THE INFLUENCE OF LOW MOISTURE STRESS ON THE GAS EXCHANGE AND THYLAKOID ACTIVITY OF LOBLOLLY PINE (Pinus taeda) AND ALEPPO PINE (Pinus halepensis)

by

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Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

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in

Forestry

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(ABSTRACT)

The objectives of this study were to determine the
influence of sublethal water stress on the physiology of
loblolly and Aleppo pine. Gas exchange characteristics,
uncoupled thylakoid electron transport capacity, and needle
osmotic potentials were measured.

Seedlings of both species were watered only when their
needle water potential fell below -1.8 and -2.2 MPa
respectively (water stress conditioning) or were kept well
watered (controls). After 10 weeks of water stress
conditioning, both regimes were allowed to dry down. During
this period of increasing water stress, photosynthesis in
both species was determined at 5 different cuvette CO₂
concentrations (approximately 200, 330, 500, 650, and 800
ppm). With Aleppo pine only, mesophyll resistances and
stomatal limitations to gas exchange were estimated.
Thylakoids were extracted from both species and their
activity was measured in a liquid phase $O_2$ electrode (Hansatech Ltd) as rate of $O_2$ consumption. Methyl Viologen ($1,1'$-dimethyl-4, 4'-$bipyridinium$ ion) was included in the reaction medium.

Photosynthesis decreased with increasing water stress but fell more slowly in the conditioned seedlings. As water stress increased, total resistance to $CO_2$ exchange increased for both regimes to a much higher level than explained by stomatal resistance alone. In the conditioned seedlings, resistances increased less precipitously than in controls. Osmotic adjustment as measured with thermocouple psychrometers occurred in both species. Decreases in photosynthesis (both species) and increases in mesophyll resistance (Aleppo pine) were not accompanied by a decrease in whole chain uncoupled electron transport capacity.
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DEDICATION

In memory of Dr. Dimitrios Mitsopoulos

my teacher and first mentor in scientific thinking.
# TABLE OF CONTENTS

ACKNOWLEDGEMENTS.............................................. iv

DEDICATION....................................................... v

INTRODUCTION.................................................. 1

LITERATURE REVIEW............................................. 5

Osmotic Adjustment.............................................. 7
Stomatal Resistance to Transpiration and
Photosynthesis.................................................. 8
Non-Stomatal Limitations to Photosynthesis................... 11
Transport and Carboxylation Limitations to
Photosynthesis.................................................. 13
Chloroplast Parameters and Water Stress
Drought Resistance as Affected by Moisture Stress
Conditioning..................................................... 22

METHODS AND MATERIALS....................................... 25

Testable Hypotheses............................................. 25
Plant Material..................................................... 26
Water Stress Conditioning..................................... 27
Osmotic Potential............................................... 28
Gas Exchange Measurements.................................... 28
Chloroplast (Thylakoid) Isolation and Assay............... 30
Data Analysis..................................................... 33

RESULTS.......................................................... 35

Loblolly Pine Gas Exchange.................................... 35
Thylakoid Activity of Loblolly Pine.......................... 37
Osmotic Potentials of Loblolly Pine.......................... 37
Photosynthetic Recovery after Rewatering of
Loblolly Pine................................................... 40

Aleppo Pine Gas Exchange....................................... 40
Thylakoid Activity of Aleppo Pine........................... 46
Osmotic Potentials of Aleppo Pine........................... 46
Chlorophyll Content of Aleppo Pine.......................... 46
Mesophyll Resistance and Gas Phase Limitation
of Aleppo Pine.................................................. 48
Photosynthetic Recovery after Rewatering
of Aleppo Pine.................................................. 48
DISCUSSION ......................................................... 53
Gas Exchange .................................................... 53
Osmotic Adjustment ........................................... 55
Comparison of Photosynthetic Declines .................. 57
Thylakoid Activity .............................................. 60
Chlorophyll Content ............................................ 62
Photosynthetic Recovery ..................................... 63
CONCLUSIONS ..................................................... 64
LITERATURE CITED ................................................ 66
VITA .............................................................. 73
LIST OF TABLES

Table 1. Mean photosynthesis of water stress conditioned and control seedlings of 32-week-old loblolly pine seedlings at three different levels of water stress........................................36

Table 2. Mean photosynthesis of 32-week-old loblolly pine seedlings as affected by CO$_2$ concentration and various levels of water stress.................37

Table 3. Uncoupled whole chain electron transport activity of thylakoids from 32-week-old loblolly pine seedlings at different water stress levels and overall means as affected by water stress conditioning........................................39

Table 4. Photosynthetic rates of 32-week-old loblolly pine seedlings at the beginning of the experiment (initial Pn) after rewatering (recovery Pn) and percentage photosynthetic recovery at ambient CO$_2$ levels as influenced by water stress conditioning........................................41

Table 5. Mean gas exchange of well watered 32-week-old Aleppo pine seedlings as affected by water stress conditioning........................................43

Table 6. Mean gas exchange of well watered 32-week-old Aleppo pine seedlings as affected by CO$_2$ concentration........................................44

Table 7. Photosynthesis of 32-week-old Aleppo pine seedlings as affected by CO$_2$ concentration and water stress conditioning when moderately and severely stressed........................................45

Table 8. Uncoupled whole chain electron transport activity of thylakoids from 32 week-old-Aleppo pine seedlings at different water stress levels and overall means as affected by water stress conditioning........................................47

Table 9. Aleppo pine seedling chlorophyll contents of both conditioning treatments during the final drought down cycle........................................49

Table 10. Mesophyll resistances and gas phase limitations of Aleppo pine seedlings when well watered as affected by water stress conditioning........................................50
Table 11. Photosynthetic rates of 32-week-old Aleppo pine seedlings in the beginning of the experiment (initial Pn) after rewatering (recovery Pn) and percentage photosynthetic recovery at ambient CO$_2$ levels as influenced by water stress conditioning.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Pn (initial)</th>
<th>Pn (recovery)</th>
<th>Recovery</th>
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<tbody>
<tr>
<td>Normal</td>
<td>200</td>
<td>150</td>
<td>20%</td>
</tr>
<tr>
<td>Stressed</td>
<td>150</td>
<td>120</td>
<td>20%</td>
</tr>
</tbody>
</table>

 ix
LIST OF FIGURES

Fig. 1 Methyl Viologen chemistry in relation to the photosynthetic electron transport and O₂ consumption........................................32
LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Ca</td>
<td>external [CO₂]</td>
</tr>
<tr>
<td>chl</td>
<td>chlorophyll</td>
</tr>
<tr>
<td>Ci</td>
<td>internal [CO₂]</td>
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<tr>
<td>e⁻</td>
<td>electron</td>
</tr>
<tr>
<td>Gl</td>
<td>conductance</td>
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<tr>
<td>lg</td>
<td>gas phase limitation</td>
</tr>
<tr>
<td>Pn</td>
<td>net photosynthesis</td>
</tr>
<tr>
<td>PSU</td>
<td>photosynthetic unit</td>
</tr>
<tr>
<td>PS I, II</td>
<td>photosystem I, II</td>
</tr>
<tr>
<td>Rm</td>
<td>mesophyll resistance</td>
</tr>
<tr>
<td>Rn-s</td>
<td>non-stomatal resistance</td>
</tr>
<tr>
<td>Rs</td>
<td>stomatal resistance</td>
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<tr>
<td>Ts</td>
<td>transpiration</td>
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<td>WUE</td>
<td>water use efficiency</td>
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INTRODUCTION AND JUSTIFICATION

Loblolly pine (*Pinus taeda* L.) is the most important economic pine species in the southeastern U.S.. Out of 34,103,000 ha of all pine forests in this region (which comprise 41 % of the total timberland) 9,012,000 and 5,749,000 ha are occupied by natural and planted loblolly pine stands respectively (Bechtold and Ruark 1988). Loblolly pine habitat is characterized by periodic summer droughts. Aleppo pine (*Pinus halepensis* Mill.) is possibly the most xerophytic pine in the Mediterranean area. This area is characterized by uneven distribution of precipitation during the year, with few rainy days and high temperatures during the summer. Aleppo pine is considered a fire species and salt tolerant. It occurs mainly on limestone derived soils with a pH in the neutral to alkaline range. Along with *Pinus brutia* Mill., Aleppo pine is a leading resin producer in the Mediterranean basin (Panetsos 1981).

Since water stress is a major environmental factor limiting both species, drought tolerance mechanisms merit an in depth investigation because knowledge of these mechanisms will help us improve the survival rate and productivity of both species. Aleppo pine occupies a generally drier and saltier environment and it is reasonable to hypothesize that it has developed more effective drought tolerance mechanisms
than loblolly pine. By comparing the acclimation mechanisms of the two species, we will be able to better understand the mechanisms of drought resistance.

Physiologists and plant breeders require a better understanding of the control factors involved in complex processes such as drought tolerance so that genetic screening and selection can become more efficient. There is a need to identify the different component processes of drought tolerance that are most important and their relative contribution in restricting the overall rate of net CO₂ assimilation. There is also a need to investigate the effect of a given moisture change in any one of the component processes and on the overall rate of the drought tolerance mechanism.

There has been a considerable amount of research on the effect of water stress conditioning on drought tolerance mechanisms of some forest trees, especially on the control mechanism of stomatal aperture and transpiration rate. This work has been based, mainly, on water relations and water potential parameters of the whole tree or the foliage. Seiler and Johnson (1988) investigated the effect of water stress conditioning on drought tolerance of three loblolly pine families during stress. They found that photosynthesis decreased greatly with reduced needle water potential but in conditioned seedlings photosynthesis remained at higher levels. Under well watered conditions transpiration was
reduced greatly but photosynthesis was not and consequently conditioned seedlings had a greater water use efficiency. Seiler and Johnson (1988) did not investigate the physiological basis for this response.

