"THE RELATION OF GROWTH TO LEAF AND SOIL WATER POTENTIALS IN SOME GRAMINEAE"

Thesis presented in part fulfilment of the requirements for the Degree of Doctor of Philosophy of the University of London

by

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January, 1967

ABSTRACT

The relationships have been examined between the water potential in the rooting medium, and plant water potential, plant water balance, leaf growth and assimilation in plant of Zea mays and Lolium perenne growing in a sandy soil and in solutions of Polyethylene glycol 4000.

Plant water potentials were measured by the Weatherley osmometer and an examination of the reliability and accuracy of the apparatus was made by comparing two distinct methods of using it, viz, either by the measurement of the rate of evaporation from a drop of water or by determining the solution which is in vapour equilibrium with the leaf.

Comparisons with the Schardakow and Slatyer vapour equilibration methods of measuring plant water potential were also made. The osmometer was shown to be the most accurate method but subject to error, caused by leaf resistance, when the measurement of evaporation from a drop of water was the method employed.

The moisture characteristic and capillary conductivity of the soil used in the experiments were determined.

Transpiration rate and plant water potentials fell

with decreasing water potential in the rooting medium and the influence of soil and plant resistances in controlling the plant water potential is discussed. Transpiration in both species was reduced similarly by decreasing leaf water potential and the role of the stomata in control by transpiration is considered. Leaf extension growth, leaf area and net assimilation rates were depressed by decreasing plant water potential and all aspects of growth of Zea were more sensitive to stress than Lolium. Leaf extension was shown to be related to turgor and net assimilation rates to plant osmotic potential. Net assimilation was shown to be less affected by stress than transpiration and also to continue when leaf extension had ceased.

Possible explanations of the differences in behaviour of plants grown in soils and solutions are considered and also the modification of plant response to stress by changes in the plant osmotic potential.

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ACKNOWLEDGEMENTS

I wish to thank Dr.A.J.Rutter for his advice and critiscism throughout the period of this work and for giving so much time to reading the manuscript.

During the course of many hours of discussion Dr. Jiří Úlehla has been a source of encouragment and ideas.

I would also like to thank Dr.Khalid H.Sheikh, with whom I shared a laboratory for three years, Dr.K.Loach and Mr.P.Robins for their stimulating company.

I am indebted to my wife, whose patience has not proved wanting.

Thanks are due to the staff of the Botany Department, Imperial College, for help in preparation of this thesis, particularly Mr.A.Ironside for the photographic work and Mrs.P.Grounds for typing the stencils.

INTRODUCTION

Water enters into the economy of the plant both as a reactant and a solvent in biochemical processes, and as a controlling factor in the gross structural organization of the plant body in the turgor of the cells and tissues, (Stocker, 1960; Kramer, 1959). The structure of proteins and therefore of enzyme and membrane systems is dependant upon the formation of hydrogen bonds and other forces within the protein molecule and between it and the water which constitutes the environment (Klotz, 1958; Vennesland, 1960). The study of the chemical and physical nature of water, especially in relation to the complex of phases existing within the cell, (Bernal, 1965) does not appear to be, as yet, advanced sufficiently to offer any explanation of the many phenomena involved in cellular activity.

It is generally concluded that water content is not, in itself, an important factor in cell reactions, but that the chemical potential of water is of the utmost importance. (Stocker, 1960; Walter, 1963). Taylor and Slatyer (1961) state: "The partial specific Gibbs free energy in the system is, by definition, the 'water potential' or chemical potential of the chemical species

water in a system of many chemical species". However 'water potential', in Taylor and Slatyer's notation, refers to the sum of the osmotic and turgor potentials in the cell (see Chapter 1). Some confusion is likely to arise if a clear distinction is not made between the chemical potential of water and the water potential, (Spanner, 1964). It is not agreed in the literature that the cell's activities are necessarily directly related to the Gibbs free energy. Walter (1963, 1955) and Walter and Wiebe (1963), have argued from a model that the "hydrature", which is the relative humidity in percent of the biocolloids in the cell, is the fundamental quantity to which growth is related. Stocker (1960) considers that hydrature is "a function of the relative vapour pressure and so practically identical with the chemical potential of water". The determination of the hydrature (Walter, 1963) depends however on the measurement of the osmotic value. The osmotic value (or osmotic potential in Taylor and Slatyers terminology) seems to be a more useful quantity than hydrature in attempting to relate growth to cellular water status because it is directly related to the specific free energy and also it does not lead to confusion with hydration of proteins for example.

The osmotic potential of the cell is the potential which is responsible for drawing water into the cell, according to the classical theory which Bennet-Clark (1959) has discussed. Differences in the osmotic potentials of cells of organs of a plant and between species have been shown, but the difficulties associated with sap extraction and measuring the osmotic characteristics must introduce uncertainty into much of the data. The matric component of the potential, caused by the presence of a solid phase within the cell, is negligible for mature, vacuolated cells, (Wiebe, 1966). In the turgid cell the osmotic potential is balanced by a hydrostatic potential, turgor, which arises as a result of the relatively inextensible nature of the cell walls and the pressures of neighbouring cells in a tissue. At maximum water content, the turgor potential of the cell is at a maximum and the water potential, i.e. the sum of the turgor and osmotic potentials, is zero. With reduction in the cell water content the turgor potential decreases and with it the water potential, until at zero turgor the water and osmotic potentials are equal. Water moves into the cell along gradients of water potential and a cell below full water content will tend to take up water and restore its turgor.

In considering the relation of growth to osmotic, turgor and water potentials in the cell or tissue it is necessary to make a distinction between different aspects of the growth process and between the potentials affecting them. Slatyer (1957) showed that expansion and elongation of organs and dry weight increase ceased at about zero turgor, although dry weight gain was more sensitive to increased water stress in privet compared to cotton or tomato. Leaf extension is reduced by decreasing soil water potential, (Wadleigh and Gauch, 1948) and leaf area may be reduced by leaf senescence when plants are subjected to stress (Gates, 1955).

In studies on whole plants it cannot be assumed that the potentials measured in one position are the same as those in another part of the plant. Leaves of one plant do not wilt at the same time (Wilson, 1948) nor do different parts of the same leaf have the same potential, (Campbell, Zollinger and Taylor, 1966). Fruits do not respond in the same way as leaves to stress, (Anderson and Kerr, 1943) which suggest that water potential is not uniform throughout the plant. The sensitivity of certain aspects of growth to lower cell water, turgor and osmotic potentials depends upon the time at

which the plants are subjected to stress. Grain development in cereals is seriously reduced by water stress at the time of the anthesis but less so by stress at other times, although the effects of stress at one stage of development may be reflected at a subsequent stage, e.g. the reduction in grain yield due to suppression of tillering at an earlier stage of growth, (Aspinall, Nicholls and May, 1964).

Carbon dioxide assimilation has been shown to respond to lowered water potentials in the plant because of effects on the cell metabolism and also because of restricted carbon dioxide supply resulting from stomatal closure. Slavik (1965) working with thallophytes where stomatal effects cannot complicate the situation, showed that decreased cell water content and osmotic potential of the cell sap reduced the rate of photosynthesis. Schneider and Childers (1941), Loustalot (1945) and Ashton (1956) clearly demonstrated that photosynthesis was reduced by stress. Translocation of photosynthates was reduced with decreasing plant water potential (Wiebe and Wihrheim, 1962) but it is interesting that even at wilting translocation was reduced only by two-thirds and did not fall to zero. Wilson and McKell (1961)

demonstrated that phosphorus applied to the leaves was not as readily absorbed or translocated when the plants were suffering from moisture stress. Normal absorption of nutrients from the soil, for example phosphate, is reduced as the soil water is depleted and soil water movement is probably responsible for the greater part of the movement of phosphate to the root, (Shapiro, Armiger and Fried, 1960).

Respiration in the cell is affected by increasing stress, although the pattern of respiratory response is less clear than that of photosynthesis (Brix, 1962). Reduction in chlorophyll formation was demonstrated by Virgin (1965) as a result of increasing stress reducing the production of protochlorophyll. Probably stress also leads to a destruction of existing chlorophyll and chloroplast structures as a result of protein changes with stress. Obviously any reduction in the activity of the photosynthetic mechanism, either the photochemical or enzymic reactions associated with carbon dioxide assimilation, will reduce the productivity of the plant. Not only is the net assimilation rate reduced, that is the balance between production and consumption of assimilates, but the type of materials produced and the proportions are affected. Starches

decrease and hexose sugars increase in response to periods of stress in cotton plants,(Eaton and Ergle, 1948). Under stress conditions tobacco plants show a decrease in nicotine content,(Van Bavel 1953), whereas in guayule, rubbers and resins increased with stress, (Wadleigh, Gauch and Magistad, 1946, quoted by Kramer, 1959). In addition lignins increased with reduction in the plant water potential but Plaut and Ordin (1964) showed that incorporation of glucose into the cell wall was reduced by stress.

The changes in metabolism which result from a reduction in the potential of water in the plant are intimately related to the changes in the cellular metabolism of proteins. Reduction in the total protein content and changes in the constituent amino acids forming the amino acid pool were reported by Petrie and Wood (1938 a and b). Enzyme systems are modified by stress. Todd and Yoo (1964) showed that saccharase, phosphatase and peroxidase activity was reduced by water stress, but that the individual enzymes were not affected to the same extent. Saccharase was more reduced than phosphatase activity, dehydrogenase did not decrease with initial reduction in the plant

water content, but rose above the control level and then fell rapidly with further increases in stress. It seems unlikely that enzyme activity is related to hydrostatic (turgor) potential, per se, but osmotic potential has a more direct effect on enzymes; as Tyler (1954) showed, succinic oxidase activity decreased with decreasing osmotic potential.

The changes in enzyme activity are paralleled by modifications in the metabolic systems of the cell. The phosphorylated intermediary compounds, for example uridine and guanosine triphosphates, increased with water stress but adenosine triphosphate decreased in the study made on Zea seedlings by West (1962).

All the changes have been regarded as due to alteration of the balance between anabolic and catabolic reactions in the cell towards a senescent condition, (Gates, 1957) but this does not explain how water stress acts in determining the balance of cellular functions.

Probably water stress acts directly on the existing cellular structure, by, for example, changes in the hydration shells of the molecules concerned and by altering the spatial distribution of the membranes and organelles within the cell, (Schnepf, 1961). Reduction in the

cell water content and potential may initiate changes in the cell by affecting he nucleic acids which control protein synthesis. West (1962) showed clearly that with decreasing osmotic potential of the growing medium ribonucleic acid accumulated in Zea seedlings and the base composition of the nucleic acid was altered. Kessler (1961) and Kessler and Frank-Tishel (1962) concluded, however, that hydrolysis of RNA increased with stress in leaves as a result of enhanced RNAase activity although synthesis of RNA with altered base composition took place. The changes in plants suffering water stress, particularly the increased rate of leaf senescence and respiration suggest that nucleic acids are involved in the response of growth to stress as they are in normal leaf senescence (Krul and Leopold, 1965).

The effects of water stress on the growth of plants seems quite clearly defined; a reduction in the expansion of the cells and organs and a change from synthetic to degradative activity in the cell. The question, however, remains to be answered as to what are the exact relationships between the processes of growth and the cell water characteristics, i.e. turgor or the water and osmotic potentials. The question is not

easily answered, because for the majority of studies the plant water characteristics have been defined only in terms of water content of the plant or its water potential, which allows for no analysis of growth in relation to turgor etc. In earlier work the stress applied to the plant was often only indicated by soil water content or potential, which is an inadequate indication of the level of stress in the cell. It was suggested earlier that leaf extension may be more closely related to cell turgor than to the water potential, which is responsible for water uptake, similarly the osmotic potential of the cell may influence the assimilation of carbon dioxide as the data of Boyer (1965), show. It is only where a full analysis of the course of events taking place in the plant is made that differences between species and aspects of growth may be understood.

Turning from the considerations of growth to water movement through, and loss from, the plant (which will be considered in more detail in the introductions to later chapters) it may be stated that reduction in plant water potential results from water uptake lagging behind transpiration. In the case of soils both the water potential and conductivity of the soil water fall with

decreasing water content. In addition the roots and leaves of the plant offer resistance to the flow of water, (Kramer, 1959; Rawlins, 1963). Increase in any of these resistances or decrease in the water potential of the rooting medium leads to a reduction in the gradient of potential between the leaf and soil and to a slower rate of water supply to the leaf.

Transpiration is governed by environmental, atmospheric, factors which may have no relation to the events taking place in the soil and hence transpiration may exceed uptake, leading to reduction in plant water potential and turgor. The stomatal aperture is reduced by falling plant turgor, (Stälfelt, 1964) which tends to restrict transpiration. Therefore although it is of interest to compare soil or rooting medium potentials to transpiration and growth and to the potentials in the plant, the relationships will at best be indirect ones. It is probably of more value to study the response of transpiration and growth to the plant characteristics. However the difficulties of measuring the cellular water status necessitates that as a first approximation the water potential of the medium is used as an indicator of the water stress imposed on the plant.

Much of the agricultural and ecological literature on plant water relations has concentrated on the problems associated with water loss from plants or communities and with attempts to relate growth or water loss directly to characteristics of the soil, (Furr and Reeve, 1945). Veihmeyer and Hendrickson, (1950) argued that water was equally available to the plant between the maximum soil water content and the point at which the plants suffered permanent wilting, but much evidence to the contrary has accumulated and Cowan (1965) has, for example, performed theoretical calculations based on a model of water movement through the soil-plant-atmosphere continuum which suggests that the distribution of the roots and rooting density, volume of soil exploited etc., affect the availability of water and that Veihmeyer's ideas are but one aspect of a range of possible interrelations between the soil and plant. Much of the evidence about plant growth under natural or seminatural conditions is based on correlations between soil water potential and the fresh or dry weight of all or part of plants, (Taylor, 1952; Hagan, Peterson, Upchurch and Jones, 1957) and attempts have been made to predict yields of crops on the basis of the transpiration or potential transpiration, (Arkley, 1963). However it would seem more valuable

to employ the type of approach used by Slatyer (1957), where many characteristics were compared at the same time, including the soil and plant water potentials and growth.

More detailed and controlled experiments may be performed in osmotica, although such experiments are not directly comparable to those performed in soils. Absorption of osmoticum from the medium is likely to affect the relation of growth to applied stress because of changes in the cell characteristics, (Bernstein, 1961). Studies on plant response to solutions of decreasing potential have been performed by Slatyer (1961). Wadleigh and Ayres (1945) used a combination of decreasing osmotic and matric stress in studies on growth.

The aims of the present investigation may now be stated in relation to the discussion which has been given. Firstly it was intended to make a study of the major factors affecting the water potential of the plant in order to related the changes in potential to conditions in the environment. An apparatus for measuring plant water potential, the Weatherly Osmometer, was employed and results obtained with it were critically examined. The soil water characteristics were examined in order to

relate the soil water potentials and conductivity to water uptake by the plant. Changes in the leaf water potentials and transpiration rates were studied in soil and polyethylene glycol solutions.

Secondly the relation of aspects of growth to plant and soil water potentials were studied. Leaf elongation was chosen as the simplest and most direct indicator of plant response. Thirdly plants were grown in solutions of polyethylene glycols and in addition to leaf elongation, dry weight increase and net assimilation were studied in relation to medium and plant water potentials. In each case two monocotyledons were used, Zea mays (corn or maize), because it is widely employed in physiological studies and had been used for similar experimental work by Mongelard (1965) and Lolium perenne, perennial rye grass. The cell characteristics of both species were examined and attempts made to relate transpiration and growth to the turgor and osmotic potentials of the leaves.

Chapter 1. An Examination of Methods to Determine the Status of Water in Plants.

A. Principles

The state of water in the plant may be expressed in terms of the thermodynamics of water. Moore (1959, page 141) states that "a complete thermodynamic description of a solution, except for its amount, can be expressed in terms of the temperature, the pressure and the chemical potentials of the various components." In the water relations of the soil, plant atmosphere system, it is the energetics of the constituent parts of the catena which is important and the ideal is, therefore, to relate the processes in the system to the chemical potential as well as the amounts of water.

Taylor and Slatyer (1961) have discussed the thermodynamic basis of water potential measurement and proposed a terminology for soil and plant studies which relates the energy state of water to the partial Gibbs free energy. The development of the Gibbs free energy function is dealt with by Moore.

The potential of water in a system is the difference between the chemical potential of water in that system and water in a standard reference state which is taken as pure free water at standard temperature and pressure. The water

potential, μw, is defined by

$$\mu w = Rw \, T \, \ln aw \qquad (1)$$

where Rw is the gas constant per gramme of water, T is the Kelvin temperature, and aw is the activity of water in the system. For practical purposes

$$aw = \frac{p}{po} \qquad (2)$$

where $\frac{p}{po}$ is the relative ~~lowering of the~~ vapour pressure and affords a method of determining the activity. These are equilibrium equations relating the terms governing the water potential.

The water potential is reduced by ~~any~~ forces acting on the system and the water potential will then be negative. The reduction in water potential may be caused by the presence of solutes, the total reduction due to the solute component is called the osmotic potential, π. It may also occur owing to the presence of the soil or plant matrix. This component of the water potential is called the matric potential, symbolized by τ. The actual extent of the reduction will depend on the physical and chemical properties of the matrix and the amount of water present in it. The exact relationship may be complicated by changes in the matrix, for example hysteresis, swelling, etc.

For the system as a whole the total reduction of water potential, which may be symbolized by ψ, is the sum of the water potentials of the components mentioned, that is

$$\psi = \pi + \tau + P \qquad (3)$$

where P is the pressure component. In this way the water potential ψ, can be written to include any force acting on the system.

In soils ψ has been called the total soil moisture stress; in unsaturated soils P is zero and τ is the major component, whilst π may be of importance in saline soils. In vacuolated plant cells, ψ is frequently called suction pressure, "Saugkraft" , diffusion pressure deficit or suction potential and is the sum of π and P, the latter being identified with the wall or turgor pressures. Discussion of these points is given by Crafts, Currier and Stocking (1949), Slatyer and Taylor (1960), Taylor and Slatyer (1961), and Weatherley (1965).

The fundamental unit of free energy is the erg g^{-1}, a unit of work. One erg g^{-1} equals 1 dyne cm^{-2} multiplied by the specific volume of water which is taken as approximately equal to 1 for dilute solutions and 10^6 dynes cm^{-2} equals one bar or 0.987 atmospheres at S.T.P. It is possible to use energy terms but pressure units have

common currency and have been adopted in the present work with the atmosphere (abbreviated to "atm".) as the unit. Because the water potential of the reference state is taken as zero the water potential of the system will either be zero or negative.

B. Methods and Materials

Water potentials have been measured in plant tissue using a variety of methods, many of which relate the plant water potential to free water by comparison with solutions of known osmotic potential. The methods employed include immersion of the tissue in water and solutions, the solution of equal potential with that of the tissue being found either by measuring the change in tissue weight or dimensions, or by measurement of solution density, refractive index etc. Ashby and Wolf (1947), Schardakow (1956). Immersion of the samples in osmotica may lead to errors in the measured water potential due to solute absorption by the tissue or contamination of the osmoticum by exudation of cell sap.

The errors due to immersion may be eliminated if a vapour phase separates the tissue from the solution, the vapour phase acting as a "semipermeable membrane". Tissues may be equilibrated over solutions of known potential and

the potential of the solution in which no tissue weight change takes place is the water potential of the tissue. (Slatyer 1958). Alternatively the vapour pressure in the gas phase in equilibrium with the experimental material may be determined and compared to that of solutions of known potential. Measuring small changes in vapour pressure is difficult because, for example, the reduction in relative humidity in equilibrium with a -10.0 atm. sodium chloride solution is 0.7%.

Measurement of the vapour pressure may be made directly (Barger 1904) or indirectly, for example, by measuring the electromotive force from a thermocouple placed in a chamber containing the sample. The thermocouple output is produced by evaporation of a drop which is formed on the junction after cooling by the passage of an electric current through the thermocouple (Spanner 1951, Monteith and Owen 1958). Alternatively the junction is a permanent "wet bulb" and the thermocouple output is determined by the rate of evaporation (Richards and Ogata 1958). The thermocouple output, in both methods, is calibrated against solutions of known potential.

Weatherley (1960) described a method which permitted the rate of evaporation from a drop of standard solution to be measured, when the drop was contained in a chamber with

the sample under study. The rate of evaporation from the drop to solutions of known potential placed in the chamber formed a calibration curve which enabled the potential of unknown material to be estimated by measurement of the drop evaporation rate. The apparatus was called a "micro-osmometer" and used for the study of biological fluids and solutions. Macklon and Weatherley (1965) have measured the water potential of plant tissues and soils with the apparatus in a modified form, which they termed a "potentiometer".

The osmometer, as it will be called, seemed to be a useful instrument, fulfilling the requirements for a vapour exchange method which was simple in construction and did not require very elaborate temperature control. The performance of the osmometer was, therefore, examined before its adoption in the work which will be described.

The osmometer method was compared with the Schardakow and Slatyer vapour equilibrium methods, as the last two methods have often been employed in the determination of plant water potentials and their relative merits could be clarified. By using the Osmometer with solutions of known potential forming the drop, a test of the accuracy of the methods could be made.

In addition the relationship between water potential and leaf water deficit was examined because, from the leaf water deficit, which is a relatively simple quantity to determine, the water potential could be estimated. (Slatyer 1961).

I. Measurement of plant water potential

a) The Weatherley osmometer

. The original design (Weatherley 1960) was modified by Macklon and Weatherley (1965) and also by Mongelard (1965) who constructed the chamber of glass and used a different form of plunger; this modified form of the apparatus will be described. Problems of contruction and materials have been discussed by the authors quoted.

1. Apparatus. Figure 1. shows the form of the apparatus and gives the dimensions. The capillary tube has a bore of 0.125 mm. diameter and is drawn into a jet with a terminal constriction. It is sealed by heating, into a B7 "Quickfit" ground glass cone with the nozzle projecting 0.5cm. below the opening of the cone. The chamber is constructed from a B7 socket sealed at the base. All glass parts are of borosilicate glass. The undrawn end of the capillary fits into a length of rubber pressure tubing (4mm. wall thickness and 3mm. internal diamter) sealed with a glass stopper. The rubber is mounted in a "perspex" holder with an external

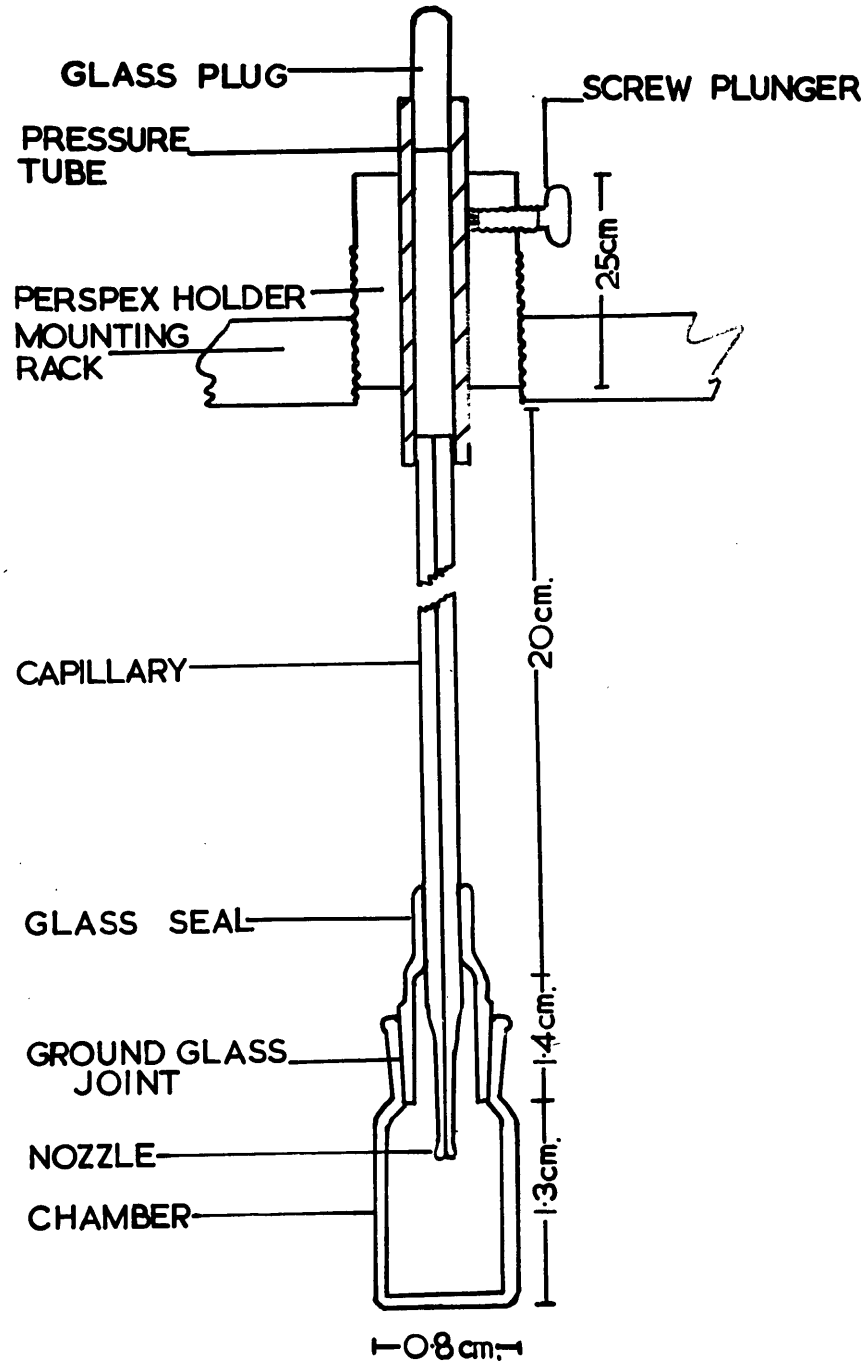


Fig. 1. The modified Weatherley Osmometer.

screw thread which fits a mounting bar above the bath and positions the osmometers. The perspex holder is fitted with a screw plunger, movement of which alters the pressure in the rubber and capillary.

The constant temperature bath is a 2x2x1 foot aquarium tank with "expanded polystyrene" insulation except at the front of the bath and an illumination port in a side panel. The bath is fitted with a toluene and mercury contact thermostat which controls a 250 watt electric heater via a "Sunvic" relay. The water is circulated by paddle wheel stirrer and maintained at 25°C for all experiments. The degree of control is better than 0.05°C and no temperature fluctuations could be observed using a travelling microscope and a mercury in glass thermometer graduated in 0.1°C.

Six osmometers are mounted at the front of the tank by screwing the mounting rack to the side of the tank. The osmometers are illuminated from the side by a 100 watt lamp. The tank is kept darkened except during periods of measurement.

The capillaries are observed through a travelling microscope fitted with a x15 ocular and 1 inch objective, mounted on a glass topped platform with a guide rail to position the carriage during its lateral movement.

