cdot s^{-1}$ in the Coastal Plain and 0.053 $cm \cdot s^{-1}$ in the Piedmont source. This trend followed the same pattern as the predicted control needle conductance shown in Table 12 .

Under the MWS and SWS treatments, needle conductance in the Coastal Plain source droped significantly to 0.032 $cm \cdot s^{-1}$; whereas, in the Piedmont source conductance droped significantly, but less so than the Coastal Plain, averaging 0.044 and 0.039 $cm \cdot s^{-1}$ at the MWS and SWS treatments, respectively. The Texas source showed only a slight, non-significant drop in conductance under MWS, averaging 0.042 $cm \cdot s^{-1}$, but conductance fell to 0.030 $cm \cdot s^{-1}$ under the SWS treatment. These trends also agreed well with regression results shown in Table 12 .

Transpiration

Initial mean daily transpiration rates (average of days 1 through 3 since last watering) as affected by seed source and moisture stress conditioning are shown in Table 17 . Both moisture stress conditioning and seed source significantly influenced transpiration; however, the effect of these factors on transpiration were not independent of each other. Therefore, the interaction between moisture stress conditioning and seed source was examined.

Table 16. Mean loblolly pine needle conductance as affected by seed source and 19 weeks of moisture stress conditioning.[1]

Treatment	Seed Source: Coastal Plain	Piedmont	Texas
	(cm/s)		
Control	0.060 A	0.053 A	0.044 B
Moderate stress	0.032 C D	0.044 B	0.042 B
Severe stress	0.032 C D	0.039 B C	0.030 D

[1]Means followed by the same letter do not differ significantly (alpha = 0.05); comparisons can be in all directions; means are averaged over all vapor pressure deficits and water potentials.

Table 17. Mean daily transpiration rate of loblolly pine seedlings as affected by seed source and 19 weeks of moisture stress conditioning.[1]

Treatment	Seed Source: Coastal Plain	Piedmont	Texas
	(g H2O/g•hr)		
Control	0.44 A B	0.49 A	0.40 B C
Moderate stress	0.28 E	0.31 D E	0.34 C D E
Severe stress	0.31 D E	0.38 C D	0.33 C D E

[1]Means followed by the same letter do not differ significantly (alpha = 0.05); means can be compared in all directions.

Transpiration rate in control seedlings was lowest in the Texas source and averaged 0.40 q $H_2O \cdot q^{-1} \cdot hr^{-1}$ which was significantly lower than the Piedmont source, which had the highest transpiration rate of 0.49 q $H_2O \cdot q^{-1} \cdot hr^{-1}$.

As a result of moisture stress conditioning, daily mean transpiration rate was reduced dramatically. This was particularly true for the Coastal Plain and Piedmont seed sources, where under MWS conditions, transpiration was reduced approximately 37 percent, averaging 0.28 and 0.31 q $H_2O \cdot q^{-1} \cdot hr^{-1}$., compared to 0.44 and 0.49 g$H_2O \cdot g^{-1} \cdot hr^{-1}$, respectively (Table 17). Under SWS conditions, transpiration was higher, but not significantly , in both the Coastal Plain and Piedmont sources when compared to MWS values.

The Texas source showed a slightly different trend in response to conditioning. Mean daily transpiration rates are decreased, but never significantly , when compared to the Texas source controls. The Texas source demonstrated only a 17 percent decrease in transpiration in response to conditioning. Additionally, transpiration rate in the Texas source was lowest under the SWS treatment, averaging 0.33 q $H_2O \cdot q^{-1} \cdot hr^{-1}$, while the other seed sources showed minimum rates under the MWS treatment (Table 17).

Water-Use Efficiency

Water-use efficiency (mg CO2 fixed per g H2O transpired) was significantly affected by the conditioning treatments (Table 18). Initial water use efficiency (days 1 through 3 since last watering) was significanlty higher in both conditioning treatments averaging 21.5 and 18.2 mg $CO_2 \cdot g^{-1}$ H_2O in the MWS and SWS treatments, respectively, compared to 12.5 mg $CO_2 \cdot g^{-1}$ H_2O in the controls.

Seed source did not significantly affect water-use efficiency ranging from a low of 16.7 mg $CO_2 \cdot g^{-1}$ H_2O in the Texas source, to a high of 18.6 mg $CO_2 \cdot g^{-1}$ H_2O in the Coastal Plain seed source.

Experiment Two

Growth Parameters

Year-old loblolly pine biomass after eight weeks of moisture stress conditioning are shown in Table 19 . Shoot dry weight was reduced only 20 percent by the moisture stress conditioning. Stressed trees averaged 4.76 g compared to 5.94 g in control trees. Root growth, however, was reduced significantly by moisture stress conditioning. Stressed seedling root weight was decreased 36 percent, averaging only 1.43 g, compared to 2.24 g in control plants.

Table 18. Mean water-use efficiency of loblolly pine seedlings as affected by 19 weeks of moisture stress conditioning.[1]

Treatment	Water use efficiency
	(mg CO2/g H2O)
Control	12.5 A
Moderate stress	21.5 B
Severe stress	18.2 B

[1]Means followed by the same letter do not differ significantly (alpha = 0.05); means are averaged over seed sources.

The greater decrease in root growth resulted in a significant decrease in root/shoot ratio. Stress conditioned seedlings averaged a root/shcot ratio of 0.31, compared to 0.38 in control plants, a 17 percent reduction (Table 19).

Needle Water Potential Components

The effect of eight weeks of moisture stress conditioning on needle water potential components is shown in Table 20 . Despite the fact seedlings were watered the day before water potential measurements were taken, the moisture stress conditioned trees had significantly lower needle water potential, averaging -1.35 MPa compared to -0.90 MPa in control trees.

Osmotic potential was reduced by 0.46 MPa as a result of moisture stress conditioning. Stressed seedlings averaged -1.96 MPa, which was significantly lower than -1.50 MPa measured in control seedlings.

Despite the lower needle water potential, turgor potential in stressed plants was equal to control seedlings with both treatments averaging approximately 0.60 MPa.

Table 19. Loblolly pine biomass as affected by 8 weeks of moisture stress conditioning.[1]

Treatment	Shoot weight	Root weight	Root/Shoot ratio
	(g)	(g)	
Control	5.94 A	2.24 A	0.38 A
Stressed	4.76 A	1.43 B	0.31 B

[1]Means followed by the same letter within a column do not differ significantly (alpha = 0.05).

Table 20. Loblolly pine water potential components as influenced by 8 weeks of moisture stress conditioning.[1]

Treatment	Water Potential	Osmotic Potential	Turgor Potential
	(MPa)		
Control	- 0.90 A	- 1.50 A	0.60 A
Stressed	- 1.35 B	- 1.96 B	0.61 A

[1]Means followed by the same letter within a column do not differ significantly (alpha = 0.05).

Photosynthesis

Regression Analysis.

Moisture stress conditioning had a significant influence on the response of photosynthesis to decreasing water potential. Stress conditioning was found to significantly change slope estimates and when the data were analyzed together, moisture stress was a significant parameter in the model. Therefore, as in experiment one, the two treatments (control and stressed) were analyzed separately.

Equations were developed as described in experiment one and are presented in Table 21 . Modeling procedures resulted in water potential squared being the only significant variable in both equations and both models had coefficients of determination of 0.76.

Because identical equations were found for both treatments, slope and intercept parameters could be compared statistically. As a result of this analysis, slope estimates were found to differ significantly.

The predicted response of photosynthesis to decreasing water potential is presented in figure 5 . The predicted responses plotted along with their corresponding data are shown in appendix c (Figures c1 and c2). Under well watered conditions (water potentials of -1.0 MPa), predicted photosynthesis differed little between control and

Table 21. Regression models, coefficients of determination and sample sizes for the two moisture treatments.[1]

Treatment	Model	R^2	n
Control	$Ln(Ps) = 2.70 - 0.82(WP^2)$	0.76	24
Stressed	$Ln(Ps) = 2.30 - 0.38(WP^2)$	0.76	32

[1]Ps = photosynthesis, WP = water potential.

conditioned seedlings. Conditioned and control seedlings exhibited predicted rates of 7.42 and 6.53 mg $CO_2 \cdot g^{-1} \cdot hr^{-1}$, respectively. However, control photosynthesis droped rapidly with decreasing water potential; whereas, conditioned trees showed a gradual decrease, maintaining photosynthesis to lower needle water potentials.

Similar to experiment one (Figure 1), a shift in the response of photosynthesis to decreasing water potential occurred such that equivalent rates of photosynthesis were found at lower needle water potentials in stress conditioned plants. For example, the rate of photosynthesis which occurred at -1.4 MPa in control trees did not occur until approximately -1.85 MPa in prestressed trees (0.45 MPa shift).

However, since slopes differ greatly between controls and stressed plants, the magnitude of this shift was dependent on needle water potential. With further decreases in needle water potential the shift became even greater. This can be seen at a needle water potential of -1.6 MPa, where the predicted rate of photosynthesis in control plants was 1.89 mg $CO_2 \cdot g^{-1} \cdot hr^{-1}$; this same rate of photosynthesis in stressed plants did not occur until a needle water potential of -2.15 MPa (0.55 MPa shift).

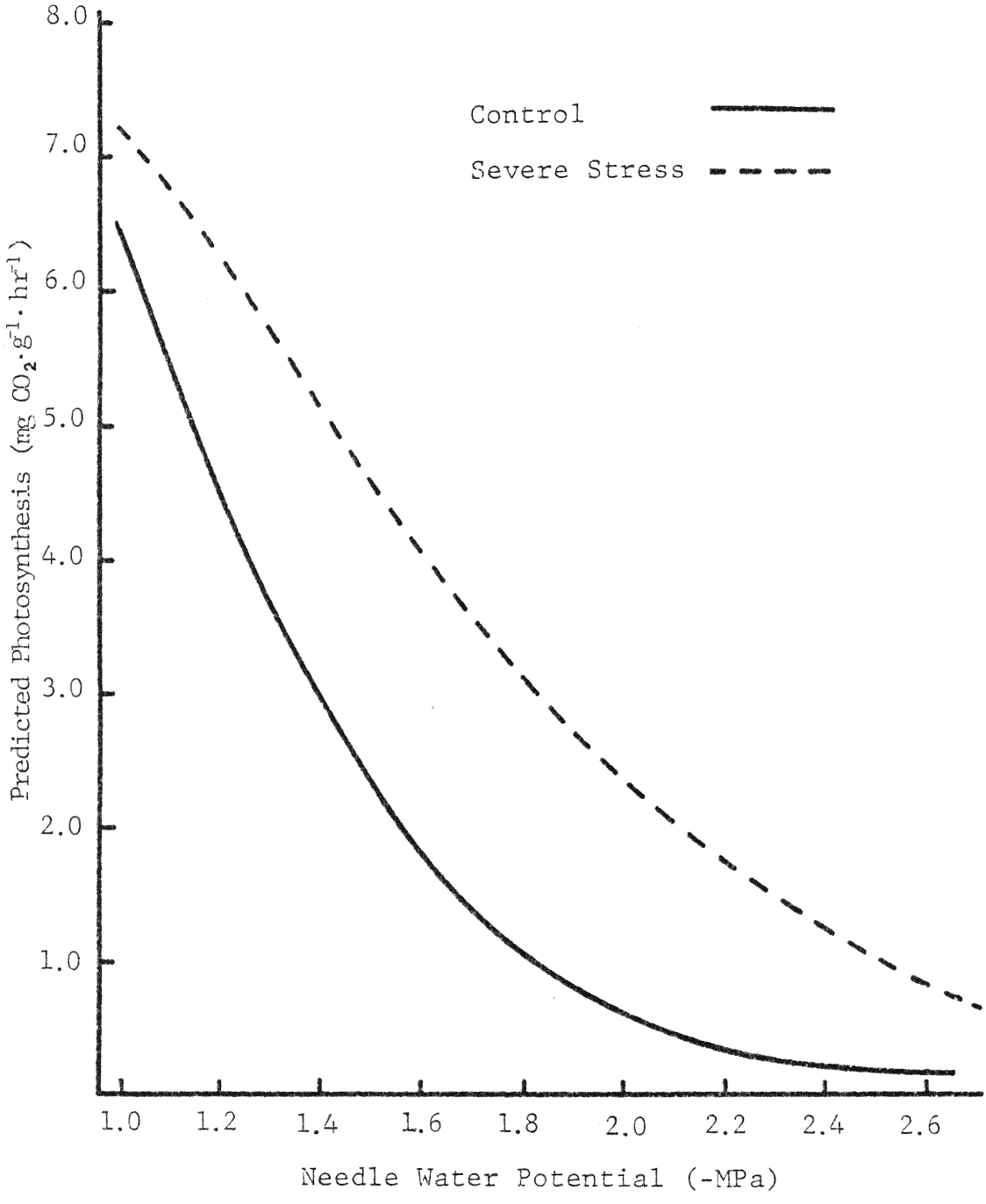


Figure 5. Predicted loblolly pine photosynthesis as affected by needle water potential and 8 weeks of moisture stress conditioning.

Analysis of Variance.

As expected, based on regression results, a highly significant water potential X moisture stress conditioning interaction occurred in the analysis (table 22).

Initial photosynthesis (water potentials greater than -1.2 MPa) was higher (but not significantly) in conditioned seedlings, averaging 7.02 compared to 5.87 mg $CO_2 \cdot g^{-1} \cdot hr^{-1}$ in controls. As water potential decreased, photosynthesis in conditioned trees did not decrease significantly until water potentials below -1.70 MPa was reached. At water potentials between -1.40 and -1.69 MPa control photosynthesis decreased sharply to 2.20 mg $CO_2 \cdot g^{-1} \cdot hr^{-1}$, while conditioned seedlings exhibited an average photosynthesis rate of 7.36 mg $CO_2 \cdot g^{-1} \cdot hr^{-1}$.

As needle water potential decreased further, conditioned trees also showed a large drop in photosynthesis, but they continued to maintain higher photosynthetic rates compared to control seedlings at all levels of needle water potential. At needle water potentials between -2.3 and -2.59 MPa, conditioned trees had an average photosynthesis rate of 1.46 mg $CO_2 \cdot g^{-1} \cdot hr^{-1}$, which was higher than the rate found in control plants at water potentials between -1.7 and -1.99 MPa and did not differ significantly than the control rate of 2.20 mg $CO_2 \cdot g^{-1} \cdot hr^{-1}$ found at water potentials between -1.4 and -1.69 MPa.

Table 22. Loblolly pine photosynthesis rate as affected by water potential and 8 weeks of moisture stress conditioning.[1]

Water Potential Class	Treatment	
	Control	Stressed
(-MPa)	--- (mg CO2/ g•hr) ---	
< 1.2	5.87 A B	7.02 A
1.20 - 1.39	4.95 B	5.67 A B
1.40 - 1.69	2.20 C D	7.36 A
1.70 - 1.99	1.22 C D E	2.96 C
2.00 - 2.29	0.28 E	1.50 C D E
2.30 - 2.59	0.53 D E	1.46 C D E
> 2.6	--	0.53 D E

[1]Means followed by the same letter do not differ significantly (alpha = 0.05); means can be compared in all directions.

Transpiration and Water-Use Efficiency

Initial mean daily transpiration and water-use efficiency (days 1 through 3 following last watering) was significantly affected by moisture stress conditioning (Table 23). Daily mean transpiration in stressed plants averaged 0.31 g $H_2O \cdot g^{-1}$, a 30 percent reduction compared to control seedlings which averaged 0.44 g $H_2O \cdot g^{-1}$. This large reduction in transpiration occurred despite initial photosynthesis being greater in conditioned plants (Table 22) and resulted in a 67 percent increase in water-use efficiency in stressed plants. Stress conditioned plants had water-use efficiencies which averaged 23.5 mg $CO_2 \cdot g^{-1}$ H_2O, compared to only 14.1 mg $CO_2 \cdot g^{-1}$ H_2O in control seedlings (Table 23).