Minimal amount of research has been done on investigating the effects of water stress on acclimation processes of forest trees at the cell or the cell organelle level. For example, what portion of drought acclimation, if any, occurs within the chloroplasts?

Leaf desiccation is accompanied by stomatal closure which can diminish the internal CO$_2$ pressure. Non-stomatal (mesophyll) components of photosynthesis are directly inhibited by water stress as well (Cornic et al. 1983). Assigning stomatal and non-stomatal limitations to photosynthesis and investigation of the chloroplast acclimation to water stress will help in elucidating the relative importance of these processes.

In addition to the above, there is increasing interest on the possible effects of elevated atmospheric CO$_2$ levels and temperature on plants and trees. Changes of gas exchange characteristics in a possibly drier and/or CO$_2$ richer environment need some initial evaluations.

The research herein investigated, in greater detail, the effects of water stress conditioning on photosynthesis on a whole leaf basis and at the thylakoid level of loblolly pine and Aleppo pine seedlings. The specific objectives of this
study were to:

1. determine the influence of sublethal water stress on the needle water potential parameters and gas exchange characteristics of loblolly and Aleppo pine.

2. evaluate the relative contribution of the stomata and the thylakoids to inhibition of photosynthesis and acclimation under water stress of the above mentioned two pine species.

3. determine the effect of elevated CO₂ on photosynthesis, transpiration, and water-use efficiency under various water stress conditions.
LITERATURE REVIEW

Water is an essential component of plant life and plays a crucial role in most plant processes. During droughts, insufficient water is available and plants develop water deficits which in turn diminish growth, yield and survival. The mechanisms that plants possess to resist this environmental stress are commonly referred to as drought tolerance. Drought tolerance involves both dehydration postponement and dehydration tolerance. The first occurs through changes that reduce transpiration such as stomatal closure or increase the ratio of absorptive to transpirational surfaces by increasing root/shoot ratios. The second is determined by the degree of dehydration without permanent injury and depends on adaptations such as osmotic adjustment (Turner and Kramer 1980).

The expression of drought tolerance is controlled, like all plant characteristics, by the interaction of heredity and environment. If a character is responsive to environmental changes it is described as phenotypically plastic and can be the target of hardening and selection procedures. Since the concept of water stress tolerance is too broad to serve as a good basis for tree hardening and breeding, specific plastic characters which are most important to the survival of different plants in specific habitats, have to be defined. Consequently, a first option to improve the drought tolerance of a tree, is to work with
physiological and biochemical characteristics directly related to field drought tolerance. During such a procedure one must concentrate on a small number of well defined attributes. The pattern of plasticity and inheritance of these characters can be determined next, and the initial cycles of water stress hardening and genetic selection can be carried out in the controlled environment of a greenhouse, where these operations are often more efficient (Turner and Kramer 1980). Characters suitable for this manipulation can be stomatal behavior, osmotic adjustment, partial resistances of leaf gas exchange, water-use efficiency, rooting pattern, photosynthesis, photosynthetic electron transport rate, carboxylating enzymes, changes in metabolites and translocation, and protoplasmic dehydration tolerance.

A second option is to select directly for performance or yield in controlled drought stressed nurseries or uncontrolled natural sites and obtain differences among species, provenances and lines over years of testing. This procedure is closer to natural stress situations but the results are confounded by fluctuations of atmospheric conditions, it is more time consuming than the first option and the parameters used are not directly related to drought tolerance.
Osmotic Adjustment

Cell processes such as vacuolization and maintenance of turgor require water. Biochemical reactions require water as a reactant and water is the widest naturally occurring solvent. During water stress, the water available to the cell for all these functions is diminished. The process that cells use to internally regulate the water status is termed osmotic adjustment or osmoregulation. Osmotic adjustment is the lowering of cell osmotic potential and the consequent achievement of a higher turgor potential by addition of solutes (Hinckley et al. 1980).

In higher plant cells, the vacuole occupies approximately four times more volume than does the cytoplasm and plays an essential role in cell enlargement and volume regulation of the rest of the cell. Compounds such as proline, KCl and polyols, have been suggested to act as osmoregulatory substances (Borowitzka 1981). Cell organelles (chloroplasts, mitochondria) must participate in osmoregulatory activities similar to the surrounding cytoplasm. The exact mechanism and the major solutes which participate in organelle water activity regulation have not yet been elucidated (Borowitzka 1981).

During the last decade studies attribute increasingly higher significance to osmotic adjustment as a mechanism participating in drought tolerance (Kandiko et al. 1980, Osonubi and Davies 1981). It is considered as a very
important factor in maintaining tree growth and water absorption during drought. Stomatal opening, gas exchange and cell enlargement occur at a rate explained only by the active osmotic adjustment of the living tissues of the tree. Species such as English oak (*Quercus robur* L.) studied by Osonubi and Davies (1978), western hemlock (*Tsuga heterophylla* (Raf)) studied by Kandiko et al. (1980), apple (*Malus pumila* Mill.) studied by Davies and Lasko (1978) and loblolly pine maintain higher turgor and growth through solute accumulation, while in other species such as silver birch (*Betula verrucosa* Ehrl.) studied by Osonubi and Davies (1978) no solute accumulation occurred and turgor and growth parameters declined rapidly as water was retained. There are many indications that water stress tolerant species exhibit osmotic adjustment while intolerant species do not (Osonubi and Davies 1978). In loblolly pine seedlings, Seiler (1984), observed that severe water stress treatment resulted in the lowest osmotic potential although there was an interaction between seed source and water stress treatment.

**Stomatal Resistance to Transpiration and Photosynthesis**

The stomata of most plants are important in regulating transpiration under stress conditions. Stomatal closure decreases the transpiration rate during high atmospheric water vapor pressure deficits, or high evaporative demand,
so that the amount of water lost from the stomatal cavity and the leaf is minimized. Survival rate in the field is highly related to stomatal resistance. One clone of Monterey pine (Pinus radiata D. Don), which exhibited a low survival rate, had a transpiration rate up to twice, and stomatal resistance almost half, of that of another clone which exhibited high survival rate (Bennet and Rook 1978). In experiments with loblolly pine conducted by Teskey et al. (1986), different absolute humidity deficits (AHD) had no statistically significant effect on stomatal resistance. This led to increased transpiration cused by small stomatal adjustment to higher AHD. In response to falling xylem pressure potential (XPP), transpiration and stomatal conductance declined slightly to a threshold value of -1 MPa. Lower XPP values induced a rapid reduction of the above parameters and stomatal closure occurred at approximately -2 MPa (Teskey et al. 1986).

Loblolly pine clones from xeric environments show greater stomatal sensitivity and declines in transpiration as leaf water potential declines compared to clones from mesic environments (Teskey et al. 1987). This type of stomatal response decreases water loss but also reduces photosynthesis because changes in stomatal conductance are closely coupled with changes in photosynthetic rate. In these cases, the stomatal mechanism seems to be adapted to favor short term-survival over growth.
Experiments with Aleppo pine showed a general two phase response of stomata to declining XPP (Melzak et al. 1985). Stomatal conductance and transpiration remained unaffected down to a threshold value of -0.8 MPa beyond which these two parameters changed dramatically. The threshold value for closure of stomata for Aleppo pine seems greater than that of loblolly, indicating a higher potential for conserving water and better adaptation to drought (Melzack et al 1985 and Teskey et al 1986).

Stomatal resistance, under normal ambient CO₂ concentrations, is a rate limiting factor in photosynthesis. Photosynthetic rate changes in a pattern similar to that of stomatal conductance. Photosynthesis begins to decline at the same threshold value that stomatal conductance does, for both species mentioned above. This is especially true for Aleppo pine where stomatal resistance under moderate XPP is 6 to 7 times greater than non-stomatal resistances. This ratio of stomatal to non-stomatal limitation to photosynthesis (Rs/Rn-s) is not typical for C₃ species from mesic environments (Gifford 1974 cited by Melzack 1985). C₃ plants generally have a Rs/Rn-s ratio of 0.3 to 0.4 and C₄ 3 to 5. In Aleppo pine during moderate XPP, stomatal resistance is approximately 10 times as high (about 15 s/cm) in relation to a typical non-stomatal resistance of about 1.5 s/cm (Melzack 1985).

In loblolly pine, stomatal resistance seems to be
coupled to photosynthesis as well. Stomatal resistance in this case exerts a significant limitation to net photosynthesis (approximately 20-30% of total resistance is stomatal) but it is not large enough to control the rate of photosynthetic process (Teskey et al. 1986). This percentage is very different from the previously mentioned 70% of Aleppo pine.

Non-Stomatal Limitations to Photosynthesis

During water stress, declines in AHD and/or XPP diminish stomatal conductance which in turn reduces stomatal transpiration and net photosynthesis. Because of the close correlation between changes in photosynthetic rate and stomatal resistance it was long believed that stomata limited photosynthesis by reducing the rate of CO₂ diffusion through the stomatal cavity into the leaf (Brix 1962). In well watered sunflower, though, under saturating light when stomata were fully open, doubling the CO₂ gradient led to an increase in the rate of total photosynthesis, but not to the extent expected if only CO₂ concentration limited photosynthesis. So photosynthesis appeared to be limited by a nondiffusional factor (Mathews and Boyer 1984). After desiccation to leaf water potentials of -1.8 MPa no response of photosynthesis to CO₂ concentration occurred. This means that photosynthesis again was clearly not limited by the
rate of CO$_2$ diffusion through the leaf to the chloroplasts. In similar studies, photosynthetic effects were ascribed to changes in mesophyll resistances which may have involved changes in chloroplast activity as well (Mathews and Boyer 1984). This hypothesis is supported by the observation that photochemical activity in vivo and isolated chloroplast oxygen evolution in vitro are inhibited by low leaf water potentials to a degree which could limit photosynthesis. Mathews and Boyer (1984) concluded that the photochemical activity of the chloroplasts probably limited photosynthesis over the entire range of light intensities when leaf water potential was low. In this way, non-stomatal components of photosynthesis are directly inhibited by water stress while the CO$_2$ diffusion from stomata to intercellular liquid phase is diminished as well. These two simultaneous effects make it difficult to partition the inhibition of leaf gas exchange caused by lowered stomatal conductance, which in turn changes the internal partial CO$_2$ pressure, from the inhibition due to lower CO$_2$ active transport into the cytoplasm or the lower chloroplast activity.