2) Method. All glass parts are frequently cleaned with chloroform, chromic-acid cleaning mixture and distilled

water and after drying coated with "Repelcoat" silicone water repellent. Special attention must be given to the nozzle and outer surface of the capillary tube to reduce creeping of the liquid over the outside surface of the nozzle. Drying the chambers and capillaries is achieved by blowing air through the apparatus; oven or air drying should be avoided and all glass parts kept moist, as adsorption errors are important with dry glass surfaces, Lambert and van Schilfgaarde (1965).

The capillary is filled by dipping the nozzle into the solution or distilled water and increasing the pressure in the capillary by manipulation of the plunger. After expulsion of air bubbles, the plunger is reversed and the fluid drawn into the capillary to the mark, giving a liquid thread 9cm. long. The nozzle is dried by touching it on a hard filter paper.

Arrangements of samples in the chamber depends on the nature of the material. For solutions, a filter paper lining (Whatman 52) is placed around the chamber walls and a quantity of solution placed in the chamber to saturate it. Excess is shaken out. For leaf tissue, pieces of cut leaf are placed around the wall with the abaxial surface facing the drop. This arrangement has been questioned by some authors. (Macklon and Weatherley, 1965; Boyer and Knipling, 1965).

Although the calibration using solutions on paper has been applied to tissue, the resistance of the cuticle, epidermis and stomata could affect the resulting water potential estimates by introducing an extra resistance to the diffusion of water vapour from the drop to the leaf. Macklon and Weatherley have used annuli, discs of tissue with a central hole punched through the discs, into which the nozzle projects. The cut surface acts as a free solution surface, without resistance to vapour diffusion; however this may lead to errors due to cell solute contamination. Ehlig (1962) and Barrs (1964) working with thermocouple psychrometers, lined the chamber walls with leaf pieces. Because of the form of the osmometer chambers it was impossible to use discs and pieces of tissue were employed. The method of arrangement and the problem of leaf resistance are discussed in section D.II.

It was considered necessary to use the Weatherley osmometer to measure the water potentials of leaves of Lolium perenne. L. Most studies of leaf water potentials have been made on large leaves and the methods have not been applied to linear or small ones. In order to use the osmometer with Lolium, attempts were made to arrange lengths of leaves around the chamber walls, the elasticity of the material serving to keep it against the wall or by using "Vaseline" to stick

the pieces to the wall, but these methods were unsuccessful. It was found, however, that if the tissue was cut into short lengths, 0.4cm., and arranged in the chamber to form a small central well into which the drop projected, reproducible readings were obtained when the osmometers were used in the way described. A sample of 30 pieces of leaf 0.3cm. wide, has a surface area about the same as that of the calibration paper. For use of the osmometers for soil water potential determinations see chapter 2.

The chamber is placed in the bath, suspended from the mount, and left for a period of 20 minutes to attain temperature equilibrium. The meniscus in the capillary is observed through the microscope with an eyepiece scale of 200 divisions = 2.5mm. and the meniscus setting checked by increasing the pressure, expelling the liquid from the capillary and drawing it back immediately. The meniscus should return to the original reading, the nozzle construction forming a reference point, but in fact setting errors were usually $\pm$ 1 eyepiece division and these were never eliminated; flow of liquid over the nozzle may be the cause.

The evaporation rates are measured by extruding the liquid from the nozzle until it forms a drop; about 1cm. of liquid is retained in the capillary. The drops are allowed to remain in the chamber for a standard time, usually 10

minutes, and then withdrawn into the capillary. The volume of liquid lost or gained is measured by the change in position of the meniscus in the calibrated eyepiece. The rate of evaporation of the drop is then measured in consecutive 10 minute intervals.

3) Calibration. With distilled water constituting the drop, the rate of evaporation from the drop to salt solutions of known potential was measured. The solutions were placed on filter paper lining the walls of the chamber. The curves for rates of evaporation with time are given in figure 2 where the rate of evaporation is shown as the number of divisions of the eyepiece scale lost in 10 minutes. Eyepiece divisions have been abbreviated to EPD.

The rates of evaporation were irregular for periods of about 60 to 80 minutes after closing the chamber; probably the steady state was not attained earlier due to temperature fluctuation in the chambers. Calibration was performed with distilled water as the drop and filter papers saturated with molal sodium chloride solutions, from 0.0 to 0.5 molal at 0.1 molal intervals, i.e. from 0.0 to 18.04 atmospheres. For calibration at lower potentials a five minute period of evaporation was more convenient. A calibration curve of the steady state rates of evaporation from a water drop at the osmotic potential of the sodium

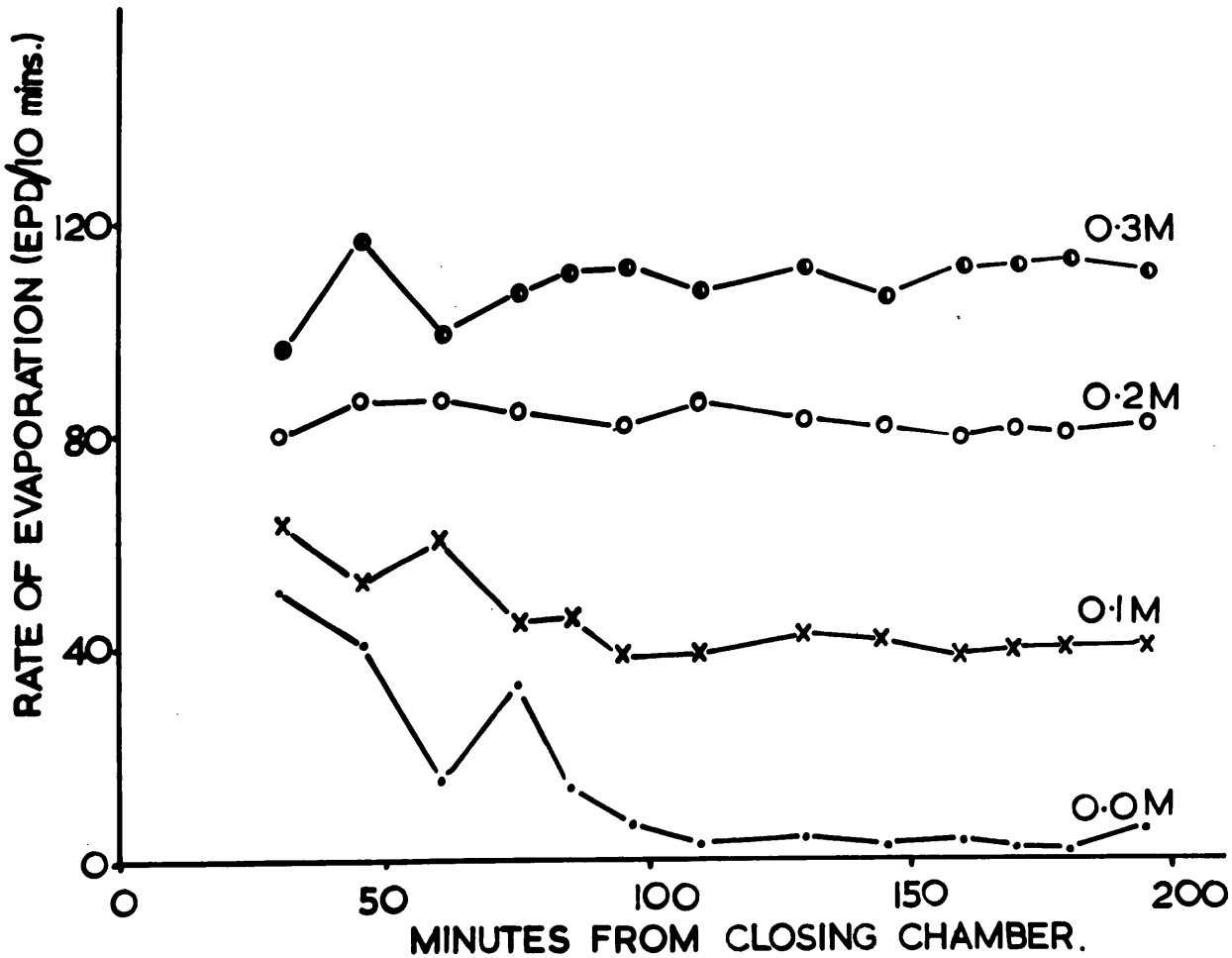


Fig. 2. Rates of evaporation from the cobalt chloride drop to salt solutions during equilibration.

chloride solutions employed, is given in figure 3. The osmometers were calibrated individually twice for each solution and the calibration curve based on these. A single calibration curve was constructed for all the osmometers and the error of using it at the highest potential was about ± 0.2 atms. and ± 0.5 atm. at the lowest potential, - 18 atm. Only those osmometers with closely comparable rates were used in the studies to be described.

The calibration curve is more or less linear over the range of calibration, although the relationship between water potential and vapour pressure is theoretically logarithmic. It should be noted that the rate of evaporation over water is not zero but about 2 EPD in ten minutes with a maximum of 5 EPD. This may result from the setting errors mentioned previously or from sinks for water vapour in the chambers, possibly the walls or "Vaseline".

b) The Modified Schardakow Method.

Schardakow (1956) introduced a method of measuring plant water potentials by equilibrating samples of tissue in sucrose solutions of known potential and determining the change in density of the test solution by comparison with the original. Drops of test solution were coloured with a dye and placed in the original solution. If the drop sank in it, the test solution density had increased or if it rose then the density

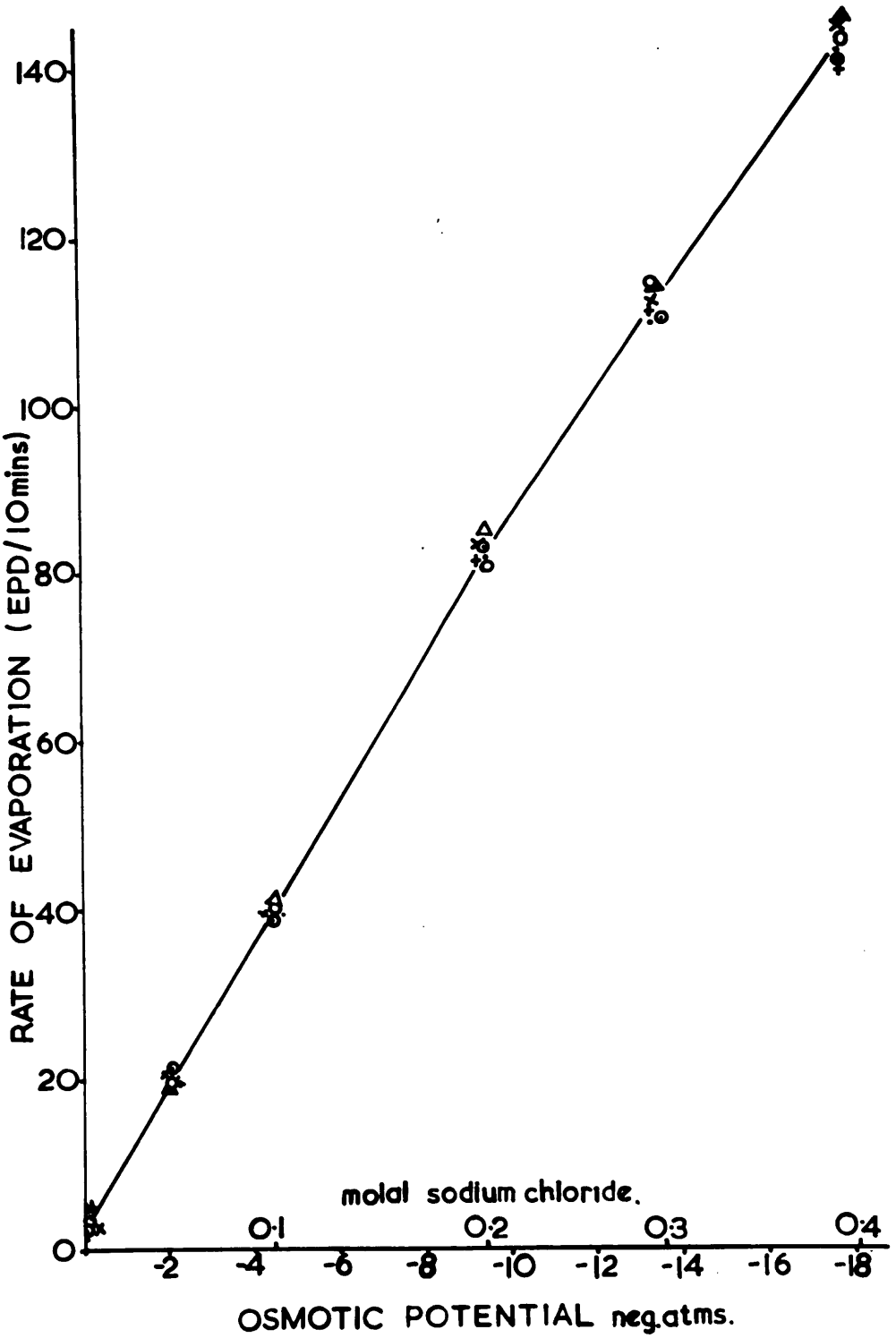


Fig. 3. Calibration curve of rate of evaporation to sodium chloride solutions.

had decreased. Dispersion of the drop indicated no change in the density of the test solution and the osmotic potential of this was taken as the water potential of the tissue. The method is discussed by Kramer and Brix (1965).

An alternative method of measuring the change in density of the solution has been suggested by Professor B.G. Grieve in a discussion at Imperial College and many of the details owe their origin to him. His help is gratefully acknowledged.

The method is based on the "dilatometer" principle of Linderstrøm-Lang (1937) for use in the study of enzyme reactions and it is discussed by Glick (1949). The density of materials, including sucrose, is determined in a gradient of increasing density, composed of hydrocarbon liquids.

1) Apparatus. The gradient is produced in a glass column, Plate 1, by adding bromobenzene (specific gravity 1.49 to 1.50) to the lower bulb and half of the column. Kerosene (s.g. 0.83 to 0.87) is then carefully added to fill the rest of the apparatus. Using a wire mesh disc on a long handle, the interface between the liquids is stirred using vertical, long strokes which decrease in amplitude as the column is mixed. A uniform and stabilized gradient of increasing density is achieved after 12 hours. The column isenclosed within 3cm. of the top, in a water filled museum jar to reduce temperature fluctuations. Growth of fungi,

which may obscure the column, is eliminated by incorporating 2 mls of Boron trifluoride ("Biobor") in to the gradient before mixing, and by removal of all sucrose drops. Cleaning the column is done most easily by introducing a fine glassrod into it; the drops of sucrose adhere and may be lifted to the surface and absorbed on filter paper.

2). Calibration of the gradient. 1.0 microlitre drops of sucrose solutions, of known osmotic potentials, are applied to the gradient using "Shandon" disposable pipettes. The drops are discharged below the gradient surface and detached by withdrawing the pipette. After 10 minutes the drop becomes static and its position in the gradient is measured with a cathetometer. A calibration curve relating density to position in the gradient is constructed before each set of determinations, as removing the drops alters the gradient.

3). Method. Triplicate samples of leaf tissue were taken and placed in a range of sucrose solutions likely to cover the water potential of the tissue. A sample consisted of 10 pieces of leaf 2mm x 1 cm long and this was placed in 0.2 mls of solution in a 1/4 x 1 inch corked tube. Samples of the test solution were taken and compared to the original on the gradient. Interpolation between solutions showing increased or decreased density gave the point of equal potential between the tissue and solution.

4. Experimental Investigations. Experiment I. The time to achieve equilibrium was determined on Zea leaves at low and high leaf water deficit, 2 and 14% respectively. The change in density was measured at intervals during the period of immersion. Experiment II. The errors of measurement were investigated by taking triplicated samples of leaves at different leaf water deficits and determining the water potentials at equilibrium.

The results of experiment I are shown in figure 4. A rise in the water potential took place at the 14% leaf water deficit in the first 3 hours of equilibration and remained constant after that time. No marked change was observed at the 2% leaf water deficit. In distilled water, exudation of solutes from the cut tissue was observed as an apparent increase in the density of water.

The results of experiment II are summarized in table 1.

Table 1. Estimated water potentials of Zea mays leaves at different leaf water deficits. (negative atmospheres.)

Leaf water deficit %	1.2	2.7	3.2	4.5	5.4	12.2	18.0
mean water potential	1.4	0.83	1.9	1.8	3.6	4.7	6.0
3 replicates							
SE of mean ±	0.1	0.2	0.2	0.2	0.3	0.3	0.9

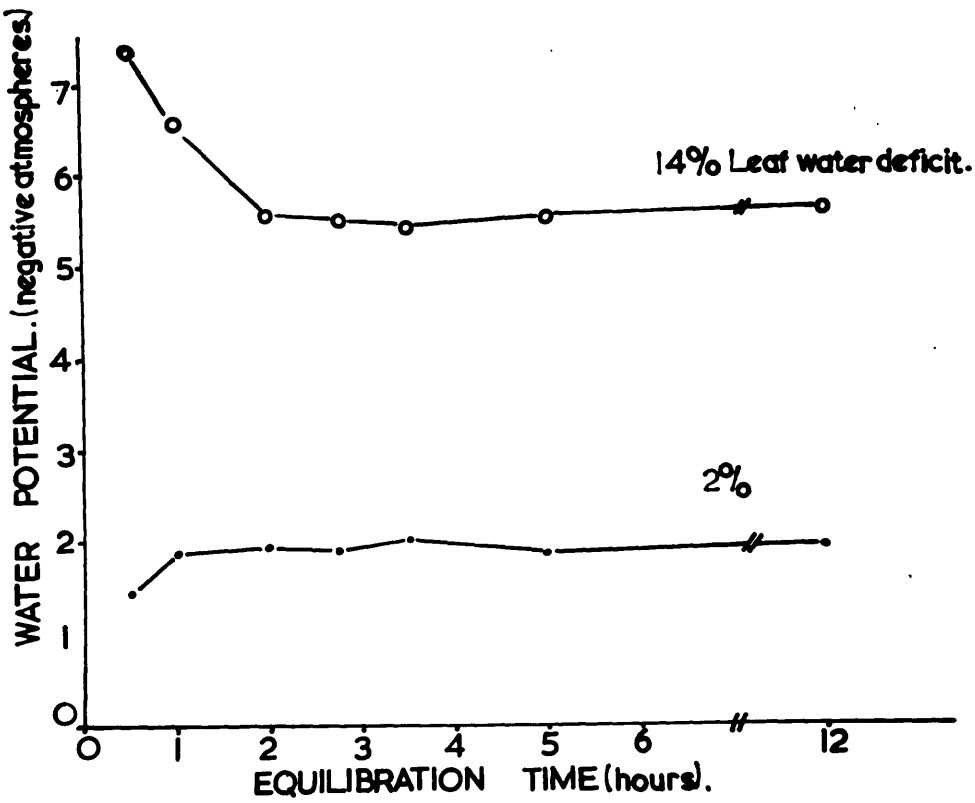


Fig. 4. Changes in water potential with time by the Schardakow method.

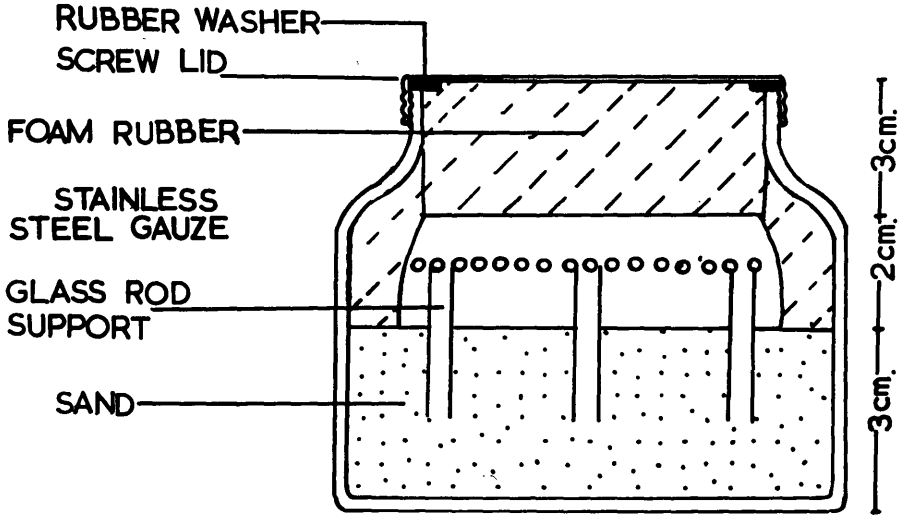


Fig. 5. Slatyer vapour equilibrium chambers.

The rise in the measured water potential over the first few hours shown in experiment I, has been discussed by Kramer and Brix (loc. cit.) and Rehder (1961) who thought it was due to the epidermal water potential being lower than the water potential of the tissue. Another possible explanation is that the tissue loses water more rapidly to solutions of low potential than it does to solutions close to its own potential. The resulting density change is observed in the low potential solutions before the higher, and the water potential, estimated by interpolation of the solution density changes, rises with time of equilibration. The exudation of sap from the cut surfaces of the tissue into the solutions may lead to errors in estimating the water potential and corrections for this have been proposed by Gaff and Carr (1964).

The error of determinations in experiment II is about 10% of the mean; no correction for sap exudation has been applied.

The method adopted to measure water potentials was to equilibrate samples of tissue, as described, and measure the density changes in sucrose solutions of increasing molality after 3 hours equilibration. This method was also applied to Lolium perenne leaf samples; 20 pieces of leaf 0.3 cm. long constituted a sample and the equilibration period was

again determined as 3 hours.

c). The Slatyer Vapour Equilibrium Method. The water potential of samples of leaf tissue is determined by equilibrating samples of leaf tissue over sodium chloride solutions of known potential in small chambers. The change in weight of the samples is found and the osmotic potential of the solution in which no change in weight occurs, is taken as the water potential of the tissue. Slatyer(1958) modified the method of Archichovski and it has been used to relate leaf water potential to leaf water deficit by Weatherley and Slatyer(1957).

1). Apparatus. The equilibrium chambers, figure 5, were constructed from "Kilner" jars, 8 cm. high x 9 cm. diameter with a screw lid and rubber seal. The polyurethane foam and sand were saturated with sodium chloride solutions. The water bath was a 3 x 2 x 2 feet "polystyrene" insulated metal tank with a central water filled metal chamber in which the temperature was kept at 25± 0.5°C. The equilibrium chambers were placed in the inner bath.

2). Method and Use. Leaf samples of about 50 - 100 mg. were weighed on a torsion balance and placed on the stainless steel gauze above the salt solution. Five replicated leaf samples were used and equilibrated in the chambers for 24

hours and the weight of the samples determined.

3). Experiments. In experiment III the water potentials of leaves of Zea mays were determined at two leaf water deficits, 2 and 16%. The change in weight is plotted against the increasing osmotic potential, fig.6. In some cases the samples over water were found to have condensation on the surface and these were rejected. The estimated leaf water potentials were -1.1 atmospheres at 2% leaf water deficit and -4.5 atmospheres at 16%.

Although the method has mainly been used to determine tissue water potentials below about -10 atm. the results suggest that it is possible to use it at higher potentials. Because of the similarity of the Slatyer method to the Osmometer a comparison of the methods appeared to be useful.

II. Leaf Water Deficit.

Leaf water content has received considerable attention, for example, Stocker (1929), Weatherley (1950, 1951), Čatský (1963). It is usual to express the water content on the basis of the turgid water content either as relative turgidity (or relative water content), RT.

$$RT\% = \left(\frac{FW - DW}{TW - DW}\right) \times 100 \qquad (4)$$

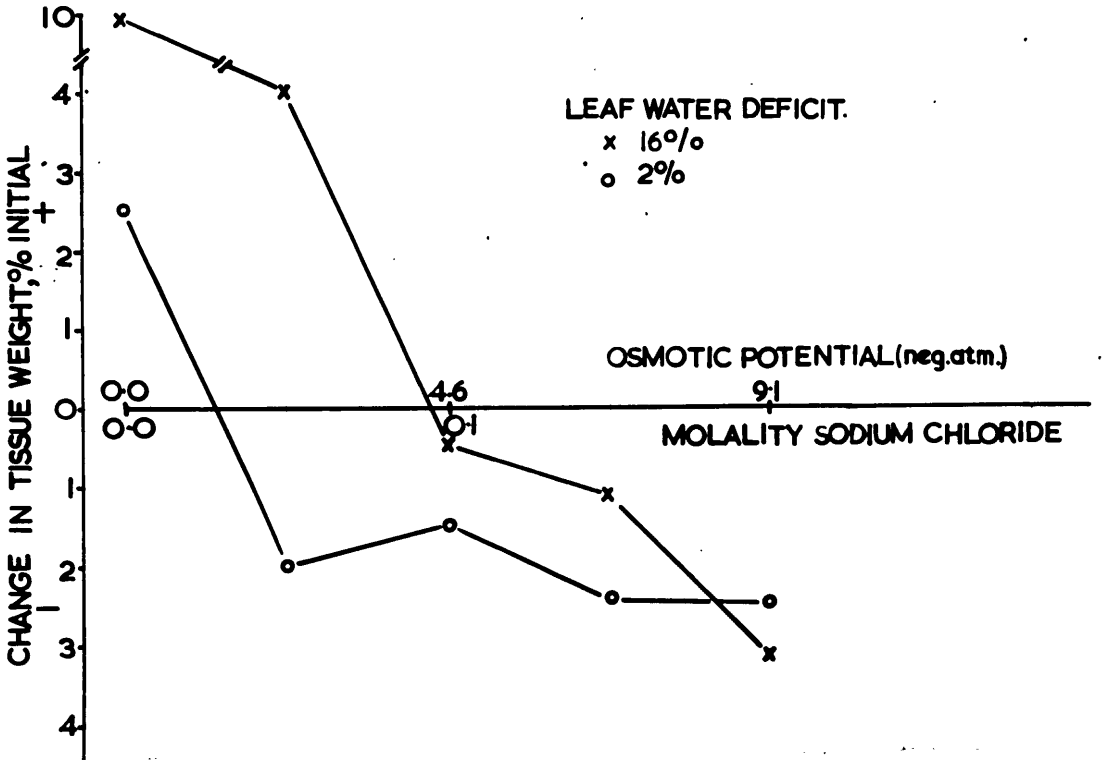


Fig. 6. Change in weight of leaf samples with solution osmotic potential.