Experiment Three

Water Potential Components

Water Stress Effects.

Water potential components of containerized, greenhouse grown loblolly pine seedlings were measured after four, nine and fourteen weeks of moisture stress conditioning. Water potential and osmotic potential were influenced significantly by an interaction between moisture stress conditioning and length of conditioning period. Turgor

Table 23. Loblolly pine transpiration and water-use efficiency as affected by 8 weeks of moisture stress conditioning.[1]

Treatment	Transpiration	Water use efficiency
	(g H2O/g•hr)	(mg CO2/g H2O)
Control	0.44 A	14.1 A
Stressed	0.31 B	23.5 B

[1]Means followed by the same letter within a column do not differ significantly (alpha = 0.05).

potential, however, was influenced independently by moisture stress conditioning and length of conditioning period. Seed source had no significant influence on any water potential components.

The effect of moisture stress conditioning and length of conditioning period on needle water and osmotic potential is presented in Table 24 . Generally, water potential decreased with the severity of the water stress treatment. This was true despite seedlings always being watered the day before the measurements were taken. An exception to this trend is in week fourteen where MWS seedlings had the lowest water potentials.

Needle osmotic potential in week four and nine was always significantly lower in SWS trees compared to controls, averaging approximately 0.88 units lower. Osmotic potential in MWS trees, during weeks four and nine never differed significantly from controls, but were always approximately 0.20 units lower. Osmotic potential values within treatments did not differ significantly between weeks four and nine (Table 24).

After fourteen weeks of moisture stress conditioning, osmotic potentials in both the MWS and SWS trees differed significantly from controls. However, the MWS trees had the lowest osmotic potential, averaging -2.07 MPa, which was

Table 24. Needle water and osmotic potential in containerized loblolly pine as affected by length and severity of conditioning.[1]

Treatment	Weeks of Moisture Stress Treatment					
	Four		Nine		Fourteen	
	Water Potential	Osmotic Potential	Water Potential	Osmotic Potential	Water Potential	Osmotic Potential
	(MPa)					
Control	-0.63 C D	-1.18 D	-0.55 D	-0.90 D	-0.78 C D	-1.26 C D
Moderate stress	-0.70 C D	-1.35 C D	-0.68 C D	-1.12 D	-1.53 A	-2.07 A
Severe stress	-1.34 A B	-2.05 A	-1.27 A B	-1.79 A	-0.97 B C	-1.71 A B C

1. Means followed by the same letter do not differ significantly; meanwater potentials and osmotic potentials can be compared in all directions.

significantly lower than MWS stress seedlings during weeks four and nine. Needle osmotic potentials during week fourteen did not differ significantly from weeks four and nine in both the control and SWS treatments (Table 24).

The effect of length of moisture stress conditioning on turgor potential was independent of the moisture stress treatments. Turgor potential averaged over all water stress treatments was approximately 0.61 MPa following four and fourteen weeks of moisture stress conditioning. However, after nine weeks of conditioning turgor was significantly lower averaging only 0.42 MPa.

Water potential components for each of the conditioning treatments averaged over all measurement periods is shown in Table 25 . Needle osmotic potential was significantly lower in each of the moisture stress treatments compared to controls, averaging -1.50 and -1.85 MPa in the MWS and SWS treatments, respectively, compared to -1.11 MPa in the controls. Despite lower needle water potential, SWS trees had significantly higher turgor potential averaging 0.66 MPa compared to 0.47 MPa in controls. MWS seedlings had an average turgor potential of 0.54 MPa, again higher than controls despite lower needle water potential.

Table 25. Needle water potential components in container grown loblolly pine seedlings as affected by moisture stress conditioning.[1]

Treatment	Water potential	Osmotic potential	Turgor potential
	---------- (MPa) ----------		
Control	- 0.65 A	- 1.11 A	0.47 A
Moderate stress	- 0.95 B	- 1.50 B	0.54 A B
Severe stress	- 1.18 C	- 1.85 C	0.66 B

[1]Means followed by the same letter within a column do not differ significantly (alpha = 0.05); means are averaged over all seed sources.

Seed Source Effects.

Seed source had no significant affect on the water potential components of containerized loblolly pine when subjected to conditioning. However, osmotic potential of SWS trees followed the same pattern as experiment one (Table 5). The Piedmont source had the lowest osmotic potential with -2.08 MPa, followed by the Coastal Plain with -1.79 MPa and the Texas source with the least negative value of -1.71 MPa (Table 26).

Chlorophyll Content

As in experiment one (Tables 3 and 4) moisture stress conditioning and seed source had little effect on chlorophyll content (Tables 27 and 28). Containerized seedlings, however, had less than half the chlorophyll of seedlings in experiment one, which were grown in one liter pots. In contrast to experiment one (Table 3), chlorophyll a/b ratio was not significantly affected by moisture stress conditioning (Table 27). Seed sources generally showed the same trend as experiment one (Table 4), with the Coastal Plain source having the greatest chlorophyll content (Table 28).

Table 26. Container grown loblolly pine needle osmotic potential affected by seed source and moisture stress conditioning[1].

Treatment	Seed Source: Coastal Plain	Piedmont	Texas
	(MPa)		
Control	- 1.07	- 1.08	- 1.19
Moderate stress	- 1.51	- 1.58	- 1.39
Severe stress	- 1.79	- 2.08	- 1.71

[1]Differences between seed sources were not statistically significant within any of the moisture stress treatments.

Table 27. Container grown loblolly pine chlorophyll content and a/b ratio as affected by moisture stress conditioning[1].

Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophyll	a/b Ratio
	(mg/cm² leaf area)			
Control	13.6	6.3	19.7	2.19
Moderate stress	13.2	6.4	19.5	2.07
Severe stress	14.4	6.1	20.4	2.36

[1]No means within columns differed significantly (alpha = 0.05); means are averaged over seed sources.

Table 28. Container grown loblolly pine chlorophyll content and a/b ratio as affected by seed source[1].

Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophyll	a/b Ratio
	(mg/cm² leaf area)			
Coastal Plain	15.7	6.8	22.5	2.30
Piedmont	13.0	5.7	18.7	2.26
Texas	12.6	6.2	18.5	2.06

[1]No means within columns differed significantly (alpha = 0.05); means are averaged over all moisture stress treatments.

Growth Parameters

As in prevous experiments, seedling biomass was greatly reduced by moisture stress conditioning (Table 29). Root biomass was affected the greatest, with the SWS trees having root weights 39 percent lower than controls, averaging 0.23 g compared to 0.38 g, respectively. Height growth was affected the least, with both conditioning treatments averaging approximately 12.0 cm, only 16 percent less than the control average of 14.3 cm. Root/shoot ratio was also decreased as a result of moisture stress conditioning, ranging from 0.24 in controls to 0.22 in SWS seedlings.

Following the first growing season in the field there were no significant differences in growth parameters due to moisture stress conditioning (Table 29). Although not significantly different, SWS trees had shoot and root weights approximately 8 and 9 percent larger, respectively, than controls or MWS trees. Height growth of SWS trees were only 1 percent less than controls, averaging 29.8 cm compared to 30.2 cm in controls. Root/shoot ratios were also very similar in all three treatments, varying only 3 percent from a high of 0.37 in controls to 0.36 in SWS trees.

Table 29. Container grown loblolly pine biomass before and after outplanting as affected by moisture stress conditioning.[1]

Treatment	Height	Shoot weight	Root weight	Root/Shoot ratio
	(cm)	(g)	(g)	
	Prior to outplanting			
Control	14.3 A	1.60 A	0.38 A	0.24 A
Moderate stress	12.0 B	1.24 B	0.27 B	0.22 A B
Severe stress	11.9 B	1.07 C	0.23 B	0.22 B
	Following the first year in the field			
Control	30.2	11.9	4.2	0.37
Moderate stress	28.8	11.9	4.1	0.36
Severe stress	29.8	12.8	4.6	0.36

[1]Means followed by the same letter within a column do not differ significantly (alpha = 0.05); no means following the first year in the field differed significantly; means are averaged over all seed sources.

Seed Source Effects.

Seed source had a significant effect on the growth of containerized loblolly pine seedlings (Table 30). Following moisture stress conditoning, the Texas seed source had the greatest shoot weight averaging 1.43 g, which was 22 percent larger than the minimum value of 1.17 g in the Piedmont source.

Height growth, however, was lowest in the Texas source which averaged 12.5 cm compared to 13.0 cm in the Coastal Plain source. Root growth and root/shoot ratio were also the lowest in the Texas source while the Piedmont source had the largest values for these parameters. These trends strongly agree with results in experiment one (Table 2).

After the first growing season in the field, similar trends in growth existed for the various seed sources (Table 30). The Texas source had the greatest height and shoot weight, averaging 31.6 cm and 13.4 g, respectively. Root dry weight was again the least in the Texas source. Root/shoot ratio followed the same pattern as prior to outplanting, ranging from 0.414 in the Piedmont source to 0.305 in the Texas source.

Planting site also had a significant effect on containerized seedling growth (Table 31). Shoot and root dry weight were 35 and 42 percent less on the alluvial site

Table 30. Container grown loblolly pine biomass before and after outplanting as affected by seed source.[1]

Seed Source	Height	Shoot weight	Root weight	Root/Shoot ratio
	(cm)	(g)	(g)	
	Prior to outplanting			
Coastal Plain	13.0 A	1.31 A	0.30 A	0.23 B
Piedmont	12.6 A B	1.17 B	0.31 A	0.27 A
Texas	12.5 B	1.43 A	0.26 A	0.18 C
	Following first year in the field			
Coastal Plain	29.1 B	12.3 A	4.46 A	0.38 B
Piedmont	28.1 B	10.7 A	4.40 A	0.41 A
Texas	31.6 A	13.4 A	4.07 A	0.31 C

[1]Means followed by the same letter within a column do not differ significantly (alpha = 0.05); Means can not be compared between time groups; Means are averaged over all moisture stress treatments.

averaging 9.7 and 3.2 g, respectively, compared to 14.8 and 5.5 g on the upland site. Root/shoot ratio was similarly reduced on the alluvial site. Height growth, however, was only slightly lower on the alluvial site averaging 29.3 cm compared to 30.0 cm on the upland site.

First year growth increment of containerized loblolly pine seedlings as influenced by moisture stress conditioning is shown in Table 32 . Height growth after outplanting was significantly greater for SWS seedlings which averaged 17.9 cm compared to 15.9 cm for control seedlings. Shoot and root dry weight increase during the first growing season, although not statistically different, was also greatest for SWS seedlings.

Seed source also had a significant effect on the first year growth increment (Table 33). The Texas seed source averaged 19.1 cm of height growth, which was significantly higher than both the Coastal Plain and Piedmont sources. The Texas source also had the greatest dry weight increase during the first growing season in the field. Root biomass varied little among seed sources, but was the least in the Texas source (Table 33) as was all other root growth parameters (Table 30).

Table 31. Container grown loblolly pine biomass and height as affected by planting site.[1]

Planting Site	Height	Shoot weight	Root weight	Root/Shoot ratio
	(cm)	(g)	(g)	
Upland	30.0 A	14.8 A	5.5 A	0.39 A
Alluvium	29.3 A	9.7 B	3.2 B	0.34 B

[1]Means followed by the same letter within a column do not differ significanlty (alpha = 0.05); Means are averaged over all moisture stress treatments and seed sources.

Table 32. Container grown loblolly pine growth increment after one year in the field as affected by moisture stress conditioning.[1]

Treatment	First year growth increment		
	Height	Shoot weight	Root weight
	(cm)	(g)	(g)
Control	15.9 A	10.0 A	3.8 A
Moderate stress	16.9 A B	9.8 A	3.5 A
Severe stress	17.9 B	11.4 A	4.2 A

[1]Means followed by the same letter within a column do not differ significantly (alpha = 0.05); Means are averaged over all seed sources.

Table 33. Container grown loblolly pine growth increment after one year in the field as affected by seed source.[1]

Treatment	First year growth increment		
	Height	Shoot weight	Root weight
	(cm)	(g)	(g)
Coastal Plain	16.1 B	10.5 A B	4.0 A
Piedmont	15.4 B	8.8 B	3.8 A
Texas	19.1 A	11.9 A	3.7 A

[1]Means followed by the same letter within a column do not differ significantly (alpha = 0.05); Means are averaged over all moisture stress treatments.

Survival

The effect of moisture stress conditioning, seed source and planting site on the survival of containerized loblolly pine is presented in Table 34 . Survival in the study was very high, averaging 97 percent overall. However, several factors did influence survival significantly.

Moisture stress conditioning had no significant effect on survival, but was nearly 2 percent greater in SWS conditioned seedlings compared to controls or MWS seedlings. In fact no SWS trees died on the alluvial planting site.

Survival did differ statistically among seed sources. Survival in the Texas source was the least averaging 95.6 percent; whereas, the Coastal Plain source had the highest survival of 98.4 percent. Planting site also had a significant influence on seedling survival, with the upland site averaging 95.8 percent compared to 98.7 percent on the alluvial site (Table 34).

Experiment Four

Osmotic Potential

The osmotic potential of needles stored frozen for 8 weeks as influenced by moisture stress conditoning and seed source is shown in Table 35 . A significant interaction between moisture stress conditioning and seed source occurred in the experiment.

Table 34. Containerized loblolly pine seedling survival[1] as affected by moisture stress conditioning, seed source and planting site.

Stress Treatment			Seed Source			Planting Site	
Control	Moderate stress	Severe stress	Coastal Plain	Piedmont	Texas	Upland	Alluvium
(% survival)							
96.8 A	96.6 A	98.4 A	98.4 A	97.8 A B	95.6 B	95.8 A	98.7 B

1. Means followed by the same letter within the stress treatment, seed source and planting site groups do not differ significantly.

Table 35. Osmotic potential of needles stored frozen for eight weeks as affected by moisture stress conditioning and seed source.[1]

Treatment	Seed Source		
	Coastal Plain	Piedmont	Texas
	(MPa)		
Control	- 2.44 E	- 2.35 E F	- 2.09 F
Moderate stress	- 2.81 C D	- 2.82 C D	- 3.01 B C
Severe stress	- 3.46 A	- 3.23 A B	- 2.63 D E

[1]Means followed by the same letter do not differ significantly (alpha = 0.05); means can be compared in all directions.

All three seed sources had significantly lower needle osmotic potentials as a result of 12 weeks of moisture stress conditioning, ranging from a 0.88 MPa decrease in the Piedmont source to a 1.02 MPa decrease in the Coastal Plain source. However, the Coastal Plain and Piedmont source had the lowest osmotic potentials when subjected to SWS conditioning, averaging -3.46 and -3.23 MPa, respectively; whereas, the Texas source had the lowest osmotic potential at the MWS treatment, averaging -3.01 MPa.

Similar to previous experiments (Table 5 and 26), the Texas source had the least negative osmotic potential when conditioned (-3.01 MPa, compared to -3.46 and -3.23 MPa, in Coastal Plain and Piedmont sources, respectively). Osmotic potential values recorded in this experiment were of the same order of magnitude as experiment one (Table 5), which also measured freezer-stored needles.

Chlorophyll Content

Chlorophyll content did not vary greatly as a result of moisture stress conditioning (Table 36) or seed source (Table 37). Moisture stress conditioning had no significant effect on any chlorophyll parameters and comparison with other experiments (Tables 3 and 27) revealed no clear pattern. Likewise, variation among seed source resulted in

no statistical differences. However, in agreement with the other experiments (Tables 3 and 27), the Coastal Plain source again had the greatest chlorophyll content. Overall chlorophyll content of growth chamber grown seedlings was greater than containerized seedlings (Table 27), but was less than seedlings grown in one liter pots in the greenhouse (Table 3).

