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The Effect of Light Intensity and Osmotic Water Stress on the Water Potential of *Populus tremuloides*

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THE EFFECT OF LIGHT INTENSITY AND OSMOTIC
WATER STRESS ON THE WATER POTENTIAL
OF POPULUS TREMULOIDES

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SUMMARY

The effects of osmotic moisture stress and light intensity on the growth

characteristics and internal water relationships of quaking aspen (Populus

tremuloides Michx.) were investigated using a sand culture technique.

Quaking aspen was selected as the test species because this species is "very

intolerant" to shade and can be propagated easily from root cuttings. Moisture

stress was varied from 0.5 to 14 atm. using various amounts of polyethylene glycol,

average molecular weight 1540, to regulate the osmotic pressure of the nutrient

solutions. Three light intensity levels — low, 900 foot-candles; medium, 1800

foot-candles; and high, 3600 foot-candles — were used. The temperature and relative

humidity were held constant.

The results of this study indicated that the plants grown at low light inten-

sity had plenty of water available for growth. The moisture content of the leaves

and stems of the plants grown at low light intensity was higher than the moisture

content of the plants grown at high light intensity. However, the leaf water

potential values at low light intensity were slightly higher than at high light

intensity. Apparently, the plants' ability to take up water from the nutrient

solutions at low light intensity was not restricted by the water potential values

of the leaves. The data obtained in this study suggested that water potential is

not an important factor which restricts the growth of quaking aspen in the shade

of other trees.

The effect of moisture stress on the growth of the plants appeared to be related

to cell turgor pressure. At each of the three levels of light intensity, cell turgor

pressure decreased and became negative as moisture stress was increased. Thus, the

decrease in the average dry weight of the plants as moisture stress was increased

was associated with reduced turgor pressure.

The data suggested that cell turgor pressure may be involved in quaking aspen's inability to grow in the shade of other trees. The plants grown at low light intensity appeared to cease growth at lower moisture stress than did the plants grown at high light intensity. The calculated cell turgor pressures became negative at a lower level of osmotic moisture stress at low light intensity than at high light intensity. Also, cell turgor pressures were lower at low light intensity than at high light intensity. These observations indicated that the plants grown at high light intensity were able to grow better at higher moisture stress levels than the plants grown at low light intensity.

The data on shoot/root ratios suggested that moisture stress became the factor controlling the growth of the plants at high light intensity. At this light intensity, the ratios decreased as osmotic moisture stress was increased. At medium and low light intensities, however, these ratios showed no definite trend as moisture stress was increased. On the other hand, the amount of light appeared to be the primary factor limiting the growth of the plants at low light intensity. The shoot/root ratios at low light intensity were significantly higher than the ratios at high light intensity, further indicating that the imposed moisture stress did not have an apparent effect on the growth of the plants at low light intensity.

The data obtained from this study suggest that both reduced cell turgor pressure and the assumed factor of reduced photosynthesis may be directly related to quaking aspen's inability to grow and develop in the shade of other trees.

INTRODUCTION

The effects of water deficits on plant growth have been the subject of extensive investigations. These studies, in general, point out that the rates of the various physiological processes control the quantity and quality of plant growth and are closely related to the water balance of plants and cell turgidity.

Tolerance is a term commonly used in silviculture to express the capacity of a tree to grow in the shade of other trees. Therefore, trees classified as intolerant will not develop in the shade as well as tolerant trees. Many explanations have been offered to explain the differences observed between species in their ability to grow under conditions of low light intensity such as found under a forest canopy. One of these explanations is that shade intolerant species do not have the ability to take up adequate amounts of moisture. This viewpoint has never been clarified.

The overall problem of shade tolerance of a species has broad implications, and all cannot be covered by a single research program. This present study was limited, therefore, to an investigation of the effects of light intensity and moisture stress on plant-water relationships. The main objective of the program was to determine whether a shade intolerant tree has the ability to take up adequate amounts of moisture at low light intensity.

HISTORICAL REVIEW

LIGHT INTENSITY

PLANT GROWTH

Tree growth can be influenced by the effects of light intensity on photosynthesis, stomatal opening, and chlorophyll synthesis. The effects of light intensity on cell enlargement and differentiation also influence height growth, leaf size, and structure of leaves and stems (1).

High light intensities favor increased root development and decreased shoot/root ratios. Leaves of plants grown at high light intensities are thicker than leaves grown at low light intensity because high light intensities favor development of long palisade cells, which often form two or three layers. Leaves grown at high light intensity show more stomata, thicker cell walls and cuticle, fewer and larger chloroplasts, and a higher ratio of internal to external leaf surface when compared with similar leaves grown in the shade.

When light intensity is too low, photosynthesis is inadequate to replace the loss of metabolizable substrates due to respiration. At low light intensities, the rate of photosynthesis is proportional to the light intensity. As light intensity increases, the rate of photosynthesis increases and then remains fairly constant. Apparently, some factor other than light, such as available moisture or temperature, becomes limiting, and the rate of photosynthesis remains constant even though light intensity increases. If the limiting factor is increased, then the rate of photosynthesis will again increase with increasing light intensity (2).

Some recent literature has been published concerning the effect of light intensity on the growth of aspen and aspen-poplar hybrids. Gatherum, et al. (3)

studied the effects of clonal material and light intensity on photosynthesis, respiration, and growth of aspen-poplar hybrids. They found that net and gross photosynthesis increased nearly linearly with light intensity from 560 foot-candles up to 3000 to 5000 foot-candles and then decreased slightly or remained constant up to 6000 foot-candles. They also observed differences among clones in net and gross photosynthesis per plant.

Gifford (4) studied root and top growth of aspen cuttings in a controlled environmental chamber. The cuttings were exposed to two light intensities (2000 and 600 foot-candles) at 18°C. His data showed an increase in total average plant dry weight from 4.51 grams at the lower light intensity to 8.08 grams at the higher light intensity. The plants were grown for 50 days. Gifford attributed the growth differences to differing rates of photosynthesis.

"Tolerance" is a term commonly used to express the capacity of a tree to develop and grow in the shade of other trees (5). A tolerant tree grows well in the shade of other trees, and an intolerant one does not. In early forestry practices, tolerance was generally considered to be a light related phenomena. Fricke (6), however, challenged the concept that tolerance was primarily concerned with light conditions in forests. He was convinced that the degree of soil desiccation caused by competition of the roots of the older trees was more important. As a result, two schools of thought developed which differ as to the major cause of growth failure under a forest canopy. One school ascribes importance to the factor of low light intensity, while the other school holds that root competition for soil moisture and the resulting moisture stress are of predominant importance.

At first thought, it seems reasonable to assume that shade tolerant species should be more efficient in carrying on photosynthesis at low light intensities than shade intolerant species. Nevertheless, when careful studies were made to

determine the degree of superiority, very slight differences appeared. These slight differences in photosynthetic rate were not sufficient to account for the difference between the tolerant and intolerant species ($\underline{5}$). These results support the concept that moisture stress and water uptake may be important factors in the ability of a tree to grow under a forest canopy.

INTERNAL WATER DEFICIT

Pierpoint ($\underline{7}$) measured the internal water deficit of seedlings using a plant pressure bomb. He found that red pine twigs growing in the shade had so-called plant moisture pressure values of -13 atm. to -16 atm.; whereas twigs growing in direct sunlight had values of -19 atm. to -20 atm.

Pierpoint's results suggest that leaves growing in the shade should have a higher (numerically lower, but negative) water potential than leaves growing in direct sunlight. However, he did not determine the complete internal water balance.

Strothmann ($\underline{8}$) recently studied the influence of two light intensity levels and three soil moisture levels on the growth of red pine seedlings. Soil moisture levels used varied from field capacity (less than 1 atm.) to a soil moisture stress of about 5.0 atm. Thus, the seedlings were not subjected to extreme moisture stress. (The so-called permanent wilting point is 15.0 atm.) Light intensity levels used were 82 and 8% of full sunlight, which corresponds to about 4500 foot-candles and 440 foot-candles.

Strothmann found that the elimination of either form of competition improved all aspects of growth. Removal of competition for light invariably produced a larger growth response than did removal of competition for moisture. A decided interdependence or interaction between the two factors was evident in all facets of growth observed. Unfortunately, Strothmann did not measure the internal water

relationships of the plants. Thus, he could not determine if the water potential values of the leaves were an important factor for the growth of the plants he studied.

PLANT-WATER RELATIONS

BASIC CONCEPTS

Kramer, et al. (9) and Currier (10) have reviewed the literature concerning the energy status of water in plants. The energy state of water is expressed by the term water potential, Ψ or WP, which is defined as the difference between the chemical potential of the water in the system and that of pure free water at the same temperature. This is expressed by the relation

$$\Psi(\text{or WP}) = \frac{\mu_w - \mu_w^o}{\overline{V}_w} \qquad (1)$$

where

μ_w = net chemical potential of water in the system,

μ_w^o = chemical potential of pure, free water, and

$\overline{V}_w$ = partial molal volume of water.

The units of water potential are conveniently expressed as bars or atmospheres. (The term $\overline{V}_w$ converts from energy units, erg/mole, to pressure units, dyne/cm.^{2} or atm.) The term water potential replaces the commonly used expression diffusion pressure deficit (DPD). Water potential values will be negative. The expression

Diffusion Pressure Deficit = Osmotic Pressure-Turgor Pressure

or

$$\text{DPD} = \text{OP-TP} \qquad (2)$$

is replaced by

$$WP = -(OP-TP) \qquad (3)$$

Cells absorb water from neighboring cells or from the growth medium when the water potential is less than that of the surroundings and lose water when the water potential is greater than that of the surroundings.

The relationships involving relative cell volume, turgor pressure, osmotic pressure, and water potential from incipient wilting to full turgidity are shown in Fig. 1 (11, 12). Water potential is shown in the absolute sense ($|WP|$) for convenience and to avoid confusion about the negative sign. The decrease in osmotic pressure results from dilution of the cell sap by water absorption. As water diffuses into the cell, the volume increases and turgor pressure develops. The cell wall resists extension, and finally the turgor pressure becomes equal to the osmotic pressure of the cell sap. When this occurs, the water potential becomes zero and equilibrium is established. Water potential can be regarded as a measure of the driving force which causes water to move into any system or from one part of the system to another. Wilson (13) gives a further analysis of the components of water potential.
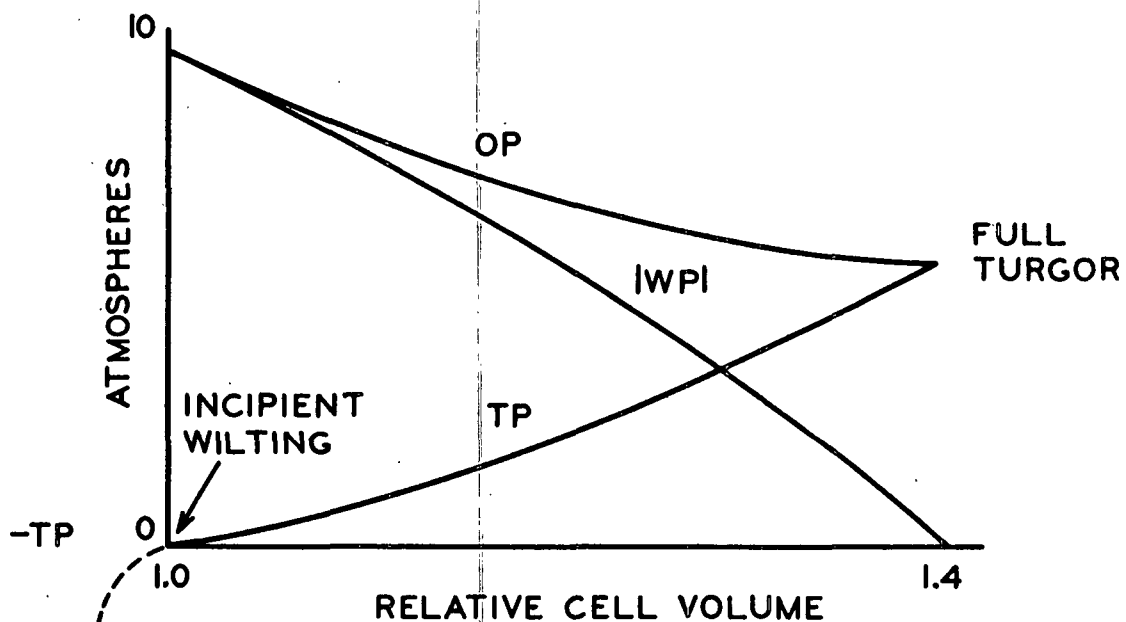
Figure 1. Osmotic Relationships of Plant Cells of <u>Nitella</u>

WATER STRESS AND PLANT GROWTH

The most important aspect of plant-water relations is the internal water balance, because the degree of water stress controls the physiological and biochemical processes and conditions which determine the quantity and quality of plant growth.

Articles by plant physiologists have frequently emphasized the close relationship between water stress and plant growth. Several comprehensive review articles and monographs concerning plant-water relationships are available. These reports include studies by Slayter (14), Steward (15), Kramer (16-18), Kramer and Kozlowski (1), Kozlowski (19), and Pierre, et al. (20). The book edited by Hagan, et al. (21) is an excellent reference source and comprehensive review.

Factors Influencing Osmotic Pressure of Plant Cells

Meyer and Anderson (22) have stated that changes in the osmotic pressure values of plant cells are brought about by changes in either the water content or the solute content of the individual cells. The rate of photosynthesis is believed to be an important factor in determining the osmotic pressure of plant cells, especially of leaf tissue. Increased photosynthetic activity results in increases in the solute concentration of the cell sap and is believed to be responsible for the observation that leaves in the sun generally have a higher osmotic pressure than leaves in the shade.