At this point, the terminology used to describe the relative importance of the different catenary resistance components merits some discussion. The importance of these components, in limiting photosynthetic rate, is described quantitatively by the resistance analogue. This bears a mechanistic meaning and applies to diffusion processes as
the water vapor or $\text{CO}_2$ diffusion through the boundary layer or stomatal cavity. It is only applicable when photosynthesis occurs under $\text{CO}_2$ limiting conditions which means that photosynthesis is proportional to the cell wall concentration of $\text{CO}_2$. In fact, the mesophyll or liquid phase resistance is a complex term of diffusive and chemical components which can be further subdivided. It was suggested by Jones (1973) that the term "photosynthetic limitations" should be used, instead of resistances, to describe quantitatively the limiting processes of photosynthesis especially when the latter is not mainly diminished by the supply of $\text{CO}_2$. This occurs when carboxylating enzymes are operating at saturation which means that the photosynthesis: [$\text{CO}_2$] internal (Pn:Ci) curve begins to depart from linearity (Jones 1973).

Transport and Carboxylation Limitations to Photosynthesis

The diffusion form of carbon within the cell wall, cytoplasm and chloroplast is most probably the bicarbonate ion and the enzyme which catalyzes the formation and the dissociation of $\text{HCO}_3^-$ within the chloroplasts is carbonic anhydrase. It also appears that a delicate balance between the different transport forms of carbon, hydrogen ions and enzyme activity is maintained in chloroplasts (Yocum 1975).

Finally, photosynthesis is limited at the last step of the whole process, which is the incorporation of the $\text{CO}_2$
molecule into the plant metabolism via the Calvin-Benson cycle. The acceptor of CO₂ is Ribulose -1, 5- diphosphate, (RuBP), in a reaction catalyzed by the enzyme Ribulose -1, 5-diphosphate carboxylase, (Rubisco) (Larcher 1975). At low intercellular partial CO₂ pressure p(CO₂), CO₂ assimilation rate is limited by the activity of Rubisco at a given low p(CO₂). The initial slope of photosynthesis – p(CO₂) curve, \([dPn/dp(CO₂)]\) as Pn \(\rightarrow 0\), which equals the mesophyll conductance or its reciprocal the mesophyll resistance, is correlated with the kinetics of Rubisco and is determined by the RuBP saturated rate of the enzyme. The final rate of Pn attained is a function of the rate of regeneration of the substrate (RuBP). The slope of the line and consequently the kinetic parameters of the enzyme depend on the p(O₂) and the temperature (Caemmerer and Farquhar 1981). Contrary to Caemmerer and Farquhar's results, Oquist et al. (1980) found that the activity of extracted Rubisco from artificially frost hardened seedlings of Pinus sylvestris was not much affected by the cold hardening treatment. Activation of the enzyme is probably light dependant since the enzyme was found to be sensitive to high vs low light intensities (Bjorkman et al. 1972). The enzyme is also sensitive to different levels of nitrogen nutrition (Wong 1979), but seems to be rather insensitive to water stress (Beadle and Jarvis 1977). In this last case, though, the researchers hypothesized that the water stress effect on Rubisco in vivo
was masked *in vitro* as a result of rehydration prior to assay.

At high internal p(CO₂) (p>220 ppm), and saturating irradiance, RuBP regeneration becomes limiting and that changes the dependence of Pn on p(CO₂). The Pn:Ci curve begins to depart from linearity. RuBP regeneration itself depends on the supply of ATP and NADPH in addition to the activation status of regulatory enzymes of the Calvin cycle. NADP reduction to NADPH depends exclusively on the electron transport capacity of the grana. So the Pn rate depends both upon the electron transport capacity, Rubisco activities and the rate of RuBP regeneration (Caemmarer and Farquhar 1981).

**Chloroplast Parameters and Water Stress**

There is a dual effect of water stress on the chloroplasts. First, it affects their development in young leaves. The macromolecular differentiation of chloroplasts during greening of etiolated tissue is sensitive to temperature and water status. Chlorophyll (Chl) accumulation and Chl a/b ratio during greening of etioplasts is sensitive to water stress. Leaf chlorophyll a/b ratios of stressed Jack bean (*Canavalia ensiformis* L. DC.) plants were higher during the 4 hour lag phase of chlorophyll accumulation. This was explained by a more reduced rate of chlorophyll b production than chlorophyll a. A 50%
reduction in the amount of Photosystem II (PS II) was observed in stressed plants during the lag period but the rate of appearance of Photosystem I (PS I) P700-chlorophyll a protein did not change in stressed tissues (Alberite et al. 1976). Bourque and Naylor (1971) had already proposed that the principal block in chlorophyll synthesis occurs in the metabolic pathway to protochlorophyll and that the enzymatic steps sensitive to water stress appear to be capable of adapting to stress when relative humidity is above 50%. They also proposed that the reduced chlorophyll accumulation could be attributed to the absence of translocational influx of energy sources.

In addition to the above, Bourque and Naylor (1971) hypothesized an indirect interaction of water stress with stomata mediated through the suppressed chloroplast efficiency. The driving force for stomatal activity is the actively transported potassium ions in and out of guard cells (Humble and Raschke 1971). Cyclic photophosphorylation is thought to be the basic energy source for this active transport (Zeiger 1983). So the initiation of stomatal control over the transpiration of the stressed leaves occurs after the detected lag period in chlorophyll development and accumulation which occurs when young leaves develop during water stress conditions (Bourge and Naylor, 1971). During this period probably the enzyme system responsible for synthesizing delta-aminolevulinic
acid (ALA) (a chlorophyllide precursor) is formed de novo in a fashion similar to that found by Castelfranco and Beale (1983) during regreening of etiolated seedlings.

Secondly, water stress affects mature chloroplasts. Conifers possess larger and fewer photosynthetic units (PSU, total light-harvesting chlorophyll to P700 reaction center) per unit leaf area than angiosperms do (Alberte et al. 1976). This results in fewer electron transport chains and a reduction in NADPH and ATP production and consequently in lower CO₂ fixation rates. Shade leaves have larger PSU than sun leaves which results in lower saturation irradiances; this seems to be an adaptive mechanism to the long periods of low irradiance of the understory (Alberte et al. 1976). Since the ability of chloroplasts to alter the size of the photosynthetic antennae in order to cope with low irradiance or temperature was found, it is also possible that the chloroplasts exhibit a similar adaptive mechanism to water stress as well. Water stress reduces the light harvesting complex Chlorophyll a/b-protein (Alberte and Thornber 1977). The photosynthetic inhibition due to this reduction may be partially alleviated by larger PSU. Mohanty and Boyer (1976) found that the quantum yield of sunflower chloroplasts isolated from leaves with low water potentials is reduced. The activity of chloroplasts was measured as the initial rate of photoreduction of sodium 2, 6-dichlorophenolindophenol (DCIP) in red light, which was
assayed spectrophotometrically. The total chlorophyll content of the leaves and the chloroplast pigments remained constant at different leaf water potentials. A possible explanation may be that changes in quantum yield are caused by suppression of the ability of Chlorophyll-membrane complex to convert absorbed radiation to chemical energy. This inhibition also must involve lower electron flow at the thylakoid level. In maize chloroplasts, Alberte and Thornber (1977) found that water stress resulted in an increase of chlorophyll a/b ratio with a simultaneous reduction in the lamellae content of the light harvesting Chlorophyll a/b-protein and smaller photosynthetic units (total Chlorophyll/P700). These losses can be attributed either to higher rates of catabolism (through photooxidation) of the complexes or to retardation of synthesis during dehydration.

Etiolated corn plants regreened when rewatered as light harvesting chlorophyll a/b-protein was resynthesized when exogenous CO₂ became available again (Alberte et al. 1975).

Whole-chain-electron transport (H₂O to NADP) and the rate of partial photoreactions decrease during autumn and winter (Oquist et al., 1980). Oquist and Martin (1980) demonstrated that a block occurs between the two photosystems in winter stressed Pinus sylvestris. Chlorophyll bleaching was also observed parallel to the decreasing rate of photosynthetic electron transport.
Chlorophyll centers are preferentially destroyed during autumn and winter. This destruction is at least partly due to photooxidation and a similar mechanism may be involved during water stress to reduce the probability of photoinhibition so that the photochemical conversion of radiant flux slows down to match the reduced availability of CO₂ during dehydration.

Bjorkman and Powels (1984) found that water stress predisposes leaves to photoinhibition and that water stress effects are in part attributable to photoinhibition. Leaves of Nerium oleander growing in full light, subjected to water stress, exhibit reduced photon yield and light-saturated capacity of photosynthetic CO₂ uptake, decline in electron transport activity of thylakoids and quenching of variable fluorescence at 692 nm. The above reactions are very similar to well watered shade leaves which are then exposed to strong light. Bjorkman and Powels (1984) concluded that light is the primary agent causing damage to the photosynthetic system under water stress. Photoinhibition may occur even in well watered leaves under bright light and some repair mechanism, which reverses this effect, may be retarded or ceased under water stress. The decline in whole-chain electron transport rate was caused by lower PS II activity. Additionally, inactivation of a factor other than PS II must be involved as well since exposure of plants to severe water stress under low light reduced electron
transport activity (Bjorkman and Powels 1984).