Growth Parameters

The effect of moisture stress conditioning on the growth of seedlings is shown in Table 38 . Shoot and root dry weight were decreased 51 and 54 percent, respectively, as a result of SWS conditioning. The MWS treatment resulted in a 44 percent reduction in shoot dry weight and a 52 percent reduction in root dry weight. These results agree with the previous experiments (Tables 1, 19 and 29) in that root dry weight was affected more by stress conditioning than shoot dry weight. Unlike the other experiments (Tables 1, 19 and 29), root/shoot ratio was not reduced significantly, and was lowest at the MWS treatment.

Seed source resulted in no significant difference in growth parameters (Table 39). Similar to other experiments (Tables 2 and 30) the Texas source had the lowest shoot dry weight. However, because the Texas source also had the

Table 36. Chamber grown loblolly pine chlorophyll content and a/b ratio as affected by 12 weeks of moisture stress conditioning.[1]

Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophyll	a/b Ratio
	(mg/cm² leaf area)			
Control	19.6	8.4	28.1	2.29
Moderate stress	17.3	7.7	25.0	2.29
Severe stress	20.0	9.2	29.2	2.20

[1]No means within columns were significantly different (alpha = 0.05); Means are averaged over all seed sources.

Table 37. Chamber grown loblolly pine chlorophyll content and a/b ratio as affected by seed source.[1]

Seed Source	Chlorophyll a	Chlorophyll b	Total Chlorophyll	a/b Ratio
	---------- (mg/cm² leaf area) ----------			
Coastal Plain	21.0	9.5	30.5	2.25
Piedmont	18.9	8.4	27.3	2.24
Texas	17.0	7.4	24.4	2.29

[1] No means within a column were significantly different (alpha = 0.05); means are averaged over all moisture stress treatments.

Table 38. Chamber grown loblolly pine biomass as affected by 12 weeks of moisture stress conditioning.[1]

Treatment	Shoot weight	Root weight	Root/Shoot Ratio
	(g)	(g)	
Control	3.80 A	1.04 A	0.29 A
Moderate stress	2.14 B	0.52 B	0.25 A
Severe stress	1.85 B	0.48 B	0.26 A

[1]Means followed by the same letter within a column do not differ significantly (alpha = 0.05); Means are averaged over all seed sources.

lowest root dry weight, this source did not have the lowest root/shoot ratio as in the other experiments (Tables 2 and 30).

Root Growth and Morphology Parameters

The effect of moisture stress conditioning on various root growth and morphology parameters is shown in Table 40 . Root growth, as measured by the increase in length during the conditioning period, was greatly affected by moisture stress. The SWS treatment resulted in a 77 percent decrease in root growth, where seedlings averaged only 171.0 cm of growth, compared to 732.5 cm in control seedlings. The MWS treatment resulted in an average of 231.3 cm of growth, which did not differ significantly from SWS seedlings.

As an indication of root morphology, root length to root volume, and root length to root dry weight ratios were examined. Root length/volume ratio did not differ significantly as a result of moisture stress conditioning , but showed a steady decrease from a value of 181.8 $cm \cdot cm^{-3}$ in controls to 148.6 $cm \cdot cm^{-3}$ in SWS seedlings. Root length/weight ratio was significantly lower as a result of SWS conditioning, where seedlings averaged 684.7 $cm \cdot g^{-1}$ compared to 921.0 $cm \cdot g^{-1}$ in controls. These results indicated a progression from fine, thin roots in controls, to stockier, thick roots as a result of stress conditioning.

Table 39. Chamber grown loblolly pine biomass as affected by seed source.[1]

Treatment	Shoot weight	Root weight	Root/Shoot Ratio
	(g)	(g)	
Coastal Plain	2.84	0.711	0.25
Piedmont	2.69	0.733	0.27
Texas	2.29	0.611	0.27

[1]No means within columns differed significantly (alpha = 0.05).

Table 40. Loblolly pine root growth and morphological characteristics as affected by 12 weeks of moisture stress conditioning.[1]

Treatment	Increase in length	Length/Volume Ratio	Length/Weight Ratio
	(cm)	(cm/cm^3)	(cm/g)
Control	732.5 A	181.8 A	921.0 A
Moderate stress	231.3 B	161.3 A	795.6 A B
Severe stress	171.0 B	148.6 A	684.7 B

[1]Means followed by the same letter within a column do not differ significantly (alpha = 0.05); Means are averaged over all seed sources.

The increase in root volume which occurred during the 12 week conditioning period was affected by the interaction between seed source and the severity of conditioning (Table 41). In all three seed sources the increase in root volume decreased dramatically as a result of moisture stress conditioning. However, volume increase in control seedlings was significantly higher in the Coastal Plain source which averaged 7.47 cm^3, compared to 4.10 and 4.14 cm^3, in the Piedmont and Texas sources, respectively.

Experiment Five

New root growth of containerized seedlings after transplanting did not vary greatly among moisture stress treatments or seed sources. The majority of variation in new root growth was due to differences in needle water potential, which alone explained over 42 percent of the variation. There were no discernable differences in needle water potential after transplanting among moisture stress treatments or seed sources.

Since water potential had such a large influence on root growth, an analysis of covariance was performed with water potential as a covariate. Least-square means (adjusted for the average covariate) of new root growth and new root/old root ratio as affected by moisture stress conditioning are

Table 41. Change in loblolly pine root volume as affected by 12 weeks of moisture stress conditioning and seed source.[1]

Treatment	Seed source		
	Coastal Plain	Piedmont	Texas
	(cm^3)		
Control	7.24 A	4.10 B	4.14 B
Moderate stress	2.40 B C	1.60 C	1.38 C
Severe stress	0.73 C	1.92 C	1.57 C

[1]Means followed by the same letter do not differ significantly (alpha = 0.05); Means can be compared in all directions.

shown in Table 42 . Control and MWS seedlings had the greatest amount of new root growth averaging 0.173 g and 0.160 g, respectively, compared to only 0.122 g in SWS trees. New root dry weight per gram of original roots (new root/old root ratio) did not differ significantly as a result of moisture stress conditioning. However, SWS seedlings did have the highest ratio averaging 0.277 compared to 0.240 in the controls.

Seed source had a significant effect on new root growth (Table 43). The Coastal Plain and Piedmont sources both averaged nearly 0.170 g, compared to only 0.110 g in the Texas source. New root/old root ratio did not vary significantly among seed sources; however, the Coastal Plain had the highest ratio averaging 0.285, compared to a low of 0.248 in the Texas source.

Table 42. Containerized loblolly pine root growth the first 28 days following planting as affected by moisture stress conditioning.[1]

Treatment	New roots	New root/Old root
	(g)	(g/g)
Control	0.173 A	0.240 A
Moderate stress	0.160 A	0.275 A
Severe stress	0.122 B	0.277 A

[1]Values presented are least-square estimates of the mean; means followed by the same letter do not differ significantly.

Table 43. Containerized loblolly pine root growth the first 28 days following planting as affected by seed source.[1]

Seed source	New roots	New root/Old root
	(g)	(g/g)
Coastal Plain	0.169 A	0.285 A
Piedmont	0.166 A	0.258 A
Texas	0.110 B	0.248 A

[1]Values presented are least-square estimates of the mean; means followed by the same letter do not differ significantly.

DISCUSSION

Photosynthesis

Effects of Water Stress

Initial rates of net photosynthesis for loblolly pine seedlings in experiments one and two (Figures 1 and 5) are typical of those reported previously for loblolly pine and other pine species. Initial rates in this study ranged from 5 to 7 mg $CO_2 \cdot g^{-1} \cdot hr^{-1}$. When grown and measured under similar environmental conditions, Strain et al. (1976) reported rates ranging from 4 to 8 mg $CO_2 \cdot g^{-1} \cdot hr^{-1}$ for loblolly pine. Drew and Ledig (1981) found field grown loblolly pine seedlings to have somewhat higher photosynthesis rates of approximately 10 mg $CO_2 \cdot g^{-1} \cdot hr^{-1}$. Rates of photosynthesis in both Caribbean pine and Scots pine seedlings have also been reported within the magnitude of rates reported in this study with loblolly pine (Bacon and Bachelard 1978, Gowin et al. 1980).

Photosynthesis in both experiments one and two decreased sharply in response to decreasing needle water potential (Figures 1 and 5, Tables 8 and 22). This is not surprising since water stress has a great effect on photosynthesis both at the stomatal and chloroplast level (Kramer 1983a).

The response of photosynthesis to decreasing water potential was not fixed in loblolly pine, but was changed dramatically by the moisture stress treatments. In both experiments, conditioning seedlings to low water potentials shifted the response such that equivalent rates of photosynthesis occurred at lower needle water potentials.

This photosynthesis response offers great potential for improving the initial growth and establishment of outplanted loblolly pine seedlings. Conditioned seedlings would be able to maintain photosynthesis, and therefore growth, to lower water potentials and subsequently would be less affected by water stress resulting from outplanting.

Although there are no previous reports on the acclimation of photosynthesis to low water potentials in tree species, a very similar response has been found in sunflower (Matthews and Boyer 1984). Matthews and Boyer (1984) found a 0.3 to 0.4 MPa shift in the response of photosynthesis to decreasing leaf water potential when plants were acclimated to a continuous low water potential of -0.7 to -1.1 MPa. In this study, SWS seedlings (watered only when needle water potential fell below -1.4 MPa) exhibited a 0.4 MPa shift in experiment one and a larger 0.5 MPa shift in experiment two (Figures 1 and 5), and MWS seedlings averaged a 0.15 MPa shift (Figure 1).

Needle osmotic adjustment is likely a major factor contributing to this shift in the photosynthesis response. With the exception of the Coastal Plain seed source in experiment one (Table 5), significant reductions in osmotic potential of at least 0.45 MPa occurred as a result of SWS conditioning (Tables 5, 20, 25 and 35). This adjustment would allow for maintenance of turgor to water potentials 0.45 MPa lower than control seedlings, and subsequently a maintenance of photosynthesis. The nearly equal 0.4 and 0.5 MPa shift in photosynthesis which occurred in experiments one and two supports this conclusion.

However, a maintenance of turgor is probably not the only factor contributing to the acclimation of photosynthesis to low water potentials. In sunflower, the acclimation of photosynthesis to low leaf water potentials was found to be greater at the chloroplast level than at the stomatal level, and similarly, the inhibition of chloroplast activity at low water potentials also contributed to decreases in photosynthesis under water stress (Matthews and Boyer 1984). Many other examples exist of non-stomatal inhibition of photosynthesis by water stress (Collatz 1977, Kramer 1983a) and it is likely that similar effects occur in loblolly pine.

Evidence for non-stomatal inhibition of photosynthesis by low needle water potential in loblolly pine comes from examination of the needle conductance data in conjuction with the photosynthesis response. First, initial rates of photosynthesis under watered conditions were as high or higher than controls as a result of conditioning (Tables 8 and 22); however, needle conductance was significantly lower in conditioned trees (Figures 2, 3 and 4, Table 13). Therefore, although needle conductance in the conditioned seedlings was roughly one half of what control seedlings exhibited, no reduction in photosynthesis resulted.

Second, photosynthesis in the controls was nearly zero at needle conductance values approximately equal to initial conductance values found in SWS conditioned trees. At water potentials between -1.70 and -1.99 MPa, needle conductance in control seedlings averaged 0.040 $cm \cdot s^{-1}$, initial values in SWS trees averaged 0.046 $cm \cdot s^{-1}$ (Table 13). However, photosynthesis in control seedlings was nearly zero, and averaged only 0.41 mg CO_2 $g^{-1} \cdot hr^{-1}$ at this conductance level; whereas photosynthesis in SWS trees averaged 6.07 mg CO_2 $g^{-1} \cdot hr^{-1}$ (Table 8). This strongly supports a non-stomatal factor inhibiting photosynthesis in control seedlings.

Third, photosynthesis in SWS trees does not fall in response to water stress at the same rate as needle conductance. At water potentials between -1.70 and -1.99 MPa needle conductance in SWS trees fell approximately 13 percent to a value of 0.040 cm s^{-1} (Table 13). However, photosynthesis at this same level of stress, fell over 40 percent, to a value of 3.27 mg CO2 $g^{-1} \cdot hr^{-1}$.

Calculations of mesophyll resistance lend further support to the possibility of non-stomatal inhibition of photosynthesis. Mesophyll resistance to CO2 (R'm) were estimated using the equations presented by Osonubi and Davies (1980) :

$$R'm = \frac{CO2\ int}{P}$$

where P is the net photosynthesis rate in mg CO2 $cm^{-2} \cdot s^{-1}$ and CO2 int is the intercellular CO2 concentration which was calculated from :

$$CO2\ int = CO2\ atm - P \cdot R's$$

where CO2 atm is the concentration of CO2 in the atmosphere (approximately 340 ppm). The stomatal resistance to CO2 movement (R's) was calculated by multiplying the stomatal resistance to water vapor (inverse of needle conductance minus boundary layer resistance) by the relative

diffusivities of H2O and CO2; a value of 1.59 was used. The boundary layer resistance was assumed to be equal to 0.3 $s \cdot cm^{-1}$ (Bennett and Rook 1978).

Mesophyll resistance as calculated by Osonubi and Davies (1980) includes the resistances to CO2 movement in the mesophyll cells as well as the efficiency of the CO2 fixation processes and can be thought of as a residual resistance. These values are not to be considered as absolute values and are only presented to show relative differences among the treatments. Average rates of photosynthesis and conductance values were used in all calculations.

At water potentials between -1.00 and -1.19 MPa, the calculated mesophyll resistance in control seedlings was considerably higher than either of the conditioning treatments. A value of 95.8 $s \cdot cm^{-1}$ was found for controls compared to 85.5 and 65.2 $s \cdot cm^{-1}$ for the MWS and SWS conditioned seedlings, respectively. These values are within the same order of magnitude for Monterey pine, where Bennett and Rook (1978) found values of 27 and 76 $s \cdot cm^{-1}$ for two different clones.

Based on these calculations, moisture stress conditioning appears to have reduced the mesophyll resistance to CO2 diffusion. These changes could be due to increases in

carboxylation efficiency or change in resistance to CO2 movement, possibily by membrane alterations.

As needle water potential decreased the mesophyll resistance increased very quickly in control seedlings; at water potentials of -1.70 to -1.99 MPa, a resistance of 1433 $s \cdot cm^{-1}$ was calculated, compared to 310 and 145 $s \cdot cm^{-1}$ in MWS and SWS conditioned seedlings, respectively. This large increase is likely the result of a failure in the photosynthetic mechanism rather than changes in resistances to CO2 movement. Whatever the cause, it appears that very large resistances have developed beyond those of stomatal resistances in the control seedlings; whereas, in conditioned seedlings, the resistances are much lower and likely the result of acclimation.

Considering these data it is very likely that non-stomatal inhibition of photosynthesis in response to water stress does occur in loblolly pine. However, no direct evidence such as inhibition in isolated chloroplasts or thylakoid membranes was obtained. Since the large reduction in needle conductance which occurred as a result of conditioning (Table 13) did not result in a decrease in photosynthesis (Table 8) and initial calculated mesophyll resistances are much lower in conditioned seedlings, it also appears that some cellular acclimation, in addition to

osmotic adjustment also occurred as a result of conditioning.

Effect of Seed Source

Although never very dramatic, significant differences in photosynthesis did occur among the three seed sources. The Texas seed source generally had the lowest photosynthetic rate at all moisture stress treatments and water potential (Table 7). This is reflected in the fact that when the photosynthesis rate for the three seed sources was averaged over all moisture stress treatments and water potentials, the Texas seed source had significantly lower photosynthesis than either of the other two seed sources (Table 9).