When water becomes scarce, the growth rate decreases. The reduction in available moisture initiates a shift in the starch-soluble carbohydrate equilibrium in the leaves toward the side of the soluble carbohydrates (22, 23). This increase in the amount of soluble carbohydrates results in an increased osmotic pressure of the cells.

Turgor Pressure

One way in which water deficits decrease growth is to decrease cell turgor. Turgor pressure is considered to be a primary cause in the process of cell enlargement. However, the exact mechanism by which turgor pressure affects cell enlargement is not known (21). Ordin (24) found that turgor pressure affected both cell wall metabolism and cell elongation. He suggested that some aspect of cellulose synthesis may be involved in the elongation response of cells to turgor pressure.

Apparently, sufficient turgor pressure must exist to keep the cytoplasmic membrane pressed firmly against the cell wall if deposition of new wall material and cell enlargement is to continue. Slayter (25) stated that plant growth will nearly cease when turgor pressure equals zero. He found that turgor pressure of plants could become negative as moisture stress was increased. Negative turgor pressure develops as dehydration proceeds beyond the point where turgor pressure equals zero.

Excessive dehydration of a plant should be reflected in a permanently wilted appearance of the leaves. Thoday (26) observed an inward folding of cell walls during severe wilting and attributed this to the development of a negative turgor pressure. However, rigid leaves may not always show wilting until a large stress is imposed. Slayter (25) found that the growth of cotton plants nearly ceased when turgor pressure equaled zero. At this point, the total moisture stress was approximately 34 atm. Ultimate wilting did not occur, however, until the total moisture stress was nearly 50 atm.

Internal Water Balance Adjustment

Plants are capable of adjusting their internal osmotic pressure in response to changes in the external osmotic pressure of the root medium. An increase in internal osmotic pressure can develop as a result of the absorption of osmotically active solutes from the substrate, metabolic changes in materials already in the

plant, or reduction of internal water content (27-31). An excellent discussion and literature review are contained in the book edited by Hagan, et al. (21).

The contribution of absorbed solutes to the increase in internal osmotic pressure depends upon the nature of the solutes used to control the moisture stress of the external solution surrounding the roots. Solutes that are readily absorbed by the plant, such as sucrose, NaCl, and KNO$_3$ can contribute a considerable amount to the increase in internal osmotic pressure. Other solutes which are not readily absorbed by the plant, such as mannitol and polyethylene glycol, will not contribute significantly to the increase in the internal osmotic pressure. The contribution of tissue dehydration to the increase in internal osmotic pressure also depends upon the nature of the solutes. Slayter (29) found that with sucrose, NaCl, and KNO$_3$ as the osmotic control agents, the contribution of tissue dehydration to the change in internal osmotic pressure was insignificant. On the other hand, with mannitol as the control agent, tissue dehydration accounted for 30-40% of the total change in osmotic pressure. Slayter commented that the remaining 60-70% was probably due to changes of materials already in the plant or an accumulation of other solutes from the solution.

Janes (31) used polyethylene glycol (PEG) (molecular weight 400) to regulate the osmotic pressure of nutrient solutions. Janes' results supported the mechanism of internal osmotic pressure adjustment described by Slayter (29). Of the total osmotic adjustment found by Janes, he attributed about 2% to uptake of polyethylene glycol, 50-75% to changes in the amount of soluble organic substance (such as an increase in soluble carbohydrates), and the remainder to tissue dehydration.

Ruf, et al. (32) used PEG (molecular weight 1540) to study the components of osmotic adjustment of plants to changes in the root medium osmotic pressure. They found that tissue dehydration accounted for about 34% of the total change in cell

sap osmotic pressure. Uptake of polyethylene glycol by the plants contributed less than 7% of the total increase in cell sap OP.

AGENTS FOR CONTROLLING OSMOTIC MOISTURE STRESS

Many different chemicals have been used to regulate the osmotic moisture stress of nutrient solutions for plant growth-water stress studies. Some of the advantages and disadvantages of polyethylene glycol and other agents are discussed below. Polyethylene glycol appears to be the most satisfactory agent for most purposes.

POLYETHYLENE GLYCOL

Janes (31, 33) employed polyethylene glycol of various molecular weights (400, 600, 1000, 1540, and 4000) to control the osmotic pressure of nutrient solutions in growth-moisture stress studies on bean, celery, and tomato plants. He reported that the PEG caused no injury to the plants and was not subject to microbial degradation. Measurable amounts of PEG were not found in the plants at nutrient solution osmotic pressure levels below 5 atm. After nine days in PEG 1540 at a moisture stress level of 14.4 atm., the PEG was 0.1% of the fresh weight of the plants. He considered the amount of PEG in the plants insignificant. Collander (34) found that the rate of penetration of PEG through cell membranes was extremely slow, thus indicating that the amount of PEG taken up by plants should be quite low.

Several workers have reported that PEG solutions exhibited some toxic effects on plants. However, Lagerwerff, et al. (35) found that by purifying the PEG solutions by dialysis, he was able to remove the toxic effects. The toxicity was attributed to the presence of foreign ions that were removed by the dialysis treatment. Both Jackson (36) and Lesham (37) also reported that PEG solutions exhibited

some toxic effects on plants. However, they made no attempt to purify the solutions as suggested by Lagerwerff.

On the other hand, workers have reported that PEG solutions were not toxic to plants. Ruf, et al. (32, 38) utilized PEG (molecular weight 1540) to develop moisture stress in plants grown in nutrient solutions. They reported that PEG was nontoxic to plants at concentrations up to 16 atm. osmotic pressure. Manohar and Heydecker (39) also found no evidence of toxicity, even in the absence of purification by dialysis. Taylor (40) concluded that PEG (molecular weight 200) was superior to mannitol, sucrose, glucose, NaCl, and $CaCl_2$ for plant growth studies at all moisture stress levels. Kramer (41) agreed with Taylor's conclusion that PEG was the most satisfactory agent for regulating osmotic pressures of nutrient solutions.

Wahab and Woolley (42) used PEG (molecular weight 4000) to control moisture availability. They reported that the response of the plants to the PEG treatments was similar to the response of plants subjected to reduced soil moisture.

OTHER AGENTS

Slayter (29) used several agents in studying the nature of water uptake by regulating the osmotic pressure of the root medium. Sucrose and mannitol were selected as organic substrates, and KNO_3 and NaCl were selected as inorganic substrates. Evidence suggested that sucrose was absorbed by plant roots and was readily metabolized in the plant, whereas mannitol was absorbed very slowly and only in small amounts by the plant. KNO_3 and NaCl were also readily absorbed into the plant. Slayter found that at a given nutrient solution osmotic pressure, the decrease in transpiration was approximately the same for each solution. Thus, the use of sucrose, mannitol, KNO_3, or NaCl to create an osmotic water stress resulted in decreased transpiration of approximately the same magnitude. These results provide further

evidence that osmotic effects are controlling, and that other factors such as mechanical interference with water transport are negligible for the systems studies.

Boyer (30) used NaCl to create an osmotic moisture stress for studies on cotton plants. The addition of NaCl to the nutrient medium increased the ratio of fresh weight to dry weight. Thus, moisture contents were higher in plants grown in the presence of NaCl. The increased moisture contents were probably associated with an increase in the osmotic pressure of the plant tissue caused by absorption of the NaCl. Such an increase in the osmotic pressure of the plant would cause a corresponding decrease in water potential, and thus, more water would be taken up by the plant.

Thimann and coworkers (43) studied the penetration of ^{14}C-labeled mannitol into potato disks. They found no evidence of penetration into the cells by mannitol. Although a small amount of mannitol entered the free space rapidly, the penetration of mannitol into the interior of the cells was extremely slow. Collander (34) reported that mannitol has a scarcely detectable permeation power of cell membranes.

Jackson (36) stated that mannitol would still seem to be the preferred osmotic agent. Mannitol does not exhibit any toxic effects on root hair growth, nor does it affect the differential permeability of the plasma membrane. Ingelsten (44) used mannitol to control nutrient solution osmotic pressures for growth studies on wheat. He reported that, at concentrations below 0.5M, mannitol did not damage the growth of wheat.

A further discussion of mannitol is presented later in this report in the section entitled Preliminary Experimental Work on page 26.

METHODS OF MEASURING WATER POTENTIAL

Several techniques are available for determining the degree of internal moisture stress in plants. These techniques include both direct and indirect methods of measurement and have been discussed in detail by Kramer and Brix (45) and Barrs (46). Indirect methods include measurements of stomatal aperture, leaf temperature, and transpiration. Such indirect methods, although helpful in many cases, do not provide a quantitative basis for comparing the degree of moisture stress in various kinds of plants.

The direct methods of determining water potential include volumetric and gravimetric techniques, vapor equilibration, electric psychrometers, and determination of a change in density of the solution in which tissue is immersed. Of these techniques the psychrometer and solution density methods are the most applicable to different types of plant tissue and have been widely used.

Measurements made with electric psychrometers are based on determining the rate at which pure water evaporates from a thermocouple junction which has been sealed into a chamber containing the tissue. The water potential of the plant tissue is found by comparing the evaporation rate when tissue is present with that which occurs when the tissue is replaced by solutions of known potential. Barrs (46) presents a detailed discussion of the techniques involved in using the psychrometer method. The advantages of this method include applicability to a wide variety of plant materials as well as to soil. Small samples can be used and preliminary estimation of the water potential is not necessary. Disadvantages of this method include the need for expensive and elaborate equipment and precise control of the experimental conditions. Also, the initial assembly and calibration of the apparatus requires a considerable amount of time.

One method of measuring the change in density of the solution in which samples of plant tissue have been immersed is the dye method, which is discussed in detail in the two references previously mentioned (45, 46). As pointed out in these references, the dye method is sometimes referred to as the Shardakov method (often spelled Schardakow or Chardakov).

The dye method is a solution immersion technique requiring the preparation of two series of test tubes containing sucrose solutions covering a range of osmotic pressures. One series is designated the control solution series and the other the test solution series. Leaf tissue is immersed in each test solution. The test solutions having osmotic pressures lower than the water potential (absolute value) of the leaf tissue will be diluted by water loss from the leaves, whereas the test solutions having osmotic pressures greater than the water potential (absolute value) of the leaf tissue will be concentrated due to water uptake by the leaves. After leaf tissue immersion for a prescribed period of time, each test solution is colored lightly with a small amount of powdered dye such as methylene blue; hence, the name dye method. Density changes in the test solutions are detected by the rise or fall of drops of the colored test solutions which are carefully introduced into the corresponding uncolored control solutions. The water potential value of the tissue being tested is assumed to lie between the osmotic pressure of the solution in which the drop rises and that in which the drop sinks. The only purpose of the dye is to make the drops visible after they are placed into the uncolored solutions. The dye method has certain advantages for many researchers. This technique is simple and easily learned, requires no elaborate or expensive equipment, and can be used in both the laboratory and field. Certain sources of error must be considered, however. Dye method values can change after progressively longer immersion times. Also, contamination of the test solutions by solutes escaping from cut cells or from the surface of leaves can cause errors.

Kramer and Brix (45) compared water potential values of tomato and tobacco leaves obtained using the dye method with the values obtained using an electric psychrometer technique. The values obtained by the two methods agreed quite well and did not differ significantly except at the highest measured water stress in tomato. Apparently, the dye method does give values that agree favorably with values obtained using the electric psychrometer.

The water potential values obtained from the dye method and psychrometer method are, at best, only average values for the tissue tested. As pointed out by Kramer (45), however, there is no method to measure the actual water potential in the protoplasm of plant cells. Furthermore, it is not known how much difference there is between the average water potential and that in the protoplasm.

METHODS OF MEASURING CELL SAP OSMOTIC PRESSURE

Two main procedures have been used to measure vacuolar osmotic pressure. One involves extraction of the cell sap and the other involves measurements on intact cells in more or less undisturbed tissue segments. These procedures have been reviewed by Barrs (46) and by Slayter and Shmueli (47).

The most widely used method involves a determination of the freezing point of extracted cell sap. Although freezing points can be determined with reasonable accuracy, considerable error may be associated with the method of sap extraction. Barrs (46) and Broyer (48) have reviewed the methods of sap extraction and the errors associated with those methods. Sap pressed from living tissue is filtered by the semipermeable plant membranes and is not representative of vacuolar sap. Killing the tissue prior to expressing the sap, however, destroys the semipermeability of the membranes and eliminates possible filtration effects. Tissue may be killed by heating, freezing, or exposure to toxic vapors such as chloroform. All

three methods appear to be equally effective. Barrs concluded from a review of the
literature that possible effects caused by dilution of vacuolar sap by water held
in the cell wall after killing the tissue are negligible.

The conventional technique for determining the freezing point depression of
cell sap employs a Beckman thermometer with its bulb immersed in the sap in a small
tube. The small tube is jacketed by a larger one that is immersed in a freezing
mixture. The cell sap is stirred continuously as the temperature falls and super-
cooling usually occurs. As ice begins to form, the temperature rises to a brief
plateau before declining again. The true freezing point of the expressed cell sap
can then be determined by either of two techniques. One technique involves noting
both the minimum temperature reached and the maximum temperature after ice formation.
The true freezing point may then be calculated by the conventional methods described
by Loomis and Shull (49). The other technique involves plotting the temperature
versus time curve and extrapolating back to zero time from the maximum temperature
reached after ice formation. This latter procedure is described by Bennet-Clark (50).
The first technique is the more common method used. However, the minimum temperature
reached must remain on the scale of the Beckman thermometer in order to be determined.
The second method does not require that the minimum temperature be noted. However,
the extrapolation procedure is somewhat time consuming.

Once the freezing point has been determined, the osmotic pressure of the
expressed cell sap may be calculated using the procedures described by Loomis and
Shull (49).

PRESENTATION OF PROBLEM

The development of water potential in plants grown under varying conditions of light intensity and moisture stress is especially important. Leaf water potential is considered to be the measure of a plant's internal ability to remove moisture from the soil or from nutrient solutions.