Photoinhibition is a deleterious process that occurs as a natural consequence of the function of a rapidly turned over Q₈ protein, whenever excess light is absorbed causing overreduction of the bound plastoquinone pool (Kyle et al 1984). In this case the binding site of the Q₈ protein cannot be filled by an oxidized quinone and the excess electrons (through radical mediated reactions) render the Q₈ protein nonfunctional. Photoinhibition and loss of photosynthetic capacity results when the rate of damage exceeds the rate of removal and replacement of the nonfunctional protein (Arntzen et al. 1984).

Photoinhibition can result under low levels of input to PS II (low light intensities) if the transfer of energy away from PS II (i.e. photosynthetic electron trasport) is limited. This may occur when the stomatal aperture of C3 plants is diminished in response to environmental stresses as drought, chilling and high salinity (Osmond 1981).

Contrary to Bjorkman and Powels (1984), Sharkey and Badger (1982) working with Xanthium strumarium mesophyll cells found that electron transport was not affected by water stress although photophosphorylation was sensitive to lower water potentials. They observed stimulation of electron transport in the absence of uncouplers. This indicates that water stress can uncouple electron transport which in turn may diminish photophosphorylation and ATP
supply. However the ATP level did not fall significantly
during stress and so the reduction of photophosphorylation
capacity may not limit CO$_2$ dependent O$_2$ evolution in that
case. Instead, some other factors may have limited ATP
utilization.

Berkowitz and Gibbs (1983) proposed that the inhibition
of photosynthesis during water stress is due to concomitant
reduction in osmotic potential which mediates its effects on
the photosynthesizing apparatus via stromal acidification.
A decrease in the extent of the alkaninization of the
stroma, will result in a decrease in the rate of carbon
fixation. KCl, which is a stromal alkalating agent,
 alleviates the effect of low osmotic potential inhibition to
photosynthesis. The osmotically induced acidification is
not evident in the dark. The light induced stromal
alkalization, which is required for Calvin cycle operation,
is absent in a stress situation. Berkowitz and Gibbs (1983)
hypothesized that if the stromal [K$^+$] doubles in response to
a reduction in chloroplast volume, it could initiate an
increased K$^+$ export coupled to H$^+$ import via an K$^+$/H$^+$
antiport, and so the stroma would become more alkaline.

Sen Gupta and Berkowitz (1988) proposed that
chloroplast volume reduction may mediate the inhibition of
photosynthesis under water stress. Stromal volume
maintenance, despite decreasing water potentials and plant
dehydration, may be one mechanism of photosynthetic
acclimation to water stress.

Santukumari and Berkowitz (1990) using three different wheat genotypes found that differences between these genotypes in terms of relative sensitivity of photosynthesis to low water potentials could be explained by the difference in extent of the in situ protoplast volume reduction at low water potentials. This is due to the differential ability of these genotypes to maintain protoplast volume during decreasing water potentials.

Drought Resistance as Affected by Moisture Stress Conditioning.

Outplanted nursery seedlings often encounter drought, resulting in the development of various degrees of dehydration. Trees are most prone to stress in their first summer after planting because they have shallower root systems and cannot compete with weed roots for water at depth. When in competition with weeds for a fixed and limiting soil water supply, the trees that obtain a greater proportion of the available water at the expense of the weeds will have an advantage in growth. Sands and Nambiar (1983) observed reduced growth rates with outplanted seedlings of radiata pine (Pinus radiata D. Don) which paralleled the development of water stress. They also found circumstantial evidence that a major cause for differences in growth rates were due to differences in the severity of
water stress and that weed competition for water and nutrients may be an important contributing factor. Bennett and Rook (1978) attributed differences in survival among clones of radiata pine to the greater ability of successful clones to limit transpiration by decreased stomatal conductance. Seedlings of Banksia serrata conditioned to water stress (hardened) by exposure to sublethal drought treatments, express a higher drought resistance (Tibbits and Bachelard 1981). This may be achieved by maintaining slower growth rates, lower stomatal conductance and lower osmotic potentials, through utilization of osmotic adjustment or by producing long thin roots. Moisture stress conditioned loblolly pine seedlings reduced transpiration 33%, with no reduction of initial photosynthesis, resulting in an approximately 70% increase in Water Use Efficiency (Seiler and Johnson, 1985). Stomata of preconditioned black alder (Alnus glutinosa) seedlings did not fully open immediately following rewatering, leading to conservation of water and maintenance of prolonged metabolic functions during drought periods (Seiler, 1985). Matthews and Boyer (1984) working with sunflower (Helianthus annuus L.) found that photosynthesis is reduced at low leaf water potentials but repeated water deficits can decrease this reduction, resulting in photosynthetic acclimation. They also found that inhibition of chloroplast activity accounts for most of the inhibition of photosynthesis at low water potentials and
that acclimation at the chloroplast level accounts for most of the acclimation of photosynthesis. This was especially true for PS II activity (which is generally diminished during water stress) was less influenced by low water potentials in chloroplasts isolated from acclimated leaves than from control leaves.
METHODS AND MATERIALS

Testable Hypotheses

In order to assess the effect of water stress on some physiological parameters of loblolly pine and Aleppo pine seedlings, the experiments were designed to test the following general hypotheses:

1. Ho: The relative contribution of stomatal and mesophyll resistances to Photosynthesis in Aleppo pine does not change during exposure to experimental drought.
2. Ho: Uncoupled electron transport capacity of the chloroplasts (thylakoids) of loblolly pine and Aleppo pine is not modified during exposure to experimental drought.
3. Ho: The response of photosynthesis to elevated CO₂ in loblolly and Aleppo pine is not modified during exposure to experimental drought.
4. Ho: Water stress conditioning does not modify any of the above relationships.
Plant Material

Aleppo pine seeds were collected from naturally occurring stands at Chalkidiki, Greece. Loblolly pine seeds were obtained from the Virginia Department of Forestry and were a nursery mix intended for use on the Virginia coastal plain (a mesic environment). Seeds of both species were moist stratified at $20^\circ$C for approximately two months. They were then planted into 1 liter plastic pots filled with a loamy sand soil obtained from the New Kent Forestry center (Seiler and Johnson 1988). The pots were placed in a glasshouse on the Virginia Tech Campus (Blacksburg, VA.).

Seedlings were grown for an initial six month period. During this time, seedlings were kept well watered and under natural light conditions supplemented with high pressure sodium vapor lights to maintain a 16-hour photoperiod (150 umol m$^{-2}$ s$^{-1}$ photosynthetic photon flux density [PPFD], following natural sunset). Temperature in the greenhouse ranged from $35^\circ$C during the day to $18^\circ$C during the night.

Loblolly pine seedlings developed an adequate number of secondary needles which were desirable for gas exchange measurements. Aleppo pine, however, does not normally develop secondary needles during the first year of its growth (Panetsos 1981). Therefore seedlings were subjected to a period of short days for two weeks (10-hour photoperiod) followed by two weeks of cold at 2 $^\circ$C in hopes of forcing a second growth flush with secondary needles.
Nevertheless, Aleppo pine seedlings did not develop secondary needles. Seedlings of both species were then subjected to 10 weeks of water stress conditioning.

**Water Stress Conditioning**

Sixty seedlings of both species were divided into two groups which received one of two levels of water stress conditioning:

1. No water stress; seedlings kept well watered to maintain mid-day needle water potential above -0.7 MPa;

2. Severe water stress; loblolly and Aleppo pine seedlings were watered only when mid-day needle water potential averaged -1.8 and -2.2 MPa, respectively.

Loblolly pine seedling water potential was monitored on single fascicles measured with the Scholander pressure chamber (Scholander et al. 1965). Aleppo pine seedling water potential was monitored on small lower branches since it was not possible to measure on primary needles. Water potential measurements were taken from five randomly selected seedlings for every combination of species and water stress conditioning. After the water stress conditioning period of 10 weeks, all the seedlings were well watered and then subjected to a drought during which physiological measurements were taken. At the end of this drought, seedlings of both regimes were rewatered and photosynthetic recovery after 24 hours was determined.
Uncoupled thylakoid activity of Aleppo pine seedlings was also determined after 24 hours of recovery.

**Osmotic Potentials**

After the 10 week conditioning period, needle osmotic potential was determined using thermocouple psychrometers (75 series, J. R. D. Merrill Specialty Equipment, Logan, Utah). Following rewatering of both treatments, needles from five seedlings per species treatment combination were collected. The needles were then placed into small stainless steel sample chambers (81-250 dimensions 75 series, J. R. D. Merrill Specialty Equipment, Logan, Utah) and sealed to the psychrometers. The chambers and the psychrometers were then submerged for three minutes in liquid nitrogen to insure complete rupture of cell membranes. They were then placed into a 30 °C water bath for 7 hours, after which the psychrometers were read for water potential. These measurements were assumed to equal the osmotic potential of the sample (Brown 1972).

**Gas Exchange Measurements**

Seedling net photosynthesis (Pn), transpiration (Ts), whole needle conductance (G1) and water use efficiency (W.U.E) were measured every other day during the drought cycle with a portable photosynthesis system (LI-6200, LI-COR Lincoln, NE). Pn was measured as umoles of CO₂ consumed per
gram dry weight tissue per second (umoles CO₂ g⁻¹ s⁻¹), Ts was measured as mmoles of water transpired per dry weight tissue per second (mmoles H₂O g⁻¹ s⁻¹), and conductance as moles per gram and per second (moles g⁻¹ s⁻¹). Gas exchange was measured at five different cuvette CO₂ concentrations. Different levels of external CO₂ (Ca) were introduced into the closed system of LI-COR cuvette by injecting a predetermined amount of CO₂ with a syringe into the cuvette. Loblolly pine Pn measurements were taken by incrementally increasing the cuvette [CO₂] from approximately 200 ppm to approximate concentrations of 330, 500, 650 and 800 ppm. Aleppo pine Pn measurements were taken during a continuous drawdown of cuvette [CO₂] from 800 ppm to 650, 500, 330 and 200 ppm. Davis et al. (1987) demonstrated that such a technique gives the same results as measurements over a wide range of [CO₂] in the cuvette.