This trend could be partially explained by the differences in chlorophyll content. Although never statistically different, the Coastal Plain seed source had the greatest amounts of both chlorophyll a and chlorophyll b in all three experiments, and the Texas source generally had the least amount of total chlorophyll (Tables 4, 28 and 37). Higher chlorophyll content could result in increased light absorption and ultimately in improved photosynthesis. However, the relative amounts of other photosynthetic components including the light harvesting chlorophyll complex and electron transport components would need to be

determined before any increase in photosynthetic gas exchange can be attributed to increased chlorophyll content (Alberte et al. 1976).

The acclimation of photosynthesis to low needle water potentials in response to the moisture stress conditioning also differed among seed sources. Acclimation (expressed as the difference in photosynthesis between SWS and control seedlings at various needle water potentials) was greatest in the Coastal Plain and least in the Texas seed source (Table 7).

This effect can in part be explained by differences in the osmotic potential and adjustment among the seed sources. Generally, the Texas seed source had the least negative osmotic potential in control seedlings and showed the least osmotic adjustment (Tables 5, 26 and 35). The Texas seed source only exhibited an average of 0.52 MPa adjustment between controls and SWS seedlings (averaged over all experiments), compared to well over a 0.80 MPa adjustment in the other seed sources. Less osmotic adjustment in the Texas source would result in a loss of turgor at higher water potentials in moisture stress conditioned seedlings and subsequently less photosynthetic acclimation to water stress.

No consistent trend between experiments exists in the degree of osmotic adjustment between the Coastal Plain and Piedmont sources. Therefore, it is not possible to make a conclusion as to whether the differences in osmotic adjustment contributed to the greater photosynthesis and acclimation which was found in the Coastal Plain compared to the Piedmont seed source (Table 7).

As discussed previously, it is very likely that the acclimation of photosynthesis to low water potentials may be the result of acclimation at the chloroplast level, and this may have contributed also to differences between seed sources.

Initial predicted conductance values in both the Coastal Plain and Piedmont seed sources were reduced greatly by moisture stress conditioning (Table 12), without causing a decrease in photosynthesis (Table 7). This again suggests some change beyond the stomatal level influencing photosynthesis. In the Texas seed source needle conductance values more closely paralleled changes in photosynthesis (Table 7 and 12), suggesting less acclimation beyond stomatal influences.

Differences in non-stomatal acclimation between any of the seed sources cannot be definitely concluded without data utilizing isolated chloroplasts, thylakoid membranes or saturated CO2 environments to eliminate stomatal influences.

Needle Conductance

Moisture Stress Conditioning Effects

The effect of moisture stress conditioning was to decrease needle conductance, and increase the response to changing vapor pressure deficit (Figures 2, 3, and 4, Table 11). Depending on the treatment and vapor pressure deficit, initial conducatance values under watered conditions were reduced 30 to 45 percent in conditioned seedlings.

This response was not surprising since long-term water stress greatly reduces stomatal conductance (Davies et al. 1981). Stomatal conductance generally recovers within a few days following the relief of water stress (Davies et al. 1981, Newville and Ferrell 1980). In this study, no recovery in needle conductance occurred in conditioned seedlings, even after a week of high needle water potential. However, needle conductance in control seedlings following the stress cycle began recovering within two days (unreported data).

It is not known how long the reduction of needle conductance would last in conditioned seedlings. However, the reduction appears more stable than what is generally reported due to abscisic acid (AbA) build up during water stress. It is generally believed that the AbA increase during water stress plays a major role in stomatal closure

and that residual AbA after stress relief is the cause of a delay in full recovery (Davies et al. 1981, Johnson and Ferrell 1982).

The more permanent reduction found in this study could possibly be due to greater osmotic adjustment in mesophyll or epidermal cells compared to cells in the stomatal apparatus (guard cells and subsidiary cells). This could cause an overall reduction in guard or subsidiary cell turgor relative to surrounding cells resulting in stomatal closure. However, no data are available to verify this possibility.

Control of water loss through changes in leaf conductance is an important factor influencing the drought tolerance of tree species (Bennett and Rook 1978, Kozlowski 1982). The decreased needle conductance as a result of conditioning is therefore an important factor controlling water loss and subsequent drought tolerance. The decreased needle conductance resulted in significantly lower transpiration (Table 17) and therefore, improved water conservation in conditioned seedlings.

The decreased needle conductance alone was a significant factor improving the drought tolerance of conditioned seedlings. However, improved drought tolerance through lowered needle conductance is generally at the expense of

photosynthesis (Bennett and Rook 1978, Hinckley et al. 1978). In this study, conditioning resulted in no decrease in photosynthesis and often times an increase (Table 8, Figure 1). This response conveyed a great advantage to conditioned seedlings which were able to maintain high photosynthesis, while conserving water through decreased needle conductance.

Based on analysis of variance results, vapor pressure deficit significantly influenced the response of needle conductance to needle water potential (Table 14). Averaged over all seed sources and moisture stress treatments, low vapor pressure deficit generally had the highest needle conductance. This response has been found in numerous other species (Losch and Tenhunen 1981).

However, the results of the regression analysis indicated that this response was not independent of moisture stress conditioning, but varied greatly among treatments. It is therefore somewhat surprising that the three-way interaction between moisture stress conditioning, vapor pressure deficit and water potential class was not significant in the analysis of variance. This was probably the result of variation occurring within the water potential classes, which caused an inflated error term compared to the regression analysis.

By comparing figures 2 through 4, it is obvious that the stomatal response to changing vapor pressure defict was greatly modified by moisture stress conditioning. SWS conditioned seedlings were the most responsive to changing vapor pressure defict, while controls were unaffected by changing vapor pressure deficits.

A stomatal response to vapor pressure defict has obvious advantages for the conservation of water. When vapor pressure deficit is low and evaporative demand decreased, stomatal conductance increases allowing for a maintenance of photosynthesis. However, when vapor pressure deficit is high and evaporative demand is great, needle conductance decreases and water is conserved. In this way the greatest water-use efficiency is maintained.

A similar response has been found by several other investigators (Johnson and Ferrell 1983, Osonubi and Davies 1980, Rawson et al. 1977, and Schulze et al. 1972). However, these investigators found an enhanced response to vapor pressure deficit during developing water stress and not after prolonged conditioning with water stress.

The stomatal response to vapor pressure deficit is believed to be the result of peristomatal transpiration (enhanced cuticular transpiration from guard cells and subsidiary cells) (Losch and Tenhunen 1981). Changes in

vapor pressure deficit affect the demand for water flowing through the epidermis and stomatal complex. When vapor pressure deficit is high, evaporative demand from guard cells and subsidiary cells increases disproportionally when compared to surrounding epidermal cells, resulting in decreased turgor in guard cells relative to surrounding cells, causing stomatal closure. Under conditions of low vapor pressure, more water is available to guard cells and subsidiary cells since evaporative demand is lessened, resulting in higher turgor pressure and stomatal opening.

Moisture stress conditioning resulted in an enhancement in the needle conductance response to vapor pressure deficit. However, the physiological mechanism governing this response is unclear. Possibly, changes in osmotic potential are contributing to this response. As discussed previously, if osmotic adjustment in response to conditioning was greater in mesophyll or surrounding epidermal cells, they would maintain an advantage over the guard cells for water supply and in turgor potential.

If this was the case, a decrease or increase in evaporative demand from guard cells (through changes in vapor pressure deficits) would be a very important factor governing guard cell turgor. In control seedlings, where cell water relations have not been influenced by

conditioning, the stomatal apparatus may have an adequate supply of water at all times and therefore changes in evaporative demand would have little influence on gaurd cell turgor.

Water supply to the guard cells depends not only on water potential gradients, but also on the relative resistances to water movement in adjacent cells (Maier-Maercker 1979). Therefore, another possibility is that conditioning increased hydraulic resistances so that water supply to guard cells or subsidiary cells was slowed. Changes in evaporative demand would then have a larger impact on the turgor potential of the stomatal apparatus.

The response of needle conductance to decreasing water potential appears to be a threshold response (Table 13) similar to what has been reported in other species (Ackerson 1980, Johnson and Ferrell 1982, Newville and Ferrell 1980). Control and MWS conditioned seedlings exhibited large decreases in conductance at water potentials between -1.70 and -1.99 MPa, while SWS conditioned seedlings were able to maintain conductance until water potentials of -2.00 to -2.29 MPa were reached (Table 13). The shift to lower needle water potentials found in SWS seedlings is likely due to osmotic adjustment and has been reported in other species previously subjected to drought (Ackerson 1980).

However, in comparison to the photosynthesis results (Figure 1), no clear shift in the response of needle conductance to decreasing needle water potential occurred due to conditoning. Although no definite conclusions can be made, this does suggest that closure of needle stomata may be more closely associated with changes in needle water potential than changes in needle turgor or that guard cells failed to exhibit strong osmotic adjustment in response to stress conditioning. In contrast, other studies have shown a close association of leaf turgor to stomatal closure (Davies et al. 1981).

The large, abrupt decrease in conductance which commonly occurs in response to decreasing water potential has been attributed to a rapid increase in AbA (Blake and Ferrell 1977, Newville and Ferrell 1980, and Zabadal 1974).

By examining the regression responses (Figures 2, 3 and 4) it is not obvious that a threshold response of needle conductance to decreasing water potential occurs. This is probably the result of a smoothing of the data points caused by the regression fit and the relatively high variation at high needle water potentials. Additionally, it appears that vapor deficit influenced this response. Under low vapor pressure deficit a more gradual decrease in conductance occurred with decreasing needle water potential (Figure 3)

in both MWS and SWS seedlings. This response can also be seen by examining Table 14, where under low vapor pressure deficit (1.63 KPa) a more gradual decrease in conductance occurred in response to decreasing water potential.

This effect is largely the result of an increase in the sensitivity of stomata to vapor pressure deficit at the lower needle water potentials. In both conditioning treatments, seedlings showed a greater response to decreasing vapor pressure deficit at lower needle water potentials (Table 11), with maximum increases in predicted needle conductance (in response to low vapor pressure deficit) occurring at water potentials of -2.3 MPa and -2.5 MPa. As water potential decreased further, differences again diminished. As a result of this response, conditioned seedlings under low vapor pressure deficit, demonstrated a slower, less abrupt decrease in conductance in response to decreasing water potential (Tables 11, 14 and Figure 3) .

Other researchers have found a similar interaction between water potential and the response of needle conductance to vapor pressure deficit. In both Douglas-fir (Johnson and Ferrell 1983) and birch (Osonubi and Davies 1980) the stomatal response to changing vapor pressure deficit increased under water stress. However, the stomatal response to vapor pressure deficit in both pearl millet and

ground-nut were suppressed by water stress (Black and Squire 1979).

The increasing sensitivity to vapor pressure deficit at lower needle water potentials found in this study could possibily be the result of changes in water supply. At lower needle water potentials, where water supply to stomata begins to become limiting, changes in evaporative demand are likely to cause larger changes in guard cell turgor, resulting in a larger change in stomatal aperture.

Effect of Seed Source

Needle conductance values differed significantly between seed sources. Generally, the Coastal Plain source exhibited the highest and the Texas source the lowest needle conductance values (Table 12, 15 and 16). This is not surprising since the Texas seedlings originated from a xeric site and numerous studies have shown trees from xeric sites to have lower conductance values or transpiration rates than trees from mesic sites (Bilan et al. 1977, Ferrell and Woodard 1965, Kozlowski 1982, Newville and Ferrell 1980, Unterscheutz et al. 1974, Zavitkovski and Ferrell 1968). This response has obvious importance for the conservation of water on xeric sites. The lower needle conductance in the Texas control seedlings resulted in significantly lower transpiration (Table 17).

Changes in needle conductance as a result of moisture stress conditioning also varied among seed sources. The Texas source had the lowest needle conductance values in control seedlings. However, conductance in the Texas source was decreased little in response to the MWS treatment and it was only under SWS conditioning that any reduction occurred (Table 12 and 16). In fact, under the MWS treatment, the Texas source had the highest predicted conductance values (Table 12) and when averaged over all water potentials and vapor pressure treatments, it had nearly the greatest actual conductance (Table 16). This effect is difficult to explain physiologically. However, since the Texas source already had lower stomatal conductance, presumably as a result of natural selection from its semiarid environment (Bilan et al. 1977), its stomatal mechanism may have less flexibility for further acclimation to moisture stress, or may simply be less affected by moisture stress conditioning.

Water Potential Components

Significant osmotic adjustment occurred as a result of moisture stress conditioning in all experiments (Table 5, 20, 25 and 35). Averaged over all experiments SWS seedlings had osmotic potentials over 0.70 MPa lower than control seedlings. Although no similar results have been reported

for loblolly pine, similar shifts in osmotic adjustment due to water stress have been documented in numerous other woody plant species (Davies and Lasko 1978, 1979, Jones and Higgs 1979, Kandiko et al. 1980, Osonubi and Davies 1978, 1981).

Osmotic adjustment can be an important component in the overall drought tolerance of plant species. As a result of osmotic adjustment, turgor and turgor related processes can be maintained to lower leaf water potentials (Hanson and Hitz 1982, Kramer 1983a). In experiments where turgor potential was estimated, turgor was either greater than or equal to control seedlings even though SWS seedlings had lower needle water potentials. (Table 20 and 25).

It appears from these results that osmotic adjustment is occurring in loblolly pine and as a result seedlings should be able to maintain normal physiological processes and growth to lower needle water potentials. This effect can be seen in the photosynthesis data where conditioned seedlings maintained photosynthesis to lower needle water potentials (Figures 1 and 5) and as discussed previously, it is likely that this effect is at least partially due to osmotic adjustment.

Although seedlings were watered the day before measurements were taken, conditioned seedlings generally had lower needle water potentials; therefore, it is likely that

a portion of this osmotic adjustment is passive adjustment, or simply due to solute concentration during dehydration. In apple trees, Davies and Lasko (1979) found a significant portion (67 percent) of diurnal osmotic adjustment due to dehydration. However, adjustments which occur due to slowly developing stress (such as the moisture stress conditioning used in these studies) are more likely to be true, active adjustments (Kramer 1983a). Additionally, the shift in photosynthesis to lower needle water potentials which occurred in these studies would probably not occur if all adjustments were simply due to dehydration.

Large changes in needle osmotic potential occurred after only four weeks of moisture stress conditioning and further conditioning resulted in no further reduction in osmotic potential (Table 24). Therefore, if changes in osmotic adjustment is all that is desired from conditioning, four weeks or less, appears adequate in loblolly pine. However, it is possible that other acclimation processes, such as changes in mesophyll resistances, continued after four weeks. Turner and Jones (1980) have reported that osmotic adjustments are generally limited to 0.5 to 0.8 MPa. This could explain why no further decreases in osmotic potential occurred after four weeks in loblolly pine.

Storage of needles at (-5°C) had a definite influence on measured needle osmotic potential. Osmotic potentials were generally 1.0 to 1.5 MPa lower in freezer stored needles (Tables 5 and 35) compared to fresh needles (Tables 20, 24, 25 and 26). Although needles were stored sealed in 10 ml plastic test tubes, dehydration during storage is likely the cause of this effect. Ice crystals were observed on the needles and inside the tubes following removal from storage. The unusually low osmotic potential found in the Coastal Plain control seedlings in experiment one (Table 5) could be the result of severe dehydration due to leaks or cracks in one or more of the test tubes in that treatment.

As a result of these problems, storage of needles frozen for more than a few days is not recommended if accurate osmotic potential measurements are desired. However, if relative differences between treatments are only desired, this technique worked well in most cases.