During periods of drought shaded leaves are injured more by the water deficit than unshaded leaves (51, 52). This observation has been explained by assuming that shaded leaves cannot develop as low a water potential as unshaded leaves which are carrying on more photosynthesis. The assumption has also been made that plants which will not grow well under low light intensity cannot develop as low a water potential as the same plants grown under a higher light intensity. Such plants would not be able to compete satisfactorily for available moisture. Little work has been done, however, to completely describe the internal water balance of plants grown under different light intensities.

The main objective of this study was to determine if differences exist in a plant's internal ability to remove moisture from nutrient solutions at different levels of light intensity and different conditions of moisture stress. In order to accomplish this objective, a complete description of the plant's internal water balance was necessary. Suitable growth measurements were also necessary in order to compare plant growth and water uptake.

It should be emphasized that the overall problem of "shade tolerance" of a species has broad implications, and all cannot be covered by a single research program. It is hoped, however, that this study will add valuable information to certain aspects of this broad problem.

MATERIALS AND METHODS .

PLANT MATERIAL

TEST SPECIES .

Quaking aspen (<u>Populus tremuloides</u> Michx.) was used as the test material.
The reasons for this choice were that quaking aspen is "very intolerant" to shade
(2), is common to a large portion of the United States, and has increased consider-
ably in economic importance in the last 20 years. Also, quaking aspen can be grown
quite easily in the greenhouse and has a relatively fast growth rate.

PREPARATION OF TEST PLANTS

Plants from the same clone were used. The use of these genetically identical
plants assured a minimum variation between individuals.

The test plants were obtained from root cuttings. Roots were cut into six-
inch lengths, and both ends of each length were sealed with paraffin to prevent
decay. The cuttings were then placed in a sand-vermiculite mixture. Root sprouts
began to develop in about three weeks. When the root sprouts reached about one
inch in height, they were cut from the old root, transplanted into clay dishes,
and allowed to develop roots. At least 144 plants were required for each growth
run. To insure maximum uniformity of the starting materials, several hundred
sprouts were obtained prior to each run. The most uniform plants were then selected
to be transplanted into the growth chamber. At this time, the test plants were
approximately three inches long including the roots.

GROWTH CHAMBER

PHYSICAL DESCRIPTION

An automatic, subirrigated sand culture technique similar to that described by Swan (53) was used. Basically, the system employs glass growth containers containing silica sand which are attached to pressurized carboys containing the nutrient solutions. A time clock activates a valve on a compressed air line which in turn causes the solutions to be pumped into the growth containers every four hours. After five minutes the valve closes and the solution drains back into the carboys. For maximum control of the environmental conditions, the experimental work was carried out in a growth chamber described by Einspahr (54).

LIGHTING ARRANGEMENTS

The lighting in the growth chamber consisted of 36, F96/T 12/VHO CW fluorescent tubes running lengthwise and at each end two, F48/T 12/VHO CW tubes located cross-wise. Supplementary lighting in the red and infrared spectra was provided by 16, 75-watt iodine quartz lamps.

EXPERIMENTAL CONDITIONS

LIGHT INTENSITY

The three different levels of light intensity which were used are low, 900 foot-candles; medium, 1800 foot-candles; and high, 3600 foot-candles. The horizontal variation of light intensity in the growth chamber was $\pm$ 5%. Light intensity was measured at the top of the growth pots using a cosine-corrected photocell.

Light intensity was controlled by regulating the number of fluorescent and incandescent lamps which were turned on. The ratio of incandescent wattage to total

wattage was held constant at 15%. Thus, light quality was constant, and the risk of changing spectral composition was eliminated.

The day length was held constant at 16 hours. Each growth chamber run was conducted at a given light intensity, and thus comparisons of results between light intensities were comparisons between runs. Care was taken to insure that light intensity was the only variable from one run to the next.

TEMPERATURE AND HUMIDITY

The ambient temperature in the growth chamber was held constant at 77-78°F., and the relative humidity was 66-67%.

NUTRIENT SOLUTIONS

Concentration of Nutrients

Olson's (55) combination of required elements which includes the necessary minor elements was used in making up the nutrient solutions. The concentrations of the elements used in this study were modified as suggested by Einspahr (54) to be 60% of the levels used by Olson. The concentrations of the major elements in the nutrient solutions are given in Table I. These nutrient solutions had a pH range of 4.2 to 4.4 at the beginning of each growth run and were made up using deionized water.

TABLE I

CONCENTRATION OF MAJOR ELEMENTS
IN NUTRIENT SOLUTIONS

Nutrient	Standard Solution, p.p.m.
N	158
P	65
K	93
Ca	46
Mg	21

Concentration of Polyethylene Glycol

Polyethylene glycol (Carbowax 1540) was used for developing water stress in the plants. The concentrations of PEG required in the nutrient solutions were determined experimentally by freezing point depression techniques and are presented in Table II. The freezing point depression measurements and osmotic pressure determinations are discussed in Appendix I.

TABLE II

CONCENTRATION OF PEG 1540 IN
NUTRIENT SOLUTIONS

Osmotic Pressure, 20°C. atm.	PEG Concn., g./l.
0.5	0..0.
3.0	72.5
5.0	122.5
7.0	162.5
10.0	198.0
15.0	232.5

For each growth chamber run, a standard PEG solution (50% by weight) was pretreated to remove possible foreign ions by running the solution through a Barnstead high purity, mixed-bed, ion-exchange column. This standard solution was then diluted volumetrically to obtain the desired concentrations.

Solution Replacement

Each growth run required 30 days for completion. For the first 14 days, the plants were grown on the standard nutrient solution. This solution was changed on the 14th day, and PEG was added to give the desired moisture stress. On the 25th day of the run, the nutrient solutions including PEG were changed again. Water was added periodically to the bottles containing the nutrient solutions to maintain a constant PEG concentration and uniform height of nutrient solution.

STATISTICAL PROCEDURES

A randomized complete-block design was used in the growth chamber with six treatment (moisture stress) levels and six blocks. Each growth container contained four plants.

Standard analysis of variance and Duncan's multiple range test techniques as described by Steel and Torrie (56) and Hicks (57) were used to analyze all final data. Linear and curvilinear regression techniques were used when necessary as guides for graphing the data (58).

GROWTH DATA

After thirty days of growth, all plants were washed from the growth containers, and the green weight was obtained for the tops and roots. Leaf samples used in determining the osmotic pressure of the expressed cell sap were removed, weighed, and frozen. The roots and the remainder of the tops were then used for moisture content and dry weight determinations. The dry weight of the tops was corrected for the dry weight of the missing leaves by using the moisture content of the tops to calculate the dry weight of the missing leaves. Fresh weight/dry weight and shoot/root ratios were calculated. The growth data were averaged for each growth container prior to the statistical analyses. Thus, the four plants in each container yielded one average value for that container.

OSMOTIC DATA

Leaf water potential values were determined using the dye method. This method is discussed by Kramer and Brix (45) and Barrs (46) and in a detailed mimeographed article by Knipling (59). The dye method is simple, easily learned, and requires no elaborate or expensive equipment. Water potential values obtained using the dye

method provide a reasonable estimate of the average water potential of the leaves tested. A further description of the dye method is given in Appendix I.

Leaf cell sap osmotic pressures were calculated from freezing point depression measurements. Leaves were frozen at -16 to -20°C., allowed to thaw, and pressed in a plant press at 2000 lb./in.^{2} to express the sap. The extrapolation technique described by Bennet-Clark (50) was used to obtain an estimate of the true freezing point of the expressed cell sap samples. Osmotic pressures were calculated by the method described by Loomis and Shull (49). A further description is given in Appendix I. The calculated cell sap osmotic pressures were corrected to a standard temperature, 20°C.

The turgor pressure of the leaf cells was determined indirectly by determining water potential and cell sap osmotic pressure and calculating turgor pressure using the relationship WP = -(OP-TP).

UPTAKE OF POLYETHYLENE GLYCOL

Polyethylene glycol concentration in tissue extract was determined by the turbidimetric techniques described by Hyden (60) and utilized by Ruf, et al. (32) and Janes (31). As a pretreatment, the cell sap samples were deproteinized by the improved method of Somogyi (61).

The procedure involved adding $ZnSO_4$, $Ba(OH)_2$, and $BaCl_2$ to the expressed cell sap samples and centrifuging the resulting mixture. After adding trichloroacetic acid to an aliquot of the supernatant liquid, the resulting turbidity was measured photoelectrically using a Coleman spectrophotometer. Preliminary tests showed that control cell sap samples used as blanks did not form a turbid mixture, thus indicating that the interfering substances had been removed. A calibration curve was determined by adding known amounts of polyethylene glycol to expressed cell sap and measuring the turbidity.

PRELIMINARY EXPERIMENTAL WORK

Several preliminary growth runs were made to determine the necessary techniques. Originally, mannitol was used as the agent to control moisture stress. However, mannitol was found to be unsatisfactory for the current work due to the presence of fungal activity. For treatment times of less than one week, mannitol appears to be satisfactory. In the current work, however, an application time of eleven days was used before changing the nutrient solutions. In this amount of time, there was a considerable growth of fungi on the mannitol in the nutrient solutions. Captan and streptomycin were tried in an attempt to control the fungi, but they were not particularly successful. The streptomycin appeared to possess some phytotoxic properties when added to the nutrient solutions for the entire thirty days of a growth run. Other investigators have tried actidione and mycostatin with limited success (41). Apparently, there is no completely satisfactory method to prevent the growth of fungi on mannitol solutions.

After encountering these problems with mannitol, the decision was made to use polyethylene glycol, thus gaining certain advantages. The problem of the fungi was eliminated, and good methods were available for determining the amount of polyethylene glycol taken up by the plants. It would seem to the author that poly-ethylene glycol is the best agent to date for controlling osmotic moisture stress.

RESULTS AND DISCUSSION ·

Data from growth runs at low, medium, and high light intensity (900, 1800, and 3600 foot-candles, respectively) will be presented. These data include information on growth, moisture contents, and osmotic relationships. Additional data on osmotic adjustment and turgor pressure will be discussed. The original data are presented in Appendix I with the analysis of variance of each set of data.

Duncan's multiple range test (50) was used to determine which of the values presented in the tables in this section were significantly different at the 5% level. The statistical comparisons obtained from Duncan's multiple range test were indicated by letters following the tabulated data. Numerical values in each column followed by the same letter were not significantly different.

GROWTH DATA

The following high light intensity data on ovendry weight, shoot/root ratio, fresh weight/dry weight ratio, and moisture content of tops and roots are a combination of the data from two identical growth runs. The reason for combining these data was that there were several missing values from one of the runs because several plants were damaged during the transplanting operation and had to be discarded. The reliability of the growth data was dependent upon the uniformity of starting materials and the survival rate of the plants in the growth chamber. By combining these data from the two runs at high light intensity, the problem of the missing values was eliminated, and the growth data were strengthened.

The osmotic pressure of expressed cell sap and water potential data were not combined because there were no missing values for these data. Within the limits of this study, the osmotic relationships were dependent upon the imposed moisture

stress rather than the survival of the plants in the growth chamber. Thus, combining the osmotic data was not necessary to strengthen it.

DRY WEIGHT

The relationship between plant growth and osmotic moisture stress at each light intensity level is shown in Fig. 2. The observed decrease in the average dry weight of the plants with increasing moisture stress appeared to be linear. Regression techniques were used to determine the best fit of the line through the data. The regression coefficients and the correlation coefficients are listed in Appendix III. The 95% confidence interval for each experimental point is shown by the vertical line segment on the graph. This same confidence interval applies to each experimental point within a given light intensity.

The relationships shown in Fig. 2 suggest an interaction between light intensity and moisture stress concerning their effect on plant growth. In other words, the response of plant growth to moisture stress depends upon the level of light intensity being used. Strothmann (8) reported a similar interaction. Plant growth decreased more at high light intensity than at either medium or low light intensity. Thus, moisture stress had a greater effect on the plants growing at high light intensity than on the plants growing at either medium or low light intensity.