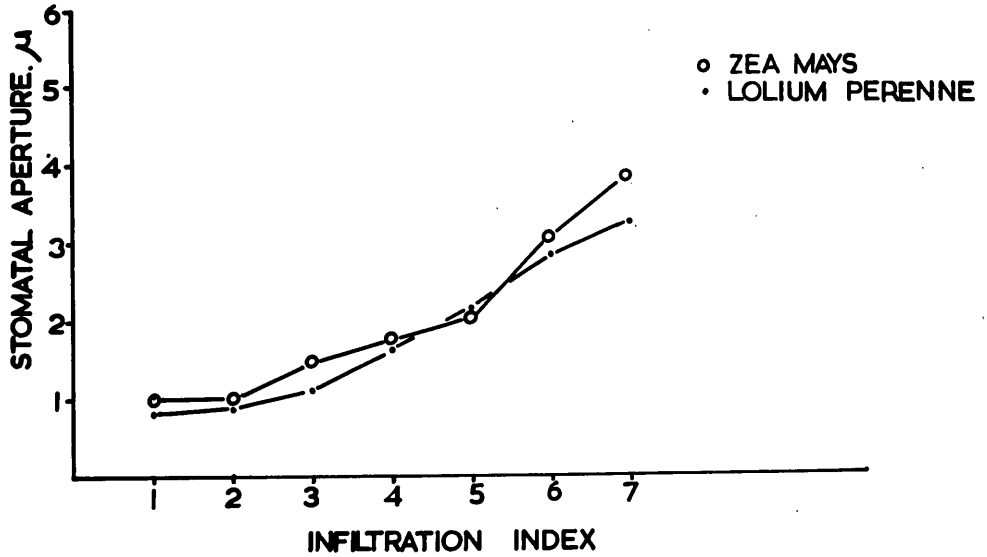


Fig.7. Stomatal infiltration index at different stomatal apertures

or as leaf water deficit

$$LWD\% = \frac{(TW - FW)}{TW - DW)} \times 100 \qquad (5)$$

TW is the weight of the tissue sample at maximum water content after a period of time in contact with free water, FW is the fresh weight of the sample, that is at sampling, and DW is the dry weight of the sample. Leaf water content will be expressed as leaf water deficit.

1). Method. 4 pieces of Zea mays leaf, 1 cm. x 1 cm. were taken as a sample, with a saturated weight of about 50 milligrams. The fresh weight of the samples was found by rapid weighing on a torsion balance and the samples were then allowed to come to full water content, with the bases of the tissue pieces in about 3 mms. of distilled water in corked tubes. The leaf water deficit of Lolium leaves was determined in the same way on six samples each of 4 pieces of leaf, 1.5 cm. long.

2). Experiments. Experiments had shown that the process of water uptake by the tissue was divisible into two phases, an initial period of rapid water uptake, and a subsequent period of slow increase in weight. This is discussed by Čatský (1963) and Barrs & Weatherley (1962) who suggest that low temperatures reduced the second phase. This was found to be so; when the tissue was kept at 4°C, uptake was

completed within 6 hours of sampling. Saturation of the mesophyll was considered negligible when only the base of the tissue was placed in water. The samples were dried between absorbent paper, "Kimwipe", by lightly pressing the hand over the paper, because drying under a constant weight, (Ashby and Wolf, 1947), did not remove the water from between the raised veins of the leaves. This error is potentially more serious for Lolium than Zea.

The replicates agreed to within 2% at low deficits and about 4% at high leaf water deficits.

III. Measurement of Osmotic Potential in Leaves.

Macklon and Weatherley (1965) used the osmometer to measure the osmotic potential of Ricinus leaf samples and the apparatus has been used for this purpose in the present studies.

Following the method of Ehlig (1962), samples of leaves, freshly cut, or those on which the water potentials had been determined, were frozen rapidly either in the osmometer chambers or wrapped in aluminium foil. A jet of carbon dioxide was found to be most suitable for this purpose. The chambers or foil wrapped samples were placed in a sealed dry chamber to exclude water vapour. The killed tissue could be kept at -15°C for a period or immediately thawed in the

air tight chambers. The tissue was arranged in the chambers in the same way as for water potential measurements.

The time to achieve a steady state rate of evaporation from the drop was about 80 to 100 minutes for both Lolium and Zea tissues which had been frozen and thawed. No marked decline in the evaporation rate was observed during the equilibration process and in fact the samples behaved like a saturated filter paper surface. The standard error of the means of three replicate samples was about 3% of the mean for Zea and 11% for Lolium.

IV. Measurement of Stomatal Aperture.

The response of stomata to changing plant water stress was studied in two ways, by the infiltration method and the impression method.

a). The infiltration method. This depends on the penetration of liquids into the stomatal pore, a process which is governed by the surface properties of the guard cells, the surface tension of the solution and its viscosity. (Heath, 1959). By using a series of liquids or a mixture of liquids in different proportions, coloured with a strong dye, the degree of stomatal opening may be assessed visually. The series used was described by Willis and Jeffries (1963)

and consists of a series of solutions and dyes as shown in table 2

Table 2. Infiltration series for stomatal aperture estimation.

Number in series	1	2	3	4	5	6	7
Volume Petroleumether	1	4	2	1	1	0	95% ethanol
Volume absolute alcohol	0	1	1	1	4	1	
Dye 100mg/100mls.	Waxoline blue			Gentian Violet			

The stomatal aperture was determined by immersing a sample of leaves in each solution, agitating for 45 seconds and washing in water. The degree of penetration was assessed visually on the basis of the colouring of the leaf mesophyll. Complete colouration was called 100% and no penetration 0%. When the discrete points of colour below the individual stomata merged into patches this was regarded as 50% penetration. The infiltration index was the number of the solution in which 50% penetration occured, 1 indicating very low penetration and hence small stomatal apertures and 7 a very large aperture.

b) The impression method.

This was described by Sampson (1961) and Zelitch (1961). Silicone rubber solution, mixed with a catalytic hardener (ICI silicone and hardener "D") in proportions such that

the silicone hardened in about 2 minutes, was painted on the leaf surface. The rubber forms a negative of the leaf surface which may be removed and painted with cellulose acetate in acetone. The resulting transparent film, which is a replica of the leaf surface, was observed by transmitted light. The stomatal apertures could be measured with a microscope fitted with an oil immersion lens used without immersion oil.

Two leaf impressions were taken, about 3 x 1 cm in area from similar positions on the leaf, and constituted a sample. The width of about fifty stomatal pores was measured from randomly selected microscopic fields on each sample.

Experiment IV. The impression method was used to calibrate the infiltration method for Lolium and Zea leaves. The stomatal apertures were determined on samples, from leaves growing under different conditions of light and transpiration, by the impression method and the infiltration index was found on parallel samples. The relationship between the infiltration index and stomatal aperture is shown in figure 7.

The apertures were never smaller than about 0.5 to 0.75 μ and solutions 1 and 2 penetrated these apertures.

Solutions 3, 4 and 5 covered the range of apertures most commonly encountered, about 1 to 2 µ. Solutions 6 and 7 penetrated very widely open pores which were rarely encountered.

The impression method is valuable as it does not destroy the sample material, the impressions are permanent and provided that the negatives and positives are handled with care, do not distort the structures replicated. Also the actual pore size is measured, whereas the infiltration method only indicates relative pore size. Infiltration involves destructive sampling but integrates the apertures of a large number of stomata.

The curve relating infiltration index to stomatal aperture should be regarded with caution for Lolium as the stomata in this species are situated on the sides of furrows on the leaf surface and observation by the impression method is difficult.

V. Materials

The experiments were performed on Zea mays L. var. Inra 200 or 321, hybrid dwarf corn, grown in "vermiculite" with frequent applications of a modified Rothamsted culture solution. (Hewitt 1952), with calcium at 5 ppm and iron as ethylene diaminetetra-acetic acid chelate. All plants

were kept, before experiments, in the dark and at high humidity overnight.

The other plant used in the investigations was Lolium perenne. L. var. Aberystwyth S23 strain, (pasture type), grown in soil and watered with Rothamsted solution. The plants will frequently be referred to only by the genus but the species and varieties are those stipulated here.

C. Investigations on the Measurement of Plant Water Potential Using the Weatherley Osmometer.

The apparatus, method of use and calibration have been described in Section B Ia. In this section the process of equilibration in the osmometer chamber is described and discussed in the light of Macklon and Weatherley's (1965) and, particularly, Mongelard's (1965) conclusions on the use of the osmometer and interpretation of the measured water potentials. An examination of the sampling and handling errors will be described and also tests of the measured water potentials by the production of tissues of known potential.

I. The course of equilibration in the chamber.

a). Experiment V. Samples of Zea leaf were cut from adjacent parts of a leaf blade, placed in the osmometer

chambers and allowed to equilibrate in the water bath for 20 minutes. Readings of evaporation rate were then made during consecutive ten minute periods with the drop extruded until a constant rate of loss from the drop was attained. The experiment was repeated at different leaf water deficits. The rates of loss, in EPD per 10 minutes, are shown in figure 8 for the period of observation. The water potentials corresponding to the rates of evaporation were determined from the calibration curves and are also shown.

The steady state of evaporation was achieved between 160 and 180 minutes in most cases and this time was found in repeated determinations on a wide range of Zea leaf materials of varying age and leaf water deficit. Slight falls in the rate of evaporation were detected after 12 hours equilibration. The initial rates of evaporation were always found to be higher than the rates after 180 minutes.

The time taken to attain a steady state of evaporation from the drop to samples of Lolium leaves in the chambers, was observed on leaf tissues at different water deficits. A decline in the rate of evaporation was observed with the steady state achieved after 4 hours, although a slight decrease in the evaporation rate was noted after 24 hours equilibration.

b) Discussion. The rate of evaporation from the drop to the

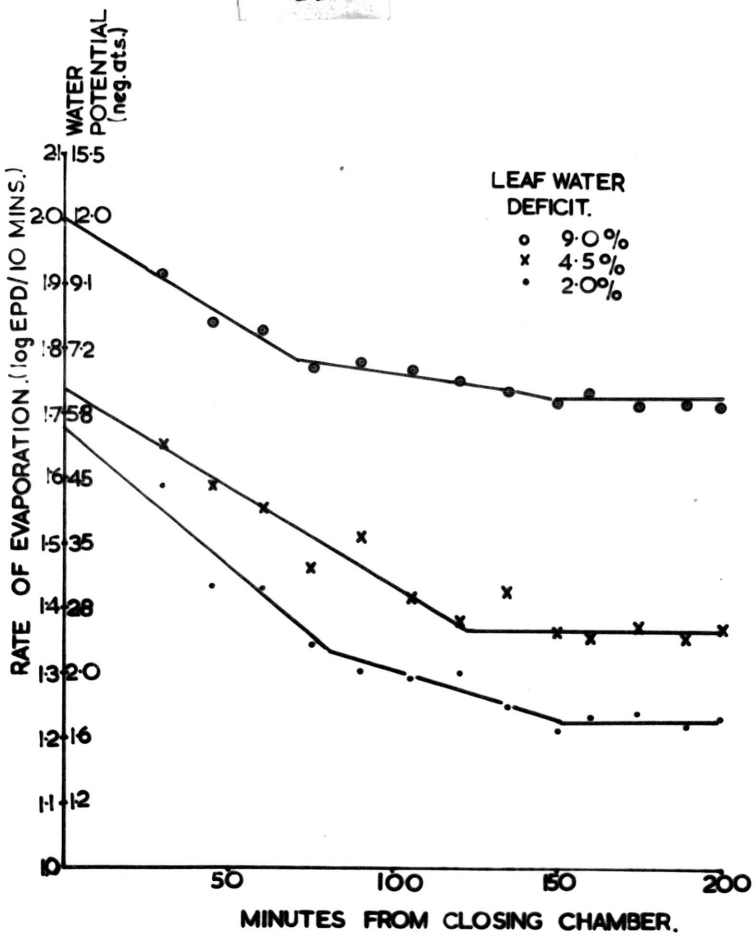


Fig. 8. Change in the rate of evaporation with time to leaf samples of different deficits.

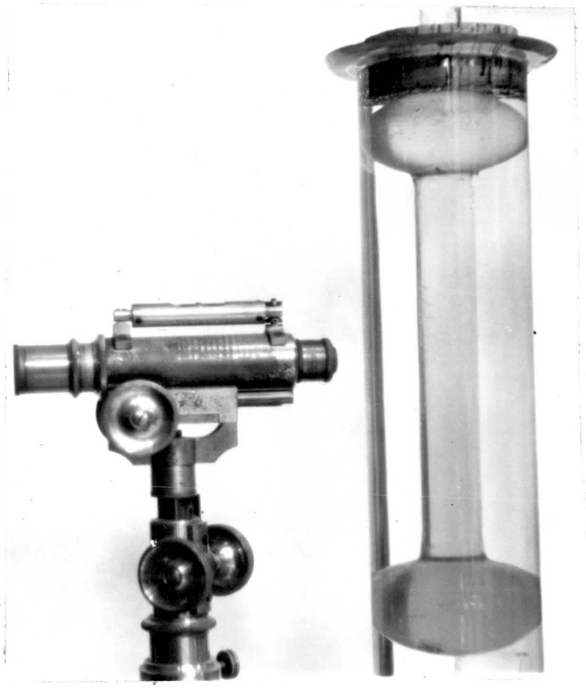


Plate 1. Gradiant column used in the determination of the densities of sucrose solutions.

leaf falls markedly over the initial 3 hour period but the rate of evaporation to filter papers saturated with sodium chloride solutions, although not achieving the steady state for $1\frac{1}{2}$ hours, shows only a small overall decline in this time. Similar changes in the rate of evaporation during the time that tissue was equilibrated in the osmometer chambers, were observed by Mongelard in his studies of Zea mays. He drew attention to the falling rate of evaporation from the drops in the initial 3 hour period with leaf samples in the chamber. He suggested that extrapolation of the evaporation rate to the time at which the tissue was cut, the zero time, would yield an estimate of the water potential of the tissue, by comparison ~~of the extrapolated rate~~ with the calibration curve. The water potential of the tissue estimated by the steady state evaporation rate, i.e. after 180 minutes, was always higher than the potential estimated by extrapolation of the evaporation rate to zero time. In this work two estimates of leaf water potential will be distinguished; that derived by extrapolating the evaporation rate curve to zero time and that from the steady state rate of evaporation. The two water potentials will be called the "zero time" and "steady state" estimates respectively and abbreviated to "ψ zero" and "ψss".

Mongelard regarded ψ zero as the potential responsible

for drawing water through the plant because he found , with Zea plants grown in soil, that the ψss values of the leaf were higher than the water potential of the soil, an impossible situation if water was to move from the soil to the plant, whereas ψ zero values were lower. He thought that the change in the evaporation rate of the drop with leaf tissue in the chamber was a consequence of a redistribution of water between the cell vacuole, cytoplasm and wall. As the cell surfaces would be expected to control conditions in the chamber the ψ zero values would be the potential of water in the cell wall or at its surface. Movement of water from high potential in the cell to low potential in the wall would take place during the period that the tissue was in the chamber, until the potentials were uniform throughout the tissue. This process, which was thought to take place in about 180 minutes, would then lead to a rise in the tissue water potential with a resulting fall in the evaporation rate. The value of ψss Mongelard regarded as representing the potential of the cytoplasm and vacuole after equilibration had taken place.

Mongelard performed experiments on leaf pieces which had stood with the basal portion in mannitol solutions and on which the two estimates of water potential were determined after increasing time of equilibration. It was thought that

the water potentials of the leaves should come into equilibrium with those of the solutions. He found that with solutions of low potential, -10 to -18 atmospheres, the ψ zero values were in close agreement with the potential of the solution but the ψss values were higher and only approached an equilibrium with the solutions after about 72 hours. The basal portions of the leaf pieces approached the mannitol solution potentials more rapidly than the middle or tip of the leaf.

On the basis of the agreement between the ψ zero estimates and solution osmomotic potentials, and also because of the lack of agreement between the steady state estimates and the solution potential, Mongelard concluded that the ψzero water potential was that associated with the movement of water through the leaf. He also concluded that the ψ ss estimates were probably not the true potentials responsible for drawing water through the plant. Against this, however, was the fact that if leaves were stood in water or mannitol solutions of high potential (-4.8 atm.) the ψ zero values were much lower than the solution potentials and in water they never rose to equal the ψss values and were never completely eliminated.

Because of the importance of Mongelard's conclusions in the study of soil and plant water relations an examination

was made of his techniques and conclusions. The sampling and systematic errors were investigated for both water potential estimates at different levels of water stress. Mongelard's attempts to produce known potentials in leaves by equilibration with osmotica were repeated and extended. The experiments led to the view that ψ zero was not a useful or meaningful potential and some calculations of events taking place in the chambers will support this.

II. Sampling Errors.

a). Agreement between replicates. Experiment VI. Replicate samples were taken from mature Zea leaves and ψ zero and ψss determined. The experiments were performed at three leaf water deficits.(LWD).

Table 3 .Estimates of water potential at three leaf water deficits. (negative atm.) mean of 6 replicates.

LWD%	ψ zero	ψss
3.6	8.5	2.7
5.4	12.1	4.0
8.9	8.9	6.1
16.4	11.0	8.0

SE of ψ zero means = ± 0.71
SE of ψ ss means = ± 0.18

The ψ zero values were much more variable than the ψ ss and they were also always lower than the ψ ss values. The ψ ss values fell regularly with increasing leaf water deficit but the ψ zero estimates did not.

b) Differences between parts of the leaf.

Another major source of error in determination of water potentials in leaf tissue is the choice of sampling site. Differences may be observed in wilting between Zea leaves, the older leaves wilt and roll before the younger and the tips become flaccid and roll inwards before the leaf bases. The magnitude of the differences in water potential between base and tip of the mature leaf was examined.

Experiment VII. Mature Zea leaves, 25 cm. long, were allowed to wilt on the plant by cutting the base of the plant and drying the whole plant in the air. Samples from the terminal and basal 5 cm. were taken and ψ zero and ψ ss determined. The experiment was performed at three leaf water deficits. The results are given in table 4.

Table 4. Estimates of water potential at different water deficits and positions. (negative atm.)

	Plant I		II		III	
	tip	base	tip	base	tip	base
LWD%	3.6	2.5	6.1	2.8	14.5	11.0
ψ zero	12.7	9.7	10.0	12.1	11.3	11.2
ψ ss.	3.6	2.8	4.9	2.1	7.6	6.7

SE of ψ zero, means of 3 replicates = ± 1.3 atm.

SE of ψ ss means of 3 replicates = ± 0.2 atm.

LSD between ψ ss means = 0.9 atm.

(P = 0.05 **t** with 12 degrees of freedom)

The ψ zero values, when subject to an analysis of variance showed increasing cant change with decreasi - nlant water potential or with the position within the leaf. leaf water ; ss values fell with increasing plant water deficit and with position in the leaf (P = 0.001), the leaf tip values of ψ ss being lower than the base of the leaf. The ψ ss values at the leaf tip and base show a greater discrepancy at intermediate plant water deficits than at low or high deficit.

c). Differences between leaves of a plant.

Experiment VIII. The variability of ψ ss and ψ zero was examined by wilting whole plants in the manner described above. Samples were taken from the middle of the youngest

leaf (leaf 7), a mature leaf (leaf 5), and an old but not senescent leaf (leaf 3). The experiment was performed on three plants dried to different degrees. The results are given in table 5.

Table 5. Water potentials of different ages of leaf at three plant water deficits. (Negative atmospheres)

	Plant I			II			III		
	LWD	ψzero	ψss	LWD	ψzero	ψss	LWD	ψzero	ψss
Leaf 7	2.1	10.0	3.3	4.2	11.5	4.0	22.0	15.6	8.1
Leaf 5	2.7	12.6	2.2	5.5	12.0	3.4	20.0	13.6	9.3
Leaf 3	3.1	9.6	2.7	4.6	8.5	3.3	18.0	9.9	9.4

LSD between ψ ss means (based on 2 replicates) = 0.83 atm

(P = 0.05, t with 9 degrees of freedom.)

The ψ zero estimates of potential were not significantly different, as shown by an analysis of variance, between leaves or between the different plants. The ψ ss values fell significantly with increasing plant water deficit; no significance may be attached to the differences between leaves although the ψ ss values of water potential tend to be lower in leaf 7, the youngest. The water potential of leaf 7 falls less than that of other leaves, with increasing leaf water deficit.

The lower ψ ss values of leaf 7, when the leaf water

deficit is low, may be due to extension growth of the youngest leaf whilst in the osmometer chamber. Cell extension would result in a fall in turgor and therefore in water potential with a consequent increase in absorption of water vapour from the drop. The youngest leaf ψss values seem to change less with increasing deficit than the older leaves, which agrees with the observations of the progress of wilting in the plant. Similarly the base of the leaf is less responsive to increasing plant water stress than the leaf tip.

In the observations described the ψ zero values were erratic and bore no relationship to leaf water deficit whereas the ψss values fell with increasing leaf water deficit and were found to change between positions in the leaf and between leaves.

III. Handling Errors.

Experiment IX was performed to examine the possible errors arising from loss of water incurred whilst samples were placed in the chambers. The rate of water loss from tissue samples 3 x 1 cm was determined; one replicated sample was weighed in the air with the epidermis intact, another was weighed with the tissue cut into 2 mm wide strips joined at one margin. The divided samples lost 5% of the initial fresh weight in 2.5 minutes, and 10% in 5 minutes. The undivided lost 5% in 4.0 minutes and 10% in 15 minutes.

A change of 2 to 3% in the leaf water deficit of Zea leaves results in a change of about 1 atm. in the water potential estimated by ψ ss. Obviously the rate of water loss under laboratory conditions is sufficiently high to introduce errors of some magnitude in a few minutes.

The effect of handling time on the measurements of water potential was examined in experiment X. Tissue samples were dried in the laboratory for 35 seconds (the shortest time possible), 1, 2, 3 and 5 minutes before they were placed in the osmometer chambers. The curves of rates of evaporation from the drops with time of equilibration are given in figure 9.

The ψ zero values did not change with the increasing lengths of time that the tissue was dried. Drying for 2 minutes had no effect on the ψ ss values but the water potentials fell after drying for 3 and 5 minutes. Handling time was therefore reduced to a minimum; about 1.5 minutes served to cut and place the samples in six osmometers.

IV. The Influence of the State of Tissue Division on Water Potential.

One possible cause of the long period of equilibration necessary to achieve a steady state of vapour diffusion from the drop to the leaf, was thought to be the resistance offered by the epidermis and stomata of the leaf. This will be dis-

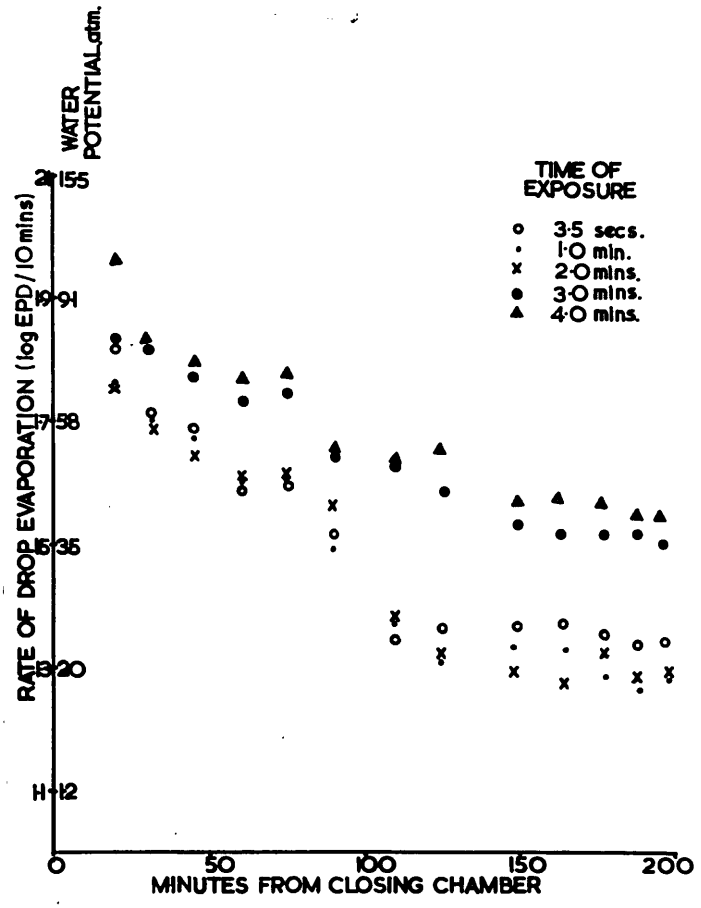


Fig. 9. The effects of handling time on the evaporation rate.

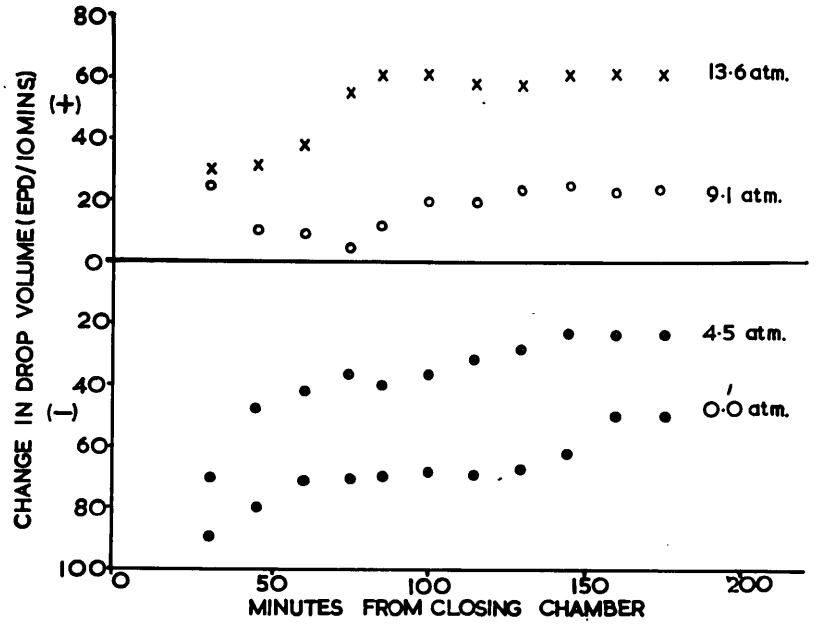


Fig. 10. Changes in the volume of sodium chloride drops during equilibration.

cussed at greater length in section D. Increasing the area of the cut surface of the leaf would be expected to reduce the resistance of the leaf and alter the water potentials estimated by the osmometer. Therefore the effect of the size of the cut surface of the sample on water potentials was examined in experiment XI.

Six 3 x 12 cm leaf samples were taken, three replicates were left with the epidermis intact and three were cut into 2 mm wide strips which remained joined at one of the longest edges. The cut surface was increased by 7.5 compared to the uncut samples. The experiments were repeated at five different water deficits and the results are given in table 6.

Table 6 Estimates of water potential for entire and subdivided samples (negative atm.)

Water potential	ψ zero	ψ ss	ψ zero	ψ ss	ψ zero	ψ ss	ψ zero	ψ ss	ψ zero	ψ ss
Cut	4.9	2.4	7.8	2.6	9.7	2.5	10.9	4.8	9.8	6.1
Uncut	5.9	2.2	7.2	2.4	8.4	2.7	10.3	4.5	9.5	6.3
Leaf water deficit	3.0		4.2		4.4		7.5		9.2	

SE ψ zero means = ± 0.8 atm. and ψ ss means = ± 0.3 atm

An analysis of variance showed that increasing the cut surface of the tissue seven fold had no effect on either the ψzero or ψss estimates. The ψss values decreased regularly with increasing leaf water deficit but the ψzero values showed no regular changes with the leaf water deficit.

V. Equilibration of Leaves with Water and Osmotica.

An examination was made of Mongelard's suggestion that the discrepancy between the ψzero and ψss values of water potential was due to a difference of potential between the cell wall and vacuole. By standing tissue in water or solutions of mannitol and preventing transpiration, it should have been possible for the water potentials of the wall and cell to come into equilibrium with one another and with the potential of the osmoticum and therefore to obtain the same values of ψzero and ψss. Alternatively if either of the two estimates of water potential was correct then it should attain the potential of the osmoticum.

a). Equilibration in water. Experimental determinations were made of the change in water potential of tissue segments stood in distilled water. In experiment XII 7 cm. lengths of mature Zea leaves were cut and placed with the cut end in 2 cm. of water in corked tubes. The initial water potentials were determined and those of samples from the middle of the

leaves after 12, 26, and 40 hours treatment.