Osmotic potential was often affected by the interaction between seed source and moisture stress conditioning (Table 5 and 35). However, the only consistent pattern due to seed source in all experiments is that the Texas source had the least difference between controls and SWS seedlings and also exhibited the least negative osmotic potential when subjected to the SWS treatment (Table 5, 26 and 35).

Differences in the capability of various species to osmotically adjust is well known and differences between cultivars have been documented (Hanson and Hitz 1982, Kramer 1983a); however, no differences between seed sources in tree species has been documented.

The Texas seed source generally showed the least acclimation of photosynthesis to low needle water potentials (Table 7). This trend could be the result of the lower osmotic adjustment in the Texas seed source.

Transpiration and Water-Use Efficiency

Mean daily rates of transpiration were reduced significantly as a result of moisture stress conditioning (Table 17 and 23). Transpiration was reduced largely because of the reduced needle conductance which resulted from the conditioning treatments (Table 13). Clemens and Jones (1978) found a similar effect in *Eucalyptus* where water use was reduced by 46 percent in plants that were watered only when visibily wilted over a 98 day period. However, this decrease in water use was attributed to changes in hydraulic conductivity of the root system and not changes in stomatal conductance. Hydraulic conductivity of the root system was not measured in this study, but considering how closely changes in needle conductance

paralleled changes in transpiration, it is likely that needle conductance is the major factor affecting transpiration.

Transpiration rate in response to stress conditioning varied among the seed sources (Table 17). The Texas source had the lowest rate in control seedlings, but was reduced little in response to stress conditioning. Again, this response closely parallels the needle conductance response (Table 16), which was discussed previously. This Texas source has previously been shown to have lower transpiration rates when compared to other seed sources of loblolly pine (Bilan et al. 1977).

The significant decrease in transpiration resulted in increased water-use efficiency in both experiments in which water-use efficiency was measured (Table 18 and 23). The absolute values of water-use efficiency reported here are probably somewhat over estimated since the transpiration rates used in their calculation were daily averages and therefore, lower than peak transpiration rates which occurred during photosynthesis measurements. However, relative differences between the treatments are very significant since they imply changes in the actual physiology of the seedlings, such as changes in mesophyll resistance as discussed earlier.

Often plants tolerant of water stress actually have low water-use efficiencies when well watered, and high water-use efficieny is not always correlated with drought tolerance (Kramer 1983b). However, the increased water-use efficiency in this study is an obvious advantage, allowing plants to conserve water without decreasing photosynthesis. By conserving available water seedlings will be able to maintain photosynthesis and growth for a longer time without rain or irrigation. Conditioned seedlings were observed to maintain photosynthesis and water-use efficiency for a longer time as drought progressed (unreported data). This response is largely due to the smaller size of conditioned seedlings (Table 1) which would use their available water more slowly, but it is also partially due to decreased transpiration.

Seedling Growth and Morphology

Effects of Water Stress

Water stress has a profound impact on plant growth (Kozlowski 1982, Kramer 1983a), so it is not suprizing that seedling growth was greatly reduced as a result of moisture stress conditioning (Tables 1, 19, 29 and 38). Cell division, enlargement, and differentiation are all reduced by water deficit, with the obvious result being a reduction in plant size (Kramer 1983a).

Shoot growth is generally decreased more by water stress than root growth, resulting in an increase in root/shoot ratio (Kozlowski 1982, Kramer 1983a, Kramer and Kozlowski 1979, Seiler and Johnson 1984). This is believed to be the result of more severe stress developing in the transpiring shoots compared to roots (Kramer 1983a). There have been reports of an actual increase in root growth during mild water stress, possibily as a result of greater osmotic adjustment in the roots (Kramer 1983a, Osonubi and Davies 1978, Sharp and Davies 1979).

In this study, water stress consistently had a greater effect on root growth, resulting in a significant decrease in the root/shoot ratio of loblolly pine (Tables 1, 19, 29 and 38). Both root length and root volume were drastically reduced in response to moisture stress conditioning (Table 40 and 41). A similar response was found by Kaufman (1967) in both loblolly pine and white pine where root growth was reduced drastically after three, 7-day drying cycles. It was concluded that water stress caused root tips to mature and become quiescent (Kaufmann 1967). Similarly, roots of Aleppo pine were found to become suberized and quiescent in response to water stress (Lesham 1970).

Root length/volume ratio and root length/weight ratio were also reduced by moisture stress conditioning (Table

40). This suggests that roots of stressed seedlings were on the average somewhat thicker than control seedlings. Possibly succulent root tips were killed or became quiescent in response to stress and upon rewatering the remaining roots thickened, but tips failed to elongate. This conclusion is supported by the results of Kaufmann (1967) and Lesham (1970) where root tips became quiescent in response to water stress. Likewise, Kozlowski (1949) found loblolly pine seedlings to produce large, branched root systems when well watered, but small, unbranched roots under water stress. Osonubi and Davies (1978), however, found root morphology in English oak to change from short, thick roots to a profusion of long, thin roots when subjected to water stress. The reason for these inconsistancies is not entirely clear, but may simply be due to species differences.

This change in loblolly pine root growth in response to water stress would not likely result in an improvement in drought tolerance. The decreased root growth and root shoot ratio indicate a decrease in absorptive root surface area and an increase in transpirational area per unit of absorbing area. Additionally, if quiescent root tips became suberized in response to water stress, this would seriously reduce water entry (Kramer 1983a). These factors would all

tend to increase the susceptability of loblolly pine to water stress.

New root growth following transplanting was affected very little by moisture stress conditioning. Absolute weight of new roots was least but new roots per unit of old roots was slightly higher in SWS seedlings (Table 42). Production of new roots following outplanting is critical for seedling survival, however, it does not appear that stress conditioning significantly affected the production of new roots.

In summary, moisture stress conditioning did not appear to affect root growth or initiation in a manner conducive for improving drought tolerance. However, this may simply indicate that root growth, relative to other factors, is not a significant factor in the drought tolerance of loblolly pine.

Effect of Seed Source

In all experiments the Texas seed source had the lowest root weight and with the exception of the growth chamber grown seedlings, (Table 39) the lowest root/shoot ratio (Tables 2, 30, 39). Containerized seedlings of the Texas source also exhibited significantly lower new root growth after planting (Table 43).

This response is interesting since root growth has been implicated in the drought tolerance of this Texas seed source. Seedlings from this source have been found to produce deeper, more extensive root systems which may provide access to a greater water supply (van Buijtenen et al. 1976). However, decreased transpiration has been suggested as the major factor contributing to the drought resistance in this source (van Buijtenen et al. 1976, Bilan et al. 1977). The results of this study support that conclusion. Root growth in the Texas source was not superior and generally less, compared to the other sources, but transpiration was significantly less in the Texas source (Table 17).

Field Performance of Containerized Seedlings

Effect of Water Stress

Initial growth of containerized seedlings was reduced significantly by the moisture stress treatments (Table 29). However, first year growth increment after outplanting was significantly greater in SWS seedlings (Table 32). As a result, at the end of the first growing season in the field, SWS seedlings were only slightly behind control seedlings in height growth, and had greater shoot and root dry weights than either MWS or control seedlings. Survival, although

very high in all treatments, was almost 2 percent greater in SWS seedlings (Table 34).

These are important trends considering SWS seedlings were much smaller at the time of outplanting. Smaller containerized seedlings generally show worse field performance, both in growth and survival (McGilvray and Barnett 1981).

Based on the physiological parameters measured in these experiments (eg. photosynthesis, needle conductance, osmotic potential) moisture stressed seedlings were in better physiological condition to survive outplanting and any associated stresses. Physiological, as well as the morphological condition of outplanted seedlings are known to be associated with their ability to survive (McGilvray and Barnett 1981). Therefore, it is likely that the better growth and survival, although slight, was due to the better physiological condition of SWS stress seedlings.

A severe frost occurred immediately following outplanting damaging a majority of needles. Although no visible differences existed in the amount of damage between treatments, conditioned seedlings may have been more frost hardy in addition to drought hardy. Past studies have reported that water stress can induce cold-hardiness in containerized seedlings (Mexal and Carlson 1981).

Therefore, moisture stress conditioning could have improved growth and survival through increases in drought and cold hardiness.

Only the SWS seedlings performed as well or better than control seedlings in the field. MWS seedlings exhibited the worst performance in survival and in all growth parameters measured, with the exception of height growth increment (Tables 29, 32 and 34). MWS seedlings were no taller than SWS seedlings at the time of outplanting. Possibly their physiological condition was not improved enough to overcome the height advantage of controls; whereas, the improved physiological condition of SWS seedlings allowed them to overcome their disadvantage in height and biomass.

Effect of Seed Source

Seed source also had a significant influence on field performance. The Texas source had significantly greater height growth and shoot dry weight increase over the first growing season in the field (Table 33). However, consistent with all the other experiments, the Texas source had the least root growth and significantly lower root/shoot ratio. The lower root growth of the Texas seed source, even after a year in the field, is again in contradiction with reports of this seed source having superior root growth (Bilan et al. 1977, van Buijtenen et al. 1976).

The Texas seed source had significantly lower survival than the other two sources. This could be related to cold-hardiness or possibly seedling morphology. The Texas source is likely to be less cold hardy than the sources from Virginia since southern seed lots are generally less cold hardy (Wright 1976). Therefore, the Texas seed source may have been damaged more by the frost which occurred following outplanting. Seedling morphology may have also influenced this survival pattern. In all experiments, the Texas source generally had greater shoot biomass, but less root biomass, resulting in significanlty lower root/shoot ratios (Table 2 and 30). Despite lower transpiration rates, this type of morphology could result in the Texas source being more susceptible to water deficits immediately following outplanting, since it has much larger transpirational area and less water absorbing area.

Effect of Planting Site

Moisture stress conditioning and seed source had the same effect on both the upland and alluvial site. However, planting site also influenced growth and survival. Survival was significantly lower on the upland site, most likely as a result of drier soil conditions during the initial establishment period. The alluvial site was very wet and

remained so longer through the summer. Growth, however, was less on the alluvial site probably as a result of increased competition. The alluvial site had an abundance of honeysuckle (Lonicera japonica Thunb.) and other herbaceous vegetation in direct competition with the newly planted seedlings. Seedlings growing in the honeysuckle were always very small with browning needles.

SUMMARY AND CONCLUSIONS

Water stress is generally the single greatest factor affecting the survival and growth of newly planted tree seedlings. Drought tolerance is, therefore, an extremely important factor influencing seedling performance following outplanting. Numerous physiological and morphological parameters are known to be important in the drought tolerance of tree seedlings. However, few studies have examined in detail how factors responsible for drought tolerance can be modified by exposing seedlings to sublethal drought treatments. In this study the roles of osmotic adjustment, needle conductance, and root growth in the drought tolerance of loblolly pine was examined. Additionally, the effect of prolonged sublethal water stress on the drought tolerance of loblolly pine was evaluated.

Four open-pollinated families of loblolly pine (geographic sources included the Virginia Piedmont, Virginia Coastal Plain, and Texas) were conditioned to three levels of water stress : no stress, seedlings well watered; moderate stress (MWS), seedlings watered when needle pre-dawn water potential reached -0.8 MPa; and severe stress

(SWS), seedlings watered when pre-dawn needle water potential reached −1.4 MPa. Conditioning treatments lasted for as little as 6 weeks to as long as 19 weeks. After conditioning, all seedlings were watered and water potential components, root growth, transpiration, water-use efficiency, and survival and growth after outplanting were measured. Photosynthesis and needle conductance were also measured over a range of needle water potentials.

Averaged over all experiments, SWS seedlings had osmotic potentials over 0.70 MPa lower than controls. As a result of this osmotic adjustment, turgor in conditioned seedlings was equal to or greater than controls despite conditioned seedlings having lower needle water potentials.

Photosynthesis decreased with decreasing needle water potential; however, this response varied among the conditioning treatments. Conditioned seedlings had equal or higher rates of photosynthesis under watered conditions and exhibited higher rates of photosynthesis at lower needle water potentials as compared to control seedlings. This response is partially due to osmotic adjustment in conditioned seedlings which resulted in a maintenance of turgor at lower needle water potentials. However, changes in mesophyll resistance, possibly as a result of chloroplast acclimation to low needle water potentials, is also likely to have contributed to this response.

Needle conductance, but not photosynthesis, was greatly reduced as a result of conditioning. As a result, transpiration was reduced and water-use efficiency significantly increased in conditioned seedlings. This response conveys a great advantage to conditioned seedlings since they are able to maintain photosynthesis while still conserving water.

The response of needle conductance to changing vapor pressure deficit was increased by moisture stress conditioning. Control seedling needle conductance was changed little by changing vapor pressure deficit. In contrast, conditioned seedlings decreased conductance at high vapor pressure deficit and increased conductance at low vapor pressure deficit. This response in conditioned seedlings was particularly evident at lower needle water potentials and has obvious advantages for the conservation of water.

Root growth was decreased more than shoot growth in response to conditioning, resulting in a decrease in root/shoot ratios. Additionally, moisture stress conditioned seedlings tended to have shorter, thicker roots. These changes in root growth as a result of conditioning are not likely to result in improved drought tolerance.

First-year height growth increment of outplanted, containerized loblolly pine was increased significantly by the SWS conditioning treatment. However, as a result of their initial smaller size, total height was not significantly greater in conditioned seedlings. Shoot weight, root weight and survival were also slightly greater in conditioned seedlings.

Significant differences existed between the seed sources; however, only a few consistent trends occurred in all experiments. The Texas source, which is known for its superior drought tolerance, had significantly lower needle conductance and transpiration in control seedlings, which is consistent with previous reports. However, this source had the lowest root/shoot ratio and osmotic adjustment, showed the least acclimation of photosynthesis to low needle water potential, and had the smallest decrease in needle conductance and transpiration in response to moisture stress conditioning. This source also had significantly lower survival the first year in the field. These results suggest better water conservation in Texas control seedlings, but less ability to further acclimate to water stress through moisture stress conditioning.

Modification of drought tolerance in loblolly pine is possible through moisture stress conditioning. Improved

drought tolerance occurs through decreases in osmotic potential, needle conductance, transpiration and, likely, mesophyll resistance. Additionally, responsiveness of stomates to changing vapor pressure deficit can be increased as a result of conditioning. These changes result in an increase in water-use efficiency and a maintenance of turgor and photosynthesis to low needle water potentials.

This response offers great potential in nursery management, particularly in the production of containerized seedlings. Containerized seedling production offers the advantage of strict control of environmental factors. By controlling water availability, the physiological condition of containerized seedlings can be manipulated to improve drought tolerance prior to leaving the greenhouse. This could result in improved survival and growth when the seedlings are outplanted.

The application of moisture-stress conditioning in bare-root seedling production would require further investigation. However, through the manipulation of irrigation regimes, it is likely that similar improvements in seedling drought tolerance could be obtained.