The average dry weight of the plants at each moisture stress level and each light intensity is given in Table III. At high light intensity, the dry weights at the two highest stress levels were significantly lower than the other dry weights. At medium light intensity, the decrease in the dry weight of the plants was not sufficient to show statistical differences. At low light intensity, the dry weight of the control plants was significantly larger than the plants at the three highest stress levels.
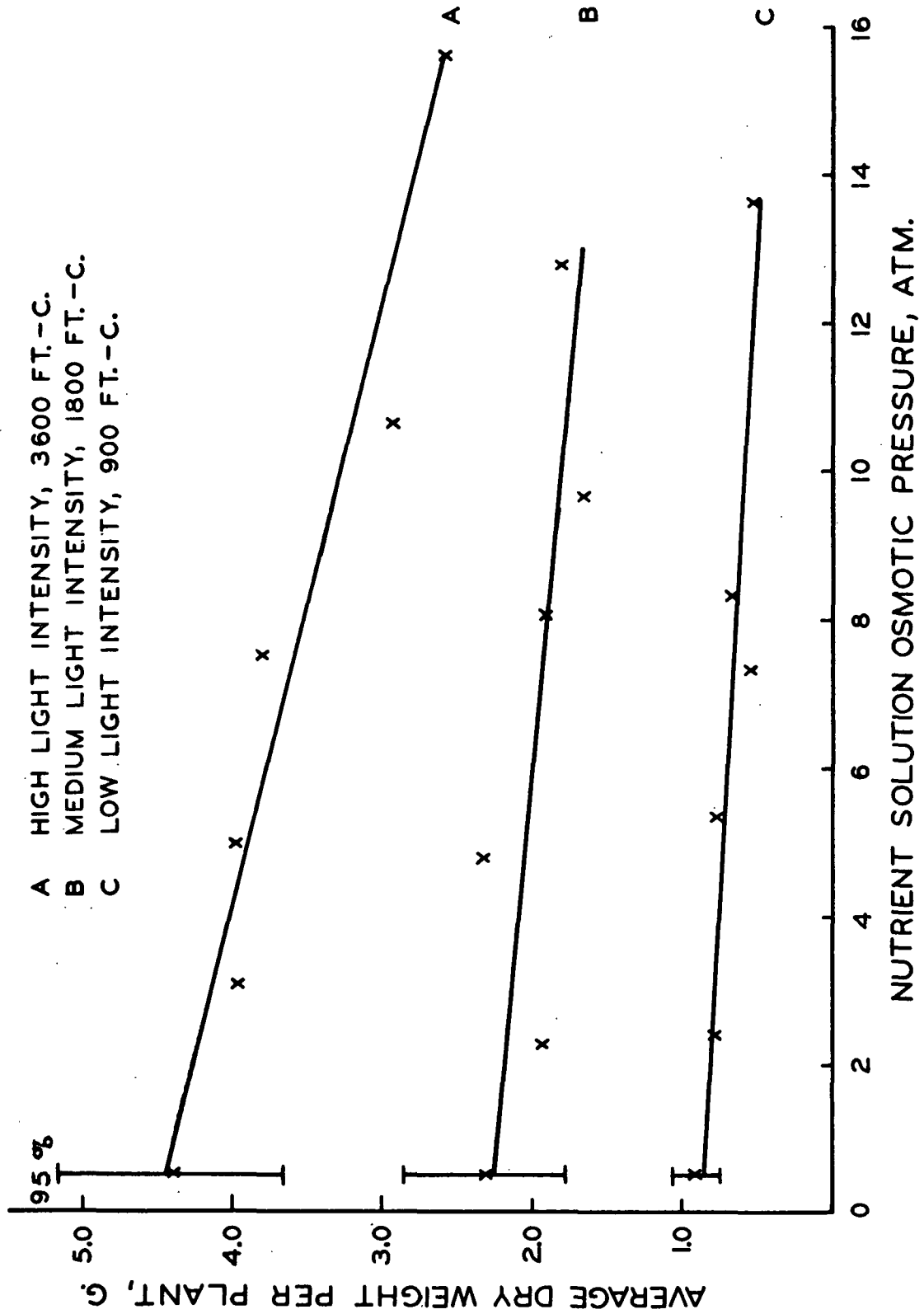
Figure 2. Growth Response to Moisture Stress

TABLE III

THE EFFECT OF MOISTURE STRESS ON PLANT GROWTH
AT THREE LIGHT INTENSITIES

3600 Foot-Candle		1800 Foot-Candle		900 Foot-Candle	
Stress Level, atm.	Dry Wt.,[a] g.	Stress Level, atm.	Dry Wt., g.	Stress Level, atm.	Dry Wt., g
0.47	4.42 a[b]	0.50	2.33 a	0.49	0.92 a
3.05	3.98 a	2.26	1.95 a	2.36	0.74 ab
4.95	3.99 a	4.78	2.34 a	5.30	0.75 ab
7.50	3.82 a	8.08	1.83 a	7.30	0.56 b
10.64	2.96 b	9.66	1.66 a	8.26	0.66 b
15.60	2.60 b	12.73	1.80 a	13.60	0.54 b

[a] Average dry weight per plant.

[b] Duncan's multiple range test (MRT), 5% significance. Values in vertical columns followed by the same letter are not significantly different (refer to p. 27).

The average dry weights of the plants at equal moisture stress levels are listed in Table IV. The values were read from Fig. 2 in order to compare the growth of the plants between light intensities at comparable moisture stress levels. At each level of moisture stress, the growth of the plants was significantly different for each level of light intensity.

TABLE IV

THE EFFECT OF LIGHT INTENSITY ON PLANT GROWTH
AT DIFFERENT MOISTURE STRESSES

Light Intensity, ft.-c.	Average Dry Weight Per Plant, g. Stress Level, atm.					
	0.5	2.0	5.0	7.0	10.0	13.0
3600	4.34 a[a]	4.22 a	3.84 a	3.46 a	2.89 a	2.70 a
1800	2.28 b	2.24 b	2.10 b	1.90 b	1.70 b	1.68 b
900	0.94 c	0.80 c	0.66 c	0.61 c	0.58 c	0.54 c

[a] Duncan's MRT, 5% (refer to p. 27).

SHOOT/ROOT RATIO

The average shoot/root ratio of the plants at each moisture stress level and each light intensity is presented in Table V. The shoot/root ratios at high light intensity tended to decrease with increasing moisture stress. The control (0.5 atm. moisture stress) shoot/root ratio was significantly higher than the other values. At medium light intensity, there were no differences, and no trend was apparent. At low light again, there were no differences, but the ratios tended to increase with increasing moisture stress.

TABLE V

THE EFFECT OF MOISTURE STRESS ON THE SHOOT/ROOT RATIOS
AT THREE LIGHT INTENSITIES
(Dry weight basis)

3600 Foot-Candle		1800 Foot-Candle		900 Foot-Candle	
Stress Level, atm.	Ratio	Stress Level, atm.	Ratio	Stress Level, atm.	Ratio
0.47	3.20 a[a]	0.50	3.95 a	0.49	3.18 a
3.05	2.17 b	2.26	4.14 a	2.36	3.76 a
4.95	1.89 b	4.78	3.97 a	5.30	3.13 a
7.50	2.20 b	8.08	4.50 a	7.30	4.20 a
10.64	1.82 b	9.66	5.10 a	8.26	4.05 a
15.60	1.84 b	12.73	3.13 a	13.60	4.00 a

[a]Duncan's MRT, 5%.

The average shoot/root ratios at equal moisture stress levels are given in Table VI. The values were read from graphs in order to compare the ratios between light intensities at comparable moisture stress levels. The ratios at the control moisture stress (0.5 atm.) at each light intensity were not different. However,

all other values at medium and low light intensity were significantly higher than the values at high light intensity.

TABLE VI

THE EFFECT OF LIGHT INTENSITY ON SHOOT/ROOT RATIOS
AT DIFFERENT MOISTURE STRESSES
(Dry weight basis)

Light Intensity, ft.-c.	Ratios Stress Level, atm.					
	0.5	2.0	5.0	7.0	10.0	13.0
3600	3.20 a[a]	2.30 a	2.00 a	1.98 a	1.96 a	1.92 a
1800	4.04 a	4.08 b	4.15 b	4.20 b	4.26 b	4.33 b
900	3.18 a	3.70 b	4.08 b	4.10 b	4.10 b	4.10 b

[a]Duncan's MRT, 5%.

The above data on the shoot/root ratios suggested that two different factors may be limiting growth at high and low light intensity. At high light intensity, moisture stress appeared to control growth; whereas, at low light intensity, the limiting factor appeared to be the level of light. However, the total dry weight of the plants at low light intensity did decrease as moisture stress was increased. The values at medium light intensity probably represented a transition from limiting light to limiting moisture, and thus an interaction between the two limiting factors was present. A similar interaction was reported by Strothmann (8).

FRESH WEIGHT/DRY WEIGHT RATIO

The average fresh weight/dry weight ratio at each moisture stress level and each light intensity is listed in Table VII. The fresh weight/dry weight ratios at high and low light intensity decreased significantly with increasing moisture stress. At medium light intensity, the decrease in the fresh weight/dry weight ratios was not sufficient to show statistical differences. The decrease in the

fresh weight/dry weight ratios with increasing moisture stress indicated tissue
dehydration.

TABLE VII

THE EFFECT OF MOISTURE STRESS ON FRESH WEIGHT/DRY WEIGHT
RATIOS AT THREE LIGHT INTENSITIES

3600 Foot-Candle		1800 Foot-Candle		900 Foot-Candle	
Stress Level, atm.	Ratio	Stress Level, atm.	Ratio	Stress Level, atm.	Ratio
0.47	6.08 a[a]	0.50	5.36 a	0.49	7.12 a
3.05	5.61 ab	2.26	4.75 a	2.36	5.96 b
4.95	5.44 b	4.78	4.55 a	5.30	5.36 bc
7.50	5.69 ab	8.08	4.62 a	7.30	5.13 c
10.64	4.80 c	9.66	4.55 a	8.26	4.70 c
15.60	4.46 c	12.73	4.65 a	13.60	5.14 c

[a]Duncan's MRT, 5%.

The average fresh weight/dry weight ratios at equal moisture stress levels,
read from graphs for ease of comparison, are presented in Table VIII. The ratios
at medium light intensity were significantly lower than the ratios at high light
intensity at the first four stress levels. At low light intensity, the ratios were
significantly higher than the ratios at high light intensity at the first two treat-
ments. The changes in the fresh weight/dry weight ratios of the plants followed the
same general patterns as the changes in the moisture content of the tops which are
discussed in the next section.

TABLE VIII

THE EFFECT OF LIGHT INTENSITY ON THE FRESH WEIGHT/DRY WEIGHT
RATIOS AT DIFFERENT MOISTURE STRESSES

Light Intensity, ft.-c.	Ratios Stress Level, atm.,					
	0.5	2.0	5.0	7.0	10.0	13.0
3600	6.01 a[a]	5.86 a	5.54 a	5.34 a	5.04 a	4.74 a
1800	5.37 b	4.82 b	4.58 b	4.58 b	4.56 a	4.56 a
900	7.12 c	6.08 c	5.36 ab	5.12 ab	4.90 a	4.78 a

[a]Duncan's MRT, 5%.

PLANT MOISTURE CONTENT — TOPS

The average moisture content of the tops of the plants at each moisture stress
level and each light intensity is given in Table IX. The moisture content of the
tops at high and low light intensity decreased significantly with increasing moisture
stress. At medium light, the decrease in moisture content was not sufficient to show
statistical differences. The decrease in plant moisture content indicated tissue
dehydration and will contribute to the osmotic adjustment of the plants (increase in
cell sap osmotic pressure with increasing moisture stress).

The average moisture contents of the tops obtained graphically at equal moisture
stress levels are listed in Table X. The moisture contents at low light intensity
were significantly higher than the values at high light intensity at each of the
six stress levels. The high moisture contents at low light intensity were probably
associated with low transpiration rates. The high moisture contents at low light
intensity indicate that the plants had plenty of water available for growth under
the conditions of low light intensity. The moisture contents of the tops at medium
light intensity were significantly lower than at high light intensity for the first

three moisture stress levels. The remaining moisture content values at high and
medium light intensity were not statistically different.

TABLE IX

THE EFFECT OF MOISTURE STRESS ON THE MOISTURE CONTENT
OF THE TOPS AT THREE LIGHT INTENSITIES
(Fresh weight basis)

3600 Foot-Candle		1800 Foot-Candle		900 Foot-Candle	
Stress Level, atm.	Moisture Content, %	Stress Level, atm.	Moisture Content, %	Stress Level, atm.	Moisture Content, %
0.47	79.75 a[a]	0.50	75.60 a	0.49	84.83 a
3.05	75.27 b	2.26	72.52 a	2.36	80.72 b
4.95	74.04 bc	4.78	71.40 a	5.30	78.90 bc
7.50	74.32 b	8.08	70.92 a	7.30	77.95 bc
10.64	72.39 c	9.66	70.64 a	8.26	76.67 c
15.60	70.32 d	12.73	72.72 a	13.60	77.09 c

[a]Duncan's MRT, 5%.

TABLE X

THE EFFECT OF LIGHT INTENSITY ON THE MOISTURE CONTENT
OF THE TOPS AT DIFFERENT MOISTURE STRESSES
(Fresh weight basis)

Light Intensity, ft.-c.	Moisture Content, % Stress Level, atm.					
	0.5	2.0	5.0	7.0	10.0	13.0
3600	79.68 a[a]	76.51 a	74.65 a	73.81 a	72.62 a	71.42 a
1800	75.60 b	72.90 b	71.25 b	70.94 a	70.62 a	70.50 a
900	84.83 c	81.77 c	78.42 c	77.62 c	77.15 c	77.08 c

[a]Duncan's MRT, 5%.

PLANT MOISTURE CONTENT — ROOTS

The average moisture content of the roots at each moisture stress level and
each light intensity is presented in Table XI. The moisture contents of the roots
at high light intensity decreased significantly with increasing moisture stress.
At medium light intensity, the decrease in moisture content was not sufficient to
show statistical differences. At low light intensity, only the moisture content at
the fifth treatment level was significantly lower than the remaining values.

TABLE XI

THE EFFECT OF MOISTURE STRESS ON THE MOISTURE CONTENT
OF THE ROOTS AT THREE LIGHT INTENSITIES
(Fresh weight basis)

3600 Foot-Candle		1800 Foot-Candle		900 Foot-Candle	
Stress Level, atm.	Moisture Content, %	Stress Level, atm.	Moisture Content, %	Stress Level, atm.	Moisture Content, %
0.47	88.17 ab[a]	0.50	89.56 a	0.49	88.24 a
3.05	88.66 ab	2.26	88.30 a	2.36	88.01 a
4.95	87.01 ac	4.78	87.64 a	5.30	86.12 ab
7.50	89.47 b	8.08	88.62 a	7.30	86.56 a
10.64	85.40 cd	9.66	89.14 a	8.26	83.71 b
15.60	83.82 d	12.73	86.54 a	13.60	86.90 a

[a]Duncan's MRT, 5%.

The average moisture contents of the roots at equal moisture stress levels
are given in Table XII. The moisture contents of the roots were not significantly
different between light intensities at each moisture stress level.