All other environmental variables were held as constant as possible during these measurements with the exception of cuvette RH% which ranged between 40 and 20% due to variations at greenhouse conditions. Light level averaged 700 umoles m⁻² s⁻¹ and temperature averaged 30°C. Water vapor data (Ts and G1) were not included for loblolly pine due to wide variations in estimated internal CO₂ concentrations; indicating problems with their estimates. For Aleppo pine, water vapor data will be reported only when seedlings from both water stress conditioning treatment were
well watered since as seedlings became water stressed and Ts fell, the LICOR reported values (Ts, G1 and Ci) became inconsistent. Leuning and Sands (1989) propose some explanations for possible inconsistencies with LICOR water exchange data. These problems are expected to exacerbate when measurements are taken from drying seedlings seedlings with low Ts rates.

Mesophyll resistances were estimated for well watered Aleppo pine by constructing photosynthesis (Pn) vs internal CO₂ (Ci) curves. The reciprocal of the slope of the Pn:Ci curve was taken as the estimate of mesophyll resistances (Jones 1985).

Gas phase limitation to Pn (lg) for Aleppo pine was determined according to Jones (1985) assuming that the CO₂ concentration is zero at the carboxylation site within the chloroplast (equations 8 and 11) and Teskey et al. (1986). The equation used was 

\[ lg = \frac{[Ca-Ci]}{Ca} \]

**Chloroplast (Thylakoid) Isolation and Assay**

Thylakoid isolation was done according to Martin et al. (1978). The preparation medium contains 0.4 M sucrose 0.05 M HEPES buffer, pH=7.6, 0.01 M NaCl, 0.005 M MgCl₂ and 20% (w/v) PEG 4000. A second resuspension medium consists of 1 M sucrose and 0.05 M HEPES buffer, pH=6.9 (NaOH). PEG is the osmoticum in the isolation media and the competitive agent against phenolics, tannins and terpenes which inhibit
chloroplast activity and which are released during the cellular disruption of conifer needles. Uncoupled thylakoid whole chain electron transport was measured as the reduction of $[O_2]$ in reaction medium containing methyl viologen ($H_2O \rightarrow MV$) by a Clark type electrode (Hansatech Ltd, England). MV acts by diverting electrons from photosystem I (PS I) by competing with oxidized ferredoxin (Richmond and Halliwell 1982). The subsequent reduced MV reacts with molecular $O_2$. Two moles of water split by photosystem II (PS II) produce 1 mole of $O_2$ and 4 $e^-$ which, by the above mentioned reaction, reduce 4 $O_2$ so the net balance is 3 moles of $O_2$ consumed for 1 mole of $O_2$ evolved (Figure 1).

Previous experimentation, using ATP production uncouplers, showed that thylakoids prepared by applying the above protocol were already uncoupled so the rate measured corresponds to the uncoupled whole chain electron transport (Oquist, personal communication). The term uncoupled refers to the fact that the proton gradient between the stroma and the intrathylakoid space no longer exists and consequently no ATP is produced. This in turn means that addition of ADP to the reaction solution would not stimulate electron transport rate. The dissipation of the proton gradient and the concomitant production of ATP which is the rate limiting step in electron transport under normal conditions cannot be accounted for in this case.

Chlorophyll determinations were done for Aleppo pine
Fig. 1 Methyl Viologen chemistry in relation to the photosynthetic electron transport and O₂ consumption.
only according to Arnon (1949). Chloroplasts were isolated from well watered plants and then under various levels of moisture stress as the seedlings dried down.

Data Analysis

The experiment was analyzed as a completely randomized design with two water stress conditioning treatments. Species were analyzed separately due to their widely different growth rates and because Aleppo pine, unlike loblolly pine, did not develop secondary needles. Only subjective comparisons will be made between species. Four replicates were sampled for all variables measured except for water potential components, where five replicates were used. In most cases it was not possible to collect gas exchange data over a continuous water potential spectrum due to the rapid dry down of the seedlings during the experimental drought. As a result, water potentials were grouped into three levels; well watered, mildly dry and severely dry conditions.

Water potential groups were analyzed separately since water stress effects were obvious and have been clearly documented with past studies. Within each water potential group, differences between conditioning treatments and CO₂ levels were compared with Analysis of Variance. The model used was:

Dependent Variable = [CO₂] + W.S. conditioning treatment

When the interaction was significant, means for both water stress conditioning groups at the five CO₂ levels were reported. Least square means test was used to perform comparisons when cell size were not equal (Searle et al., 1980). Otherwise paired t-test or Duncan's multiple range test was used.
RESULTS

Loblolly Pine Gas Exchange.

When both regimes were well watered (W.P. > -1.0 MPA), there was no interaction between [CO₂] and the water stress conditioning treatment for Pn (Table 1). Averaged across all CO₂ concentrations, there was, however, a significant water stress treatment effect on Pn. Twenty four hours after rewatering, control seedlings had significantly higher Pn (36% greater) as compared to the water stress conditioned seedlings (Table 1). When moderately stressed, (W.P. between -1.0 and -1.5 MPA), water stress conditioned plants had higher Pn levels (Table 1). As seedlings became more severely stressed (W.P. less than -1.5 MPA) differences between the two stress groups were no longer evident and photosynthesis was very low (Table 1).

When seedlings were well watered, Pn increased 2.3 times as [CO₂] increased from 335 ppm (ambient) to 729 ppm (Table 2). As seedlings became moderately stressed (water potentials between -1.0 and -1.5 MPA) Pn still responded greatly to increasing [CO₂]. In fact, at a [CO₂] level of 729 ppm, Pn in moderately stressed seedlings was 45% greater than well watered plants at ambient (335 ppm) CO₂ levels. No interaction was observed between CO₂ level and water stress conditioning at any water stress group.

At 808 ppm CO₂, Pn in severely stressed seedlings (W.P. ≤ -1.6 MPA), recovered only partly to well watered Pn rates
Table 1. Mean photosynthesis of water stress conditioned and control seedlings\textsuperscript{1} of 32-week-old loblolly pine seedlings at three different levels of water stress.

<table>
<thead>
<tr>
<th>Water stress Treatment</th>
<th>Water potential (MPa)</th>
<th>(\text{umuoles CO}_2 \text{ g}^{-1} \text{ s}^{-1})</th>
<th>(\text{umuoles CO}_2 \text{ g}^{-1} \text{ s}^{-1})</th>
<th>(\text{umuoles CO}_2 \text{ g}^{-1} \text{ s}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;-1.0</td>
<td>0.0856 *\textsuperscript{2}</td>
<td>0.0217 *</td>
<td>0.0046 NS</td>
</tr>
<tr>
<td>Control</td>
<td>0.0627</td>
<td>0.0450</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conditioned</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{1} Conditioned seedlings were not watered until their midday needle water potential reached an average of -1.8 MPa; control seedlings were kept well watered; values are averaged across CO\textsubscript{2} levels.

\textsuperscript{2} NS - non significant, statistically different at an alpha=.05 level as determined by a t-test.
Table 2. Mean photosynthesis of 32-week-old loblolly pine seedlings as affected by CO₂ concentration and various levels of water stress.

<table>
<thead>
<tr>
<th>Water potential (MPa)</th>
<th>CO₂ level</th>
<th>Photosynthesis</th>
<th>CO₂ level</th>
<th>Photosynthesis</th>
<th>CO₂ level</th>
<th>Photosynthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;-1.0</td>
<td>(ppm±SD)</td>
<td>(umoles CO₂ g⁻¹ s⁻¹)</td>
<td>(ppm±SD)</td>
<td>(umoles CO₂ g⁻¹ s⁻¹)</td>
<td>(ppm±SD)</td>
<td>(umoles CO₂ g⁻¹ s⁻¹)</td>
</tr>
<tr>
<td></td>
<td>205±4</td>
<td>0.0203 A²</td>
<td>219±3</td>
<td>0.0042 A</td>
<td>210±2</td>
<td>-0.00320 A</td>
</tr>
<tr>
<td></td>
<td>335±4</td>
<td>0.0517 B</td>
<td>347±3</td>
<td>0.0189 A</td>
<td>355±4</td>
<td>0.00098 B</td>
</tr>
<tr>
<td></td>
<td>467±6</td>
<td>0.0743 C</td>
<td>498±8</td>
<td>0.0419 B</td>
<td>513±3</td>
<td>0.00430 C</td>
</tr>
<tr>
<td></td>
<td>580±12</td>
<td>0.0988 D</td>
<td>621±9</td>
<td>0.0634 C</td>
<td>649±6</td>
<td>0.00750 D</td>
</tr>
<tr>
<td></td>
<td>729±15</td>
<td>0.1214 E</td>
<td>785±1</td>
<td>0.0749 D</td>
<td>808±4</td>
<td>0.01091 E</td>
</tr>
</tbody>
</table>

1. Values are averaged across water stress conditioning treatments.

2. Means within a column followed by a different letter differ significantly as determined by a Duncan's Multiple range test.
at 335 ppm CO₂ (Table 2). An external CO₂ concentration of 808 ppm should be well above that required to create an internal CO₂ of 330 ppm (ambient external levels), thereby eliminating any stomatal limitation to Pn. This suggests that there is significant non-stomatal limitations (i.e. increased mesophyll resistance) occurring in water stressed loblolly pine.

**Thylakoid Activity of Loblolly Pine**

No significant differences in the thylakoid activity were detected due to the conditioning treatment or as seedling W.P. decreased. The low rates on the first day could be due to the room temperature of the freshly prepared isolation media (Table 3). Normally thylakoids were isolated into solutions held at 4 °C, but on this day freshly prepared solutions had not yet been adequately chilled.