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Appendix A

LOBLOLLY PINE SEEDLING PHOTOSYNTHESIS DATA FOR EXPERIMENT ONE AND PREDICTED REGRESSION RESPONSES

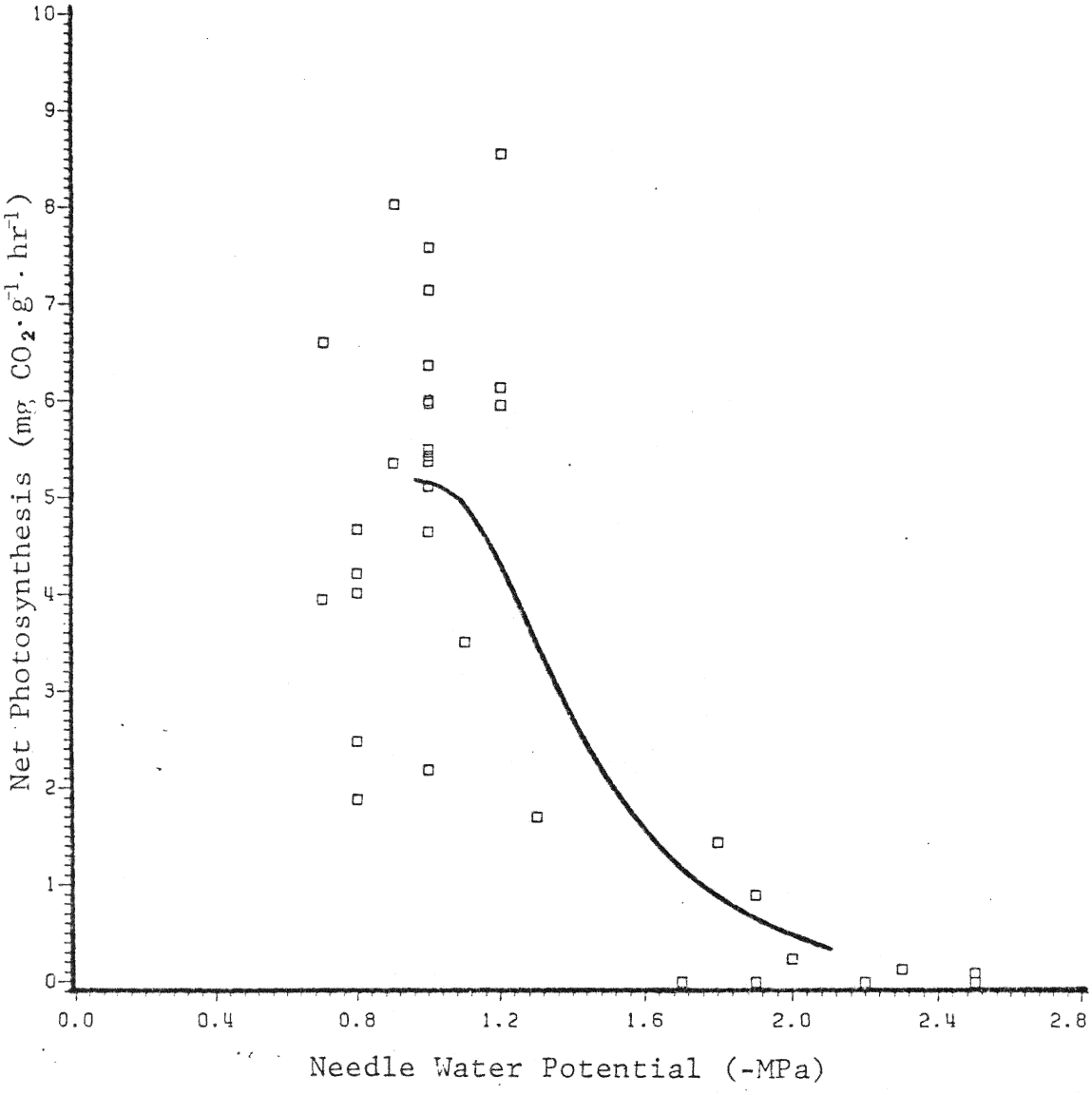


Figure a1. Control loblolly pine photosynthesis and predicted regression response as affected by needle water potential.

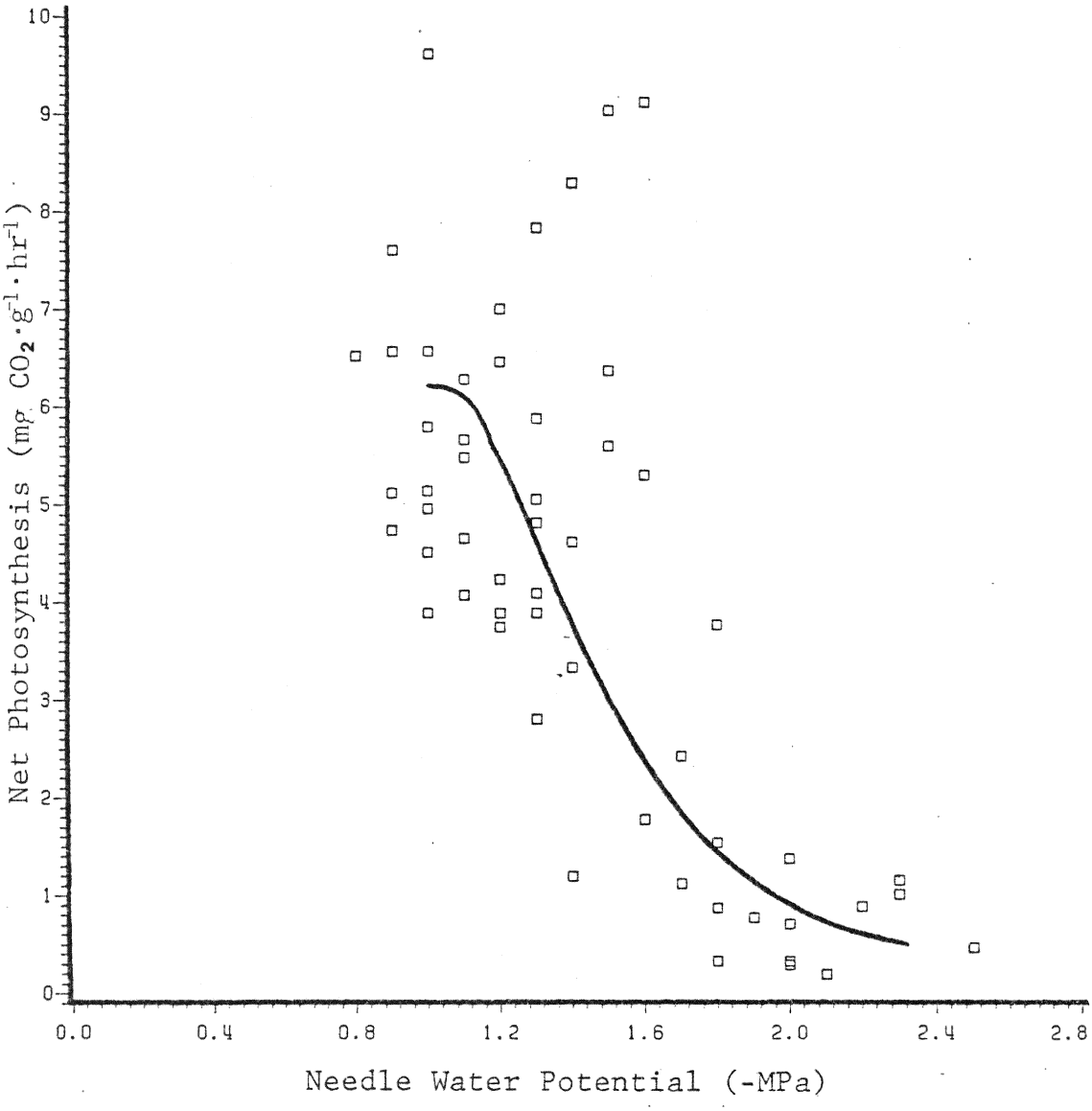


Figure a2. Moderate conditioned loblolly pine photosynthesis and predicted regression response as affected by needle water potential.

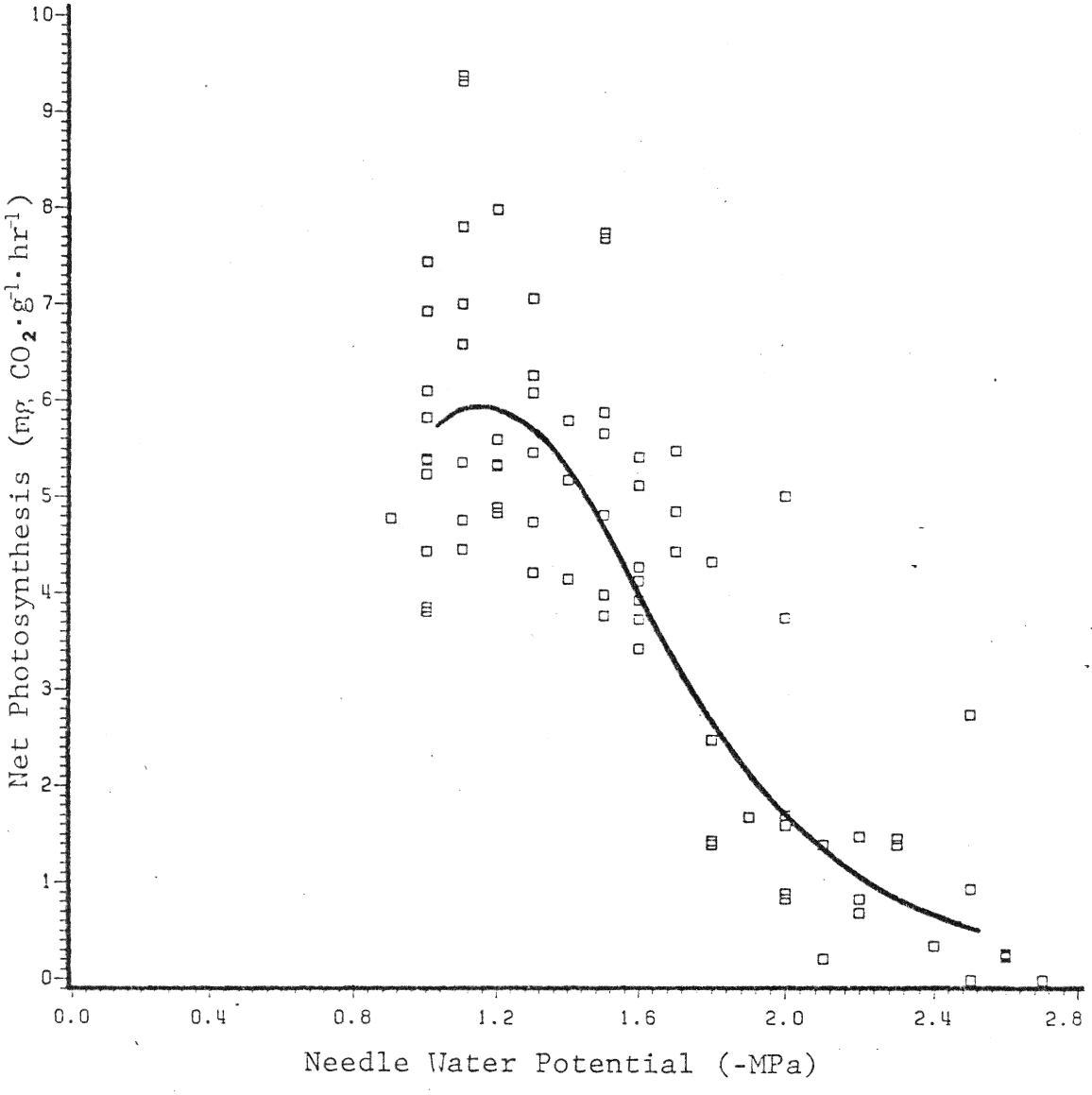


Figure a3. Severe conditioned loblolly pine photosynthesis and predicted regression response as affected by needle water potential.

Appendix B

LOBLOLLY PINE SEEDLING NEEDLE CONDUCTANCE DATA AND PREDICTED REGRESSION RESPONSES

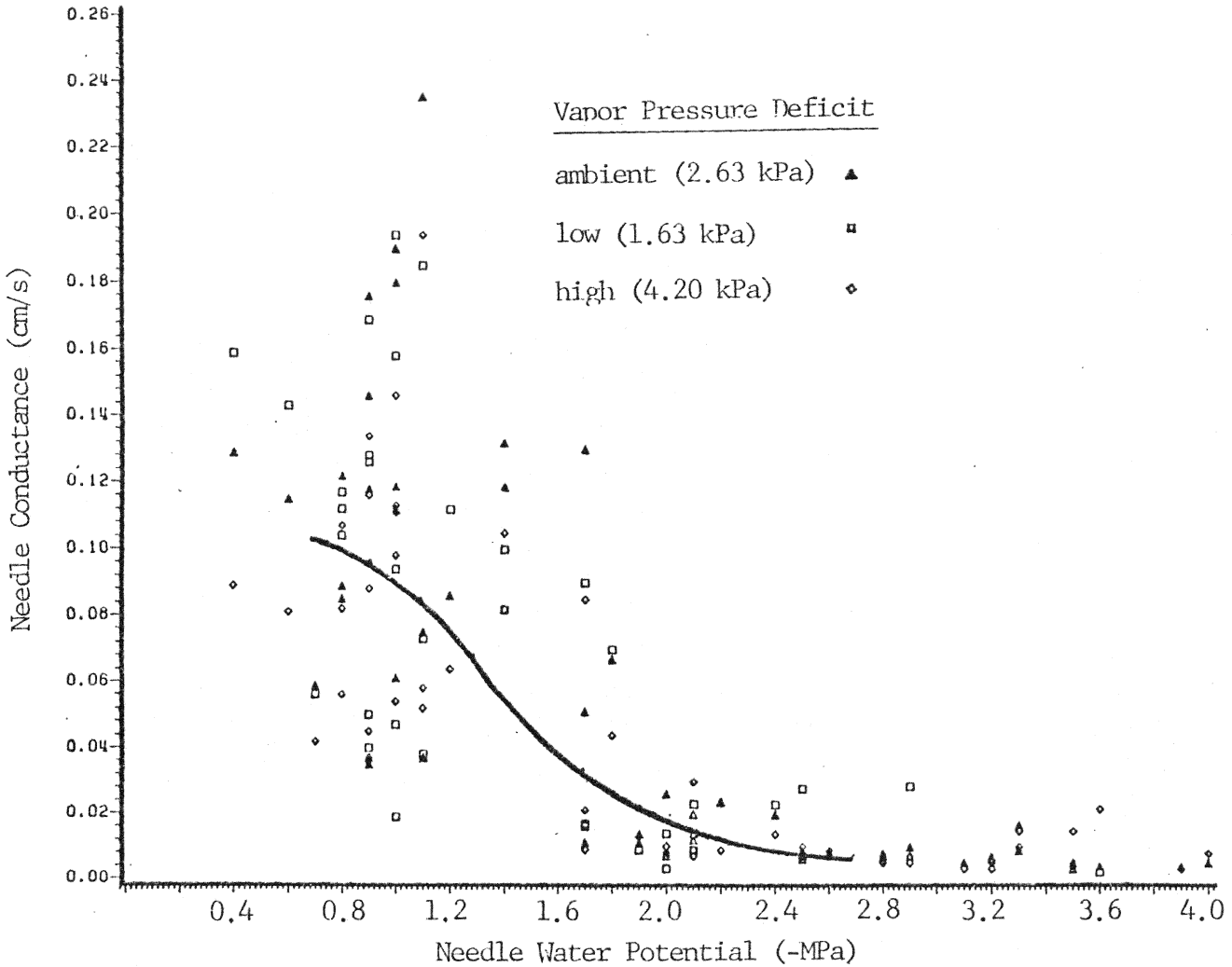


Figure b1. Control loblolly pine needle conductance and predicted regression response as affected by needle water potential and vapor pressure deficit.