TABLE XII

THE EFFECT OF LIGHT INTENSITY ON THE MOISTURE CONTENT
OF THE ROOTS AT DIFFERENT MOISTURE STRESSES.
(Fresh weight basis)

Light Intensity, ft.-c.	Moisture Content, % Stress Level, atm.					
	0.5	2.0	5.0	7.0	10.0	13.0
3600	88.88 a[a]	88.48 a	87.68 a	87.17 a	86.38 a	85.58 a
1800	89.78 a	89.38 a	88.60 a	88.06 a	87.30 a	86.50 a
900	88.44 a	87.96 a	88.06 a	86.46 a	85.58 a	84.68 a

[a]Duncan's MRT, 5%.

OSMOTIC RELATIONSHIPS

The relationships between water potential, osmotic pressure of the expressed cell sap, and turgor pressure at three light intensity levels are shown in Fig. 3, 4, and 5. Regression techniques were used to determine the best fit of the curves through the data. The regression coefficients are listed in Appendix III. Water potential values were plotted as absolute values to avoid confusion about the negative sign. When discussing changes in leaf water potential, however, the negative sign will be considered.

WATER POTENTIAL

The water potential curves at high and medium light intensity are nearly equal. The |WP| curve at low light intensity is slightly lower than the curves at high and medium light intensity.

The average water potential value of the leaves at each moisture stress and each light intensity is given in Table XIII. At high light intensity, each leaf
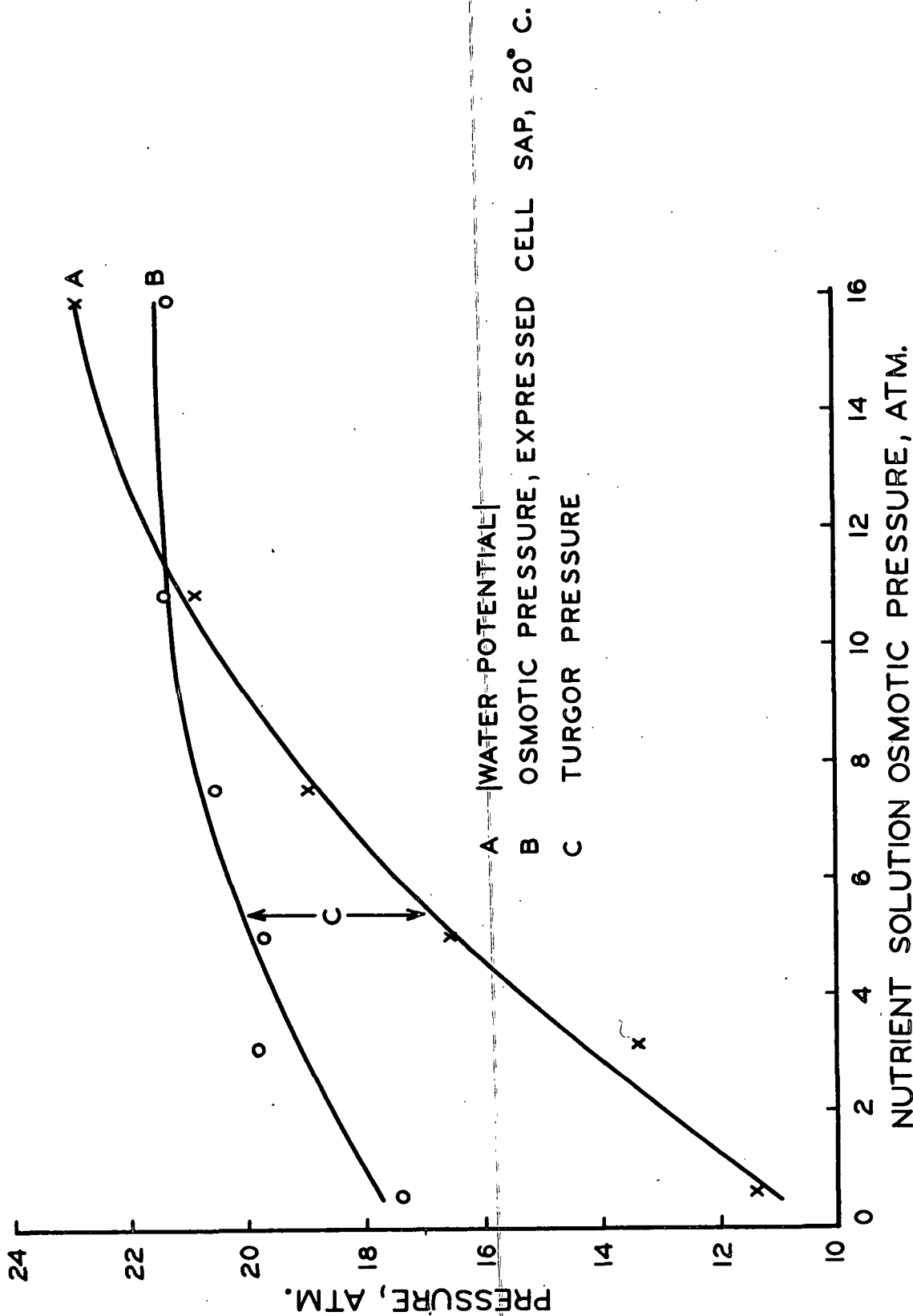
Figure 3. Osmotic Relationships of the Leaves as a Function of the Osmotic Pressure of the Nutrient Solutions at High Light Intensity
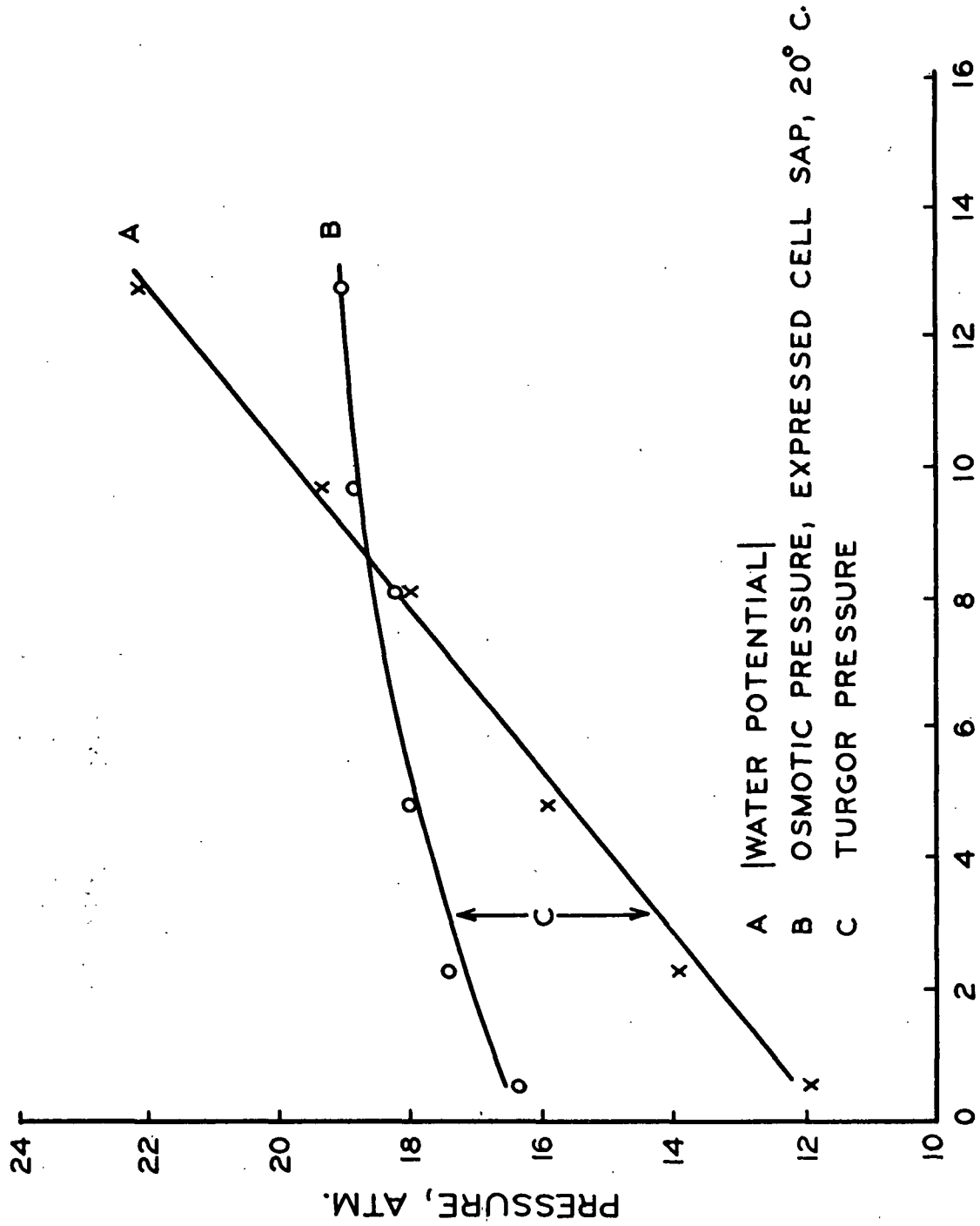
Figure 4. Osmotic Relationships of the Leaves as a Function of the Osmotic Pressure of the Nutrient Solutions at Medium Light Intensity

NUTRIENT SOLUTION OSMOTIC PRESSURE, ATM.

PRESSURE, ATM.

A |WATER POTENTIAL|
B   OSMOTIC PRESSURE, EXPRESSED CELL SAP, 20° C.
C   TURGOR PRESSURE

NUTRIENT SOLUTION OSMOTIC PRESSURE, ATM.

A |WATER POTENTIAL|
B   OSMOTIC PRESSURE, EXPRESSED CELL SAP, 20° C.
C   TURGOR PRESSURE
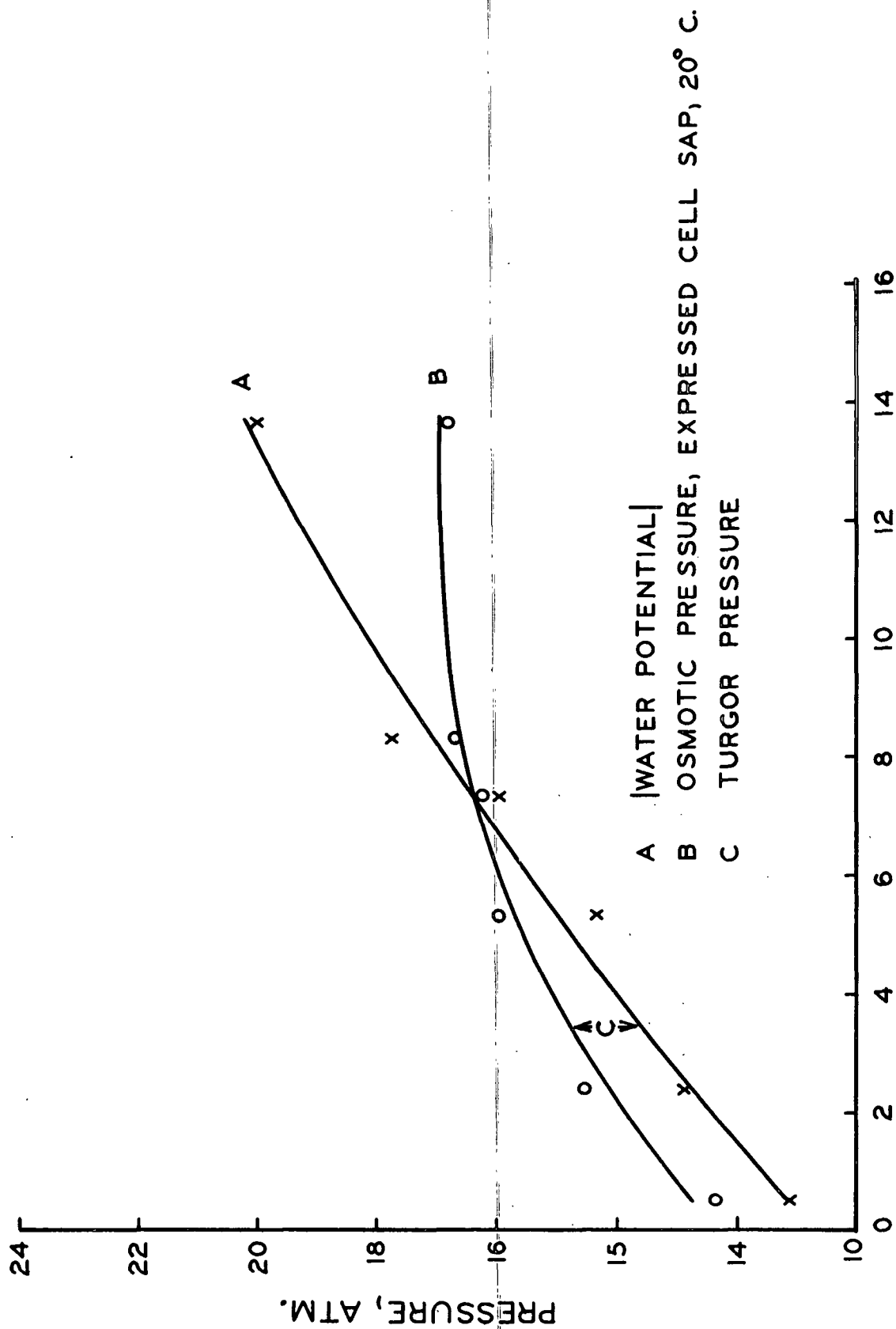
Figure 5.   Osmotic Relationships of the Leaves as a Function of the Osmotic
Pressure of the Nutrient Solutions at Low Light Intensity

water potential was significantly different from the other values. The same is also true at medium and low light intensity.