Table 7. Water potentials of leaf pieces standing in water (negative atm.)

time (hrs)	0	12	26	40
ψ zero	10.3	7.5	6.7	7.7
ψ ss	4.8	4.6	3.6	3.9

SE of ψ zero means (3 replicates) = $\pm$ 0.34 atm. and of ψ ss means = $\pm$ 0.25 atm. LSD (P = 0.05) ψ zero = 2.5 atm. ψ ss = 0.8 atm.

Both the ψ zero and ψ ss estimates of water potential changed significantly with time. ψ zero rose in the first 12 hours but never attained zero even after 40 hours when the leaves were starting to turn yellow. Similarly ψ ss values rose between 12 and 26 hours (P = 0.05) but never attained values above -3 atm., although in other experiments the maximum level of ψ ss was about -2 atm. This may be due to differences in the tissue sampled or the pre-treatment.

Saturation of the tissue by standing in water does not eliminate the discrepancy between ψ zero and ψ ss even after long periods of treatment and it may be concluded that the ψ zero estimates of potential are not due to disequilibrium within the tissue.

b) Equilibration in mannitol solutions without transpiration.

Experiment XIII. An alternative approach was to bring leaf pieces to the potentials of solutions of mannitol.

As in experiment XII, leaf segments were placed with the base in mannitol solutions. (The osmotic potentials of these were determined in the osmometer). Samples were taken from the centre of the leaf pieces, above the mannitol level in the tube, after 0, 12, 40 and 60 hours and placed in the osmometer chambers.

Table 8. Water potentials of leaf pieces treated with mannitol solutions (negative atmospheres).

Mannitol Osmotic Potential	TIME (hours) 0		12		40		60	
	ψ zero	ψ ss	ψ zero	ψ ss	ψ zero	ψ ss	ψ zero	ψ ss
0.0	7.9	2.7	6.9	3.5+	7.4	3.2	5.6	3.3
4.6	"	"	9.2	2.9	6.9	2.9	14.0	4.5
9.6	"	"	9.0	5.6	13.8	5.1+	13.0	9.5
15.5	"	"	11.4	6.4	15.5	6.8	18.0	15.1

+ signifies 1 replicate only.

The results, given in table 8, show that the tissue in water behaved as in experiment XII; the potentials were always below zero. In the mannitol solutions the ψzero estimates were not in agreement with the solution potentials

except perhaps at -15.5 atm. in which region Mongelard also found agreement. The ψ_{ss} estimates were higher than those expected from the solution potentials in the first 40 hrs. but by 60 hours had fallen to values which were in close agreement with the osmotic potentials of the solution. This agreement may be related to the senescence of the tissue, which by 60 hours was distinctly chlorotic and showing saturation of the mesophyll. The low values of ψ_{ss} in the -15.5 atm. mannitol solution after 60 hours are much lower than the potentials of the cell sap (about -8 atm.) and may be due to uptake of mannitol by the tissue.

c) Equilibration in mannitol solutions under transpiring conditions.

The poor agreement between the applied osmotic potential and the plant water potential., ψ_{ss}, may be attributed to the very long period of equilibration required for water to move from the leaf sample to the osmotic solution and experiment XIV eliminated this diffusional movement of solution and water by allowing mass flow through the tissue under transpiring conditions.

Pieces of Zea leaves, 7 cm. long, were placed with their bases in mannitol solutions of -4.6, and -15.5 atm. osmotic potential and allowed to transpire for 15 hours in open tubes. One set of leaf pieces was kept in corked tubes.

The initial values of ψzero and ψss and the values after 15 hours "equilibration" were determined on samples from the middle of the leaf. At this time the upper, cut surface of the leaf samples under transpiring conditions was covered with white crystals, presumably of mannitol.

Table 9. Effect of transpiration on equilibration in solutions.

T = transpiring NT = non transpiring conditions (negative atm.)

Sampling time (hours)	0		15			
Solution osmotic potential	ψzero	ψss	ψzero T	ψzero NT	ψss T	ψss NT
4.6	9.8	2.7	10.7	12.1	5.3	3.5
15.5	"	"	19.8	10.1	12.8	5.3

LSD between 15 hours ψzero means (P=0.05; with 8df) = 4.8 atm.

LSD between 15 hours ψss means (P=0.05; with 8df) = 1.6 atm.

The zero values are lowest in the transpiring -15 atm. solution where they are closest to the solution osmotic potential. In the -4.6 atm. solution the ψzero values are much lower than the solution potentials. The ψss values fell with the potential of the applied solution and were much lower and closer to the applied potential in the transpiring treatment than in the non transpiring conditions.

d) Equilibration in the vapour phase of osmotica. The pretreatment of leaf pieces by standing the samples in solutions may be complicated by the absorption of osmotic solute due to its metabolism by the tissue or by diffusion into it. Trip, Krotkov, Nelson (1964) and Grocnewegen and Mills (1960) have examined the metabolism of mannitol by different plant species. The difficulty of uptake of the osmoticum may be overcome by placing tissue in the vapour phase of salt solutions contained in small chambers. The method is discussed in more detail in section BIb.

The effects of equilibrating tissue in the vapour phase above solutions were examined in experiment XV. Samples of Zea leaves were placed in the equilibration chambers over sodium chloride solutions, maintained at 25°C in a water bath and sampled at 24, 40 and 60 hours. Different chambers were sampled on each occasion to reduce the effects of re-establishment of constant conditions in the chambers after opening (Carr and Gaff, 1961).

Table 10. Water potentials of tissue equilibrated over salt solutions (negative atm.)

Potential		ψ zero			ψ ss		
Time (hours)		0	24	60	0	24	60
Solution potential	0.0	8.6	5.4	6.6	1.8	2.2	2.7
	2.3	"	7.8	5.6	"	2.1	2.2
	4.5	"	7.9	11.1	"	3.1	4.1
	9.1	"	8.2	8.0	"	3.7	6.6

LSD between ψzero values (P=0.05 with 16 degrees of freedom) = 2.1 atm. and LSD between ψss values = 2.5 atm. Only applicable to data for 24 and 60 hours.

The results given in table 10 show that the ψzero values bore no consistent relationship to the osmotic potentials of the sodium chloride solutions and did not come into equilibrium with them or the ψss values. The ψss values fell with increasing time of equilibration over the -4.5 and -9.1 atm. solutions, they were in agreement at -2.3 atm. but remained low with the water treatment. After 60 hours the -9.1 atm. solution potential was not reflected in the ψss values of the tissue but was about 2 atm. too high. Equilibration could not be continued over a longer period as the tissue was becoming senescent.

This experiment, as the others in the section, showed

that the ψzero values did not correspond to the potentials of the osmotica in which the tissue was equilibrated. The high variability and lack of correlation with other leaf characteristics strongly suggest that the ψzero values do not have any physiological significance. Mongelard's studies were performed at low water potentials and it has been shown in the present work that closer agreement is found between the ψzero values and the potentials of the osmotic solutions in this range than at high potentials.

The ψss values, in contrast, are less variable; they are closely correlated with the leaf water deficit and they respond to applied osmotica. Although the very long periods required to produce tissues of known potential may suggest that the ψss values are not the true leaf water potentials, the explanation suggested by experiments XIII and XIV is that the slow response of the ψss values is due to a diffusional movement of water and solutes between the leaf and solutions. When the diffusional movement is replaced by mass flow then the change is very much quicker.

The inability to produce tissues with water potentials of zero which has been clearly shown, may be due to the growth of leaf samples whilst the water potential is being measured, or it may be due to the sap exuded by the cut

tissue acting as an alternative sink for water vapour although in the light of experiment XI this explanation seems unlikely.

D. Tests of the Weatherley Osmometer and Analysis of the Events Taking Place in the Chamber.

I. An alternative method of using the osmometer.

The data presented in section C suggest that the ψss estimates of leaf water potential are those existing within the leaf sample and tests of the ψss values were required to substantiate this. The difficulty which was experienced in producing leaf samples of known water potential by treatment with osmotic solution, (section C.V) suggested that the osmometer could not be easily or conclusively tested with leaf samples and an alternative approach was made to the problem. The method and experimental results will be briefly described.

The rate of water vapour diffusion from a drop to a filter paper or leaf in the osmometer chamber is proportional to the gradient of water vapour concentration, and inversely proportional to the resistance of the diffusion path. The calibration curve, from which the water potential is estimated, is constructed using saturated filter paper surfaces and it may not be legitimate to apply this calibration curve to

leaf materials, as the leaf epidermis and stomata introduce a resistance to vapour flow. This is considered in section D.IIb. Boyer and Knipling (1965) pointed out that if the water potentials of the drop and the leaf tissue were equal then no vapour diffusion would take place. The water potential of the leaf would then be estimated from the osmotic potential of the solution forming the drop and would be independent of leaf resistance. They called the point of equal potential the "isopiestic point" and, by using a range of sodium chloride solutions as the drop of a thermocouple, determined the leaf water potentials of several types of leaves and compared them to the water potentials estimated from water vapour diffusion measurements.

The principle was applied to the Weatherley Osmometer, the leaf water potential was determined isopiestically (this will be symbolized as ψ_{isop}) and also by the rate of diffusion of water vapour at the steady state, ψ_{ss}.

a) Measurement of leaf water potential.

Method. Samples of Zea mays were placed in the osmometer chambers in the way described and the capillaries of the apparatus filled with water or salt solutions of known potentials, whose range was chosen to cover the expected tissue water potential.

Three replicate determinations were made with water as the drop and one estimate of ψisop was made based on the interpolation between changes in drop volume of three sodium chloride solutions. This was found to be adequate for interpolation provided that the tissue water potential lay within the highest and lowest potentials of the salt solutions.

The rate of gain or loss from the drop was determined over 10 minute intervals in the way previously described.

The water potentials of leaf samples were measured in experiment XVI. Leaves of Zea werebrought to a range of leaf water deficits by drying in the laboratory. Samples were equilibrated in the chambers for 20 minutes and the ψss and ψisop values were determined. The course of the evaporation rates from the drops of different potentials during the equilibration process is shown in figure 10 and the data from ψisop and ψss are given in Table 11.

Table 11. ψss and ψisop estimates at different leaf water deficits (negative atm.) for Zea mays.

Leaf water deficit %	ψisop	ψss	SE of ψss means±	ψss - ψisop	$\frac{\psi \text{isop}}{\psi \text{ss}}$
1.9	2.3	2.1	(0.3)	-0.2	1.09
2.4	1.5	1.3	(0.2)	-0.2	1.16
2.3	1.4	1.2	(0.1)	-0.2	1.16
7.8	4.3	4.7	(0.2)	+0.4	0.914
11.0	6.3	6.2	(0.4)	-0.1	1.02
15.9	8.8	8.9	(0.3)	+0.1.	0.98

The rate of water vapour diffusion or absorption by the drops in the osmometer chambers did not become constant until 140 or 160 minutes from the start of equilibration. Loss from the drop followed the same pattern as described in section CIa and the rates of absorption by the drops of salt solution, increased with time. The ψss and ψisop estimates of leaf water potential are in good agreement, with the ψss values overestimating the ψ isop. values by about 10%, if the large underestimate at 7.8% leaf water deficit is rejected as inconsistent.

The error, (ψss- ψisop) due to leaf resistance, was also examined for Lolium perenne. The values of ψss were determined in experiment XVII on leaves at different water deficits and compared to the ψisop values determined on parallell samples. The arrangement of the leaf samples in the chamber and procedure have been described in section BIa.

The results, given in table 12, showed that the ψss values are higher than the ψisop values in all cases, the magnitude of the error increasing initially with rising leaf water deficit and falling at the highest deficits but this is probably due to the inaccuracy of the measurements.

Table 12. ψ ss and ψ isop estimates at different leaf water deficits (negative atm.) for Lolium perenne.

Leaf water deficit %	ψisop	ψss	SE ψ ss means ±	ψss- ψisop	$\frac{\psi \text{ isop}}{\psi \text{ ss}}$
4.0	3.3	3.1	(0.3)	-0.2	1.06
4.9	5.1	4.6	(0.2)	-0.5	1.11
10.2	6.9	6.1	(0.3)	-0.8	1.13
12.2	7.9	6.8	(0.6)	-1.1	1.16
30.0	13.5	11.8	(1.3)	-1.7	1.14
32.4	13.3	12.0	(2.1)	-1.3	1.11

b) Measurement of Leaf Osmotic Potentials

The osmotic potentials of samples of leaves, killed by freezing and then thawed may also be in error. When the osmotic potential is determined by the rate of water vapour diffusion (called πss) and the osmotic potential is estimated by the isopiestic method (called π isop), then the error associated with the π ss method may be estimated.

In experiment XIX the osmotic potentials of leaf samples were determined on Zea mays leaves by the procedure given in BIII. Three replicate determinations of π ss were made

with water as the drop and estimates of π isop based on the interpolation between the rates of evaporation and absorption of drops of salt solution. The data for leaves at different leaf water deficits are given in table 13.

Table 13. Osmotic potentials of Zea leaves (negative atmospheres)

Leaf water deficit %	π isop	π ss	SE means π ss ±	πss- πisop	$\frac{\pi \text{isop}}{\pi \text{ss}}$
5.0	7.3	7.3	(0.2)	0.0	1.00
11.9	10.3	10.1	(0.3)	-0.2	1.02
14.5	11.4	10.9	(0.2)	-0.5	1.05

The osmotic potential falls as the leaf water deficit rises.

Experiment XX.

The leaf osmotic potentials of Lolium perenne were also determined. πss and πisop values were obtained, in the manner described, for leaves at three different water deficits, on which the water potentials had been determined. The data for leaf water potential may be obtained from the body of table 12 where the leaf water deficits correspond to those in table 14 in which the osmotic potentials are displayed.

Table 14. Osmotic potentials of Lolium leaves (negative atm.)

Leaf water deficit %	πisop	πss	SE πss means $\pm$	πss-πisop	$\frac{\pi\text{isop}}{\pi\text{ss}}$
4.9	10.8	9.1	(1.0)	-1.7	1.18
12.2	12.6	11.5	(0.4)	-1.1	1.10
32.4	19.1	16.0	(2.1)	-3.1	1.19

The error associated with the use of the water vapour diffusion is about 20%. In each species the leaf osmotic potential falls with increasing leaf water deficit but the osmotic potentials of the frozen and thawed leaves of Lolium are lower than those of Zea at equivalent leaf water deficits. In both cases the water vapour diffusion rates over-estimate the osmotic potential although killing the tissue by freezing would be expected to eliminate the leaf resistance.

c) Discussion. Both the water potential and osmotic potential of Zea and Lolium leaves are overestimated by the water vapour diffusion method when compared to the isopiestic method. The error is more serious in Lolium than Zea which may be explained by the observation that Lolium leaves are hypostomatous with the stomata set in the furrows of the leaf, whereas Zea leaves are amphistomatous and flat.

In both species the isopiestic method should be used

to determine the leaf water potential although the error of using the water vapour method is 10% in Zea and the advantages of increased replication outweigh the disadvantages. For Lolium the error is greater but water potentials can be determined to within about 12% of the true value.

II. Factors Affecting the Rates of Evaporation in the Osmometer Chambers.

The process of water evaporation from a drop to a leaf sample in the osmometer, from the time of closing the chamber until steady state rates of evaporation were found, has been described in section CIa . Mongelard's interpretation of the falling evaporation rates was considered and tests were made of the validity of the ψ zero and ψ ss estimates of water potential. It was concluded that the ψ zero values were of no significance physiologically but no alternative explanation of the fall in evaporation rate was offered.

In this section the process of evaporation will be considered and the possible causes of the observed evaporation will be examined. The process of equilibration in the chambers will be divided into two phases, phase I, the initial period of declining evaporation rates and phase II, the period of steady state rates of evaporation.

a) Phase I, the declining rate of evaporation. The declining evaporation rate reaches a steady state in about 180 minutes and it has been demonstrated that this process is not altered by the state of the leaf or pretreatments applied to the leaf sample. It is suggested that the air entrapped in the osmometer chamber when the sample is placed in it, is initially at a low water vapour content. During phase I water vapour is absorbed from the drop and sample, which results in a rise of the water vapour concentration of the air. As the rate of evaporation from the drop is proportional to the gradient of water vapour concentration so the rate will decline as the air becomes more humid. The sample, because it is much bigger than the drop, will control the final vapour concentration in the chamber and when this is attained, the steady state evaporation is observed, i.e. phase II. If phase I is due to equilibration between the drop, tissue sample and air, then it should be possible to equilibrate a leaf sample in a chamber until phase II is established, open the chamber to the air, close the chamber, and observe a repeat of phase I during a second equilibration period. If, on the other hand, phase I is a process of equilibration within the tissue, opening the chamber during phase II should not lead to a repeat of phase I but a continuation of phase II.

This was examined by experiment XXI. Zea mays leaf

samples were placed in the osmometer chambers and the rates of evaporation measured until the steady state, phase II, was attained. The chambers were then removed from the bath, dried, and opened. Two chambers were opened from 30 seconds, 2 for 60 seconds and the leaf samples were exchanged between 2 chambers. The chambers were closed and reequilibrated in the bath for 20 minutes and the evaporation rates determined.

The results are shown in figure 11. The initial determination proceeded as previously described, with clearly defined phases I and II. After the chambers had been opened, the rates of loss on the second determination were again divisible into phases I and II. The initial rates of evaporation in phase II were highest for the chambers in which the tissue had been exchanged, the rates for 60 seconds opening were not as high as these but were higher than the chambers opened for 30 seconds. The time to attainment of phase II in the first period of equilibration was about 160 minutes and about 100 to 120 mins. in the second.

Experiment XXI confirms that phase I is due to the air in the chamber acting as a sink for water vapour. A simple calculation supports this.

If the volume of air in the chamber is known and also the initial water vapour content, then it is possible to calculate the amount of water vapour required to bring the

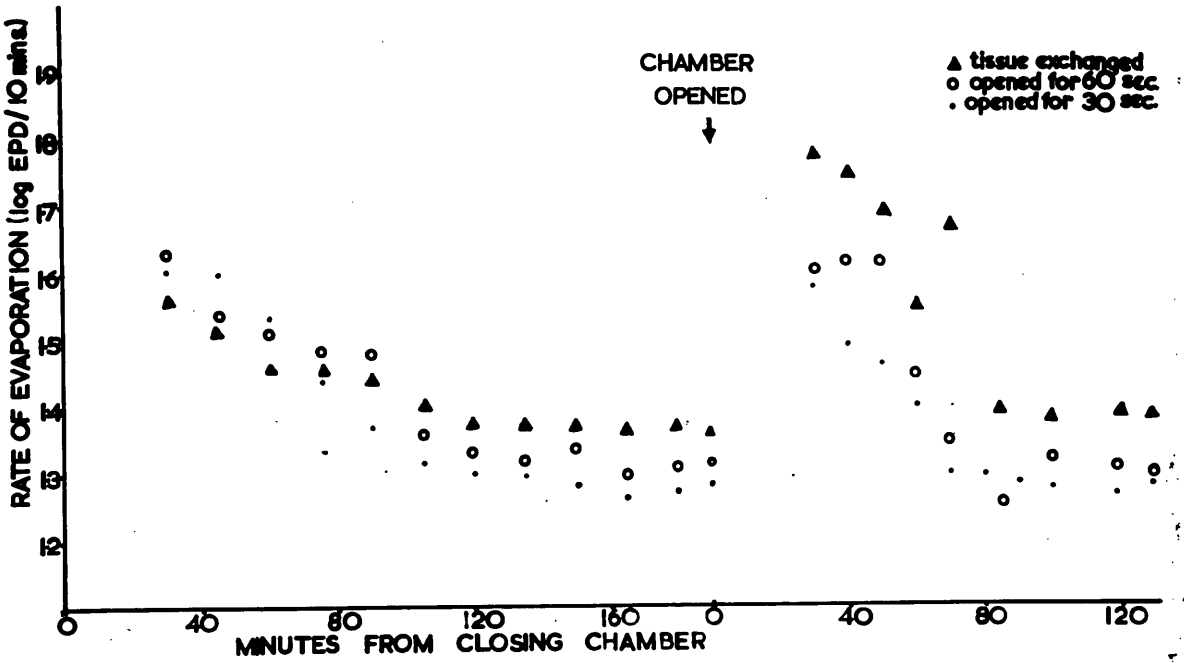


Fig.11. The effect of opening the osmometer chamber on equilibration.

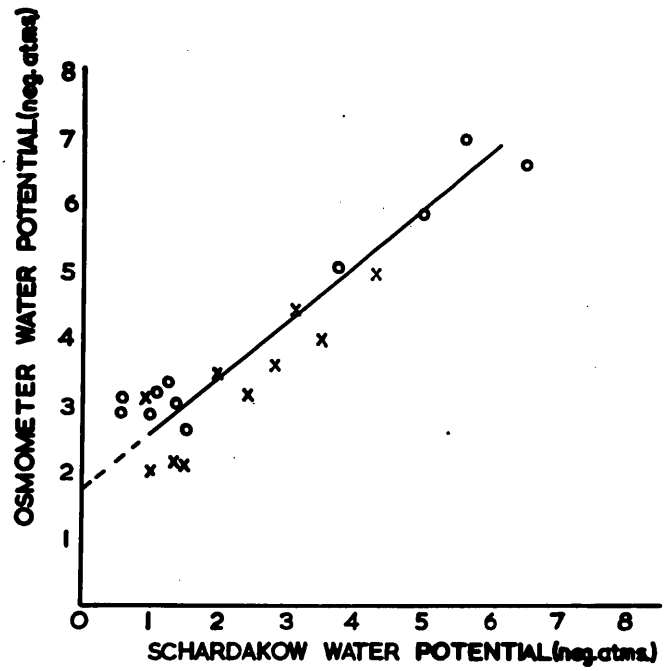


Fig. 12. Comparison of the Schardakow and Osmometer methods of measuring leaf water potential.

air to equilibrium with a sample at a given water potential. This may be compared with the actual volume lost from the drop during phase I. The following data are given: volume of chamber 1.3 mls. (calculated from the dimensions in figure 1.), the saturation vapour density of water at 25°C is 23×10^{-6} g cm^{-3} and that of the air at 40% relative humidity is 9.2×10^{-6} g cm^{-3} (assumed level of air humidity). The total amount of water required to saturate the air in the chamber is 17.9×10^{-6} cm^3 or 119 eyepiece division.

From an experiment the total amount of water lost from the drop in phase I was 240 EPD. The excess of 120 was possibly due to the relative humidity being below 40% in the actual experiment or to uptake of water from the drop by the tissue during phase I.

It cannot, however, be assumed that uptake by the tissue is responsible for the excess loss from the drop. Initially the air would be at a much lower potential than the tissue, which would lose water to the air. As the water potential of the air rises, so the leaf would lose less water and finally take water vapour from the drop. The excess loss might be due to a period of water vapour absorption by the tissue when the air and tissue are in partial equilibrium. What effect would loss of water to the chamber air have on the water content of the tissue? If the air requires about

18×10^{-6} gms. as indicated in the previous calculation, and all is supplied by the tissue, which has a volume of 60 mm^3, the tissue would lose about 0.03% of its volume. This loss would be without significance in the tissue water potential. Similarly the absorption of water vapour from the drop would not affect the tissue water content to any significant extent.

Changes in tissue water potentials were thought, by Mongelard, to arise from redistribution of water within the leaf sample and specifically that the water in the vessels and cell walls could be drawn into the vacuole, thus raising its potential. The magnitude of the effect likely to result from withdrawal of water from the vessels into the tissue, may be calculated. It is assumed that all the water held in the xylem vessels is withdrawn into the leaf and that the volume of this water is given by $v = \pi Nr$ where v is the volume, r is the mean radius of the vessels, N the total number of vessel vessels in the leaf sample and l, their length, which is taken as the length of the tissue sample. For a typical Zea leaf sample the values are: r = 0.012 mm, l = 1.2 cm, and N is 60, which gives v as 0.27 mm^3. The tissue volume is about 60 mm^3 and the withdrawal of water into the cell would cause a rise in the volume of about 0.5%. If the cell volume is 75% of the total volume, the withdrawal of all the xylem water might lead to a fall in the water deficit of the leaf

of about 0.6%. If the water content changes by 2% for each atmosphere change in water potential then a change of about 0.5 atm. might result from withdrawal of water from the xylem vessels. However, this is quite insufficient to account for any major changes in leaf water potential during phase I.

The fall in evaporation rate from the drop during the first 180 minutes with leaf tissue in the chamber, is a result of equilibration between the drop, air and leaf and not due to changes in the distribution of leaf water. However, it is possible that the initial process of equilibration may mask changes in the water potential of leaf samples but of a much smaller magnitude than Mongelard suggests.

The attainment of phase II was more rapid with saturated filter papers than with leaves in the chambers. This may be due to saturation of the air being controlled by the filter-paper because of the very large surface area. The leaf has an epidermis and therefore relatively little water vapour will come from the leaf and the drop will tend to control the saturation of the air. The air is therefore saturated very rapidly above filter papers but more slowly above leaf tissue.

b) Phase II. The steady state evaporation. In section D I, the ψ ss values were compared to the ψ_{isop} values for leaf samples, and it was shown that a leaf resistance to water vapour diffusion could have been responsible for the over-

estimation of leaf water potentials by ψ_{ss}. The magnitude of this resistance and that of the chamber may be calculated.

Following Mongelard (1965) and Rutter (personal communication to Mongelard), the water vapour diffusion process in a chamber may be described by Fick's law.

$$F = D \left(\frac{c_1 - c_2}{L} \right) \qquad (b)$$

where F is the rate of diffusion per cm^2 of surface, D is the diffusivity of water vapour in air ($cm^2\ sec^{-1}$), c_1 is the saturation vapour concentration of water at the drop temperature and water potential (0 atm. for water), c_2 is the saturation vapour concentration of water at the leaf or paper surface temperature and water potential. When F is in $cm^3\ cm^{-2} sec^{-1}$, L is the resistance to diffusion in centimetres of still air. A resistance term, R, with units of sec cm^{-1} may be substituted for L, where $R = \frac{L}{D}$. The value of ψ_{isop} is the true leaf water potential because it is free of leaf resistance errors which affect the water vapour diffusion process. The resistance of the leaf may be calculated from the observed values of F and the true values of ψ (and from these the values of c_1 and c_2.)

For the case of diffusion to a leaf sample in the chamber equation (6) may be written

$$F = \frac{D\,(c_1 - c_l)}{L_{CH} + L_{LF}} \qquad (7)$$

where c_1 is the vapour pressure at the water potential of the leaf, which is estimated by ψ isop and L_{CH} and L_{LF} are the chamber and leaf resistances respectively.

When the rates of evaporation to a saturated filter paper and a leaf are the same, the effect of L_{LF} is to overestimate the potential from water evaporation.