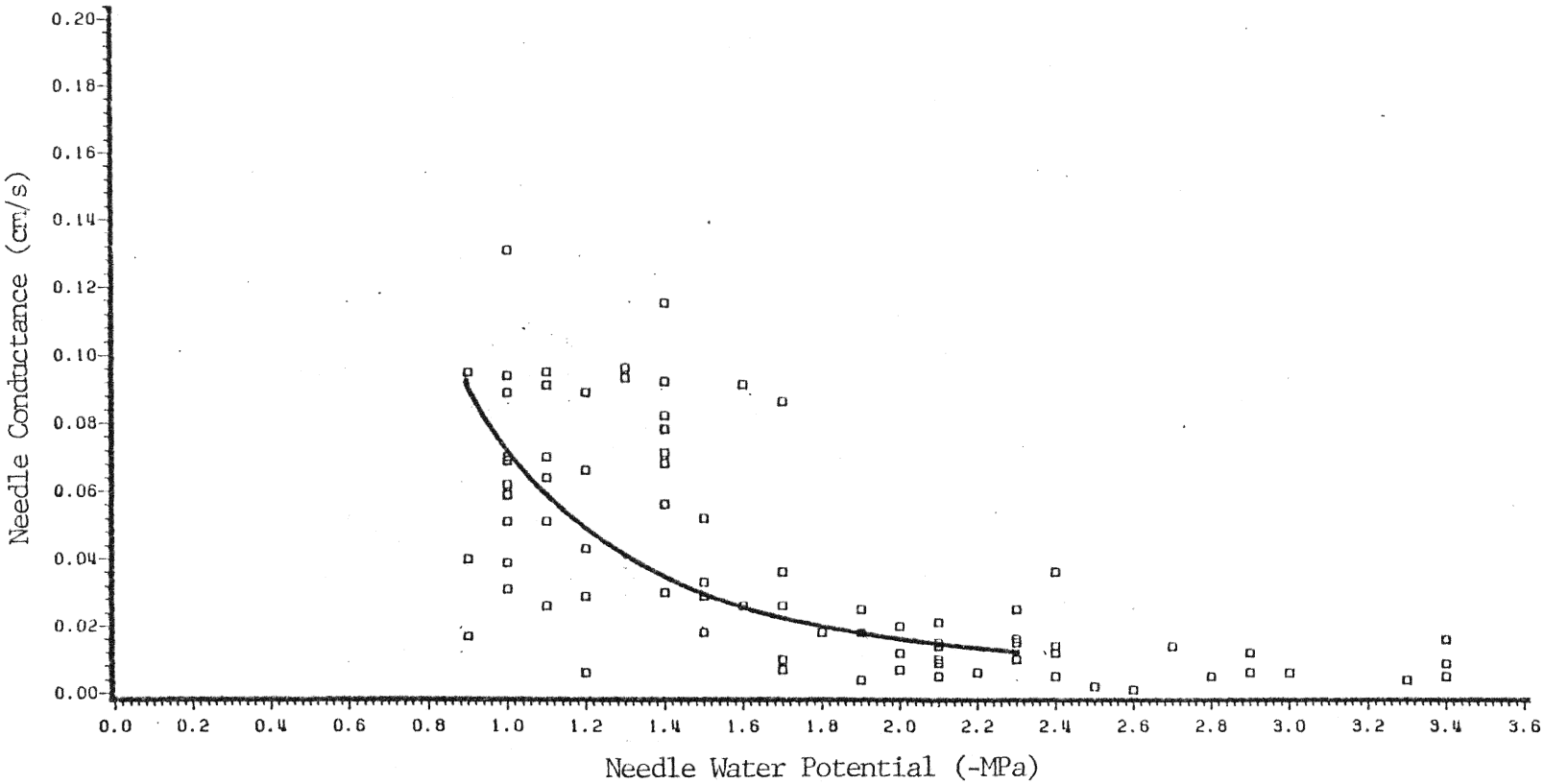


Figure b2. Moderate conditioned loblolly pine needle conductance and predicted regression response at ambient vapor pressure deficit (2.63 kPa) as affected by needle water potential.

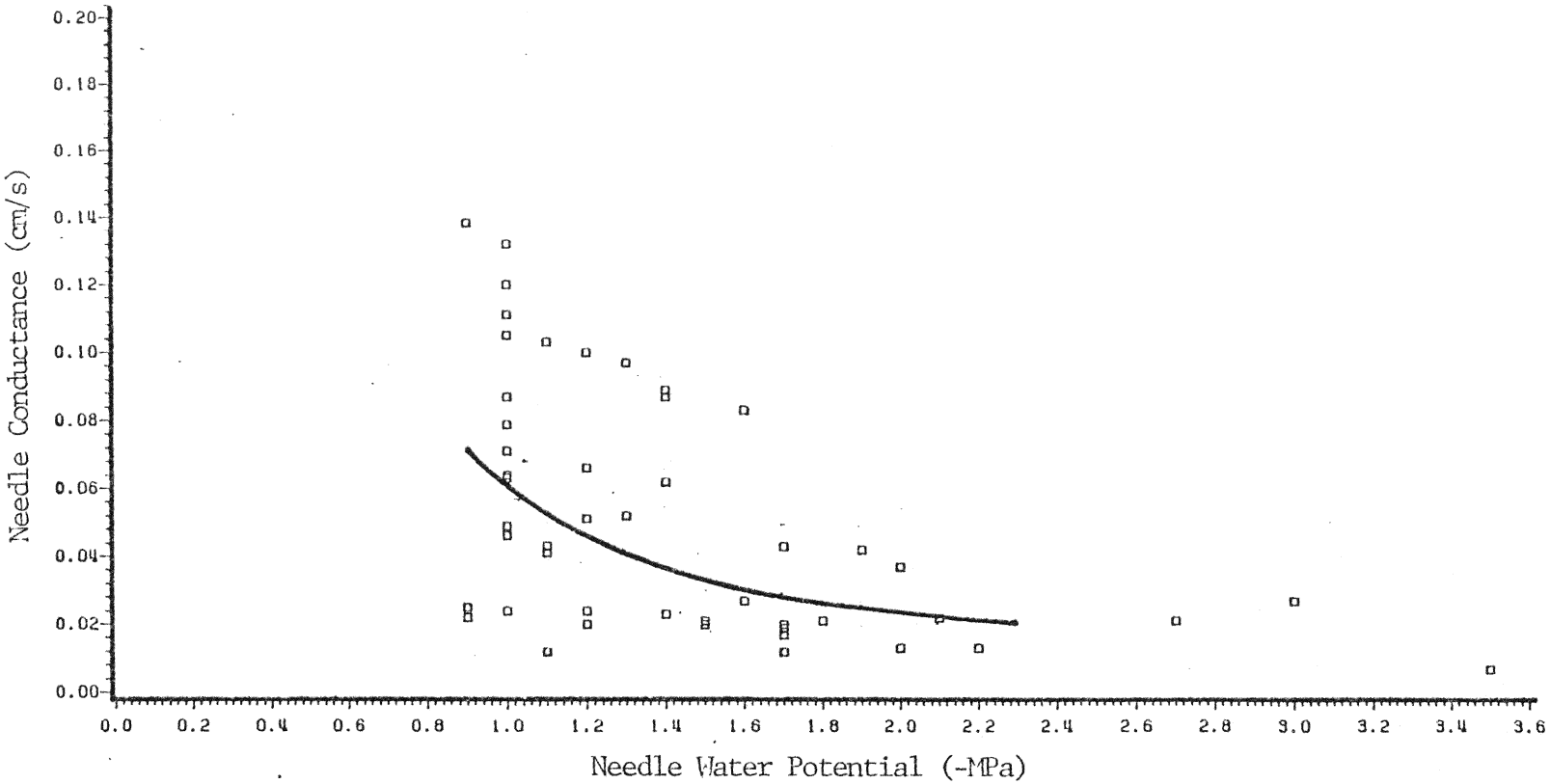


Figure b3. Moderate conditioned loblolly pine needle conductance and predicted regression response at low vapor pressure deficit (1.63 kPa) as affected by needle water potential.

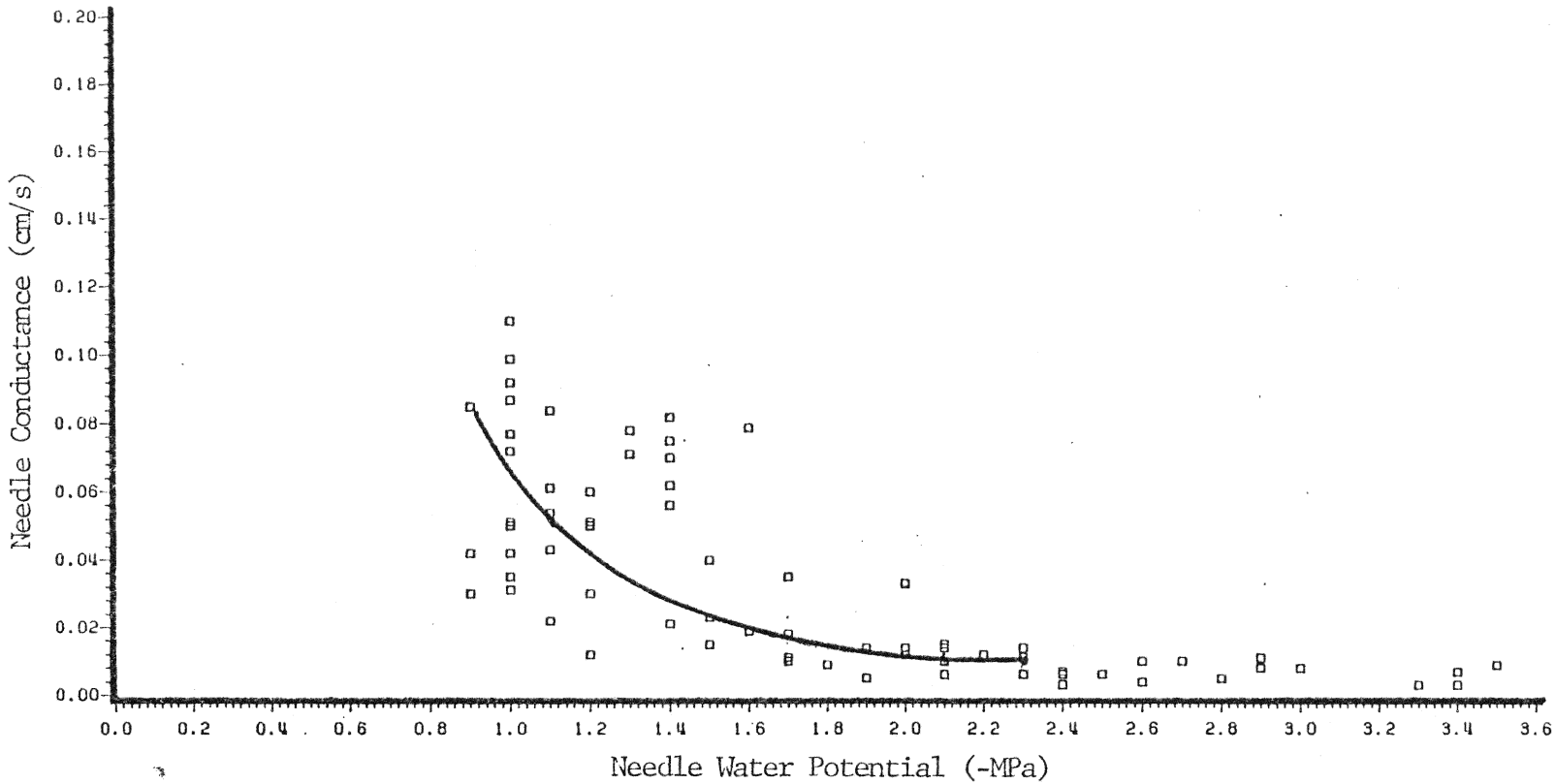


Figure b4. Moderate conditioned loblolly pine needle conductance and predicted regression response at high vapor pressure deficit (4.20 kPa) as affected by needle water potential.

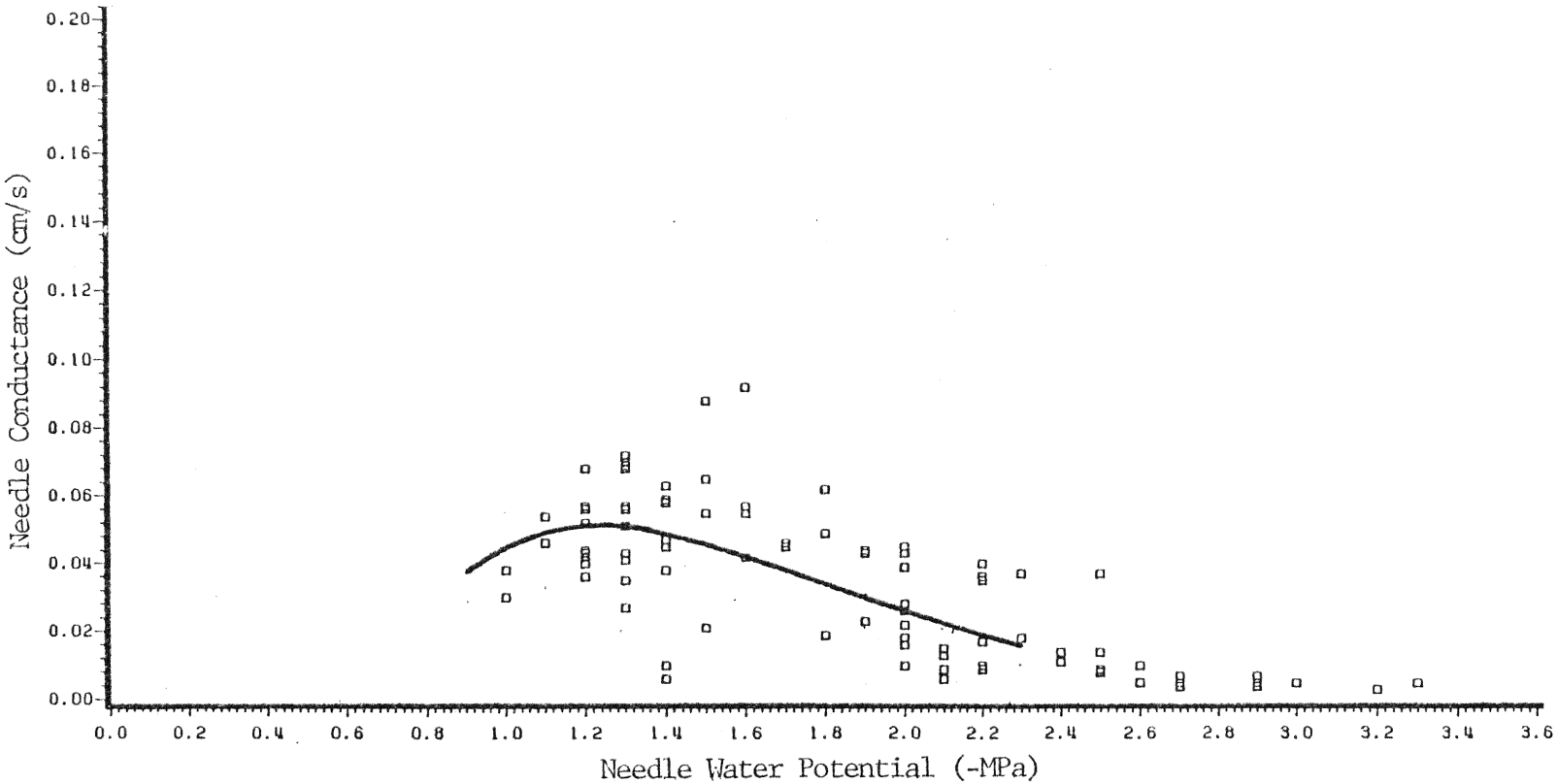


Figure b5. Severe conditioned loblolly pine needle conductance and predicted regression response at ambient vapor pressure defict (2.63 kPa) as affected by needle water potential.