TABLE XIII

THE EFFECT OF MOISTURE STRESS ON LEAF WATER POTENTIAL
AT THREE LIGHT INTENSITIES

3600 Foot-Candle		1800 Foot-Candle		900 Foot-Candle	
Stress Level, atm.	WP, atm.	Stress Level, atm.	WP, atm.	Stress Level, atm.	WP, atm.
0.52	-11.4 a[a]	0.50	-12.0 a	0.49	-11.1 a
3.08	-13.4 b	2.26	-14.0 b	2.36	-12.9 b
4.95	-16.5 c	4.78	-16.0 c	5.30	-14.4 c
7.50	-18.9 d	8.08	-18.1 d	7.30	-16.0 d
10.81	-20.9 e	9.66	-19.4 e	8.26	-17.8 e
15.87	-22.8 f	12.73	-22.2 f	13.60	-20.1 f

[a]Duncan's MRT, 5%.

The average water potential values of the leaves at equal moisture stress levels are listed in Table XIV. The values were read from Fig. 3, 4, and 5 in order to compare the water potential values of the plants between light intensities at comparable moisture stress levels. At the control moisture stress (0.5 atm.), the water potential values at high and low light intensity were not significantly different. The corresponding water potential value at medium light intensity was significantly lower than the other two values. At all higher moisture stress levels, the water potential values at high and medium light intensity were not significantly different. However, the water potential values at low light intensity were significantly higher than the values at high and medium light intensity.

TABLE XIV

THE EFFECT OF LIGHT INTENSITY ON LEAF WATER
POTENTIAL AT DIFFERENT MOISTURE STRESSES

Light Intensity, ft.-c.	Water Potential, atm. Stress Level, atm.					
	0.5	2.0	5.0	7.0	10.0	13.0
3600	-11.0 a[a]	-12.9 ac	-16.3 a	-18.2 a	-20.4 a	-22.0 a
1800	-12.3 b	-13.5 ab	-15.9 a	-17.5 a	-19.9 a	-22.2 a
900	-11.1 a	-12.4 c	-14.7 c	-16.2 c	-18.1 c	-19.9 c

[a]Duncan's MRT, 5%.

There was very little difference in the leaf water potential values of the control plants at each of the three levels of light intensity. As moisture stress increased, the water potential values at low light intensity did not decrease as rapidly or become as low as at medium and high light intensity. It is doubtful, however, that the slightly higher water potential values at low light intensity could have seriously affected the water uptake of the plants. The water potential differences were fairly small. Also, the moisture contents of the leaves at low light intensity were considerably higher than at medium or high light intensity (see previous section on moisture contents — tops). The growth of the plants at low light intensity was certainly not restricted by a lack of water in the plants.

OSMOTIC PRESSURE OF EXPRESSED CELL SAP

The response of the osmotic pressure of the expressed cell sap to increasing moisture stress at the three light intensity levels is shown in Fig. 3, 4, and 5.

The average cell sap osmotic pressure at each moisture stress level and each light intensity is presented in Table XV. At each level of light intensity, the cell sap osmotic pressure increased with increasing moisture stress. At each of

the three light intensities, the cell sap osmotic pressure of the plants grown at the lowest moisture stress level was significantly lower than the other values. The increases in the cell sap osmotic pressure as moisture stress was increased were associated with the decreased moisture contents and with increased solute concentrations in the cell sap.

TABLE XV

THE EFFECT OF MOISTURE STRESS ON CELL SAP
OSMOTIC PRESSURE AT THREE LIGHT INTENSITIES

3600 Foot-Candle		1800 Foot-Candle		900 Foot-Candle	
Stress Level, atm.	OP,[a] atm.	Stress Level, atm.	OP,[a] atm.	Stress Level, atm.	OP,[a] atm.
0.52	17.43 a[b]	0.50	16.41 a	0.49	12.39 a
3.08	19.85 bc	2.26	17.50 ab	2.36	14.56 b
4.95	19.79 b	4.78	18.06 ab	5.30	16.00 bc
7.50	20.50 bcd	8.08	18.25 b	7.30	16.24 bc
10.81	21.34 d	9.66	18.90 b	8.26	16.72 c
15.87	21.24 bcd	12.73	19.09 b	13.60	16.85 c

[a]Osmotic pressure of expressed cell sap, 20°C.

[b]Duncan's MRT, 5%.

The average cell sap osmotic pressures at equal moisture stress levels are given in Table XVI. The values were read from Fig. 3, 4, and 5 in order to compare the osmotic pressure values between light intensities at comparable moisture stress levels. At the high and medium light intensities, the osmotic pressure values at the first two moisture stress levels were not significantly different. The osmotic pressure values at low light were significantly lower than the values at high and medium light intensity. At all other moisture stress levels, cell sap osmotic pressure decreased significantly as light intensity was decreased. This decrease

in osmotic pressure as light intensity was decreased was probably due in part to lower rates of photosynthesis at the lower light intensities resulting in a lower concentration of soluble photosynthetic products in the cells of the leaves.

TABLE XVI

THE EFFECT OF LIGHT INTENSITY ON CELL SAP OSMOTIC
PRESSURE AT DIFFERENT MOISTURE STRESSES

Light Intensity, ft.-c.	Osmotic Pressure[a], atm. Stress Level, atm.					
	0.5	2.0	5.0	7.0	10.0	13.0
3600	17.8 a[b]	18.6 a	20.0 a	20.6 a	21.3 a	21.5 a
1800	16.6 a	17.2 a	18.0 b	18.4 b	18.9 b	19.1 b
900	12.7 c	13.9 c	15.7 c	16.4 c	16.9 c	16.9 c

[a]20°C.

[b]Duncan's MRT, 5%.

TURGOR PRESSURE

The relationship between cell turgor pressure and moisture stress at high, medium, and low light intensity is given in Fig. 6. The turgor pressure values for Fig. 6 were derived from Fig. 3, 4, and 5. At each light intensity used, cell turgor pressure decreased as moisture stress was increased. Thus, the decrease in the average dry weight of the plants as moisture stress was increased was associated with reduced turgor pressure. Cell turgor pressure also decreased as light intensity was decreased.

The water stress value at which turgor pressure equaled zero decreased as light intensity was decreased. Thus, the water stress value at which plant growth will cease decreased as light intensity was decreased. At high light intensity, turgor pressure equaled zero at an osmotic moisture stress of about twelve atmospheres; at

A HIGH LIGHT INTENSITY, 3600 FT.-C.
B MEDIUM LIGHT INTENSITY, 1800 FT.-C.
C LOW LIGHT INTENSITY, 900 FT.-C.

NUTRIENT SOLUTION OSMOTIC PRESSURE, ATM.

TURGOR PRESSURE, ATM.
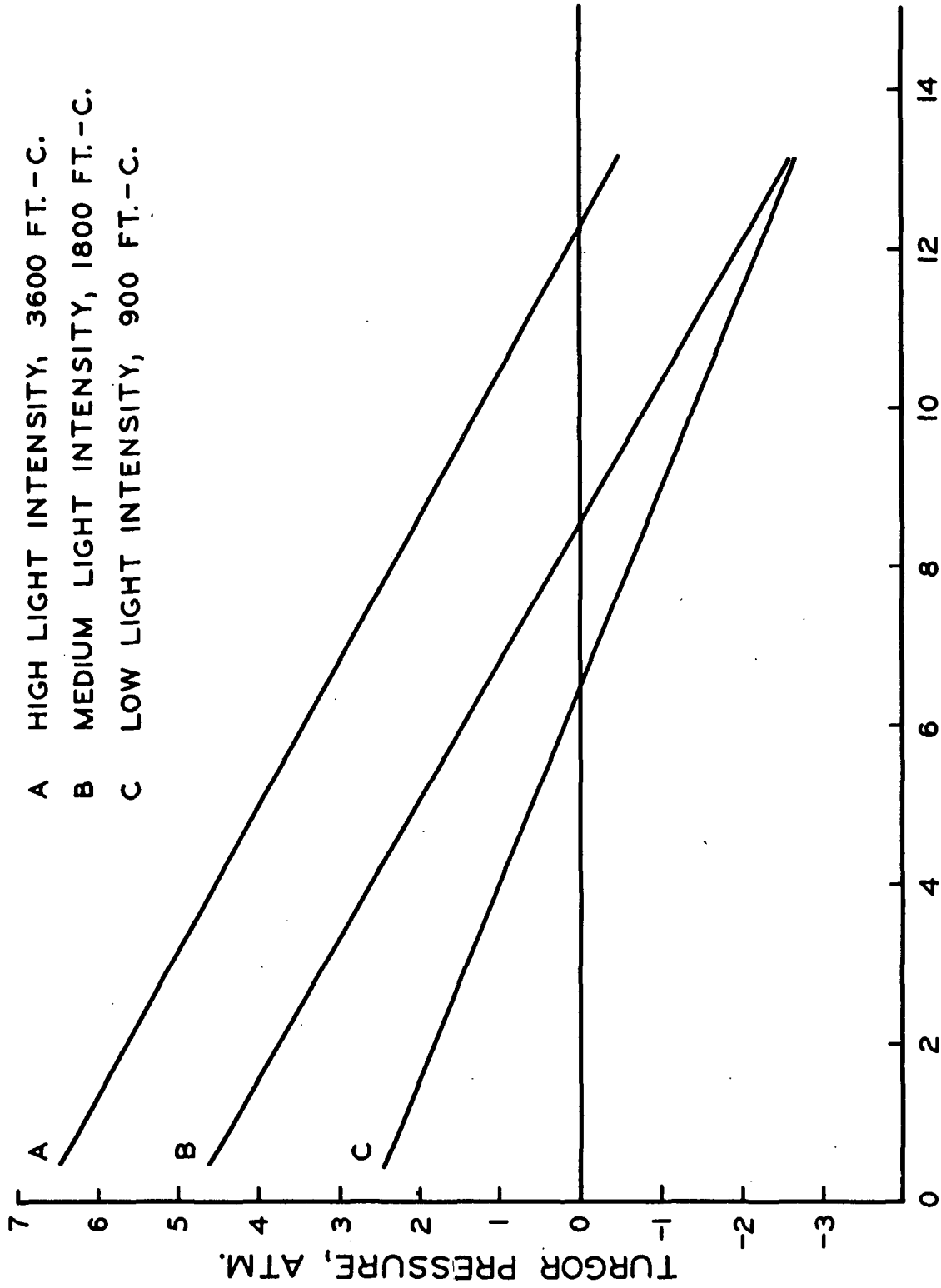
Figure 6. Response of Cell Turgor Pressure to Moisture Stress.

medium light intensity, eight and one-half atmospheres; and at low light intensity, six and one-half atmospheres.

The reduction in turgor pressure as light intensity was decreased is also important. For example, at the lowest moisture stress level, at high light intensity turgor pressure equaled six and one-half atmospheres; at medium light intensity, four and one-half atmospheres; and at low light intensity, two and one-half atmospheres. This reduction in turgor pressure, as well as the reduction in turgor pressure as moisture stress was increased, probably did have an affect on the growth of the plants. Ordin (24) found that a reduction in cell turgor pressure affected both cell wall metabolism and cell elongation.

To better understand the relationship between turgor pressure and growth, an example from the literature will be compared with the current results. Strothmann (8) studied the influence of two light intensity levels and three soil moisture levels on the growth of red pine seedlings. He found that either increasing the light intensity or increasing the available moisture improved plant growth. Increasing the light intensity produced a larger growth response than did increasing the available moisture. Strothmann did not offer a full explanation of the mechanism through which his plants reacted.

However, the results shown in Fig. 6 suggest one possible explanation for Strothmann's results. At low light intensity (Curve C) as moisture is increased (moisture stress decreased), cell turgor pressure becomes positive, and a slight growth response should result. At high light intensity, a much larger growth response should result from the same increase in moisture because the turgor pressure values are larger than at low light intensity. At a given moisture stress, for example 7 atm., plant growth at low or medium light intensity would be quite slow possibly due to the low values of turgor pressure. On the other hand, at high

light intensity, the plants would grow faster because turgor pressure is much larger
than at low or medium light intensity. Thus, one would expect a larger growth response
due to increased light intensity than to increased moisture. Of course, increasing
the light intensity would also cause a positive growth response by increasing the
photosynthetic activity.

In view of the above discussion, the turgor pressure data in Fig. 6 suggests an
interaction between light intensity and moisture stress on plant growth. In other
words, the growth response to an increase in the available moisture depends on the
level of light intensity. Indeed, Strothmann (8) found a decided interaction between
these two factors on all facets of growth that he observed.

POLYETHYLENE GLYCOL UPTAKE

The concentrations of polyethylene glycol in the expressed cell sap samples are
listed in Table XVII. The concentration of the polyethylene glycol is given in mg.
per ml. of expressed cell sap. The corresponding osmotic pressure values were deter-
mined from a standard curve developed experimentally from known dilute solutions of
polyethylene glycol and water.

At each light intensity, the concentration of polyethylene glycol in the leaves
was quite low. The contributions of polyethylene glycol to the increase in the
osmotic pressure of the expressed cell sap at the highest stress level were less
than 1% at high and medium light intensity and less than 2% at low light intensity.
For most purposes, such low concentrations of polyethylene glycol in the leaves of
the plants can be assumed insignificant and can be ignored without affecting other
osmotic data.

Increases in cell sap osmotic pressure as moisture stress is increased are
associated with changes in the moisture content, actual solute changes, and uptake

of the osmotic agent, in this case polyethylene glycol. Since the amount of poly-ethylene glycol in the leaves was insignificant, the increase in cell sap osmotic pressure must have been due to moisture content decreases and to actual solute changes in the cells of the leaves.