The ratio of the vapour pressure gradients is then proportional to the ratio of the resistances;

$$\frac{c_1 - c_l}{c_1 - c_2} = \frac{L_{LF} + L_{CH}}{L_{CH}} = \frac{\psi \text{ isop}}{\psi \text{ ss}} \qquad (8)$$

Within the range 99 to 100% relative humidity ψ and c are linearly related and therefore, rearranging equation (8) yields

$$L_{LF} = L_{CH} \left(\frac{\psi \text{ isop}}{\psi \text{ ss}} - 1 \right) \qquad (9)$$

and from this the leaf resistance may be calculated.

The calculation is performed in two stages. Firstly the chamber resistance is calculated using data for F and the water vapour concentration difference derived from the calibration curve i.e. for a saturated filter paper. An alternative calculation of the chamber resistance, based on a geometric relationship is given in order to provide a

comparison. Secondly the resistance of the leaf is calculated using equation (9).

Calculation of chamber resistance, the following data are given: D = 0.256 cm^2 $sec.^{-1}$, c_1 at 25°C - 23 x 10^{-6} g cm^{-3}, c_2 (corresponding to a -10.0 atm. sodium chloride solution) is 23 x 10^{-6} x $\frac{99.3}{100}$ g cm^{-3} and F to a -10.0 atm. solution is 85 epd per 10 minutes to 3.7 cm^2 of paper or 5.7 x 10^{-9} g cm^{-2} sec^{-1}. Substituting these values yields L_{CH} of 7.18 cm per unit area of paper or 1.94 cm for the total area.

The alternative calculation of chamber resistance is based on an analogous diffusion process, that of heat diffusion between two concentric spheres at different temperatures. For the derivation see Crank (1956) and Starling and Woodall (1957). The equation is

$$Q = Dt4 \quad \frac{ab}{b-a} \quad (c_1 - c_2) \qquad (10)$$

where Q is the total diffusion over the surface of the chamber, in time t; a radius of the drop and b that of a sphere having the same volume as the chamber. The other symbols have been given. The value of a is 0.069 cm and b 0.677 cm. The resistance of the diffusion path, equivalent to L in equation (6) is the reciprocal of $\frac{4 \pi ab}{b-a}$. L is calculated at 1.04 cm for the chamber or 5.96 sec cm^{-1} for unit area of the chamber.

A possible reason for the lower estimates of chamber resistance derived from equation (10) compared to (6) is the assumption that the chamber is a sphere; the chamber is cylin-

drical and complicated by the "dead space" in the cone, which forms the top portion of the chamber.

Calculation of leaf resistances.

The resistance of the leaf samples of Zea and Lolium were calculated from the values of ψ_{isop} / ψ_{ss} given in tables 11 and 12 respectively. Taking the mean of the ratio of Zea, 1.05, and the value of 7.18 cm for L_{CH}, L_{LF} was calculated as 0.359 cm or the resistance as 1.36 sec cm^{-1}. The data are inconsistent and if those values where ψ_{isop} is higher than ψ_{ss} are rejected (as this result is theoretically impossible) the mean ratio is 1.109 and the value of L_{LF} becomes 0.783 cm and the resistance 3.06 sec cm^{-1}.

From the data in table 12 the resistance for Lolium were calculated from the mean ratio of 1.12. L_{LF} was 0.789 cm and the resistance 3.37 sec cm^{-1}. This was derived by assuming that the value of L_{CH} obtained from a saturated filter paper lining the chamber walls could be applied to a system of different geometry such as that of Lolium samples in the chambers. Because of this assumption the calculated resistance should be taken with reserve but it is included for comparative purposes.

The resistances, expressed as a length of diffusion path, have been converted to diffusion resistances and are presented in table 15.

Table 15. Resistances of chamber and leaves

	chamber	Zea	Lolium
Resistance lengths (cm)	7.18	0.359* 0.783†	.789
Diffusion resistance ($Sec\ cm^{-1}$)	28.04	1.36* 3.06†	3.37

* from ζ_{isop} = 1.05

† from ζ_{ss} " = 1.109

The resistance of the chamber is much higher than that of the leaves and the diffusion process is therefore controlled by the chamber. In fact from equation (4) it is seen that the drop size is the controlling factor. This explains how a calibration curve obtained with wet filter papers can be used for Zea leaves without serious error. The resistance of Lolium leaves is higher than that of Zea.

At this point the use of leaf annuli, as advocated by Macklon and Weatherley, (1965) may be considered. They suggest that annuli present a large area of cut surface to the drop and therefore a calibration curve from saturated filter paper can be applied to leaf material. The annuli should therefore give values of leaf water potential, ζ_{LF}, which are free from any resistance error, but intact leaf pieces should, if a resistance error is important, overestimate the

water potential, i.e. give higher values of ψ_{LF}. Figure 4, of the paper quoted above, shows the relationship for Ricinus leaves between relative tugidity and ψ_{LF} (which is expressed as water potential deficit). The annuli gave higher values of water potential than intact leaf pieces, at a given leaf water deficit . Leaf pieces with scored epidermis gave intermediate values of water potential. This result cannot be explained on the assumption that a leaf resistance is important in the method used by Macklon and Weatherley. Possibly changes in turgor pressure produced by punching the annuli may account for the difference between the curves.

In the present work, the absolute values of leaf resistance were small, ~~particularly so for Zea and seemed to be low~~ for leaves which would be expected to have closed stomata. Comparison with published values indicated that a minimum resistance of 1.1 sec cm^{-1} was found, for cotton leaves with the stomata at maximum aperture, by Slatyer and Bierhuizen (1964). Holmgren, Jarvis and Jarvis (1965) cited a minimal stomatal resistance for Zea mays of 0.41 sec cm^{-1}. The lowest resistance found by Boyer & Knipling by isopiestic studies was about 6 sec cm^{-1} for Geranium sp. It seemed that the stomata of Zea in the osmometer chambers could not be closed although this would be expected. To clarify this situation the response of Zea leaves, in the chambers, was observed

and the resistance of the stomata calculated.

c) Stomatal Activity and Resistance in the Osmometer Chambers.

Samples of Zea leaves, at low leaf water deficit, were placed in the osmometer chambers and the stomatal apertures measured by the impression method at the beginning and end of the equilibration process. The initial and final mean apertures were 2.2 μ and 1.9 μ respectively.

The resistance for the stomatal array for Zea was calculated using the method of Penman and Schofield (1951). The data given were obtained from the material sampled at the end of experiment XXI. The stomatal numbers were 2,820 on the adaxial and 5,150 on the abaxial leaf surfaces. The mean pore length was 23.8μ and the diameter of the stomatal throat 1.9μ. An epidermal and cuticular thickness of 2.5×10^{-3} cm. was found in sections of leaf.

The resistance of a single stoma was calculated to be 2.53×10^{3} cm^{-1} and the total resistance per unit area of leaf 0.49 cm. or 1.91 sec cm^{-1}.

The stomatal resistances calculated by geometrical means, are lower than resistances calculated from the process of water vapour diffusion. The suggestion is that the stomata are not closed in the chamber and therefore offer a low resistance to water vapour diffusion. The opening of the stomata in the chamber is, perhaps, due to low carbon dioxide

levels in the chamber, as stomata open in carbon dioxide free air (Heath 1959). Possibly the tissue sample is capable of assimilating the carbon dioxide in the chamber during the periods of illumination which are necessary to observe the capillaries. The ability of Zea mays to effectively reduce the carbon dioxide concentrations of air to zero is discussed by Ketellapper (1963).

The leaf resistance did not seem to increase with increasing leaf water deficit, as the ratio of Ψ_{isop} to Ψ_{ss}, shown in section DII tables 11 and 12, did not greatly increase with increasing leaf water deficit. However the effect of low water potentials and reduced leaf turgor would be to cause stomatal closure and the resistance of the leaf should then rise. Unfortunately no observations were made on the stomata of leaf samples at high water deficits.

E. Comparison of the Osmometer with the Schardakow and Slatyer Vapour Equilibrium Methods.

A comparison of the methods was made to evaluate the use in experimental situations. The methods have been described in Section BI. Determination of the water potentials of Zea mays leaves was made by the Schardakow and Osmometer methods after 3 and 20 hours equilibration. The Slatyer

method was used to determine the potential at 20 hours only.

Experiment XXII, leaf samples from Zea plants were saturated for 4 hours with their bases in water and subsamples were then taken for water potential determinations and leaf water deficits. The water potential and deficit were also determined on leaf samples from tissue dried to about 75% of the initial weight. The results are given in table 16

Table 16. Comparison of three methods of water potential measurement. (negative atmospheres)

Method		Osmometer		Schardakow		Slatyer
Time (hours)		3	20	3	20	20
Leaf Water % Deficit	1.9	2.9	2.1	1.5	1.6	0.8
	27.4	9.5	8.9	4.6	5.0	5.2

Means of 5 replicates. LSD (P = 0.05) between method x deficit means at 3 hours. 1.83 atm.; at 20 hours, 1.75 atm.

All three methods show significantly lower water potentials in the high leaf water deficit treatment than in the low leaf water deficit treatment. The Schardakow and Osmometer methods gave estimates of water potential which did not fall significantly with equilibration from 3 to 20 hours. At high leaf water deficits, the water potentials estimated

by the Schardakow and Slatyer methods, were much higher than those estimated by tne Osmometer but the differences were not significant at the low leaf water deficits. The order of increase in the estimates of leaf water potential are Osmometer method $<$ Schardakow $\simeq$ Slatyer method.

The data are also presented here for a comparison made between the Osmometer and Schardakow methods on Zea plants growing in soils at different soil moisture stresses. The experiments are described in Chapter 2, experiments I and II. The osmometer and Schardakow methods were used to determine leaf water potentials on parallel samples from plants growing over a wide range of soil water potentials.

The relationship between the estimated potentials for the two methods, is shown in figure 12; each point is the mean of three replicates.

The Schardakow method gave higher estimates of water potential than the Osmometer, about 2 atm. higher at high potentials but about 1 atm. at the low potentials. However, the relationship between the Schardakow and Osmometer estimates of water potential in experiment XXII is not in good agreement with experiments I and II, the Schardakow being very much higher in experiment XXII than in I and II at the same water potential estimated by the Osmometer. This discrepancy has not been explained. The water potentials

estimated by the Osmometer for Zea are regarded, on the evidence of section DI, as being not more than about 10% higher than the true leaf water potential. On this basis, therefore, the Slatyer and Schardakow methods are seen to be in error. The Schardakow method is potentially a useful method for field studies but requires closer examination. The Slatyer method is unsuitable for measurements of high leaf potential, because of the difficulties of the technique.

F. Summary of Chapter 1

1. The principles and methods of measuring plant water potentials are briefly discussed and described.

2. The process of equilibration in the Weatherley Osmometer is described and the significance of the falling rate of evaporation is discussed in the light of previous theories.

3. The ψ_0 zero values of leaf water potential (estimated from the extrapolation of the falling evaporation rate curves to the time of sampling) and the ψ_{ss} values (estimated from the steady state rates of evaporation) were tested by correlating them with tissues brought to known water potentials in osmotica and above salt solutions.

4. The ψzero values were shown to be of no significance but the ψss values agreed well with the solution potentials and the discrepancy found after short periods of tissue equilibration were due to slow rates of equilibration in the tissue.
5. The falling evaporation rate is shown to be due to the establishment of equilibrium between the drop, the sample and the air in the osmometer chambers.
6. The isopestic method was used as a test of the accuracy of the ψss values and these were shown to be in error by about 10% for Zea and 12% for Lolium leaves.
7. The error in the ψss values was attributed to a leaf resistance to water vapour diffusion which was calculated and shown to be about the resistance expected for leaves with partially closed stomata.
8. The Schardakow and Slatyer vapour equilibrium estimates of leaf water potential are shown to be in error when compared to the osmometer estimates.
9. The leaf water potentials of the youngest leaf and the tip of the leaves were shown to be lower than those of the mature leaves or the leaf base.

Chapter 2. The Measurement of Soil Water Content, Potential and Capillary Conductivity.

A. Introduction

Water movement from the soil to the plant root has been described by Gardner, (1960) who writes,

$$E \sim k\ (\psi soil - \psi root) \qquad (1)$$

where E is the rate of soil water depletion, k the capillary conductivity of unit area of path through which the water flows and ψsoil and ψroot are the potentials of water in the bulk of the soil and at the root surface respectively. For a given transpiration rate, T, the maintenance of E equal to T will depend on the conditions in the soil and plant. In wet soils, where k is high and ψsoil is above -1 atm, ψroot and the resistance of the plant controls the rate of uptake (Gardner and Ehlig. 1962). As the soil moisture is depleted by transpiration, so the water content falls and with it ψsoil and k. Water extraction continues as long as the water potential of the plant, ψplant (assumed equal to ψroot), is lower than that of the soil. However, k falls very rapidly and below about -1 atm. the rate of movement through the soil to the plant root becomes limiting. E falls below T in this case and leads to a decrease in the

plant water content and a fall in ψplant. The uptake of water is maintained by the fall in ψplant and ψroot but changes also take place in the plant, closure of the stomata reduces the rate of transpiration and tends to preserve the plant water content and hence to maintain ψplant. Changes in ψplant are also reflected in modifications in the growth of the plant; the rate of leaf extension is slowed, and assimilation rates fall.

Both transpiration and growth are intimately but not necessarily directly related to the characteristics of the rooting medium and to the distribution and density of the plant's root system.

The relationship between the soil water content, its potential and capillary conductivity were therefore examined for the soil used in the experiments. The apparatus for the determination of total and matric soil water potentials will be described and also the results of determinations made on the soil used in the experiments.

B. Materials

The soil used in the work was a sandy brown earth of very poor structure, from agricultural land at Silwood Park, Imperial College Field Station. The mechanical analysis was given by Etherington (1962) as sand 76% of the dry weight,

silt 14%, clay 10% and organic matter 2.6%. The pH was about 4.8.

The soil was passed through a 3/8 inch sieve for all growth experiments and through a 4 mm. sieve for the determination of the moisture characteristic and conductivity. The soil was packed into containers at about 14% water content, as the soil was most easily handled in that state, to a bulk density as close to 1.2 g cm $^{-3}$ as possible for all determinations and experiments.

The soil will be referred to as "Silwood Soil".

C. Apparatus.

The apparatus for determining the total and matric soil water potentials is briefly described and also the apparatus for the production of known water potentials in porous materials.

I. Determination of Total Soil Water Potential: The Weatherley Osmometer.

The apparatus was fully described in Chapter 1. Macklon and Weatherley (1965) and also Mongelard (1965) used the apparatus to measure the total soil water potential. Mongelard used glass chambers constructed from B24 "Quickfit" cones and sockets, but these were mechanically unsound. However, the small chambers used for determinations on

leaves have been found suitable also for soil studies.

Soil samples were taken and placed in the chambers and a central well into which the drop hung was made in the sample with a glass rod. The rate of evaporation was determined in the way described. Steady state evaporation rates were observed after about 2 hours equilibration in the controlled temperature bath and the water potential was estimated from the calibration curve.

Because of the large number of samplings which were required in the experiments the method was not employed as a routine but was used as a check on the matric potentials produced by the methods described in Section IV and as a means of estimating the osmotic potential of the soil solution.

II. Direct Measurement of Matric Potential: the Tensiometer

The matric potentials were measured in cans of soil by tensiometers which enabled direct and continuous readings to be made with a permanent installation. The apparatus was described by Richards (1942) and the equipment used in this work by Etherington (1962). It consists of a porous ceramic cup (Doulton PA4) connected to a mercury filled manometer; the whole is filled with air free water and the cup is buried in the soil. Equilibration is achieved between the water in the soil and in the cup and the matric potential of the soil

water is measured directly by the manometer. Corrections for capillary depression were applied to the readings. The apparatus will not work satisfactorily below - 0.8 atm. because air enters the cups at about this potential.

III Indirect Measurement of Matric Potential: Resistance Blocks

The matric potentials were estimated in soils over the range 0 to -10 atm. with the resistance blocks described by Bouyoucos (1954). The electrical resistance was measured between two parallel stainless steel electrodes embedded in a cylindrical porous "plaster of paris" block. The resistance was determined with a meter designed by Dr. W.Hinson of the Forestry Commission Research Station (Hinson and Kitchin 1964). The construction and calibration of the block has been described by Etherington and the choice of material was discussed by Bourget, Elrick and Tanner (1958). Etherington's procedure was adopted, except that 10% by weight of polymethyl cellulose gum was incorporated to improve the hardness of the blocks.

The blocks were calibrated from 0. to -1 atm. in the Kaolin plate apparatus (section IVa) and from -1 to -10 atm. in the pressure membrane apparatus(section IVc). The blocks were placed in a little kaolin (to increase contact) on the plate or membrane and differential air pressures applied

across the membranes to produce known matric potentials. The resistance was determined at equilibrium and the calibration curve is given in figure 13.

IV Methods of applying known matric potentials to porous materials.

The apparatus to be described was used to produce known levels of potential in soil samples or resistance blocks. The principle is the same in each case. The sample under study is placed on a membrane of Kaolin, porous ceramic or cellophane, and the pressure below the membrane reduced, as in the Kaolin plate method, which enables the production of matric potentials from 0 to -1 atm., or the pressure above the membrane is increased by pumping gas into an air tight chamber of which the membrane forms the base. Air pressures equivalent to -2 atm. matric potential can be applied in pressure plate apparatus, and to -20 atm. or lower in the Richards pressure chamber.

Water moves through the membrane until the matric water potential of the sample comes into equilibrium with the applied pressure differential. The volume of outflowing soil water may be collected and the soil water contents of the samples determined gravimetrically after their removal from the chamber.

a) Kaolin plate apparatus. Described by Botelha da Costa and Alves(1942) and by Etherington, the apparatus consists

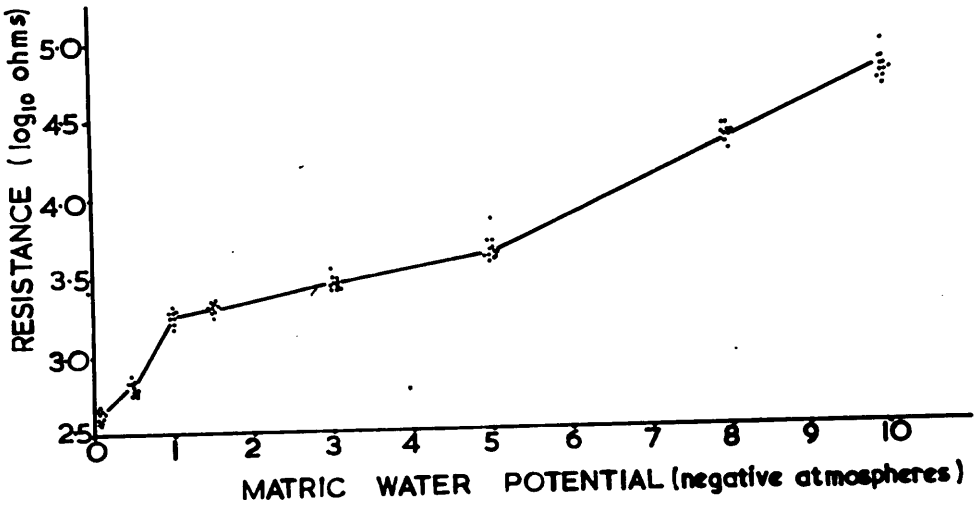


Fig. 13. Calibration curve of resistance blocks.

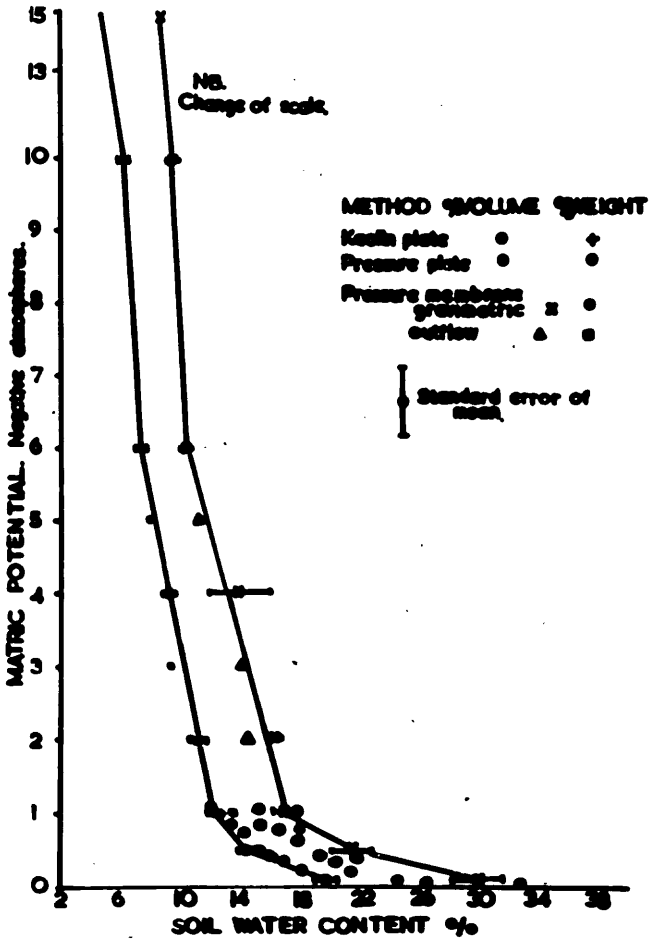


Fig.14. Moisture characteristic for Silwood soil.

of a Buchner funnel with a layer of Kaolin deposited, in the wet state, on a filter paper placed in the funnel; the whole mounted in a Buchner flask. Samples placed on the plate are subjected to a pressure differential by evacuating the flask. The outflow was observed until it ceased, when the sample was assumed to have equilibrated. The apparatus was used in the construction of the moisture characteristic (section D) and in calibration of the resistance blocks.

b) Pressure plate Apparatus. Richards(1941) described the apparatus; that used in the present work is a small, air tight chamber, 3.8 cm. by 5.0 cm. diameter, with a porous ceramic plate (Doulton 13A) forming the base and with a removable lid fitted with needle valve gas port, and a pressure guage. The pressure in the chamber was increased with a hand pump or by gas from a cylinder. The apparatus was used in the determination of capillary conductivity of the soil. For this purpose the outflow was collected in a device similar to that suggested by Tanner and Elrick (1959) which also served to remove entrapped air from beneath the porous plate.

c) The Richards pressure membrane apparatus. The apparatus was similar to that described in (b) except that the chamber was larger, 15 cm.x 6 cm. and the membrane was of "Cellophane" film. (Visking tubing was later found to be more robust). A small vessel of 1:2 epoxy propane was incorporated in the

chamber to inhibit fungal attack on the membrane.

The liquid expelled from the chamber was collected in a small vessel with a hole to permit pressure equilibration with the atmosphere. Water loss from this was negligible. The volume of outflow was determined by weighing the collecting vessel. Tanner and Elrick(1959) suggest that the condensation of water vapour on the walls of the chamber is an important source of error and to avoid this a small heating element,a 12 volt light bulb in a metal container, was placed on the chamber to raise the temperature of the walls.

The apparatus was used to bring soil samples and resistance blocks to known matric potentials below -1 atm. and also to determine the capillary conductivity and the moisture characteristic.

D. The Moisture Characteristic

The relationship between the water potential and water content of a soil was called the moisture characteristic by Childs(1941) and it is discussed by Youngs(1965). The determination of the moisture characteristic for the Silwood soil will be described only for the desorption process because this is the part of the hysteresis curve relating soil water potential and content which is of most importance to the supply of water to the plant.

a) Experimental. Soil samples were prepared by sieving and were packed into metal rings 2.2 cm. internal diameter and 1.5 cm. high to a bulk density of 1.2 g. $cm.^{-3}$ and saturated. Replicate samples were placed on the Kaolin plate and Pressure membrane apparatus and subjected to pressure differentials equivalent to matric potentials of -0.03, -0.5 and -1.0 atm. on the Kaolin plate, and -2.0, -4.0, -6.0, -8.0 and -10.0 and -15.0 atm. in the pressure membrane apparatus. Four samples were taken for water content determinations after outflow from the membrane had ceased, at each given potential.

The water content was determined by weighing the soil upon removal from the apparatus and drying at 105°C to a constant weight. The moisture content was expressed as a percentage of the sample dry weight or as a percentage of the volume of the soil.

Additional information for the moisture characteristic was obtained from the determination of capillary conductivity described in section E. In this procedure the outflow from samples in the pressure plate and membrane apparatus was measured with progressive increases in the applied pressure differential. The final water content and dry weight and volume were determined and the water content at any stage could then be calculated on a dry weight and volume basis from the data.

b) Results. The data in figure 14 are shown for the water contents on a volume and dry weight basis. The method of determination is indicated and also the process from which the points are derived.

The water content on a dry weight basis falls from 20% at -0.03 atm. to 12% at -1.0 atm. and to 5% at -15 atm. Of the 15 cm^3 of water per 100 g. of soil held above -15 atm., 8 cm^3 are held above -1.0 atm. and 5 cm^3 between -1.0 and -6.0 atm. Similarly over half the water content, expressed on a volume basis, which is held above -15 atm. is released between -0.03 and -1.0 atm.

At this point the results will be given of determinations of the total water potential measured by the osmometer method on soils brought to known matric potentials. The results are given in table 17.

Good agreement was found between the applied matric potential and the total water potential estimated by the osmometer.

Table 17 Total water potential of soils at various matric potentials.

Matric potential (neg. atm.)	0.1	1.0	6.0	10.0
Mean total water potential (neg. atm.) and Standard errors of means of 3 determinations ±	0.4 (0.1)	1.2 (0.2)	5.4 (0.3)	9.6 (0.2)

The total water potential is not markedly lower than the applied matric potential, which suggests that the osmotic potential of the soil is very small and may be safely ignored.

c) Discussion. There is good agreement between the different methods of determining water content when the values on a dry weight basis are considered but on a volume basis the methods do not agree well, particularly the pressure plate outflow data. These determinations were based only on a single series of measurements of successive volumes of outflow from one soil sample and should therefore be regarded with caution. Also the measurement of the sample volume is not as accurate as the determination of dry weight because of sample shrinkage. Over the -0.1 to -1.0atm. range the volume water content obtained from the outflow data underestimates the water content determined gravimetrically by about 3% . Because the volume water content from outflow data was based only on one sample and the gravimetric determination on 4 replicates, the curve in figure 14 relating matric tension and volume water content was drawn through the replicated determinations. The curves are similar to those given for other sandy soils by Gardner(1960). For all experimental work where the volume water content was required, the water content was determined on weight basis and the corresponding

volume water content was determined from figure 14. In addition, some direct observations of the volume water content were made to serve as a check on the reliability of this method.

E. The Capillary Conductivity of Silwood Soil.

The reduction in capillary conductivity which occurs with decreasing water content is one which has proved difficult to determine at soil water potentials below about -0.8 atm., i.e. outside the tensiometer working limit. (Gardner and Mayhugh, 1958; Nielsen and Biggar, 1961).