**Osmotic Potentials of Loblolly Pine.**

Water stress conditioned seedlings, at the end of the conditioning period, had significantly lower osmotic potentials (18% more negative) than those of controls (-1.9 and -1.6 MPa respectively ).
Table 3. Uncoupled whole chain electron transport activity from thylakoids of 32-week-old loblolly pine seedlings at different water stress levels and overall means as affected by water stress conditioning.

<table>
<thead>
<tr>
<th>Water Stress Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
</tr>
<tr>
<td>Day</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>mean</td>
</tr>
</tbody>
</table>

1. Conditioned seedlings were not watered until their midday needle water potential reached an average of -1.8 MPa; control seedlings were kept well watered.
Photosynthetic Recovery after Rewatering of Loblolly Pine.

Following the last conditioning drought cycle all seedlings were rewatered and 24 hours later Pn was measured. After the 24 hours of rehydration, Pn of conditioned seedlings were lower than control rates (Table 1 and 4). All seedlings, including controls, were then allowed to dry down (experimental drought) and rewatered again. Both sets of seedlings recovered to approximately the same in absolute values so the final percentage recovery tended to be higher for conditioned seedlings (54%) than for control seedlings (33%) (Table 4). This difference however is not significant. During the experimental drought both sets of seedlings experienced very low W.P. values. During the water stress conditioning period W.P. never fell below -1.0 and -1.8 MPa for the controls and the conditioned seedlings respectively. During the experimental drought though, W.P. fell as low as -2.5 MPa. Both sets were fully rehydrated after 24 hours (following the experimental drought rewatering) and had equal mean W.P. of approximately -0.6 MPa.

Aleppo Pine Gas Exchange

When well watered (W.P. greater than -1.2 MPa), no interaction between CO₂ level and water stress conditioning was observed for Pn. Averaged across all CO₂ levels, conditioned seedlings had slightly higher Pn but
<table>
<thead>
<tr>
<th>Water Stress Treatment</th>
<th>Initial Pn (umoles CO$_2$ g$^{-1}$ s$^{-1}$)</th>
<th>W.P.</th>
<th>Pn recovery</th>
<th>W.P. recovery</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0695 *3</td>
<td>-0.9</td>
<td>0.0256 NS</td>
<td>-0.6</td>
<td>33 NS</td>
</tr>
<tr>
<td>Conditioned</td>
<td>0.0469</td>
<td>-0.7</td>
<td>0.0247</td>
<td>-0.6</td>
<td>54</td>
</tr>
</tbody>
</table>

1. Percentage photosynthetic recovery determined by the formula P.P.R.=$\frac{(W.P. - \text{initial Pn})}{\text{initial Pn}}$ / 100

2. Conditioned seedlings were not watered until their midday needle water potential reached an average of -1.8 MPa; control seedlings were kept well watered.

3. NS - non significant, * - statistically different (p<0.05) as determined by a t-test.
significantly lower Ts and Cs, and consequently higher WUE (44% greater) than the controls (Table 5). Pn rates and WUE averaged across water stress treatments increased approximately 2 times as CO₂ level increased from 315 (ambient) to 776 ppm (a 2.5 times increase) (Table 6).

As seedlings became moderately stressed (W.P. between -1.2 and -1.7 MPa) there was a significant interaction for Pn between CO₂ level and the water stress treatment. Conditioned seedlings had Pn rates significantly greater than control seedlings. In addition, rates for conditioned seedlings increased more as the [CO₂] increased (Table 7). Pn in conditioned seedlings increased 2.4 times whereas the control seedlings only increased 2.1 times as [CO₂] increased from 306 to 772ppm (a 2.5 times increase) (Table 7). When mean W.P. fell below -1.7 MPa only CO₂ level had significant effect on Pn. Pn continued to increase as CO₂ level increased but less dramatically. When seedlings began respiring the CO₂ exchange rates were not included in the means (Table 7). Photosynthetic values are statistically non-significantly different from zero with the exception of these at the highest [CO₂] for the controls and the two highest CO₂ levels for the conditioned seedlings.

Pn of both regimes at high CO₂ concentration (where stomata should not be limiting) and low W.P. (W.P. < -1.7) are approximately 30% of the Pn at high W.P. (W.P. > -1.2) and ambient CO₂ (Tables 6 and 7). The lack of a greater Pn
Table 5. Mean gas exchange of well watered\(^1\) 32-week-old Aleppo pine seedlings as affected by water stress conditioning\(^2\).

<table>
<thead>
<tr>
<th>Water Stress Treatment</th>
<th>Photosynthesis (umoles CO(_2) g(^{-1}) s(^{-1}))</th>
<th>Transpiration (mmoles H(_2)O g(^{-1}) s(^{-1}))</th>
<th>Conductance (cm s(^{-1}))</th>
<th>WUE (umoles CO(_2) / mmoles H(_2)O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0453 NS(^3)</td>
<td>0.0108 *</td>
<td>1.327 *</td>
<td>4.760 **</td>
</tr>
<tr>
<td>Conditioned</td>
<td>0.0468</td>
<td>0.0072</td>
<td>0.994</td>
<td>6.852</td>
</tr>
</tbody>
</table>

1. Values are for seedlings with water potentials greater than -1.2 MPa and averaged across all CO\(_2\) levels.

2. Conditioned seedlings were not watered until their midday needle water potential, reached an average of -2.2 MPa; control seedlings were kept well watered.

3. NS - non-significant, * ** - statistically different, (p=.05) and (p=.1) respectively, as determined by a t-test.
Table 6. Mean gas exchange of well watered\(^1\) 32-week-old Aleppo pine seedlings as affected by CO\(_2\) concentration.

<table>
<thead>
<tr>
<th>CO(_2) level (ppm±SD)</th>
<th>Photosynthesis (umoles CO(_2) g(^{-1}) s(^{-1}))</th>
<th>WUE (umoles CO(_2) / mmoles H(_2)O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>189±4</td>
<td>0.016 A(^2)</td>
<td>1.812 A</td>
</tr>
<tr>
<td>315±3</td>
<td>0.034 A B</td>
<td>3.957 B</td>
</tr>
<tr>
<td>476±6</td>
<td>0.051 B C</td>
<td>6.186 C</td>
</tr>
<tr>
<td>613±9</td>
<td>0.061 C</td>
<td>7.713 C D</td>
</tr>
<tr>
<td>776±10</td>
<td>0.066 C</td>
<td>7.948 D</td>
</tr>
</tbody>
</table>

1. Values are for seedlings with water potentials greater than -1.2 MPa and averaged across conditioning treatments.

2. Means within a column followed by a different letter differ significantly as determined by a Duncan's Multiple Range Test.
Table 7. Photosynthesis of 32-week-old Aleppo pine seedlings as affected by CO₂ concentration and and water stress conditioning when moderately and severely stressed.

<table>
<thead>
<tr>
<th>Water Potential (MPa)</th>
<th>CO₂ level (ppm±SD)</th>
<th>Photosynthesis (umoles CO₂ g⁻¹ s⁻¹)</th>
<th>CO₂ level</th>
<th>Photosynthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>conditioned</td>
<td></td>
<td>controls</td>
</tr>
<tr>
<td>-1.2 to -1.7</td>
<td>193±5</td>
<td>0.0126 A⁴ NS⁵</td>
<td>0.0175 A</td>
<td>203±7</td>
</tr>
<tr>
<td></td>
<td>306±2</td>
<td>0.0227 B **</td>
<td>0.0334 B</td>
<td>347±6</td>
</tr>
<tr>
<td></td>
<td>474±10</td>
<td>0.0391 C *</td>
<td>0.0542 C</td>
<td>503±15</td>
</tr>
<tr>
<td></td>
<td>597±11</td>
<td>0.0467 D *</td>
<td>0.0656 D</td>
<td>631±10</td>
</tr>
<tr>
<td></td>
<td>772±14</td>
<td>0.0485 E *</td>
<td>0.0793 E</td>
<td>785±8</td>
</tr>
</tbody>
</table>

1. Conditioned seedlings were not watered until their midday needle water potential, reached an average of -2.2 MPa; control seedlings were kept well watered.
2. Seedlings with water potentials between -1.2 and -1.7 MPa.
3. Seedlings with water potentials less than -1.7 MPa.
4. Means within a column followed by a different letter differ significantly as determined by a Duncan's Multiple Range Test.
5. Ns - non-significant, *, ** significantly different means (p=.05 and .1 respectively) as determined by Least Square Means test.
recovery indicates that significant non-stomatal limitations are occurring. This is similar to the results obtained with loblolly pine.

Thylakoid Activity of Aleppo Pine

No significant decline in thylakoid activity was detected in the case of Aleppo pine seedlings. However in contrast to loblolly pine, the mean activity value of the controls (averaged across all water potentials) is more than double the rate of conditioned seedlings (Table 8).

Osmotic Potentials of Aleppo Pine.

Osmotic potentials of conditioned seedlings at the end of the treatment period were significantly lower (more negative) than those of control seedlings (−2.6 and −2.2 MPa respectively). Aleppo pine seedlings, though, exhibited a more pronounced osmotic adjustment than loblolly seedlings did (a 24% decrease in osmotic potential versus 18% in loblolly seedlings).

Chlorophyll Content of Aleppo Pine.

Water stress conditioned Aleppo pine seedlings had consistently higher total chlorophyll content per dry weight than controls. However, differences were only significant at day one following the conditioning treatment. The lack
Table 8. Uncoupled whole chain electron transport activity of thylakoids from 32 week-old-Aleppo pine seedlings at different water stress levels and overall means as affected by water stress conditioning.