TABLE XVII

CONCENTRATION OF POLYETHYLENE GLYCOL IN TISSUE EXTRACT

Light Intensity	Nutrient Solution Stress Level, atm.	PEG in Cell Sap	
		Concentration, mg./ml.	Equivalent OP, atm.
3600 ft.-c.	0.52	0.00	0.000
	3.08	0.38	0.013
	4.95	0.64	0.021
	7.50	0.58	0.019
	10.81	0.77	0.026
	15.87	0.97	0.032
1800 ft.-c.	0.50	0.00	0.000
	2.26	0.20	0.007
	4.78	0.25	0.008
	8.08	0.30	0.010
	9.66	0.39	0.013
	12.73	0.38	0.013
900 ft.-c.	0.49	0.00	0.000
	2.36	2.51	0.084
	5.30	1.89	0.063
	7.30	2.62	0.088
	8.26	2.16	0.072
	13.60	2.32	0.078

GENERAL COMMENTS

Trees growing in a forest are subject to a more complex environmental situation than were the plants grown in this study. Complicating interactions are common in the field, and care must be taken in making generalizations about the applicability of growth chamber results to field conditions. It is of interest, however, to consider some possible applications of the results of this study to field conditions.

Under field conditions, increased light intensity will increase overall plant growth including root growth. Increased root growth should automatically reduce the moisture stress by making more soil moisture available to the plant. Thus, in a soil system increased light intensity could increase overall growth both by increasing photosynthetic activity and by stimulating root growth which would make more soil moisture available. On the other hand, an increase in soil moisture by irrigation or by reducing competition from other plants increases growth only by making more soil moisture available to the plant. Thus, under field conditions, one would expect the growth response to increased light intensity to be greater than to increased soil moisture.

Information about the effect of light intensity on water potential values of plants growing in the field could be misinterpreted under certain conditions. As mentioned above, increased light intensity can increase the amount of moisture available to plants by stimulating root growth. As the water deficit is reduced, water potential values would increase. Such data might be falsely interpreted as meaning that increased light intensity caused an increase in leaf water potential values. Such a change in water potential would not be due directly to a change in light intensity but primarily to increased water availability and reduced moisture stress.

The results of this study concerning the response of turgor pressure to light intensity and moisture stress suggest that turgor pressure may be intimately involved in the overall problem of why some species will grow in the shade and others will not. Shade tolerant species may have the ability to maintain higher turgor pressures under greater water stress and thus would be able to grow better than would intolerant species. More work is needed in this area before definite conclusions can be drawn, however, concerning possible differences between shade tolerant and shade intolerant species.

CONCLUSIONS

The results obtained in this study suggest that the effect of osmotic moisture stress on the growth of the plants was related to cell turgor pressure. The data also suggest that leaf water potential is not an important factor restricting the growth of quaking aspen in the shade of other trees. On the other hand, cell turgor pressure may be involved in quaking aspen's lack of ability to grow in the shade of other trees.

No apparent differences existed in the plant's internal ability to remove moisture from the nutrient solutions as light intensity was decreased. The high moisture content values at low light intensity indicated that the plants had adequate amounts of water available for growth. The plant's ability to take up water from the nutrient solutions at low light intensity was not restricted by the leaf water potential values. Leaf water potential does not appear to be an important factor restricting the growth of quaking aspen in the shade of other trees.

Plant growth response to osmotic moisture stress was related to cell turgor pressure. The reduction in cell turgor pressure, as moisture stress increased, appeared to have a greater affect on plant growth at high light intensity than at medium and low light intensities. As light intensity was decreased, plant growth appeared to be affected more seriously by the loss of cell turgor pressure than by a possible decrease in the plant's ability to remove moisture from the nutrient solutions. The moisture stress level at which plant growth essentially ceased decreased as light intensity decreased. Cell turgor pressure may be involved in quaking aspen's lack of ability to grow in the shade of other trees.

Moisture stress became the factor controlling the growth of the plants at high light intensity. On the other hand, the amount of light appeared to be an important factor limiting the growth of the plants at low light intensity.

The increase in the osmotic pressure of the expressed cell sap of the leaves as moisture stress was increased was due to a combination of the decreased moisture content and an increase in the concentration of internal solutes in the cell sap. The contribution of polyethylene glycol to the increase in cell sap osmotic pressure was negligible.

Mannitol is not a completely satisfactory agent for controlling osmotic moisture stress, especially when treatment times of more than one week are involved. Polyethylene glycol is probably the better agent for regulating osmotic moisture stress.

The data obtained from this study suggest that both reduced cell turgor pressure and the assumed factor of reduced photosynthesis may be directly related to quaking aspen's inability to grow and develop in the shade of other trees.

ACKNOWLEDGMENTS

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APPENDIX I

MEASUREMENT OF LEAF WATER POTENTIAL

The dye method for measuring leaf water potential (WP) is a solution immersion technique which requires detecting changes in the density of the solutions in which leaves were immersed. Clean, dry glassware and fresh, uncontaminated sucrose solutions were used in setting up the method. Whole leaves were used rather than cut leaves to avoid possible contamination of the test solutions by cell sap released from cut edges. Whole leaves were removed from the plants at the beginning of the dark period, rolled longitudinally, and inserted tip first into the test solutions. Thus, the amount of leaf tissue used per unit volume of solution was as large as possible without restricting access of the solution to the leaf. Care was taken in inserting the leaves into the test solution to avoid mechanical injury to the tissue. The cut end of the petiole was kept above the top of the test solution to avoid possible contamination by solutes leaking out of the cut end.

Preliminary tests indicated that an immersion time of four hours was sufficient. At the end of this period, a small amount of methylene blue was added to the test solution. A clean medicine dropper was used to introduce each colored test drop into the uncolored control solutions. The direction of movement of the colored drop was recorded. The water potential of the leaves was assumed to lie between the osmotic pressure of the solution in which the drop sank and that in which the drop rose.

The sucrose solutions used varied by increments of 1 atm. Thus, the water potentials were determined to ± 0.5 atm. Four separate water potential measurements were made at each level of water stress.

MEASUREMENT OF CELL SAP OSMOTIC PRESSURE

Cell sap osmotic pressures were calculated from freezing point depression measurements. Approximately 30 g. of leaves, fresh weight, were required from each water stress level. The leaves were removed from the plants, placed in heavy polyethylene bags, and immediately frozen at -16 to -20°C. to kill the tissue, thus preventing filtration errors. The leaves were allowed to thaw and were pressed in a plant press at a maximum pressure of 2000 lb./in.^{2} to express the cell sap. The cell sap samples thus obtained were immediately frozen to reduce the possibility of chemical changes.

The freezing point of the cell sap was determined using a Beckman thermometer with its bulb immersed in the sap in a test tube. The tube containing the sap was jacketed by a larger tube immersed in an ice-salt water bath at -5°C. As the temperature decreased the expressed sap was stirred continuously. The sap was supercooled to a critical temperature at which a sudden release of heat of crystallization caused the temperature to rise rapidly to a brief plateau before declining again. The correct freezing point was then determined by plotting the temperature versus time curve and extrapolating back to an arbitrarily set zero time from the maximum temperature reached after ice formation as described by Bennet-Clark (50). This extrapolation procedure was necessary because preliminary experiments indicated that the minimum temperature reached did not always fall within the range covered by the Beckman thermometer. The extrapolation procedure was standardized by preliminary experiments with known sucrose solutions.

The osmotic pressure of the expressed cell sap was then calculated by the method described by Loomis and Shull (49) using the equation

$$OP, 0°C. = \frac{(22.4)(\Delta T)}{1.86} \tag{4}$$

where ΔT = the calculated freezing point depression. The osmotic pressures thus obtained were corrected to 20°C. by the equation

$$OP, 20°C. = \frac{(293)(OP, 0°C.)}{273}.$$

(5)

The osmotic pressures of standard sucrose solutions determined using the above procedures agreed to within ± 0.1 atm. of tabled values. This accuracy was considered sufficient because the osmotic pressure of the duplicate cell sap samples varied by more than ± 0.1 atm. Two separate cell sap osmotic pressure determinations were made at each water stress level.

APPENDIX II

DATA AND STATISTICAL ANALYSES

GROWTH DATA

TABLE XVIIIA

PLANT DRY WEIGHT-GRAMS
HIGH LIGHT INTENSITY

Stress Level, atm.	Blocks					
	A	B	C	D	E	F
0.47	4.62[a]	3.08	4.44	5.46	4.65	4.24
3.05	4.24	3.82	4.46	4.46	3.90	3.00
4.95	1.22	3.74	5.62	5.81	3.60	3.96
7.50	3.76	3.60	4.01	4.14	4.22	3.18
10.64	2.80	2.74	3.46	2.91	1.82	4.02
15.60	3.03	2.58	2.84	1.60	3.62	1.96

[a] Average per plant.

TABLE XVIIIB

ANALYSIS OF VARIANCE

Source	df	SS	MS	F	
Blocks	5	4.59	0.92	1.15	N.S.[a]
Stress levels	5	14.45	2.89	3.61	*[b]
Error	25	19.91	0.80		
Total	35	38.95			

[a] N.S. = Not significant.
[b] * = Significant, 5%.

TABLE XIXA

PLANT DRY WEIGHT-GRAMS
MEDIUM LIGHT INTENSITY

Stress Level, atm.	Blocks					
	A	B	C	D	E	F
0.50	1.92[a]	1.15	2.78	2.11	2.45	3.55
2.26	2.15	1.38	2.61	1.61	2.44	1.52
4.78	1.01	3.38	2.89	2.37	2.58	1.76
8.08	1.95	2.00	1.63	2.48	1.36	1.55
9.66	1.48	0.88	2.86	1.82	1.40	1.55
12.73	1.14	2.10	1.79	2.39	1.65	1.76

[a]Average per plant.

TABLE XIXB

ANALYSIS OF VARIANCE

Source	df	SS	MS	F	
Blocks	5	2.33	0.47	1.20	N.S.[a]
Stress levels	5	2.38	0.48	1.22	N.S.
Error	25	9.76	0.39		
Total	35	14.48			

[a]N.S. = Not significant.

TABLE XXA

PLANT DRY WEIGHT-GRAMS
LOW LIGHT INTENSITY

Stress Level, atm.	Blocks					
	A	B	C	D	E	F
0.49	0.66[a]	0.85	1.16	1.34	0.74	0.74
2.36	0.89	0.86	0.81	0.89	0.35	0.64
5.30	0.85	0.92	0.77	0.52	0.84	0.62
7.30	0.71	0.73	0.56	0.37	0.48	0.52
8.26	0.80	0.66	0.78	0.62	0.66	0.45
13.60	0.64	0.50	0.35	0.64	0.52	0.56

[a]Average per plant.

TABLE XXB

ANALYSIS OF VARIANCE

Source	df	SS	MS	F	
Blocks	5	0.19	0.037	1.23	N.S.[a]
Stress levels	5	0.59	0.118	3.88	**[b]
Error	25	0.76	0.030		
Total	35	1.54			

[a]N.S. = Not significant.
[b]** = Highly significant, 1%.

TABLE XXIA

SHOOT/ROOT RATIO
HIGH LIGHT INTENSITY

Stress Level, atm.	Blocks					
	A	B	C	D	E	F
0.47	2.12	3.03	2.12	4.24	3.83	3.85
3.05	2.38	2.10	2.00	2.10	2.60	1.87
4.95	1.55	1.96	2.19	1.41	1.61	2.64
7.50	1.55	2.46	1.75	2.62	2.35	2.45
10.64	1.66	1.76	1.70	2.00	1.79	2.01
15.60	2.68	1.74	1.80	2.02	0.91	1.88

TABLE XXIB

ANALYSIS OF VARIANCE

Source	df	SS	MS	F	
Blocks	5	1.32	0.26	<1	N.S.[a]
Stress levels	5	8.19	1.64	5.86	**[b]
Error	25	7.07	0.28		
Total	35	16.58			

[a] N.S. = Not significant.
[b] ** = Highly significant, 1%.

TABLE XXIIA

SHOOT/ROOT RATIO
MEDIUM LIGHT INTENSITY

Stress Level, atm.	Blocks					
	A	B	C	D	E	F
0.50	3.62	4.35	5.95	3.49	2.59	3.69
2.26	2.65	2.50	5.26	6.10	4.61	3.69
4.78	5.31	2.94	2.93	2.68	4.66	5.30
8.08	3.73	2.84	4.57	3.20	6.80	5.89
9.66	6.80	5.28	1.98	6.17	5.08	5.28
12.73	2.45	5.09	3.01	3.02	2.77	2.42

TABLE XXIIB

ANALYSIS OF VARIANCE

Source	df	SS	MS	F	
Blocks	5	1.60	0.32	1	N.S.[a]
Stress levels	5	12.86	2.57	1.19	N.S.
Error	25	54.14	2.16		
Total	35	68.60			

[a]N.S. = Not significant.

TABLE XXIIIA

SHOOT/ROOT RATIO
LOW LIGHT INTENSITY

Stress Level, atm.	Blocks					
	A	B	C	D	E	F
0.49	4.37	3.47	3.46	2.91	3.04	1.85
2.36	3.05	3.47	3.88	3.94	4.78	3.41
5.30	2.85	2.35	3.05	3.57	2.69	4.26
7.30	5.24	3.36	4.45	2.89	4.33	3.86
8.26	6.98	2.41	3.10	4.39	2.24	5.18
13.60	3.79	4.36	5.12	3.95	3.12	3.64

TABLE XXXIIIB

ANALYSIS OF VARIANCE

Source	df	SS	MS	F	
Blocks	5	4.90	0.98	1	N.S.[a]
Stress levels	5	5.46	1.09	1.08	N.S.
Error	25	25.38	1.01		
Total	35	35.74			

[a]N.S. = Not significant.

TABLE XXIVA

% MOISTURE CONTENT, TOPS
HIGH LIGHT INTENSITY

Stress Level, atm.	Blocks					
	A	B	C	D	E	F
0.47	79.74	78.49	78.46	79.82	80.82	81.18
3.05	75.94	74.51	74.14	75.20	76.40	75.44
4.95	72.65	72.94	74.27	73.75	74.77	75.83
7.50	71.09	74.86	71.71	79.00	73.55	75.74
10.64	71.14	72.80	70.88	73.24	73.82	72.47
15.60	71.96	68.12	70.86	68.08	72.68	70.22

TABLE XXIVB

ANALYSIS OF VARIANCE

Source	df	SS	MS	F	
Blocks	5	22.06	4.41	1.78	N.S.[a]
Stress levels	5	301.20	60.24	24.22	**[b]
Error	25	62.18	2.49		
Total	35	385.44			

[a] N.S. = Not significant.