The theoretical basis for measuring capillary conductivity assumes that the volume of water flowing in unit time (the flux density) across unit area at right angles to the direction of flow, is proportional to the potential gradient and to the capillary conductivity. In unsaturated soil capillary conductivity is itself a function of the soil water potential and of water content. The relationship is discussed by Gardner (1956) who has solved the equations relating the variables by assuming that if the changes in water content are small enough then capillary conductivity (and the Diffusivity) remain constant. This enables it to be determined in the pressure membrane apparatus. Miller and Elrich (1958) have described a procedure allowing the resistance due to the membrane to be separated from that due

to the soil. Criticism of the method has been made by Jackson, Van Bavel and Reginato (1963).

In view of the difficulty of studying this aspect of soil moisture relations only a single series of determinations of capillary conductivity was made over the drying curve from -0.1 to -5 atm.

a) Experimental. The method given by Gardner was followed. A sample of soil, at 14% water content, was passed through a 4 mm. sieve, and packed wet into a brass ring 15 cm. in diameter and 4.0 cm. high. This was placed on the membrane and the soil saturated. The pressure in the chamber was raised first to 10 atm until water ceased to flow from the soil. This was done to ensure thorough settling and wetting. The soil was then allowed to saturate slowly and re-equilibrated at 1 atm. pressure and the outflow discarded. The soil was then subjected to a pressure of 2 atm. and the rate of outflow recorded. Similarly the outflow was measured for increases in pressure to 3, and then 5 atm. The membrane was found to leak at more than 5 atm. over the long period of time required for complete outflow and the data were discarded for pressures above 5 atm. Data for two determinations from 1 to 2 atm. are given in the results.

Determinations of the conductivity at matric water potentials higher than -1 atm. were made using the outflow

data obtained from the pressure plate apparatus (section IVc.) The chamber was packed with soil in the way described for the pressure membrane apparatus, the soil was saturated and pre-treated by applying a pressure oflatm. The soil was then resaturated, equilibrated at -0.1 atm. and the outflow measured over the pressure increments - 0.1 to 0.2, o.2 to 0.3, 0.3 to 0.4, 0.4 to 0.5, 0.5 to 0.7, 0.7 to 0.8, 0.8 to 1.0 atm.

b) Results. The results of measurements of the outflow for successive pressure increments, for both pieces of apparatus are plotted, in figure 15 as the total volume of outflow remaining to pass the membrane or plate at that particular time i.e. $(Q_o - Q)$ where Q_o is the total outflow obtained at the end of the outflow process at a given pressure increment and Q is the outflow at any time, t. Calculation of the capillary conductivity, k, was made from equation 16 given in Gardner (1956)

$$k = \frac{B\, Q_o\, \rho\, g}{V \Delta P\, \alpha^2}$$

where k is in cm. day $^{-1}$, B the slope of the line when the $\log_{10}$ of the outflow remaining to pass the plate is plotted as a function of time, ρ is the density of water, g the acceleration due to gravity, V the volume of the sample, ΔP the pressure increment (dynes cm^{-2}) applied to the

sample and α a geometric term equal to $\frac{N\pi}{2L}$ where $N=1$ and L is the height of the sample on the membrane. Using values of B derived from figure 15 and the appropriate values of α, for the pressure plate and membrane apparatus the capillary conductivities were calculated.

The results are given in table 18.

Table 18. Capillary conductivities with decreasing matric potentials.

Matric potential increment (neg ats.)	0.1 - 0.2	0.2 - 0.3	0.3 - 0.4	0.4 -0.5
k $\frac{cm}{day}$	1.28×10^{-2}	8.5×10^{-4}	2.51×10^{-4}	1.99×10^{-4}
Matric potential increment (neg ats.)	0.5 - 0.7	0.7 - 0.8	0.8 - 1.0	1.0 - 2.0
k $\frac{cm}{day}$	1.24×10^{-4}	1.26×10^{-4}	8.49×10^{-5}	2.73×10^{-4} 2.23×10^{-4}
Matric potential increment (neg ats.)	2.0 - 3.0	3.0 - 5.0		
k $\frac{cm}{day}$	2.26×10^{-5}	3.56×10^{-6}		

The relationships between the capillary conductivity and water content and matric water potential are shown in figure 16. The capillary conductivity falls markedly with a decrease in the soil water content, and with matric potential from about 10^{-2} cm. day^{-1} at -0.1 atm. (26% water content by volme) to about -10^{-7} cm day^{-1} at 5 atm. (12% water

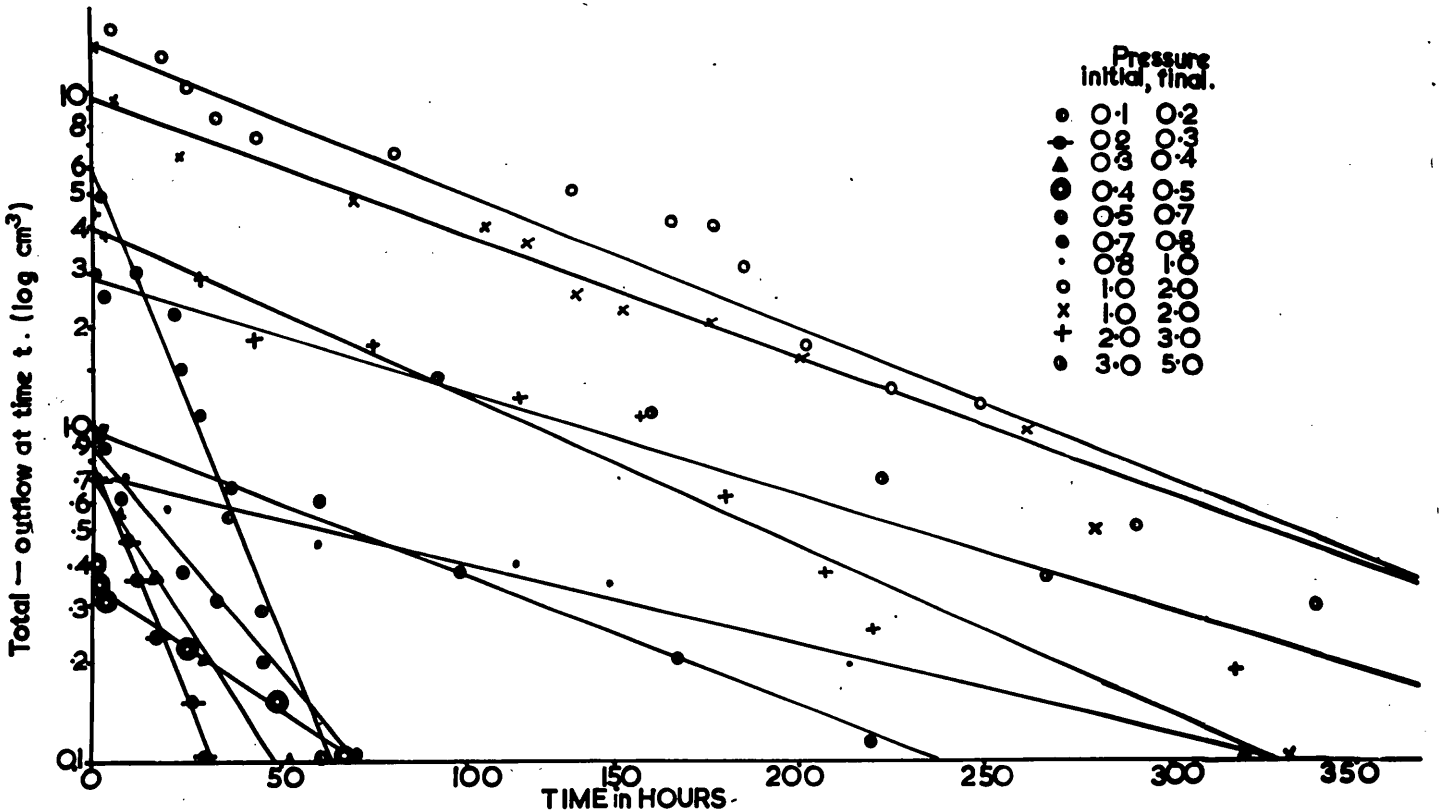


Fig.15. Outflow versus time curves at different applied pressures for capillary conductivity determinations.

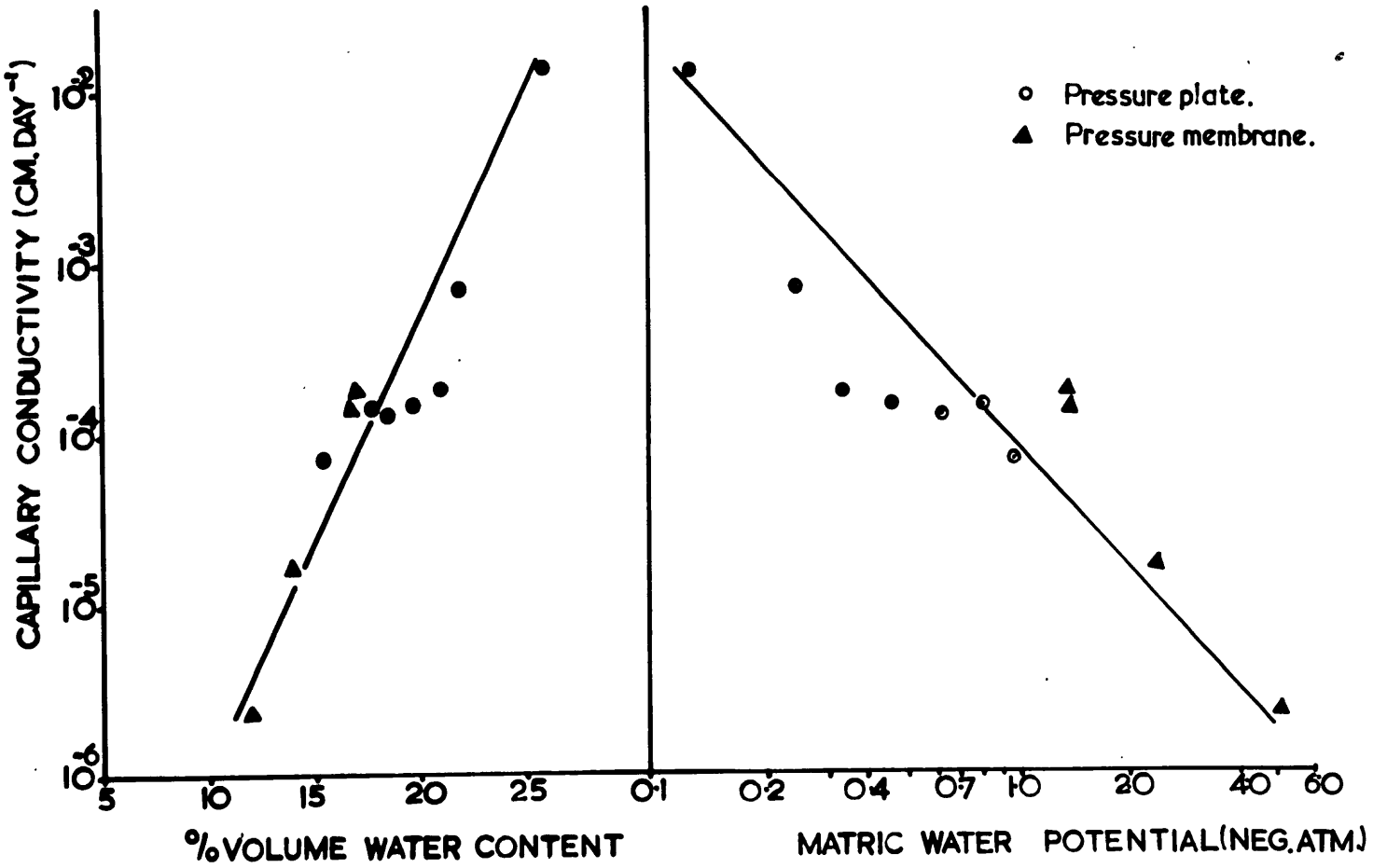


Fig. 16. Capillary conductivity as a function of soil water content and potential.

content). The data for the two methods of determination overlap and the membrane apparatus gives higher values of the conductivity at ψ of -1 to -2 atm. than the pressure plate does at -0.6 to -1.0 atm. The logarithms of the calculated conductivities fall on a straight line when considered in relation to the volume water content and matric potential. Although in the latter case a somewhat curved relationship is suggested, a straight line was fitted, by eye, to the data.

Comparison of the calculated conductivities for the Silwood soil with those published by Gardner for a pachappa sandy loam soil shows that the values agree at 0.1 atm (10^{-2} cm day^{-1}) but at lower matric potentials-3 to -5 atm., the values determined for the Silwood soil are lower than for the Pachappa, i.e. 3×10^{-6} cm. day^{-1} compared to 1×10^{-5} cm. day^{-1}.

The uncertainty introduced into the values by the resistance of the membrane and the difficulties of obtaining adequate contact between the membrane or plate and the sample, are major limitations to the method. In view of the criticisms put forward by Jackson et al. (1963). both of the theory and practical use of the method and also the comments received from Professor Childs (personal communication) only a single determination was attempted. The results are, therefore, to be regarded as indicative only of the order of magnitude of the capillary conductivities for the Silwood soil. The results will be used in subsequent calculations on the rates of movement to the plant root.

F. Discussion

The determination of soil water content on a weight basis is relatively accurate for the sieved soils used in the experiments. The water contents expressed on a volume basis are more variable than those expressed on a dry weight basis because of errors in sample volume determination. Estimates of the volume percentage from the dry weight percentage should be made with care as alteration of the bulk density and pore space of the soil may invalidate the relationships shown in figure 14.

The determination of matric potential from water content data is also subject to inaccuracies due to sampling errors in the construction of figure 14. The moisture characteristic on a dry weight percentage is based on 4 replicates which lie within a range of 1% soil moisture and to within 2% volume percentage. The potentials at which the samples were equilibrated could be determined, from the applied pressure, by manometer which could be read to within ± 0.05 atm. below 1 atm. and from the cylinder gauges which were accurate to about ±1 atm.

The tensiometers were read to less than 0.05 atm, although rates of water movemeмt between the pot and soil

may lead to slow rates of equilibrium attainment and therefore to additional errors. The resistance blocks can only be relied upon to estimate τ to about $\pm$ 0.5 to 1 atm. because of the variability between blocks. Also the rates of water movement may affect the blocks, particularly in dry soils where contact between the block and soil maybe poor.

In the determination of matric potentials in experiments, estimates were made from instruments and from figure 14 and also by gravimetric sampling.

Chapter 3. Growth and Transpiration of Zea mays in Soils and Osmotica

A. INTRODUCTION

Considerable attention has been directed to the relationship between growth, transpiration and soil water potentials for many types of soils and plants. The monographs by Crafts, Currier and Stocking (1949) and Russel (1959), and the review by Kramer (1959), deal with much of the earlier work. The emphasis previously placed on soil constants such as field capacity and permanent wilting point by, for example, Viehmeyer and Hendrickson (1950) and on transpiration ratios which relate growth to water consumption, has been replaced by an approach which regards transpiration as a process governed by atmospheric conditions, gradients of water potential and the resistances to water movement within the soil and plant, (Slatyer and Gardner, 1965). The relation between soil water potential, transpiration and growth, which has so frequently been studied (Stanhill, 1957) is not necessarily a direct one, and theoretically the water potential gradients and resistances to flow, which are related to soil conductivity, root distribution and root permeability, should be examined in order to

characterise the transpiration process. Growth is, logically, related most closely to the plant water potential. As Vaadia, Raney and Hagan (1961) have stated, the plant water potential is the most important single measure of water in the soil, plant, atmosphere continuum because it integrates the effects of variable rooting and soil water distribution and conductivity, and is a measure of the balance between the uptake process and the rate of loss (Slatyer 1957). Because the plant water potential itself is a function of the transpiration process, which is highly dependent upon the changing conditions of the atmosphere, it will not be constant with time and as Rawlins (1962) has shown, it is not constant throughout the plant because of the resistance to water movement existing in the pathway of water flow, although as a first approximation the leaf water potential may be taken as that of the whole plant. Peters(1960) has concluded that it is difficult to assign a value to the integrated length of time of internal plant stress.

In the soil, measurement of water potential and its integration over soil depth and time have been discussed by Taylor (1952) and Jarvis and Jarvis (1963), but the integration involves many assumptions about root distribution and water absorbing activity.

It is not possible to maintain actively transpiring plants under experimental conditions of a given soil water potential because not only does the soil water potential at the root surface vary, depending on the transpiration rate and soil water conductivity as shown for example by Gardner (1960), but depletion of the soil water produces variable distribution in the soil and progressive lowering of the soil water potential. Also the rate of soil water depletion by plants transpiring actively and with a limited rooting volume may be so high that the soil water potential may change in a very short time from "field capacity" to potentials at which the plants wilt. Relating growth and transpiration to soil water potential is complicated by the difficulties of assigning a single value to the whole root zone. The measured values may not be those which affect the plant because of the transmission characteristics of the soil and because the water potential may change rapidly. Similarly the plant water potential may vary within the plant because of its water transmission characteristics and also vary with transpiration during a diurnal evaporation cycle.

Measurements of plant growth based on changes in dry weight and leaf area, or values of net assimilation

rates etc. derived from such data, cannot be obtained at a single level of water potential and are usually related to an arbitrary soil or plant water potential measurement made under a particular condition or at a given time, see for example Rutter and Sands (1958). Often plant response is measured over a period of time during which the plants are subjected to a cycle of soil drying and many daily transpiration cycles. The drying cycle is allowed to proceed until a given minimum level of soil water potential is reached when the whole soil mass is returned to maximum water status. In long term experiments, of the type necessary to obtain growth data, the plants may be subjected to several cycles of drying and although minimum potentials are known, the integrated soil and plant water potentials are unsatisfactorily characterized. Gates (1955, 1957), Gates and Bonner (1959), Aspinal, Nicholls and May (1964) and Aspinal (1965), have studied plant responses to cycles of soil water stress, but the relationship between stress and plant response cannot be obtained under these constantly changing conditions of the experiments.

An alternative method of subjecting plants to water stress is that of growing plants in solutions of

known osmotic potential. For example Wadleigh and Ayres (1945), Slatyer (1961) and Boyer (1965) have used salts or sugars either as solutions or added to soils in order to decrease the total soil water potential. However, Slatyer showed that salts and metabolizable sugars entered the plant during the investigation. The use of mannitol as an osmotic solute has been examined by Groenewegen and Mills (1960) and Trip, Krotkow and Nelson (1964), and it appears that mannitol is only slowly absorbed and metabolized. High molecular weight Polyethylene glycols (Carbowaxes) have been used as osmotica because they are unlikely to be easily metabolized and are of such large molecular weight that entry into the plant should be limited. Provided that they are properly purified they are also not toxic, (Lagerwerf, Ogata and Eagle, 1961; Jackson, 1962; Ruf, Eckert and Gifford, 1963).

In the present work plants were grown in soils and aspects of growth examined in relation to soil and plant water potentials. The plant response was compared with that of plants grown in polyethylene glycol solutions in order to provide constant and controlled stress in the plant rooting medium which allowed growth to be studied over periods of up to two weeks.

B. Growth of Zea mays in soil

Three experiments were performed on Zea plants grown in cans of Silwood soil. The methods, experimental details and results will be described together because of similarity. Experiment I was designed to elucidate the relationship between leaf extension, leaf water deficit and soil and plant water potentials. Experiment II repeated the experiment, but with different presampling treatment and experiment III was performed to include transpiration.

I. Method

Experiments I and II. 4 plants of Zea mays var Inra 321, were grown in 18 cm diameter x 30 cm high bitumen painted metal cans with basal drainage holes. Holes in the sides of the cans, fitted with rubber bungs, provided access for soil sampling. The cans were filled with moist Silwood soil equivalent to 7 kg. of dry soil. For experiment III the cans were 18 cm diameter x 18 cm high, filled with 3 kg. of soil. 4 plants were grown in each can. Two resistance blocks were placed in each can and also a tensiometer in the cans intended to remain at high water potentials. The blocks and tensiometers were read, when possible, twice daily. The plants were

grown to the 6 leaf stage in experiments I and II and to the 9 leaf stage in III. Frequent applications were given of a Rothamsted nutrient solution. The plants were grown initially in a controlled environment room and later under glass. In Experiment III supplementary lighting of about 1,200 foot candles was given both during initial growth and throughout the actual experiment to ensure greater constancy of conditions. The lights operated from 9 to 17 hours daily. The leaf growth was measured by ruler from a reference point on the cans, usually twice daily. All the leaves were measured although only the data for the youngest 2 leaves will be presented.

Transpiration was measured in III, by weighing the pots on a balance which was accurate to about 0.5 g. The pots were covered with thick "Polythene" sheeting taped around the plant stems.

The methods of measuring plant water potentials by the Weatherley osmometer (using the steady state evaporation rates), soil water potentials, stomatal aperture and leaf water deficit have been described in Chapters I and II. The symbols ψ and τ soil will be used for leaf water and soil matric potential respectively.

II. Experiments

Experiments I and II were conducted in April and July 1965, and III in June 1966. In each case the moisture regime of drying cycles was designed to study the changes over the range from maximum plant turgor to wilting with the emphasis on the initial decline in soil water potential. The treatments were; control maintained at above -0.1 atm ψ soil, and treatments which were allowed to dry to minimum ψ soil values of -0.5 atm -0.1, -5.0 and -10.0 atm. Each treatment was replicated twice. However, the exact stress levels were hard to maintain and considerable variation from the desired levels occured. In experiments I and II the treatments were started at the same time and rewatered as they achieved approximately the levels of stress stipulated. The cans in III were allowed to dry from different starting times in order to achieve a range of ψ soil values on the same day. This enabled transpiration rates to be compared under the same meteorological conditions. The drying cycle occupied three days, during which transpiration and growth were measured. On the fourth day ψ plant was determined, together with stomatal apertures, transpiration and leaf elongation.

ψ plant was determined on samples of mature leaves taken in experiment I after the plants had been kept under non-transpiring conditions for 12 hours in a darkened, closed polythene bag. In experiment II the plants and cans selected for sampling were taken from the normal growing site, usually at 10.00 hours, and placed in a 20°C. controlled light and 50% humidity room for 2 hours prior to sampling. The ψ plant data of experiment III were obtained from samples of leaves taken directly from the plants, without pre-treatment, at three times during the day, 8.00 hours, 12,00 and 20.00 hours.

The contrast between the experiments was, therefore, that in I a period of 12 hours was given in which the plant might be expected to achieve equilibrium with the soil, if this were possible. In experiment II ψ plant was measured at the end of a period of constant atmospheric conditions which might be expected to eliminate large fluctuations in transpiration rate and in ψ plant resulting from variable evaporating conditions. Experiment III allowed the comparison of ψ plant with T soil at three times of the day under the same conditions on each occasion.

III. Results

The results of the three experiments are considered together.

a) Plant and soil water potentials.

The results of the determinations of ψ plant and τ soil for experiment I and II are given in figure 17. The fitted regressions are highly significantly different between the two experiments. In both experiments the ψ plant values at high τ soil were very similar and with decreasing τ soil, ψ plant fell in both cases, but in experiment I the fall was very slight compared with II. In experiment I, τ soil was lower than ψ plant when the former was below about -3 atm. whereas in experiment II τ soil was lower than ψ plant below about -5 or -6 atm. τ soil. The data for experiment III are shown in figure 18. The measurements of ψ plant were made on the cans indicated by symbols at each period. Because of the high transpiration rates τ soil was changing rapidly through the experiment. The values of ψ plant fell from 8.00 to 12.00 hours in all the treatments and rose from 12.00 to 20.00 hours when the soil water potential was above -10 atm. Below this value of τ soil, there was no recovery of ψ plant in the evening. Even at 12.00 hours, however, ψ plant

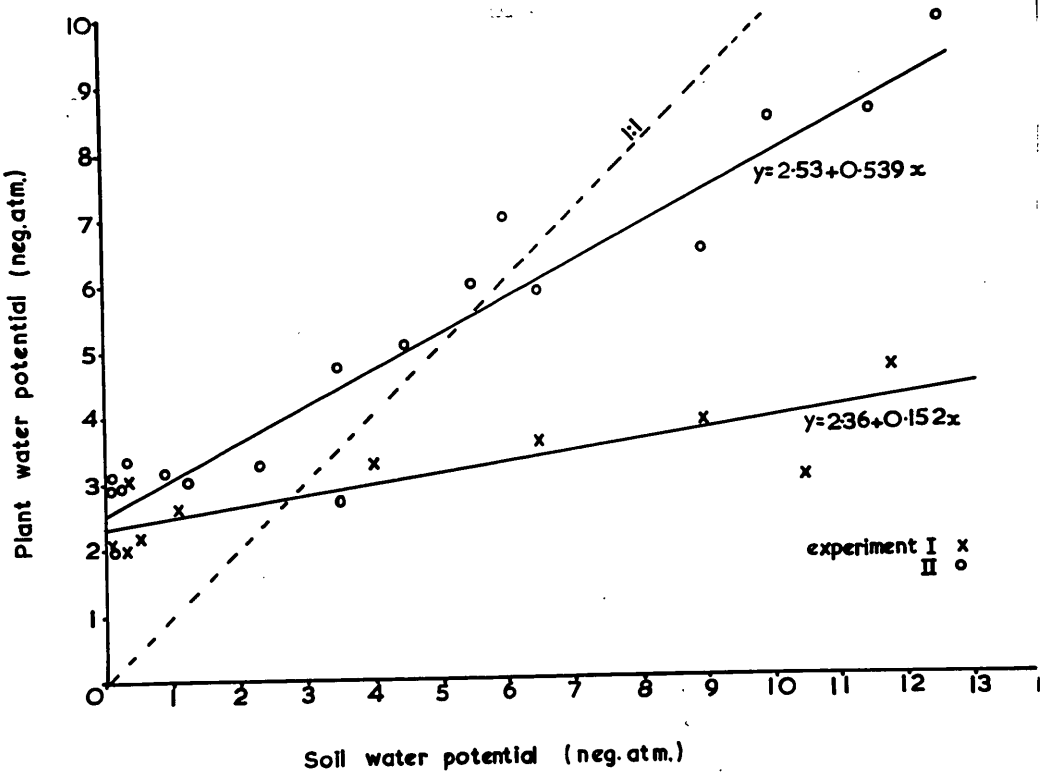


Fig. 17. Plant water potential of Zea in relation to soil water potential, experiments I and II.