<table>
<thead>
<tr>
<th>Water Stress Treatment</th>
<th>Control</th>
<th>Conditioned</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W.P.</td>
<td>activity</td>
</tr>
<tr>
<td>Day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(MPa)</td>
<td>(umoles O₂ mg⁻¹ Chl h⁻¹)</td>
<td>(MPa)</td>
</tr>
<tr>
<td>1</td>
<td>-0.8</td>
<td>224.8</td>
</tr>
<tr>
<td>3</td>
<td>-2.3</td>
<td>226.7</td>
</tr>
<tr>
<td>5</td>
<td>-3.1</td>
<td>253.6</td>
</tr>
<tr>
<td>7</td>
<td>-3.4</td>
<td>217.4</td>
</tr>
</tbody>
</table>

mean=230.6 *²               mean=116.8

1. Conditioned seedlings were not watered until their midday needle water potential reached an average of -2.2 MPa; control seedlings were kept well watered.

2. * Means statistically different as determined by a t-test (p=.05)
of significance in later days is likely due to the slight drop in chlorophyll content in conditioned seedlings. Chlorophyll content in control seedling did not decline during the drought cycle (Table 9).

Mesophyll Resistances and Gas Phase Limitations of Aleppo Pine.

Mesophyl resistances of Aleppo pine seedlings following the water stress conditioning treatments were determined using CO₂ curves. The LICOR portable photosynthesis system gave meaningful internal CO₂ values for well watered seedlings and therefore mesophyll resistances were calculated only for this W.P. range.

Aleppo pine conditioned seedlings had lower mesophyll resistance than control seedlings but differences were not statistically significant. Control seedlings did however have significantly lower In (Table 10).

Photosynthetic Recovery after Rewatering of Aleppo Pine.

With Aleppo pine, both conditioning regimes initially had similar photosynthetic rates. However, conditioned seedlings following the final stress cycle recovered to a higher rate than control seedlings. Therefore the percentage recovery is higher for conditioned (90%) as compared to control seedlings (63%) (Table 11). This difference though is not significant.
Table 9. Aleppo pine seedling chlorophyll contents of both conditioning\(^1\) treatments during the final drought cycle.

<table>
<thead>
<tr>
<th>Water Stress</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Day</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(ug Chl/g leaf dry weight)</td>
</tr>
<tr>
<td>1</td>
<td>1016.5 *(^2)</td>
<td>1229.4</td>
</tr>
<tr>
<td>3</td>
<td>1012.2 NS</td>
<td>1174.2</td>
</tr>
<tr>
<td>6</td>
<td>1021.0 NS</td>
<td>1167.9</td>
</tr>
</tbody>
</table>

\(^1\)Conditioned seedlings were not watered until their midday needle water potential reached an average of -2.2 MPa; control seedlings were kept well watered.

\(^2\) NS - non-significant, * - statistically different means (p=.05) as determined by a t-test.
Table 10. Mesophyll resistances and gas phase limitations of Aleppo pine seedlings when well watered\(^1\) as affected by water stress conditioning\(^2\).

<table>
<thead>
<tr>
<th>Water Stress Treatment</th>
<th>Mesophyll Resistance</th>
<th>Gas Phase Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( g s/ moles )</td>
<td>%</td>
</tr>
<tr>
<td>Control</td>
<td>4935 A(^3)</td>
<td>38 A</td>
</tr>
<tr>
<td>Conditioned</td>
<td>4438 A</td>
<td>48 B</td>
</tr>
</tbody>
</table>

1. Values are for seedlings with water potentials greater than -1.2 MPa.

2. Aleppo pine conditioned seedlings were not watered until their midday needle water potential reached an average of -2.2 MPa; control seedlings were well watered.

3. Means inside a column with different letters are statistically different as determined with a t-test.
Table 11. Photosynthetic rates of 32-week-old Aleppo pine seedlings at the beginning of the experiment (initial Pn), after rewatering (recovery Pn) and percentage photosynthetic recovery at ambient CO₂ levels as influenced by water stress conditioning.

<table>
<thead>
<tr>
<th>Water Stress Treatment</th>
<th>Initial Pn (umoles CO₂ g⁻¹ s⁻¹)</th>
<th>W.P. MPa</th>
<th>Recovery Pn (umoles CO₂ g⁻¹ s⁻¹)</th>
<th>W.P. MPa</th>
<th>Pn recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0335 NS²</td>
<td>-0.9</td>
<td>0.0197 NS</td>
<td>-1.2</td>
<td>63 NS</td>
</tr>
<tr>
<td>Conditioned</td>
<td>0.0321</td>
<td>-1.0</td>
<td>0.0259</td>
<td>-1.2</td>
<td>90</td>
</tr>
</tbody>
</table>

1. Percentage photosynthetic recovery determined by the formula
P.P.R.=[1-((initial Ps - recovery Ps) / initial Ps)] / 100

2. Conditioned seedlings were not watered until their midday needle water potential reached an average of -2.2 MPa; control seedlings were kept well watered.

3. NS - non significantly different means as determined by a t-test.
As with loblolly pine, both Aleppo pine treatments experienced very low W.P. values. During the water stress conditioning period W.P. never fell below -1.0 and -2.1 MPa for the controls and the conditioned seedlings respectively. During the experimental drought though, W.P. fell as low as -3.5 MPa. Both sets were fully rehydrated after 24 hours (following the experimental drought rewatering ) and had equal mean W.P. of approximately -1.2 MPa.
DISCUSSION

Gas Exchange

One day after the beginning of the experimental drought (one day after the last conditioning cycle), Pn of loblolly control seedlings was significantly higher than conditioned seedlings (Table 1). Apparently, the conditioned seedlings did not recover fully or the photosynthetic potential was no longer as high as of the previously unstressed seedlings. This is in contrast to Seiler and Johnson (1985, 1988) who found recovery of Pn rates 1 day after rewatering in loblolly pine. Similarly, prestressed red spruce seedlings were found to have rates as high as controls one day after rewatering (Seiler and Cazell 1990). Mathews and Boyer (1984) found that prestressed sunflower leaves exhibited the same Pn rates as control leaves showing no Pn advantage at this level of water stress. In contrast to loblolly pine, Pn of Aleppo pine conditioned control seedlings (averaged across all [CO2]) recovered to control rates one day after rewatering (Table 5). At the same time, Ts in the conditioned Aleppo pine seedlings was lower, resulting in significantly higher WUE compared to that of the control seedlings. This is at least partially due to the lower stomatal conductance of conditioned seedlings.

Despite this lower stomatal conductance, conditioned Aleppo pine seedlings had equally high Pn rates. Gas phase limitations were higher for the conditioned
seedlings indicating a lesser percentage of Rm to the total resistance. Rm calculated using the Pn:Ci curves tended to be lower (but not significantly different) in conditioned Aleppo pine seedlings (Table 10).

No interaction was observed between [CO₂] and water stress conditioning treatments; meaning that the increase in external [CO₂] had a parallel effect on Pn in both treatments.

As W.P. fell between -1.0 to -1.5 MPa, Pn of loblolly pine seedlings declined (from well watered rates) much more at low [CO₂], than at higher [CO₂] (Table 2). This indicates a greater percentage participation of stomatal limitation to the total limitation to Pn as compared to the mesophyll limitation. This may in turn indicate that the CO₂ fixation mechanism is not the primary limiting factor in the photosynthetic decline as W.P. begins to fall, but rather stomatal closure. Conditioned seedling Pn (averaged across all [CO₂] levels) declined much less and was significantly higher than control seedling Pn (Table 1). Matthews and Boyer (1984) found that water stress acclimated sunflower leaves, at W.P. of -1.9 MPa, photosynthesized at 40% of the rate prior to withholding water while controls had dropped to 12%. Seiler and Johnson (1985) found that conditioned loblolly pine seedlings photosynthesised twice as much as controls did when W.P. fell for both treatments to -1.6 MPa. This difference continued at lower W.P. but to
a lesser extent.

At W.P. between -1.2 and -1.7 MPa, conditioned Aleppo pine seedlings also had significantly higher Pn than controls (Table 7). This can be attributed to the fact that for conditioned seedlings total resistance increased only slightly as W.P. decreased while for controls resistances increased much more and consequently Pn was reduced proportionally more. The conditioned seedlings were most probably able to maintain stomatal opening and/or delay increases in non-stomatal limitations longer. The values of lg (measured only when well watered) indicate a much lower participation of Rs to the total resistance to Pn than indicated by the values of Melzak et al. (1985). Their experimental material, however, was much younger (one-month-old Aleppo pine seedlings) than that used in this study (ten-month-old). The same researchers also found that one-month-old Aleppo pine seedlings photosynthesised and transpired at a constant level till W.P. reached -0.8 MPa.

Osmotic Adjustment

One of the factors likely contributing to the prolonged Pn capacity of the conditioned seedlings is their osmotic adjustment. This was more clear with the Aleppo pine seedlings since the osmotic potential difference between control and conditioned seedling was 0.4 MPa while for loblolly pine seedlings it was only 0.3 MPa. This
could be due to the fact that Aleppo pine conditioned seedlings were exposed to lower water potentials during the conditioning period. Downton (1983) found that leaves of grapevine (Vitis vinifera L.) previously exposed to water stress, exhibited more negative osmotic potential of 0.5 MPa as they compare to control leaves. Mathews and Boyer (1984) found that sunflowers exhibited a 0.3 to 0.4 MPa shift in the response of photosynthesis to decreasing water potentials. Buxton et al. (1985) found that osmotic potentials decreased approximately -0.3 MPa when seedlings of Picea glauca, P. mariana, and Pinus banksiana were exposed to a mild water stress caused by -0.4 MPa solution of polyethylene glycol. Accumulation of osmotically active substances help the plant to maintain its cytoplasmic turgor and hence cell division and growth in addition to prolonged stomatal opening (Turner and Jones 1980).

Osmotic adjustment may also help in the maintenance of chloroplastic turgor (despite cell water losses) and consequently allow the continuation of Pn over a broader spectrum of water potentials (Sen Gupta and Berkowitz 1988) and for protection against photoinhibition (Downton 1983). Downton determined the chlorophyll a fluorescence yield from water stressed leaves and concluded that water stress results in a blockade of electron flow on the water oxidation side of photosystem II. The same researcher proposed that maintenance of turgor pressure associated with
osmotic adjustment prevents damage to photosystem II.