[b] ** = Highly significant, 1%.

TABLE XXVA

% MOISTURE CONTENT, TOPS
MEDIUM LIGHT INTENSITY

Stress Level, atm.	Blocks					
	A	B	C	D	E	F
0.50	73.81	74.67	69.82	73.56	82.04	79.73
2.26	77.98	70.75	73.13	71.16	71.93	70.16
4.78	70.56	60.00	75.58	74.49	71.51	76.25
8.08	68.24	72.73	70.15	74.48	71.02	68.87
9.66	71.30	70.60	72.75	69.92	68.68	70.58
12.73	69.00	74.55	73.99	73.93	72.50	72.38

TABLE XXVB

ANALYSIS OF VARIANCE

Source	df	SS	MS	F	
Blocks	5	27.97	5.59	1	N.S.[a]
Stress levels	5	99.87	19.97	1.42	N.S.
Error	25	352.07	14.08		
Total	35	479.91			

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[a] N.S. = Not significant.

TABLE XXVIA

% MOISTURE CONTENT, TOPS
LOW LIGHT INTENSITY

Stress Level, atm.	Blocks					
	A	B	C	D	E	F
0.49	84.00	82.03	84.26	85.03	85.07	88.57
2.36	82.70	82.55	80.81	79.09	78.57	80.63
5.30	78.55	82.43	78.06	78.05	78.73	77.55
7.30	76.58	79.78	80.00	77.14	75.71	78.50
8.26	71.20	80.00	80.14	75.29	78.64	74.76
13.60	77.88	79.74	77.09	72.08	78.42	77.33

TABLE XXVIA

ANALYSIS OF VARIANCE

Source	df	SS	MS	F	
Blocks	5	40.82	8.16	1.62	N.S.[a]
Stress levels	5	277.96	55.59	11.13	**[b]
Error	25	124.90	4.99		
Total	35	443.68			

[a] N.S. = Not significant.

[b] ** = Highly significant, 1%.

TABLE XXVIIA

% MOISTURE CONTENT, ROOTS
HIGH LIGHT INTENSITY

Stress Level, atm.	Blocks					
	A	B	C	D	E	F
0.47	89.32	87.24	85.62	89.56	87.14	90.13
3.05	88.22	89.53	86.71	88.13	90.00	89.36
4.95	83.37	86.30	88.03	86.82	88.55	89.00
7.50	86.58	88.94	89.26	91.33	89.50	91.21
10.64	84.02	84.98	81.91	87.55	86.82	87.09
15.60	86.18	83.62	84.22	84.28	78.24	86.43

TABLE XXVIIB

ANALYSIS OF VARIANCE

Source	df	SS	MS	F	
Blocks	5	36.47	7.29	2.02	N.S.[a]
Stress levels	5	136.81	27.36	7.57	**[b]
Error	25	90.30	3.61		
Total	35	263.58			

[a]N.S. = Not significant.

[b]** = Highly significant, 1%.

TABLE XXVIIIA

% MOISTURE CONTENT, ROOTS
MEDIUM LIGHT INTENSITY

Stress Level, atm.	Blocks					
	A	B	C	D	E	F
0.50	88.91	89.51	88.57	91.30	90.21	88.87
2.26	87.84	85.64	90.46	90.56	88.55	89.92
4.78	91.58	82.40	87.05	87.71	88.29	88.80
8.08	87.85	88.57	87.82	88.76	90.54	88.16
9.66	90.62	91.92	83.25	90.13	90.32	88.62
12.73	85.00	86.98	88.22	84.66	88.30	86.13

TABLE XXVIIIB

ANALYSIS OF VARIANCE

Source	df	SS	MS	F	
Blocks	5	16.21	3.24	1	N.S.[a]
Stress levels	5	36.84	7.37	1.58	N.S.
Error	25	116.81	4.67		
Total	35	169.86			

[a]N.S. = Not significant.

TABLE XXIXA

% MOISTURE CONTENT, ROOTS
LOW LIGHT INTENSITY

Stress Level, atm.	Blocks					
	A	B	C	D	E	F
0.49	89.11	88.12	89.28	89.62	85.90	87.42
2.36	85.65	92.45	87.18	87.14	88.00	87.66
5.30	84.83	85.09	86.90	87.22	87.86	84.84
7.30	85.22	83.87	87.08	86.43	89.41	87.35
8.26	85.71	82.42	85.09	82.31	80.48	86.25
13.60	84.41	89.00	86.92	85.56	87.50	88.00

TABLE XXIXB

ANALYSIS OF VARIANCE

Source	df	SS	MS	F	
Blocks	5	6.22	1.24	1	N.S.[a]
Stress levels	5	80.17	16.03	4.09	**[b]
Error	25	98.09	3.92		
Total	35	184.48			

[a]N.S. = Not significant.

[b]** = Highly significant, 1%.

TABLE XXXA

FRESH WEIGHT/DRY WEIGHT RATIO
HIGH LIGHT INTENSITY

Stress Level, atm.	Blocks					
	A	B	C	D	E	F
0.47	6.37	5.65	5.57	6.18	6.32	6.41
3.05	5.68	5.64	5.25	5.35	6.00	5.76
4.95	4.58	5.18	5.28	6.30	5.79	5.50
7.50	5.03	5.44	5.64	6.60	5.50	5.93
10.64	4.54	4.87	4.32	5.21	4.97	4.90
15.60	5.00	4.40	4.58	4.59	3.50	4.69

TABLE XXXB

ANALYSIS OF VARIANCE

Source	df	SS	MS	F	
Blocks	5	1.59	0.32	1.79	N.S.[a]
Stress levels	5	10.94	2.19	12.35	**[b]
Error	25	4.43	0.18		
Total	35	16.95			

[a]N.S. = Not significant.

[b]** = Highly significant, 1%.

TABLE XXXIA

FRESH WEIGHT/DRY WEIGHT RATIO
MEDIUM LIGHT INTENSITY

Stress Level, atm.	Blocks					
	A	B	C	D	E	F
0.50	4.92	5.00	4.10	5.50	6.86	5.80
2.26	5.55	4.43	4.80	4.47	4.49	4.75
4.78	4.75	3.31	5.02	5.06	4.40	4.96
8.08	4.22	4.99	5.08	5.10	4.36	3.97
9.66	4.40	5.61	4.40	4.26	4.36	4.26
12.73	4.21	4.55	5.01	4.50	4.94	4.67

TABLE XXXIB

ANALYSIS OF VARIANCE

Source	df	SS	MS	F	
Blocks	5	0.26	0.05	1	N.S.[a]
Stress levels	5	2.83	0.57	1.38	N.S.
Error	25	10.29	0.41		
Total	35	13.38			

[a]N.S. = Not significant.

TABLE XXXIIA

FRESH WEIGHT/DRY WEIGHT RATIO
LOW LIGHT INTENSITY

Stress Level, atm.	Blocks					
	A	B	C	D	E	F
0.49	6.81	6.20	7.02	7.44	6.80	8.47
2.36	6.07	6.51	6.68	5.39	5.29	5.82
5.30	5.16	6.00	5.32	5.24	5.66	4.78
7.30	4.67	5.23	5.50	5.14	5.10	5.12
8.26	3.92	5.20	5.45	4.35	4.81	4.49
13.60	5.63	5.33	4.90	4.25	5.44	5.27

TABLE XXXIIB

ANALYSIS OF VARIANCE

Source	df	SS	MS	F	
Blocks	5	1.25	0.25	1	N.S.[a]
Stress levels	5	22.47	4.49	15.11	**[b]
Error	25	7.44	0.29		
Total	35	31.16			

[a]N.S. = Not significant.

[b]** = Highly significant, 1%.

OSMOTIC DATA

TABLE XXXIIIA

LEAF WATER POTENTIAL–ATM.
HIGH LIGHT INTENSITY

Stress Level, atm.	Determinations			
	1	2	3	4
0.52	-11.5	-12.0	-11.0	-11.0
3.08	-14.0	-13.5	-13.5	-12.5
4.95	-17.0	-16.5	-16.5	-16.0
7.50	-18.5	-18.0	-19.5	-19.5
10.81	-21.5	-21.0	-20.5	-20.5
15.87	-22.5	-22.5	-23.0	-22.5

TABLE XXXIIIB

ANALYSIS OF VARIANCE

Source	df	SS	MS	F	
Stress levels	5	379.05	75.81	276.36	**[a]
Error	18	4.94	0.27		
Total	23	383.99			

[a]** = Highly significant, 1%.

TABLE XXXIVA

LEAF WATER POTENTIAL-ATM.
MEDIUM LIGHT INTENSITY

Stress Level, atm.	Determinations			
	1	2	3	4
0.50	-11.0	-12.0	-12.5	-12.5
2.26	-13.5	-13.5	-14.5	-14.5
4.78	-15.5	-15.5	-16.5	-16.5
8.08	-17.0	-18.5	-18.5	-18.5
9.66	-19.0	-19.5	-20.5	-18.5
12.73	-22.5	-21.5	-22.5	-22.5

TABLE XXXIVB

ANALYSIS OF VARIANCE

Source	df	SS	MS	F	
Stress levels	5	277.83	55.57	123.10	**[a]
Error	18	8.12	0.45		
Total	23	285.95			

[a]** = Highly significant, 1%.

TABLE XXXVA

LEAF WATER POTENTIAL-ATM.
LOW LIGHT INTENSITY

Stress Level, atm.	Determinations			
	1	2	3	4
0.49	-10.5	-11.5	-11.5	-11.0
2.36	-12.0	-12.0	-13.0	-13.5
5.30	-14.5	-14.0	-14.5	-14.5
7.30	-16.5	-16.0	-15.5	-16.0
8.26	-18.0	-17.5	-17.5	-18.0
13.60	-20.0	-19.5	-20.5	-20.5

TABLE XXXVB

ANALYSIS OF VARIANCE

Source	df	SS	MS	F	
Stress levels	5	215.62	43.12	221.78	**[a]
Error	18	3.50	0.19		
Total	23	219.12			

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[a]** = Highly significant, 1%.

TABLE XXXVIA

EXPRESSED CELL SAP OSMOTIC PRESSURE-ATM., 20°C.
HIGH LIGHT INTENSITY

Stress Level, atm.	Determinations	
	1	2
0.52	17.69	17.17
3.08	20.40	19.30
4.95	19.50	20.08
7.50	21.50	19.95
10.81	21.76	20.92
15.87	21.05	21.43

TABLE XXXVIB

ANALYSIS OF VARIANCE

Source	df	SS	MS	F	
Stress levels	5	20.50	4.10	12.69	**[a]
Error	6	1.94	0.32		
Total	11	22.44			

[a]** = Highly significant, 1%.

TABLE XXXVIIA

EXPRESSED CELL SAP OSMOTIC PRESSURE-ATM., 20°C.
MEDIUM LIGHT INTENSITY

Stress Level, atm.	Determinations	
	1	2
0.50	16.54	16.28
2.26	17.25	17.76
4.78	18.73	17.38
8.08	19.06	17.44
9.66	18.73	19.06
12.73	18.99	19.19

TABLE XXXVIIB

ANALYSIS OF VARIANCE

Source	df	SS	MS	F	
Stress levels	5	9.64	1.93	4.70	*[a]
Error	6	2.46	0.41		
Total	11	12.10			

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[a] * = Significant, 5%.

TABLE XXXVIIIA

EXPRESSED CELL SAP OSMOTIC PRESSURE-ATM., 20°C.
LOW LIGHT INTENSITY

Stress Level, atm.	Determinations	
	1	2
0.49	12.91	11.87
2.36	13.68	15.43
5.30	16.07	15.94
7.30	15.88	16.59
8.26	16.33	17.11
13.60	16.07	17.63

TABLE XXXVIIIB

ANALYSIS OF VARIANCE

Source	df	SS	MS	F	
Stress levels	5	29.32	5.86	9.13	**[a]
Error	6	3.85	0.64		
Total	11	33.17			

[a]** = Highly significant, 1%.

APPENDIX III

LINEAR AND CURVILINEAR REGRESSION ANALYSES

TABLE XXXIX

LINEAR REGRESSION ANALYSIS
PLANT DRY WEIGHT

Light Intensity	Correlation Coefficient	Regression Coefficient, $\underline{b}$	Confidence Limits of $\underline{b}$[a]
High	-0.9698	-0.1233	$\pm$ 0.0425
Medium	-0.7445	-0.0459	$\pm$ 0.0570
Low	-0.8769	-0.0265	$\pm$ 0.0202

[a]95% Confidence coefficient.

TABLE XL

CURVILINEAR REGRESSION ANALYSIS
WATER POTENTIAL AND OSMOTIC PRESSURE DATA

	$\underline{b}_1$	Confidence Limits $\underline{b}_1$[a]	$\underline{b}_2$	Confidence Limits $\underline{b}_2$[a]
High light				
WP	1.39	$\pm$ 0.53	-0.038	$\pm$ 0.031
OP	0.63	$\pm$ 0.45	-0.025	$\pm$ 0.026
Medium light				
WP	0.81	$\pm$ 0.42	-0.001	$\pm$ 0.032
OP	0.38	$\pm$ 0.36	-0.014	$\pm$ 0.027
Low light				
WP	0.87	$\pm$ 0.62	-0.012	$\pm$ 0.042
OP	0.88	$\pm$ 0.38	-0.040	$\pm$ 0.026

[a]95% Confidence coefficient.