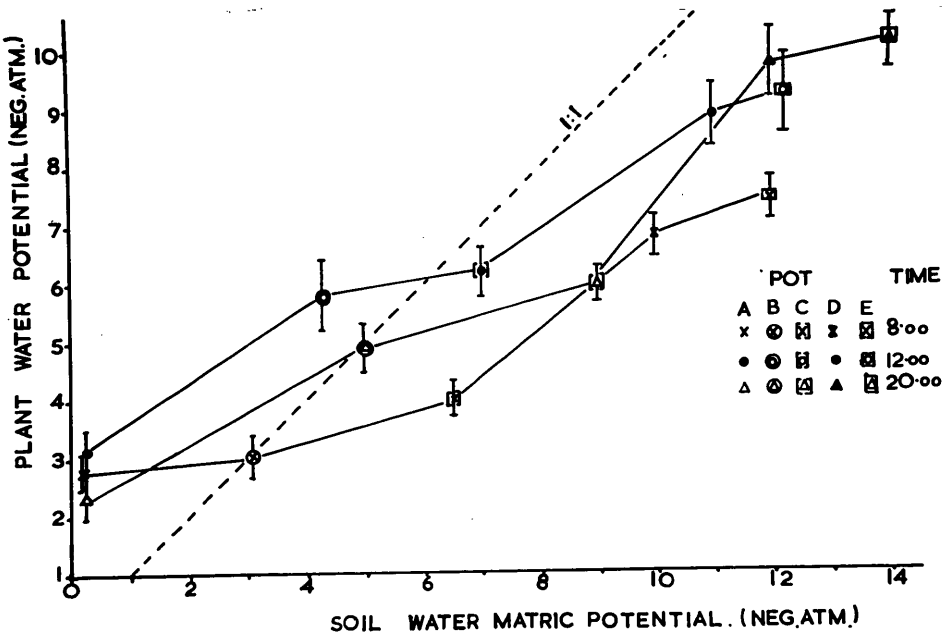


Fig. 18. Plant water potential of Zea in relation to soil water potential, experiment III.

was higher than ψ soil when the latter was below -6 atm.

The inversion of the gradient between the soil and plant below about -5 atm ψ soil was a most striking result and for plants growing under normal atmospheric conditions, one which is theoretically impossible for plants which were transpiring. The significance of these results will be discussed more fully in a later section.

b) Transpiration and soil and plant water potentials

In figure 19 the rates of transpiration for different periods during the course of the drying cycle in experiment III are shown as a function of ψ soil, the values of which were taken as the mean of values at the beginning and end of transpiration period. The transpiration is expressed relative to the rates of water loss by the control plants maintained at ψ soil higher than -0.1 atm. The relative rate declined with decreasing ψ soil. From about -0.1 to -1 atm only a slight decrease in transpiration was observed, but from -1 atm transpiration declined linearly with ψ soil to about -8 atm., where the rate of water loss tended to remain more or less constant as ψ soil was reduced to -14 atm.

The rates of transpiration differed widely between the periods, because of the fluctuations in atmospheric

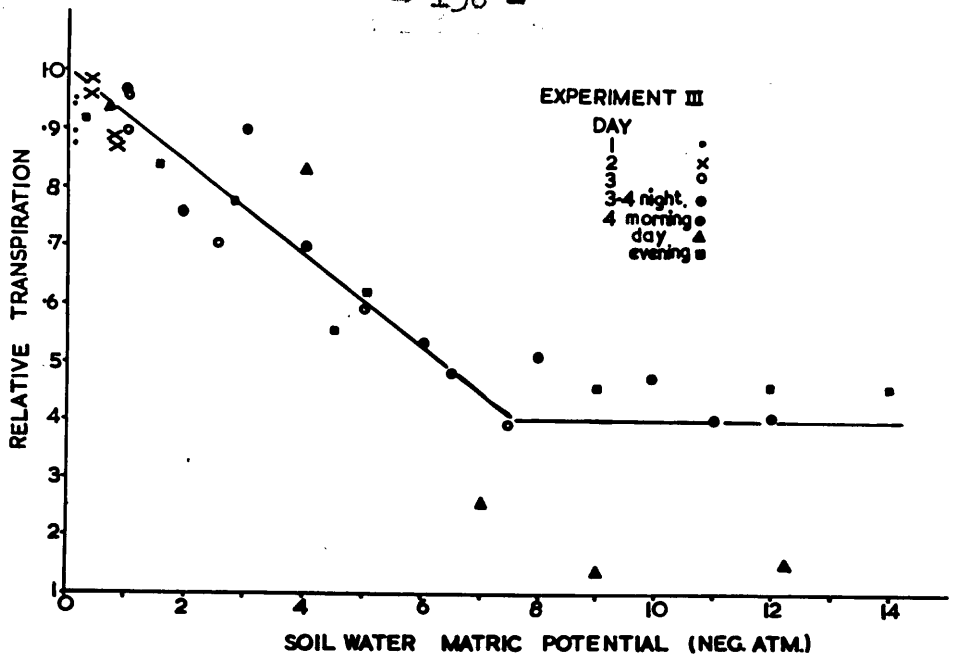


Fig. 19. Transpiration of Zea in relation to soil water potential.

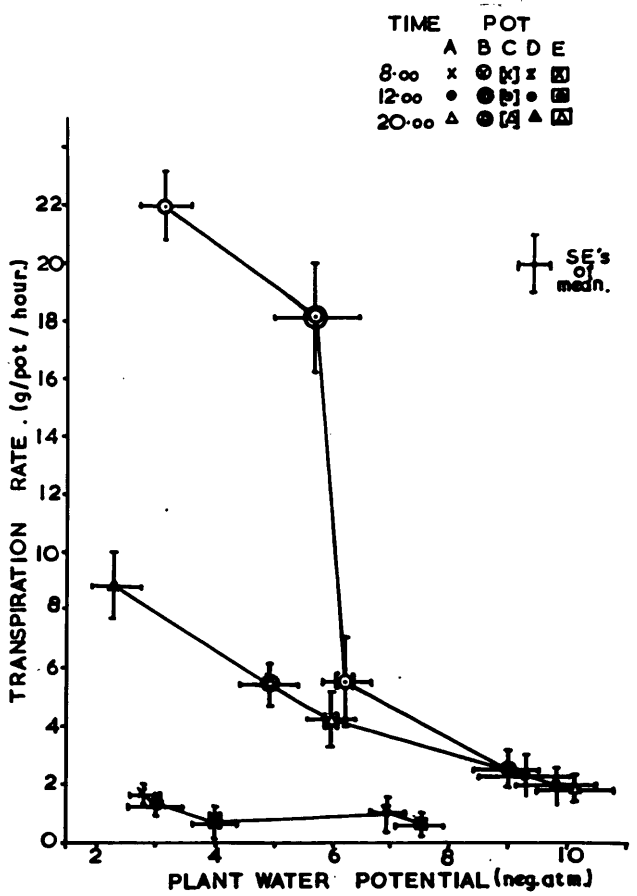


Fig. 20 Transpiration of Zea in relation to plant water potential.

conditions, but the relative rates were very similar for the different periods and conditions, except that the afternoon period on the fourth day showed very reduced transpiration compared with other periods at the same water potential. The rates of transpiration, in experiment III, during the periods in which ψ plant was measured, have been related to the ψ plant values and figure 20 shows the mean transpiration rate and the ψ plant values determined during the same period.

In the morning, before the lights were operating, low transpiration rates were measured, about 0.03 $g/dm^2/hr.$ in the control plants, and reduced transpiration was associated with ψ plant values lower than those of the plants growing in adequate soil moisture. During the day transpiration rates increased, to about $0.46 g/dm^2/hr.$ in the controls. The rate of transpiration decreased as ψ plant, with a large reduction in transpiration noticeable between -5.5 and -6.5 atm ψ plant. Transpiration in the evening period was $0.19 g/dm^2/hr.$, and a more or less linear relationship between transpiration rate and ψ plant was observed.

Transpiration continued despite the apparent inversion of the potential gradient. Because of this inversion it is

not possible to relate the rate of water loss to the to the gradiant of water po'ential between the soil and leaf.

c) Stomatal activity

Figure 21 (a) shows the relation between the stomatal aperture and γ soil, and (b) that between stomatal aperture and ψ plant in experiment III. The stomatal aperture decreased progressively with reduction in γ soil and ψ plant, reaching the nimimal aperture of about 0.5 μ at -6 atm γ soil and with ψ plant at about -6 or -7 atm. At low water potentials the stomatal aperture was independent of the time of day, i.e. light conditions, but at higher water potentials the maximum stomatal widths were observed in the morning and mid-day periods, and the closure took place in the evening. Apart from the morning period, the transpiration rate, shown in figure 21 (c), was linearly related to stomatal aperture.

d) The rate of leaf extension

The rates of extension per can, of the youngest and second youngest leaves, in experiment I, have been plotted in figure 22 relative to the rate of extension of the plants grown at less than 0.1 atm γ soil. In order to emphasize the importance of the stress between 0.1 and -1 atm, γ soil is given on a logarithmic scale. Leaf extension was reduced as γ soil decreased from about -0.1 atm and was nearly

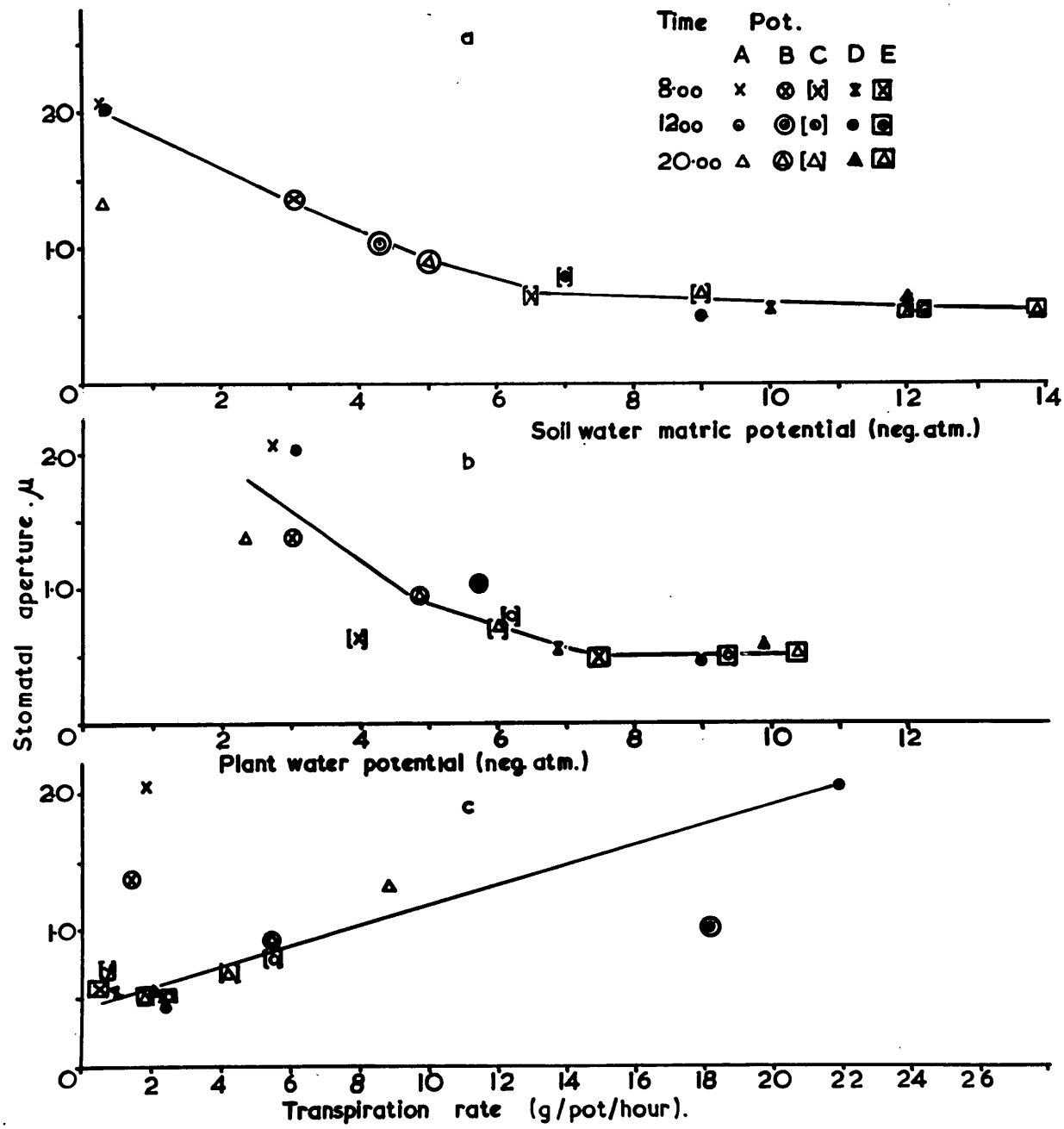


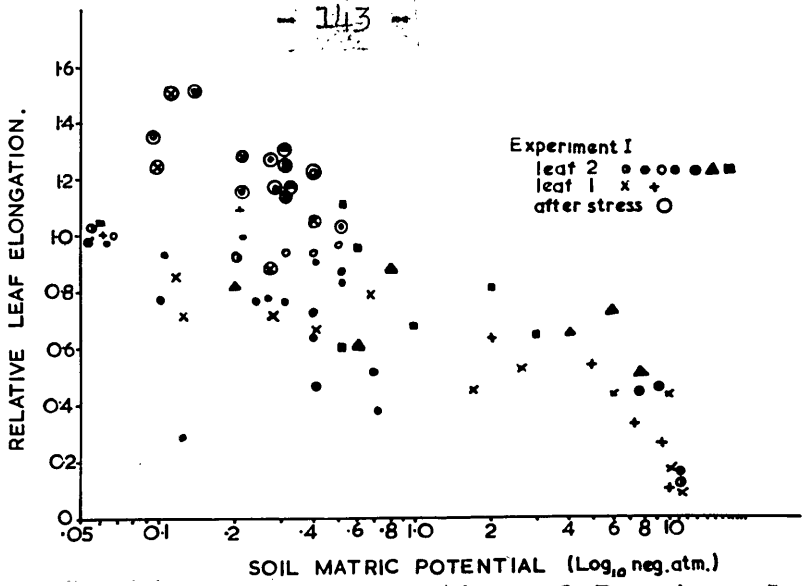
Fig. 21. Stomatal apertures of Zea with (a) changing soil water potential, (b) plant water potential and (c) transpiration rate.

zero at -10 atm. Growth was clearly affected by water stress between -0.1 and -[illegible] atm. From figure 17 it can be seen that measurement of small changes in ψ plant, over this range, are difficult, because of errors in the technique and also because ψ plant is subject to variation due to changes in transpiration rates.

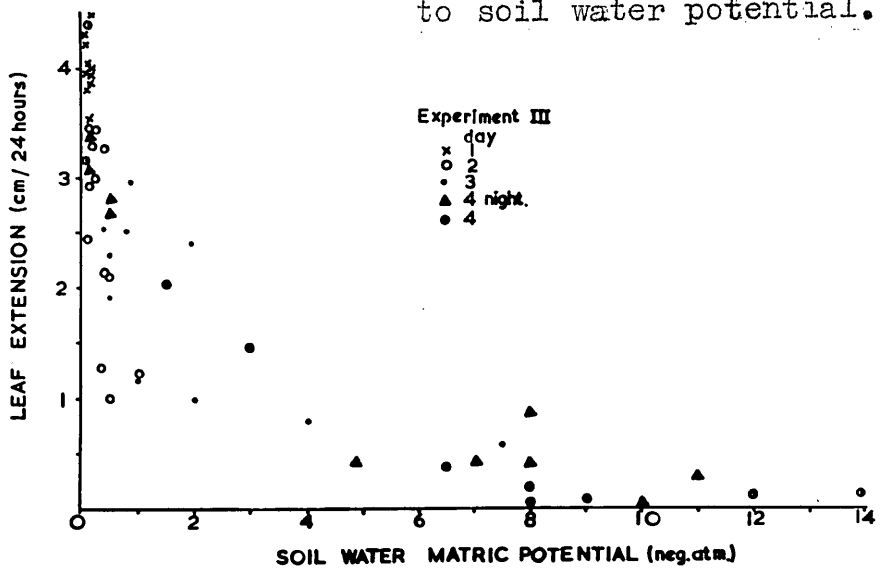
The relative rates of leaf extension of plants which have been subjected to stress and were watered to "field capacity" are also shown in figure 22. The τ soil values to which the plants were returned, measured about 12 hours after rewatering, were those to which growth is related in the figure.

The rate of growth of plants recovering from water stress were usually higher than those of plants maintained at high τ soil, although the total accumulated leaf length was reduced by stress condition. The surge of growth after a period of water stress was insufficient to overcome the effects of the stress on total leaf length.

The relation between τ soil and extension of the leaves was also examined in experiment III and the results are shown in figure 23. Here the leaf extension has been shown as rate per 24 hours; where the extension was measured over periods less than that the rate was calculated for 24 hours.



Figs. 22 and 23. Leaf elongation of Zea in relation to soil water potential.



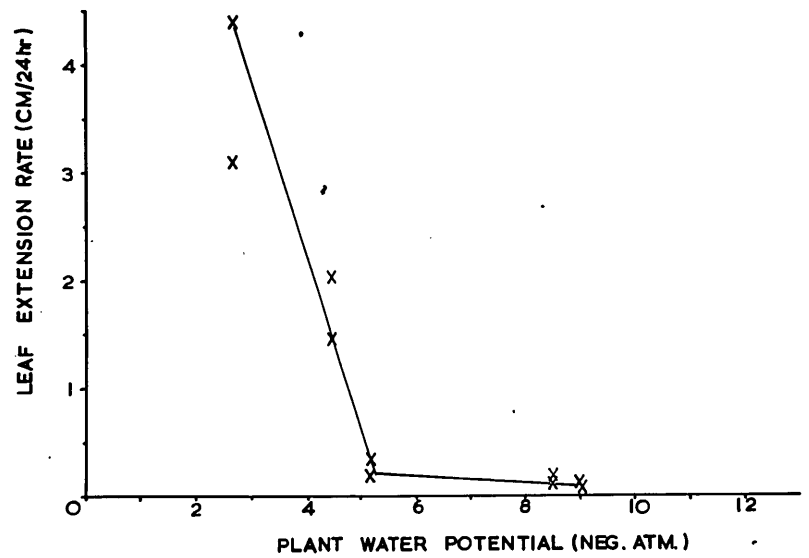


Fig. 24. Leaf elongation of Zea in relation to leaf water potential.

As in the previous experiment the rate of leaf extension was reduced by decreasing τ soil. Between -0.1 and -1 atm the rate fell sharply for any given day. From -1 to -6 atm the rate of extension decreased with decreased τ soil and ceased by about -8 atm.

The data for experiment II were very similar to those of the experiments described, with zero growth at about -8 atm τ soil. The actual rates of growth were higher, at a given level of τ soil above the potential where growth ceased, in experiment I than in II or III. The higher rates of extension in experiment I may have been due to differences in nutritional status of the plants or to higher temperatures. The transpiration rates, which could have affected the leaf water balance and therefore growth, were highest in experiment III, about 1.4 cm^3/cm^2 of pot surface/day; and about 0.4 to 0.5 cm^3/cm^2/day in experiments I and II. (The latter figures were calculated from the rate of soil water depletion). The difference between the experiments cannot, therefore, be due to an effect of transpiration rate on growth.

The relationship between leaf extension growth and ψ plant is considered only for experiment III. The date from experiment II are closely comparable. The rates of leaf extension were measured over the period during which the ψ plant values

were determined. Expressed on a 24 hour basis, the mean rate of growth of the youngest leaf per pot are shown in figure 24 as a function of the mean leaf water potential over the period.

As ψ_{plant} was reduced from the normal values of about -3 atm to -5 atm, the leaf extension rate fell from about 4 cm/24 hours to about 0.2 cm/24 hours. From -5 to -9 atm ψ_{plant} the growth of the leaves was almost zero.

C. Growth of Zea mays in Polyethylene Glycol Solutions

The apparent inversion of the water potential gradient between the soil and leaf of Zea plants, even though they were transpiring and taking up water from the soil, which was described in the previous section, was further investigated by growing plants in polyethylene glycol (PEG) solutions. The osmotic potential of the solution, ψ_{sol}, could be determined with accuracy unobtainable in the measurement of ψ_{soil}; the other advantages of using PEG solutions have been discussed in the introduction to this chapter.

I Methods

The difficulty of determining the osmotic potential of solutions of PEG's have been discussed by Lagerwerff,

Ogata and Eagle (1961) and Jackson (1962).

The solutions used in the present work were made up by dissolving Pharmaceutical grade PEG of molecular weight 4000 (Shell Chemical Company, Ltd.) in a Rothamsted nutrient solution (see Hewitt, 1952) and determining the π sol using the osmometer. As it is not possible to calculate the osmotic potential of PEG solutions from the Van't Hoff relationship the π sol was estimated experimentally.

The weight of PEG 4000 molecular weight per litre of culture solution and the mean of two determinations of π SOL are given in table 19.

Table 19 Osmotic potential of PEG solutions

g./litre culture sol	0	34	80	113	130	215	300
π sol (neg.atm)	0.4	1.1	2.0	3.3	5.4	$^{+}$8.0	12.9

($^{+}$ determined as -7.0 atm in experiment I and -8.0 atm in experiment III for two differently prepared batches of solution).

The Zea mays (var.Inra 321) plants were grown in polyethylene beakers, of 600 ml. capacity, covered with black paper for experiments I and II. The selected seedlings were placed, 4 per pot, with the roots through a perforated

black plastic cover and grown to the 6 leaf stage in culture solution. The plants in experiment III were grown in black bitumen painted trays, 25 x 25 cm x 10 cm deep, on fibre glass and "polystyrene" rafts with the trays covered with black polyethylene sheeting, sealed around the stem bases. The pots in experiments I and II were also covered when it was desired to measure transpiration. The water loss, determined by weighing, was replaced by distilled water and twice weekly a culture solution was applied in equal amounts to each container. This gave equal volumes of nutrient solution to the plants, irrespective of PEG treatment. The PEG solutions were replaced at weekly intervals.

The methods of measuring plant, LWD and stomatal aperture have been described. Leaf extension growth was measured from a datum on the edge of the pots.

II. Experiments

Experiment I was conducted in December, 1965. The Zea plants were grown under supplementary lighting from 9.00 to 20.00 hours each day, at approximately 1,200 foot candles, in a heated greenhouse. The extension growth of the youngest leaf was measured for a period of 2 days with the plants growing in culture solution only and

ψ plant was determined on mature leaves (at 11.00 hours) from plants kept in the dark for 10 hours and then about 3 hours after the lights came on. The nutrient solutions were replaced by PEG solutions of -1.1, -3.3, -5.4 and -7.0 atm, and a control treatment was provided. Each treatment was replicated 3 times. The plants were kept under conditions of very low transpiration; high humidity and darkness. ψ plant values were determined after 12 and 22 hours in the solutions. The plants were then exposed to the lights and ψ plant determined after 12 and 20 hours exposure. The leaf growth was determined for 3 days after the application of the PEG solutions.

Experiment II was conducted in February, 1965. Plants were grown as in II and ψ plant, leaf growth and transpiration rates were measured for 3 days on plants growing in culture solutions and for 4 days after transpiration to PEG solutions. The solutions used were those given for experiment I, except that the -7 atm solution was replaced by a -13 atm solution in order to study the response of the plants under conditions of severe wilting. In experiment II the plants were grown to the 5 leaf stage in culture solution, under the previously described greenhouse conditions.

A study was made of the effects of transpiration and lighting conditions on ψ plant and stomatal aperture. In period A, before the application of PEG solutions, the plants were kept in the dark for 12 hours and ψ plant, transpiration and stomatal aperture determined at the end of the period. The plants were then exposed to the lights and the same values determined after 3 hours. The plants were next subjected to the lights for 3 hours plus a high wind, produced by 2 fans, which caused the leaves to flutter strongly. The transpiration rates and other quantities were measured during the period.

The plants were placed in the PEG solutions on the next day, period B, allowed to transpire in the light for 5 or 8 hours and ψ plant and transpiration measured. In period C the plants were transferred to the dark for 10 hours and at the end of the dark period ψ plant, stomatal aperture and transpiration measured. The plants were then subjected to light for 3 hours and following this, to light plus wind for a further 3 hours. This was after a total of about 15 hours in the PEG solutions. It was hoped by this procedure to determine the effects of transpiration, solution osmotic potential and light conditions

on ψ plant and their interrelation.

Experiment III was conducted in June, 1966. The growth in dry weight and leaf area of Zea plants in PEG solutions was measured and estimates of various growth characteristics calculated. The plants were grown to the 5 leaf stage, 15 plants per tray. PEG solutions of -0.4 atm (control), -1.1, -3.3, -5.4 and -8.0 atm were applied on 1st June. Each treatment was replicated twice, divided into two blocks in the greenhouse. Harvest 1, (referred to as h_1) was taken at the time of application of the PEG solutions, and harvests 2 and 3 (h_2 and h_3 respectively) were taken after one and two weeks growth in the PEG solutions. Each harvest consisted of 8 plants. The h_1 plants were selected at random from each tray, h_2 and h_3 consisted of four plants from each replicate tray, selected at random.

The transpirational losses from the trays were replaced by giving equal volumes of culture solution to each tray and making up the excess, which arises from the different rates of transpiration with treatment, by addition of distilled water. The PEG solutions were replaced after 1 week.

The leaf area of each plant was determined by outlining

the leaf on paper, and estimating the area of the paper by weighing the cutout shapes and comparing with the known weight to area relationship for the paper.

The leaves and the roots were separated at their junction with the stem. The dry weights were measured for each plant after drying at 80°.

In order to compare the plant response to plant water potential as well as solution potential, and to relate the experiment to the others previously described, samples were taken between harvest 2 and 3 during the mid-day period for ψ plant determination.

III Results

a) The relation between ψ plant and π sol

Experiment I. The data for ψ plant, determined in the dark and light prior to PEG application, for 12 and 22 hours after the application of PEG solutions and after 12 and 20 hours in the light (i.e. after a total of 34 and 42 hours in PEG), is shown in figure 25 (a)

There was no significant change between the ψ plant values after 12 and 20 hours either in the dark or light, at the various levels of π sol. With decreasing π sol. the values of ψ plant were reduced, but ψ plant was much

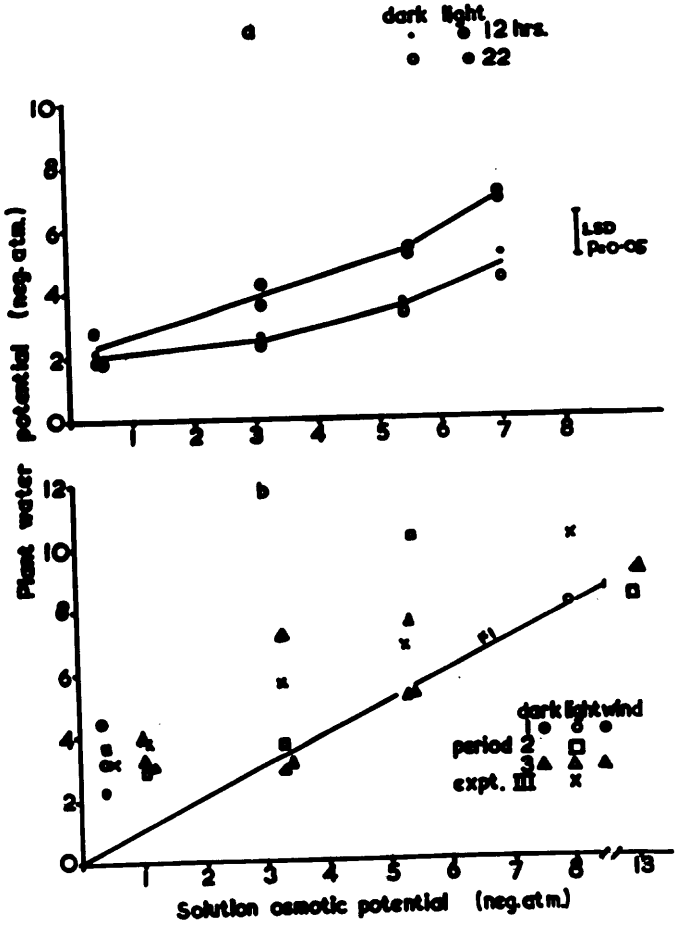


Fig.25. Leaf water potential of Zea grown in polyethylene glycol solutions.