When W.P. fell below -1.5 MPa, for loblolly pine seedlings or -1.7 MPa for Aleppo pine seedlings, photosynthetic rates became very low in both conditioning treatments and many of them were not significantly different from zero.

**Comparison of Photosynthetic Declines**

Estimates of mesophyll resistance can be obtained by allowing the seedlings to photosynthesize at high [CO$_2$] (2.5 times higher than ambient). In this case, stomatal limitation to CO$_2$ diffusion is gradually surpassed till $c_i = c_a$ (330 ppm) after which there is no stomatal limitation to Pn. External [CO$_2$] of over 600 ppm should easily create a minimum internal [CO$_2$] of 350 ppm as can be deduced by the data provided by von Caemmerer and Farquhar (1981) and Matthews and Boyer (1984).

Pn of water stressed seedlings from both species, at high external [CO$_2$], did not recover to well watered ambient external [CO$_2$] rates (Tables 2, 6 and 7). This recovery was not to the extent expected if only stomatal resistances were increasing and mesophyll limitations had remained the same during the drying cycle.

Boyer (1971) in a similar way found that in well watered sunflowers, doubling the CO$_2$ gradient led to an increase in Pn but not to the extent expected if only [CO$_2$] limited Pn.
The same researcher also found that when W.P. fell between -1.1 and -1.2 MPa the response of Pn to increased [CO₂] was almost negligible.

A linear relationship between stomatal conductance and net photosynthesis has been demonstrated for Zea mays L., Gossypium hirsutum L., Phaseolus vulgaris L., and Eucalyptus panniflora Sieber ex A. Spreng (Wong et al. 1985a, 1985b). Although the stomata are closely coupled to the photosynthetic system and impose a significant limitation to net Pn as W.P. decrease, during a drying cycle, the rate of CO₂ diffusion does not appear small enough to control the photosynthetic rate for P. taeda (Teskey at al. 1986).

The same likely holds true for the Aleppo pine seedlings, but dried seedlings at high CO₂ recover to a greater percentage than loblolly pine do indicating the possibility of lesser damage on the CO₂ fixation mechanism as a result of water stress.

Mesophyll resistance has been found to increase as water potential falls in numerous studies. Beadle et al. (1981) found that the calculated mesophyll conductance of current year shoots of Sitka spruce (Picea sitchensis (Bong.) Carr.) declined as W.P. declined and reached zero within the same range of water potentials as did net photosynthesis. Mohanty and Boyer (1976) found that increases to mesophyll resistance during water stress are not caused by a decrease
in chlorophyll amount but rather by a reduced ability of the chlorophyll membrane complex to convert absorbed radiation to chemical energy (reduced quantum yield).

Vu and Yelenosky (1988) found decreases at both activation and total activity of Rubisco extracted from water stressed leaves of "Valencia" Orange (Citrus sinensis [L.] osbeck). The activity of the above enzyme is well correlated with mesophyll conductance. So decreases at the activity during water stress are expected to be accompanied by increases in mesophyll resistance.

Bjorkman and Powles (1984) working with Nerium oleander found that mesophyll resistance increased during water stress. These increases were accompanied by phenomena similar to photoinhibition.

On the other hand though, Downton et al. (1988) found that non-uniform stomatal closure can account for the apparent non-stomatal inhibition of photosynthesis in water stressed grapevine, oleander and red-flowering gum (Eucalyptus ficifolia F. Muell). This argument does not seem as plausible in the context of the conifer needle morphology. In conifers, stomata are aligned in lines along the needle and are not spread all across its surface. Besides, the small internal needle space may allow for better exchange and diffusion of gases as compared to a broad leaf.
Thylakoid Activity

Decreased photosynthetic rates and likely increases in mesophyll resistance (as they are indirectly determined in this study) as W.P. decreased, seem not to be accompanied by reductions of the total uncoupled electron transport rate as indicated by our data with both species (Tables 3 and 8). Uncoupled thylakoid activity measured as umoles O₂ consumed mg⁻¹chl h⁻¹ did not decline as water stress increased. This is in agreement with Sharkey and Badger (1982) findings that CO₂ dependant O₂ evolution (whole chain coupled electron transport) was very robust. Instead they found that photophosphorylation (but not total ATP) was decreasing as W.P. decreased. Contrary to the above, Bjorkman and Powels (1984) working with Nerium oleander found that water stress treatment preferentially inhibited the PS II-driven electron transport. Berkowitz and Gibbs (1983) argue that stromal acidification which occurs during the stromal osmotic adjustment is responsible for the reduction of photosynthetic capacity by depressing the activity of stromal enzymes which are involved in the photosynthetic carbon reduction cycle. Sen Gupta and Berkowitz (1988) believe that it is the maintenance of stromal volume which allows continued Pn rate during cell water loss.

Both of the above effects are expressed at the level of whole chloroplast in situ. In this experiment only
uncoupled thylakoids were isolated and consequently these effects cannot be expressed. The regulatory contribution of photophosphorylation on the electron transport rate cannot be assessed under the experimental conditions used here. An additional reason for the non-detection of any e⁻ rate reduction might be that thylakoid isolation and reaction media were not prepared isoosmotic to the needle water potentials. Perhaps thylakoid activites were depressed in the seedling with low water potentials but when isolated into the media they quickly recovered.

Conditioned Aleppo pine seedlings thylakoid activity did not decline either but controls activity, averaged across all water potentials was significantly higher (more than double) compared to that of conditioned seedlings. Whole chain uncoupled thylakoid electron transport activities were consistently 20-100% higher in Aleppo pine than they were in loblolly pine. A possible explanation, in addition to the species difference, may be that in the case with Aleppo pine, primary needles were the source of thylakoids whereas in the case of loblolly secondary needles were used.

Loblolly pine seedling thylakoid activities are similar to those found by Alscher et al. (1989) for red spruce (Picea rubens Sarg.).

Aleppo pine conditioned seedlings had Pn rates as high as control seedlings but lower stomatal conductance (Table 5). This again suggests that changes in mesophyll
resistance are not accompanied by parallel changes in thylakoid activity. The hypothesis that "Light reactions" limit photosynthesis under water stress can not be supported by these data. The "dark reactions" seem to be the next possible area for investigation and especially the enzymes that operate the regeneration of Ribulose bisphosphate. Phosphorylation process and membrane conformation during water stress are additional areas of future research.

Chlorophyll Content

Aleppo pine conditioned seedlings at the beginning of the experimental drought had significantly higher chlorophyll content than the controls (20% more). Chlorophyll of conditioned seedlings declined as W.P. fell, but remained consistently higher than the controls (Table 9). On the other hand conditioned seedlings had lower thylakoid activity (on a per chlorophyll basis, 50% less) but also lower (non-significantly) Rm, lower stomatal conductance, and significantly higher stomatal limitations to Pn (all parameters were measured when well watered). A possible explanation for this equally high Pn rate, but lower thylakoid activity in conditioned seedlings is their increased chlorophyll content. The increased CO₂ fixation efficiency based on higher chlorophyll content is supported by the fact that when conditioned seedlings had higher chlorophyll (at the highest W.P. at day 1) they also had
slightly higher Pn (and lower Rm) as compared to controls although they also had lower stomatal conductance. Alberte and Thornber (1977) found that all the chlorophyll lost during water stress could be accounted for by reduction in the lamellar content of the light-harvesting chlorophyll a/b protein which is a rather specific target for water stress. Chlorophyll content is not always found to decline. Mohanty and Boyer (1976) observed only very small reductions in water stressed soybeans. Vu and Yelenosky (1988) observed non significant changes between leaves of control and water stressed Orange trees.

Photosynthetic Recovery

Aleppo pine seedlings of both water conditioning regimes recovered twice as much upon rewatering as loblolly pine did (Tables 4 and 11). Aleppo pine control seedlings had photosynthetic rates half of loblolly control seedlings in absolute numbers. This indicates that possibly the CO₂ fixing mechanism of the primary needles of Aleppo is better adapted to water stress and undergoes less percentage damage at lower W.P. than the secondary needles of loblolly or that Aleppo pine can recover more quickly.

In general Aleppo pine seedlings, bearing primary needles, seem to possess photosynthetic mechanism less sensitive to water stress than loblolly pine seedlings with secondary needles.
CONCLUSIONS

As water potential decreased during an experimentally imposed drought, photosynthesis (Pn) of Aleppo and loblolly pine seedlings decreased in both the control (previously kept well watered) and water stress conditioned (previously exposed to water stress) regimes. This was due to increases in mesophyll resistance (Rm) (as measured indirectly by raising the level of ambient CO₂). However, in both species, conditioned seedlings maintained Pn to lower water potentials. Pn of Aleppo pine conditioned seedlings, after 24 hours of rehydration (beginning of drought cycle), recovered to rates as high as control seedlings despite significantly lower stomatal conductance. This resulted in substantially higher water use efficiency. Loblolly pine conditioned seedlings, however, exhibited significantly lower Pn rates (at the first day of measurements) as compared to controls.

The increases in Rm (during the drought cycle) were not accompanied by reductions in the uncoupled electron transport capacity of isolated thylakoids of both species. Possibly the thylakoid activity in vitro (as measured by our protocol) does not correspond to the activity in vivo or the uncoupled thylakoid activity is actually insensitive to increasing water stress. Photophosphorylation which is the rate limiting step to electron transport under normal
circumstances is not taken into account in this case. The higher chlorophyll content of conditioned Aleppo pine seedlings is partly responsible for their equally high Pn rate (as compared to controls) despite conditioned seedlings having only half the uncoupled thylakoid electron transport capacity.

Conditioned seedlings of both species had lower osmotic potentials than well watered seedlings by the end of the conditioning period. The difference was more pronounced with Aleppo pine seedlings.
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VITA

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