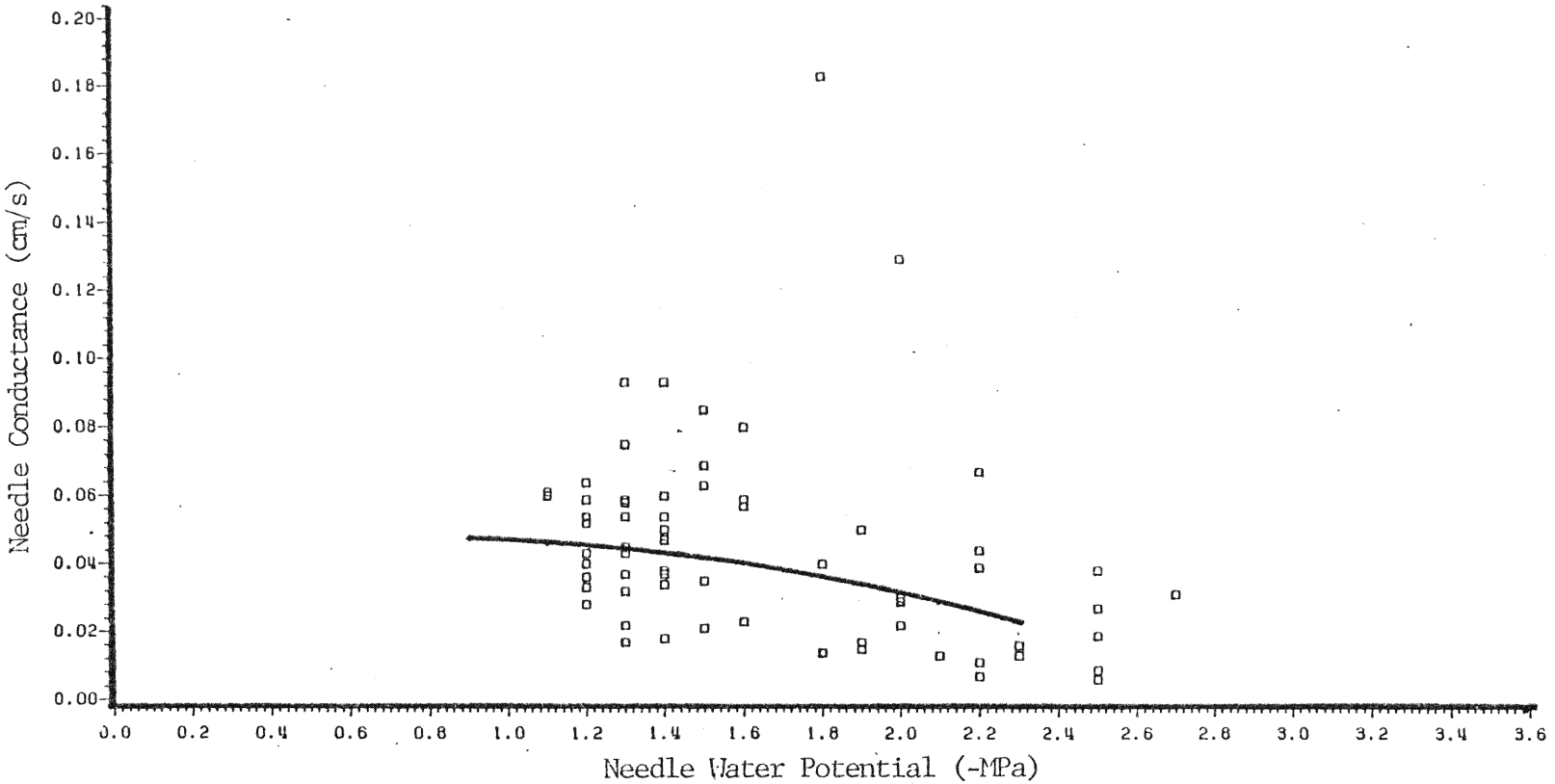


Figure b6. Severe conditioned loblolly pine needle conductance and predicted regression response at low vapor pressure deficit (1.63 kPa) as affected by needle water potential.

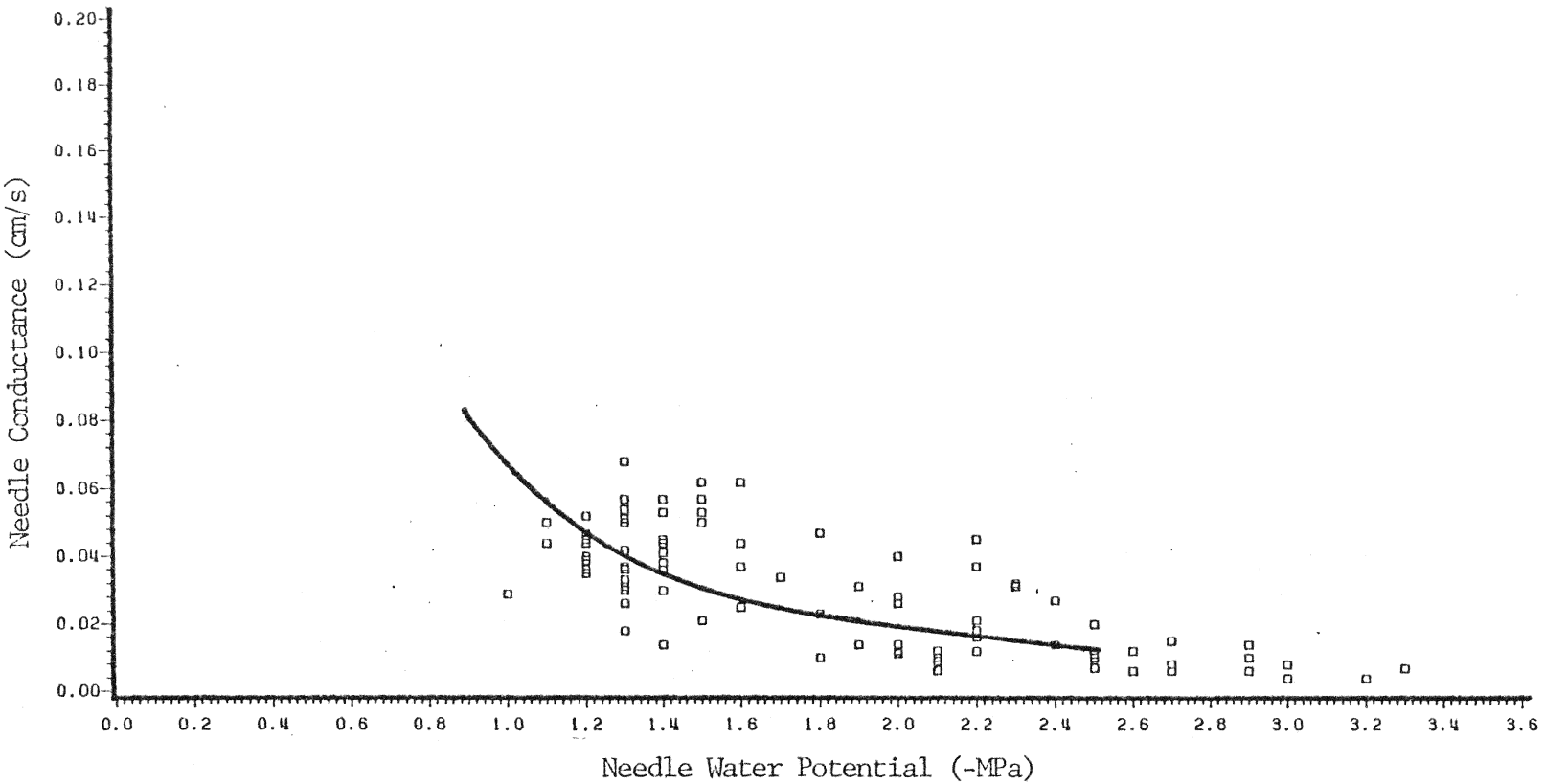


Figure b7. Severe conditioned loblolly pine needle conductance and predicted regression response at high vapor pressure deficit (4.20 kPa) as affected by needle water potential.

Appendix C

LOBLOLLY PINE SEEDLING PHOTOSYNTHESIS DATA FOR EXPERIMENT TWO AND PREDICTED REGRESSION RESPONSES

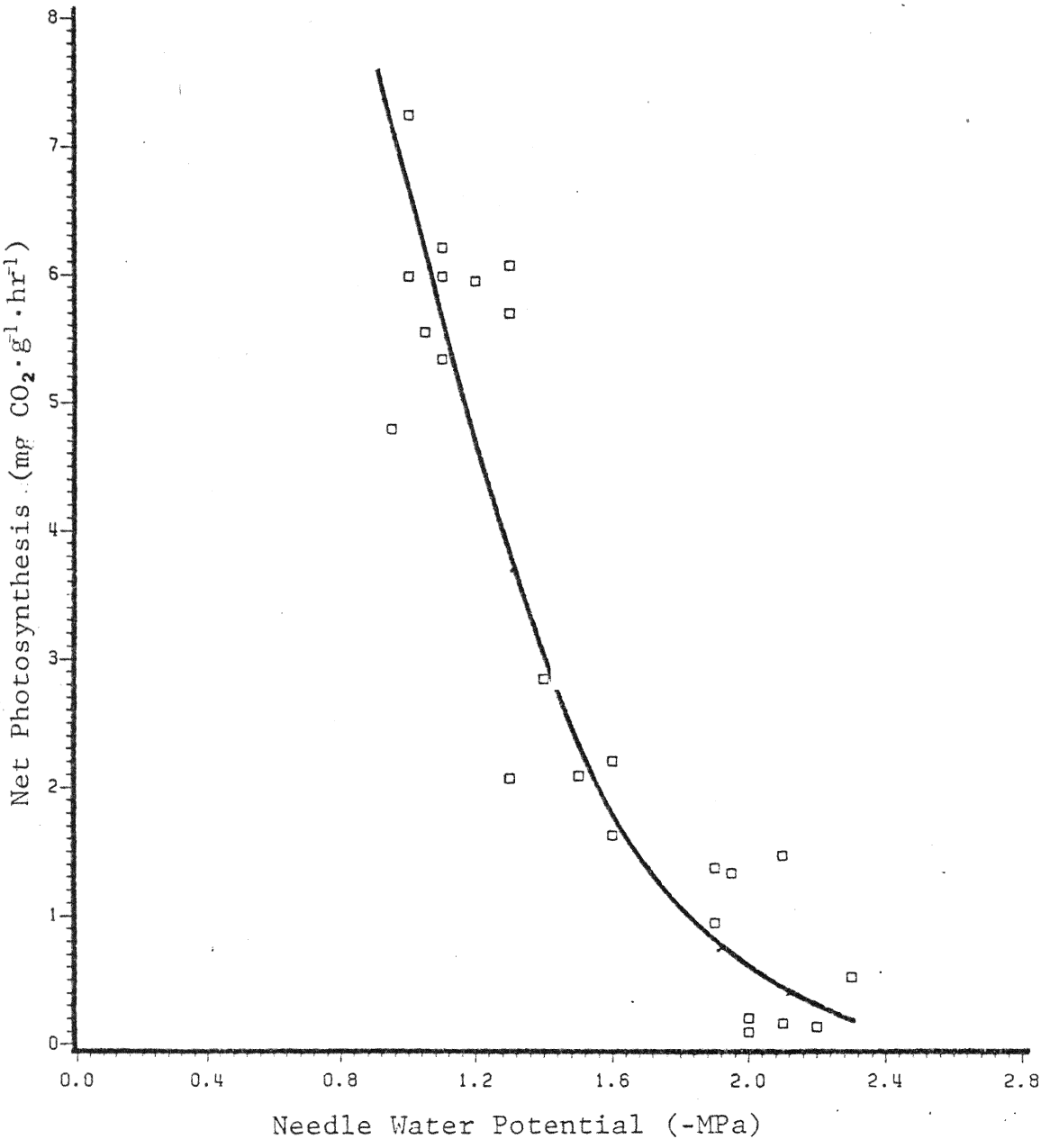


Figure c1. Control loblolly pine photosynthesis and Predicted regression response as affected by needle water potential.

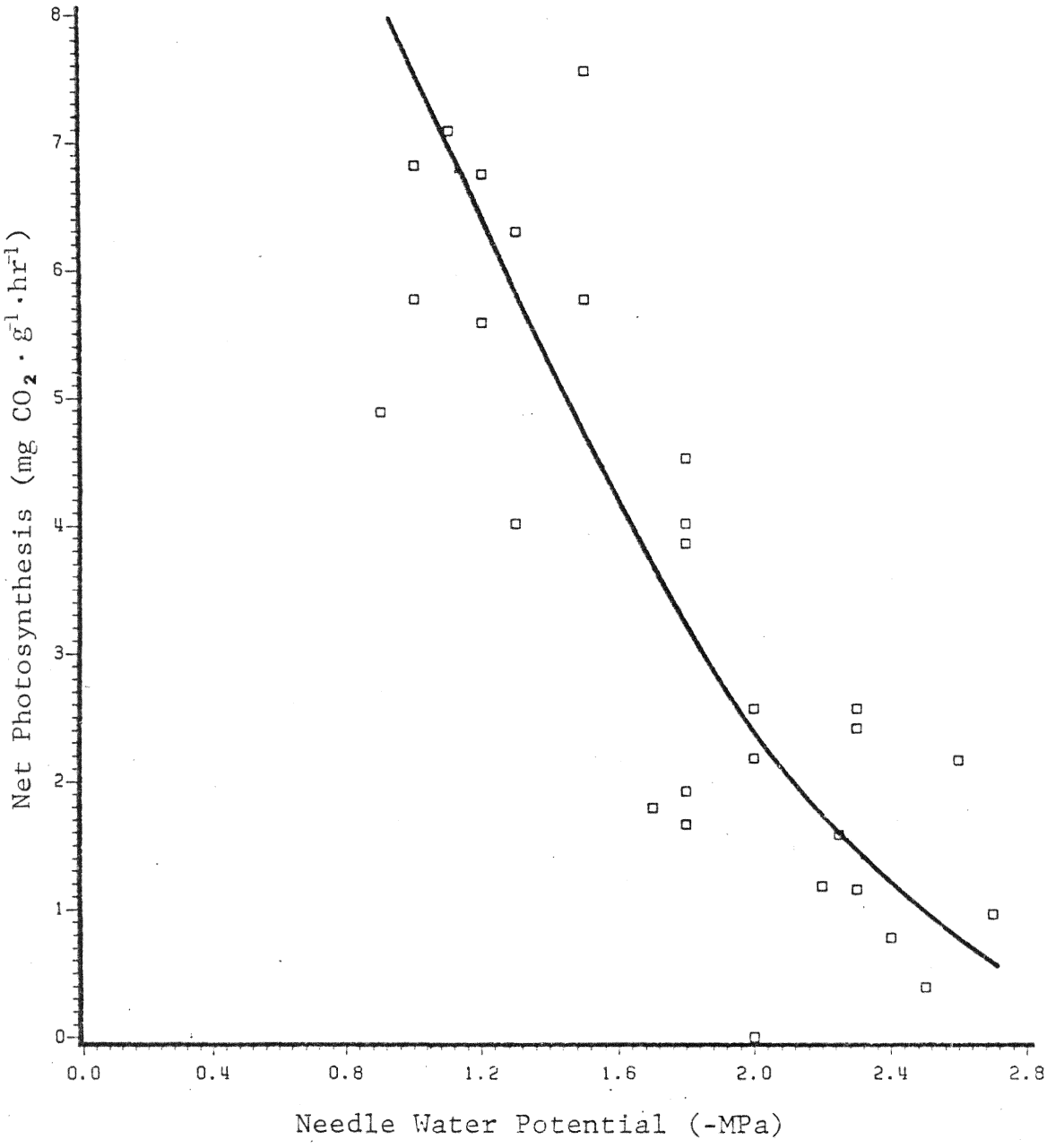


Figure c2. Severe conditioned loblolly pine photosynthesis and predicted regression response as affected by needle water potential.

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