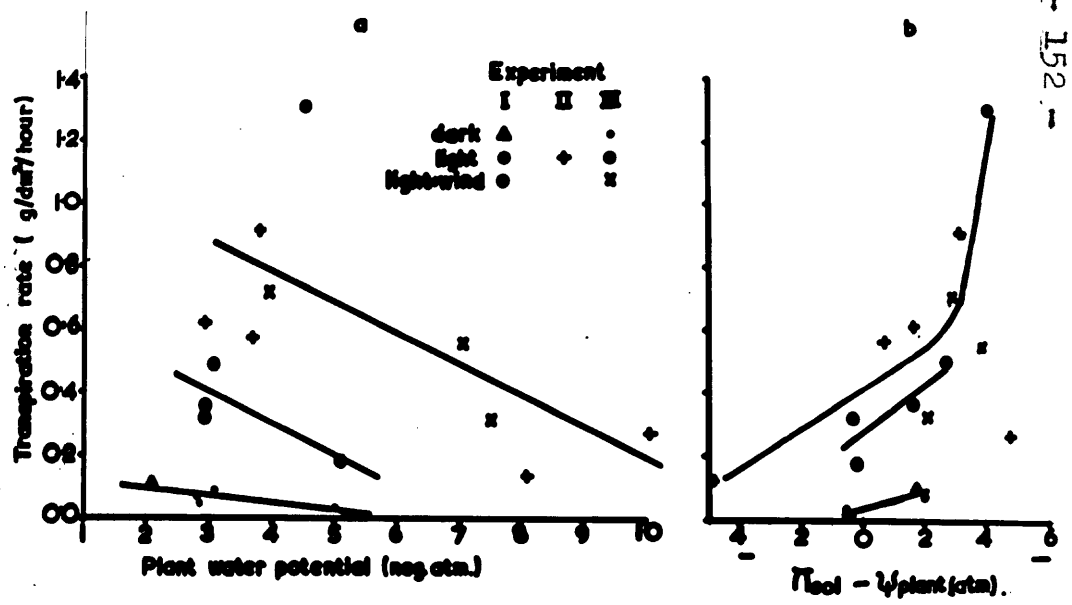


Fig.26. Transpiration of Zea in relation to plant water potential and the gradient of potential between the solution and leaf.

higher in the plants grown in the dark than in those grown in the light at a given π sol, below -0.5 atm. In the dark the gradient of water potential between the solution and leaf was inverted, but in the light ψ plant was very similar to the solution potential. In the -0.4 atm solutions the plant water potential, in the light, and dark, was always about -2 to -3 atm.

Experiment II. The data are given in table 20 for determinations of transpiration, ψ plant and stomatal aperture for Zea plants grown in culture solution under different light and transpiration conditions during period A.

Table 20 Transpiration; ψ plant and stomatal apertures under different conditions.

Length of Treatment		Dark	Light	Light and Wind
		12 hours	3	3
Transpiration mg/dm^2/hr.		102	490	1318
LSD (P = 0.05, t with 21 d.f. n = 8)		4.5 mg.		
ψ Plant		2.3	3.2	4.5
LSD (P = 0.05 t with 3 d.f. n =2)		0.67		
Stomatal aperture μ	Lower epidermis	1.32	1.54	1.33
	Upper epidermis	1.23	1.57	1.32

The data are given in table 21 for period B in experiment II for plants growing in culture and PEG solutions in the light.

Table 21 Transpiration, ψ plant and stomatal apertures of plants in culture and PEG solutions in the light.

π sol (neg.atm)		0.5*	1.1*	3.3+	5.4	13.0+
Transpiration mg/dm^2/hr.		912	618	575	263	141
LSD (P = 0.05 t with 5 d.f.) = 1.3 mg.						
ψplant (neg.atm)		3.6	2.8	3.7	10.3	8.2
LSD (P = 0.05 t with 6 d.f.) = 2.21 atm.						
Stomatal aperture μ	Lower	1.81	1.20	0.98	0.51	0.52
	Upper	1.62	1.64	0.92	0.48	0.39

+ after 8 hours in PEG

* after 5 hours in PEG

The data for the relationship between ψplant and transpiration rate of plants grown for 15 hours in PEG solutions during period C, are summarized in table 22.

Table 22. The effects of light and wind conditions on transpiration, ψ plant and stomata for plants in PEG solutions.

		Dark			Light			Light and Wind		
π Sol.(neg.atm)	1.1	3.3	5.4	1.1	3.3	5.4	1.1	3.3	5.4
Transpiration mg/dm^2/hr.	95	53	40	363	323	170	708	550	316
LSD (P = 0.05 t with 9 d.f.) = 2.3 mg.									
ψ plant (neg.atm)	3.1	2.8	5.1	2.9	2.9	5.2	3.9	7.1	7.5
LSD (P = 0.05 t with 5 d.f.) = 0.48 atm.									
Stomatal aperture μ Upper	1.0	0.8	-	1.4	0.9	0.8	1.82	1.1	0.6
Lower	-	-	-	-	-	-	1.60	1.2	0.8

To further illustrate the relationship of the variables, the data for ψ plant under different conditions have been presented in figure 25 (b).

When plants were exposed to light after a period of darkness, the transpiration rate increased, by about 3 times, but no significant change was observed in ψ plant at any level of π sol. Transfering the plants to wind conditions led to a fall in the ψ plant values below the level of π sol.

In the -0.5 and -1.1 atm solutions, ψ plant was always

lower than π sol. In the dark the ψ plant values, at lower values of π sol, were in approximate equilibrium with the solution potentials. There was no evidence that ψ plant exceeded π sol even after periods of 15 hours in the dark. The difference between the behaviour of plants in experiments I and II under non-transpiring conditions was possibly due to the fact that the determinations of ψ plant in I were made on plants which were only subjected to PEG solutions in the dark and under very low transpiration conditions. Possibly the rate of water movement from the plant to solution in experiment I was very slow, giving the appearence of equilibrium attainment, although the change from 12 to 20 hours was in the direction of an increase in ψ plant rather than a lowering.

In experiment II the ψ plant values attained approximate equilibrium with π sol, except in the -13 atm treatment, during the dark period but did not rise above it.

b. Transpiration and water potentials

The relation between the determined values of ψ plant and transpiration have been plotted in figure 25 (a) and the relation between transpiration and the difference in potential between leaf and solution (π sol $-\psi$ plant) in figure 26.

In the dark transpiration rates were small, and decreased with decreasing ψ plant. Increases in the rate of transpiration were accompanied by reduction in ψ plant and as ψ plant declined with treatment so transpiration was reduced. The transpiration declined as the gradient of water potential was reduced. The highest rates of transpiration were associated with the largest gradient, and reduction of the gradient by only one or two atmospheres caused considerable reduction in transpiration rate. A gradient of zero was still, however, associated with transpirational losses.

The data, for Experiments II and III of the rates of transpiration of plants growing in PEG are collected in figure 27. The rates have been expressed relative to the plants grown at -0.4 atm. In Experiment II the rates of transpiration were determined on a leaf area basis, but in experiment III the rates were for trays only. The transpiration data for Experiment III are based only on the first 3 days after application of the PEG solutions, before leaf area reduction would have affected the rates of transpiration.

The transpiration rate fell with decreasing ψ sol. From -0.4 to -1.1 atm the transpiration rate was reduced by about 10% and fell to about 40% at -5.4 atm. The

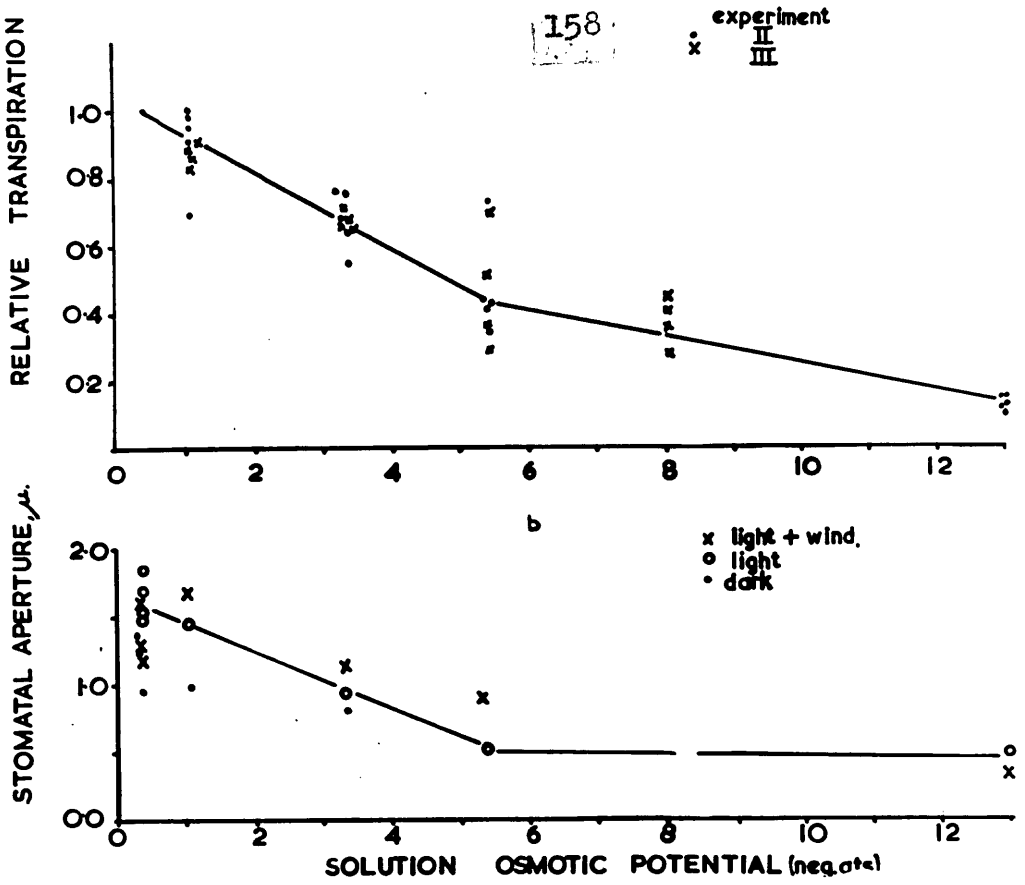


Fig. 27 (a) Transpiration and (b) stomatal aperture of Zea in relation to the solution osmotic potential.

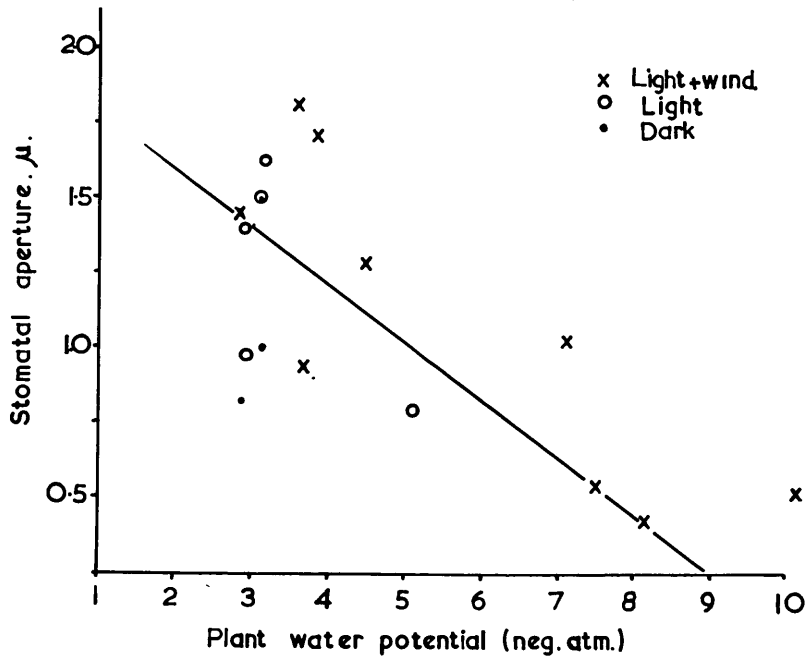


Fig. 28. Stomatal apertures of Zea in relation to plant water potential.

relative rate of transpiration was reduced more rapidly with changes in π sol from -0.4 to -5.4 atm than from -5.4 to -13 atm. In the 13 atm treatment the plants were severely wilted but transpiration was still about 10% of the control rates.

c. The stomatal aperture

The stomatal apertures, determined by the silicone rubber method, were determined on plants growing in PEG and culture solutions, under different light conditions. The results, figure 27 b, have been shown as a function of ψ sol . The stomatal aperture declined with decreased π sol and reached a minimum of about 0.5μ at about -6 atm. In the -0.5 atm solution, as might be expected, stomatal aperture was smallest in the dark and highest in the light with light plus wind treatments intermediate.

The response of the stomata to ψ plant is shown in figure 28. The stomatal aperture was reduced as ψ plant fell from the maximum value of about -3 atm. Variation due to the stomatal light response occured but stomatal apertures were minimal at about -8 atm ψ plant.

d. Leaf extension growth

The data of the rates of extension of the youngest leaves for plants growing in PEG in experiments I and III

are presented in figure 29 (a). The mean rate per pot has been expressed relative to the control plants' growth rate over the same period. Figure 29 (a) shows the dependence of extension on π_{sol}. Both experiments agree in essentials. Growth was unaffected by decreasing π_{sol} from -0.4 atm to -1.1 atm, but declined when π_{sol} was -3.4 atm. At -5.4 atm growth in experiment II was almost reduced to zero. In experiment I growth was still about 20% of the control at -5.4 atm. Growth was zero at -7 atm. The curves in the figure are the means of periods of several days, but no change in the response to π_{sol} was observed over the experimental period.

The growth of the youngest leaf was examined in relation to the measured values of ψ_{plant}. The leaf growth during the periods in the two experiments when ψ_{plant} determinations were made, has been plotted in figure 29(b)as a function of the mean water potential during the period of growth, or where only one measurement of ψ_{plant} was available, as a function of this.

In experiment I growth during the dark and light periods, after 22 and 52 hours in the PEG solutions, was compared with growth in experiment II in the light 3 and

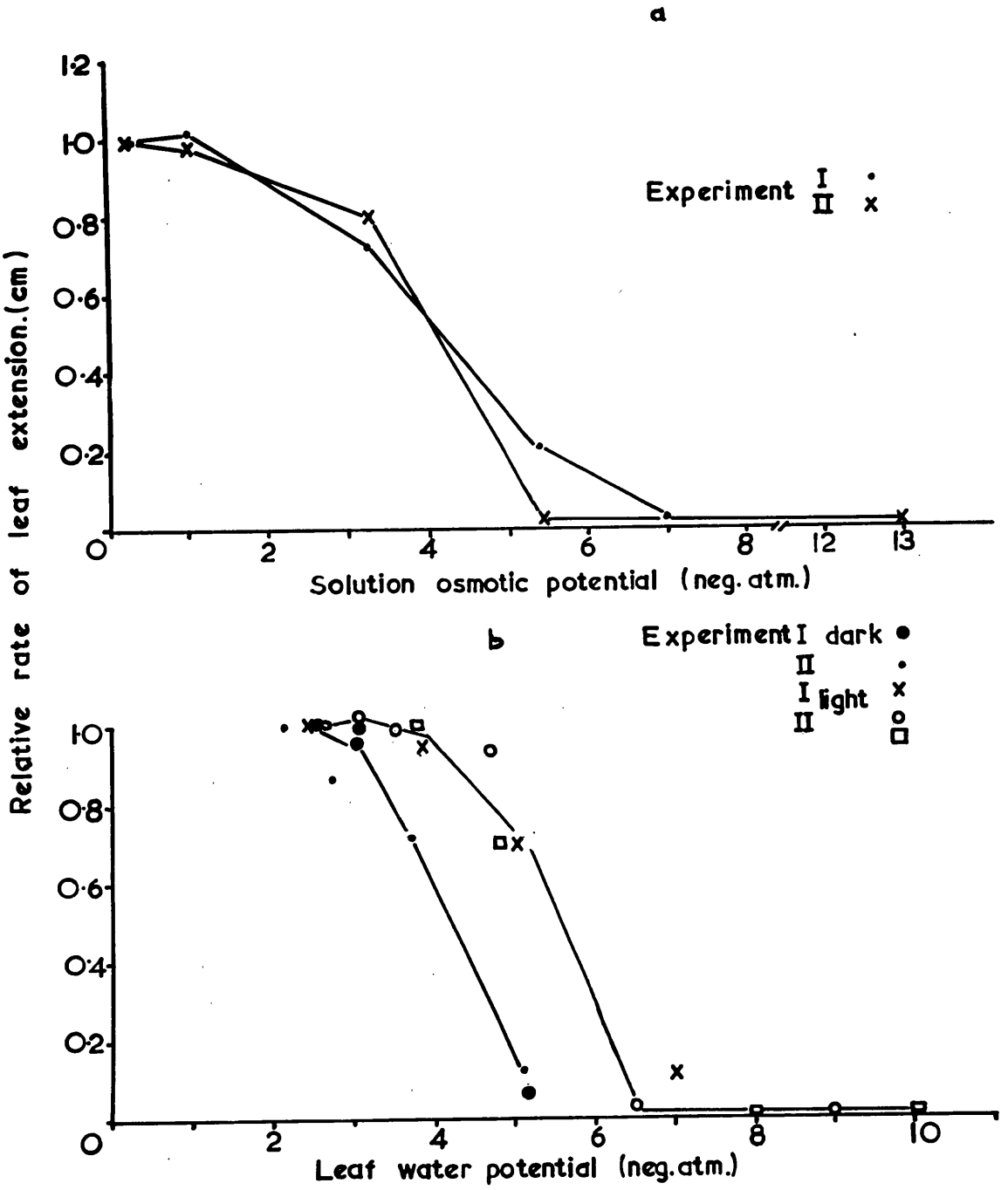


Fig. 29. Leaf extension of Zea in relation to (a) solution osmotic potential and (b) leaf water potential.

32 hours after PEG application and in the dark after 21 hours growth in PEG solutions. Maximum growth was associated with ψ plant values in the range from -2 to -3 atm in the dark and -2.5 to -4 atm in the light. Below -3 atm in the light, growth was reduced and became zero at -5 and -6.5 atm in the dark and light respectively. In the dark the form of the growth curve with decreasing ψ plant was similar to that in the light but growth appeared to be more sensitive in the dark than in the light to decreasing water potential.

In the short term studies of growth no recovery of leaf extension was noticed after the initial depression following the application of PEG solutions, and even after two weeks growth in solutions the plant leaf growth was inhibited both in number and size. This is shown in Plate 2 by the plants from the final harvest in experiment III.

e) Growth in leaf area and dry weight

The growth of plants under water stress may be described, following Evans and Hughes (1961), by the size and form of the plant at a particular instant in time, or by the processes taking place over a time period. Examples of the former are; the dry weight distribution

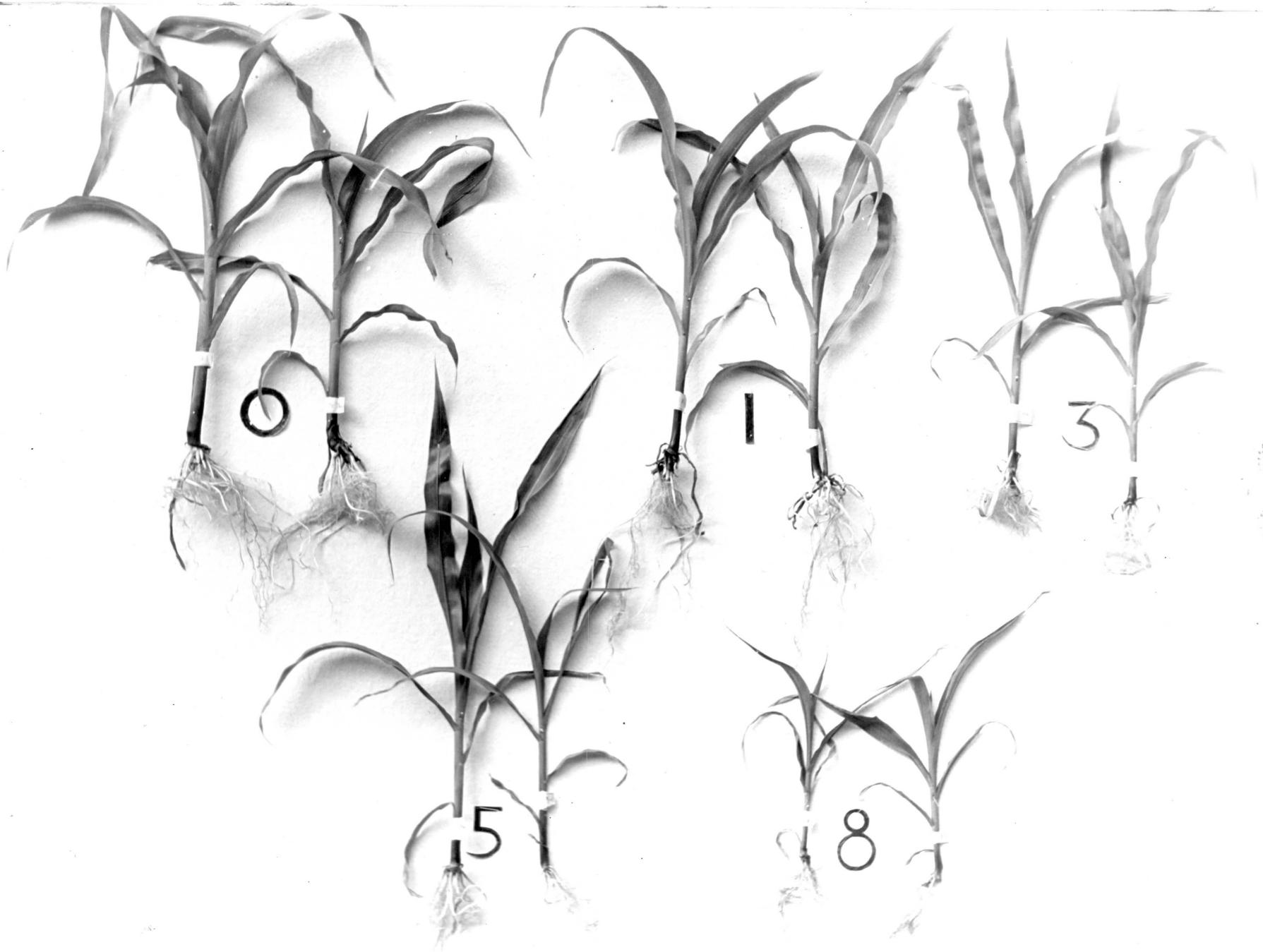


Plate 2. Zea mays after 2 weeks growth in polyethylene glycol solutions.

between the leaves, root and stem, and the ratio of leaf area to weight; of the latter the relative growth rate or rate of dry matter accumulation per unit area of leaf.

Of the measurements of size and form the following will be considered in the section; the dry weight distribution between the leaf, stem and roots and the ratio of leaf plus stem weight to that of the root (shoot root ratio); the ratio of leaf area to total plant dry weight (leaf area ratio) and the ratio of leaf area to leaf dry weight (specific leaf area).

The following symbols will be used; t the time, L the leaf area, W the total dry weight, subscript LF, ST, R refer to the leaf, stem and root weights respectively and subscript 1, 2 or 3 refer to harvests 1, 2 and 3 respectively.

Then: Leaf area ratio (LAR) $= \frac{L}{W}$

Specific leaf area (SLA) $= \frac{L}{W_{LF}}$

Shoot root ratio (SRR) $= \frac{W_{ST} + W_{LF}}{W_R}$

The processes of growth which will be considered are the overall rate of increase in dry matter, the relative growth rate; the relative rate of leaf area increase,

and the rate of accumulation of dry matter per unit area of leaf, the net assimilation rate.

Evans and Hughes have discussed the derivation of the growth rate estimators and these may be written;

Relative growth rate, Rw,

$$Rw = \frac{1}{W}\frac{dw}{dt}.$$

$$= \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

Relate rate of leaf area increase, R_L,

$$R_L = \frac{\log_e L_2 - \log_e L_1}{t_2 - t_1}.$$

Net assimilation rate, E,

$$= \frac{1}{L}\frac{dw}{dt}.$$

$$= \frac{\log_e L_2 - \log_e L_1}{t_2 - t_1} \quad \frac{W_2 - W_1}{L_2 - L_1}$$

In deriving E the assumption is made that W and L are linearly related between harvest. Evans and Hughes discuss this and also the derivation of an alternative equation which assumes that W is related to L^2. They concluded that provided $\frac{L2}{L_1}$ was not greater than 2 the estimates of E were nearly the same from both assumptions. In the present experiments $\frac{L\,2}{L\,1}$ was never greater than 2 and so

the equation given was used to calculate E. In deriving the values of R_W, R_L and E the unit of time employed was one week and the values are expressed as $g/g_2/week$, $cm^2/cm^2/week$, and $mg/cm^2/week$ respectively.

Whitehead and Myerscough (1962) have related R_W to R_L in the form $\alpha = \frac{R_W}{R_L}$ where α is the ratio of the rate of dry matter accumulation to rate of leaf area development. Where $\alpha = 1$ the leaf area ratio is constant between two harvests, where α is greater than 1 the ratio has decreased and where less than unity the leaf area ratio has increased. Using values of α derived from the experiments the adaptation and response of the plants to decreased water potentials could be examined.

The data were analysed by variance analysis of the total leaf area and the leaf, stem, root and the total plant dry weight. The date for h_2 and h_3 were obtained by harvesting 4 plants from the 2 replicated trays of each treatment. The replicates were divided into two blocks. However, for the analysis the experimental design was treated as a random one which gave greater number of degrees of freedom for the residual variance.

The R_W, R_L, E, SLA and LAR estimates were derived by taking the mean plant weight and leaf area per replicate

tray and calculating the desired quantity. This yielded 2 replicates of each for each harvest and treatment and the analyses were performed on these.

1. Dry weight

The $\log_{10}$ mean dry weight of the whole plants at each harvest and treatments is shown in figure 30(a) as a function of the rooting medium osmotic potentials. The form of the analysis of variance is shown in table 23, analyses were performed on the $\log_{10}$ data.

Table 23 Analysis of Variance for Harvests 2 and 3.

Source	d.f.	Dry weight	Leaf Area	Leaf	Stem	Root
Treatments	4	** *	***	**	**	***
Blocks	1	ns	ns	ns	ns	ns
B x T	4	ns	ns	ns	ns	ns
Harvest	1	***	***	***	***	***
T x H	4	***	ns	***	***	**
Residual	65					
Total	79					

Significance levels P = 0.05 (*), 0.01 (* *), 0.00 (***)
ns = not significant on P = 0.05

The mean dry weight per plant increased between each harvest, for all treatments, although the rate of increase was reduced by decreasing Ψ_{sol}, which was

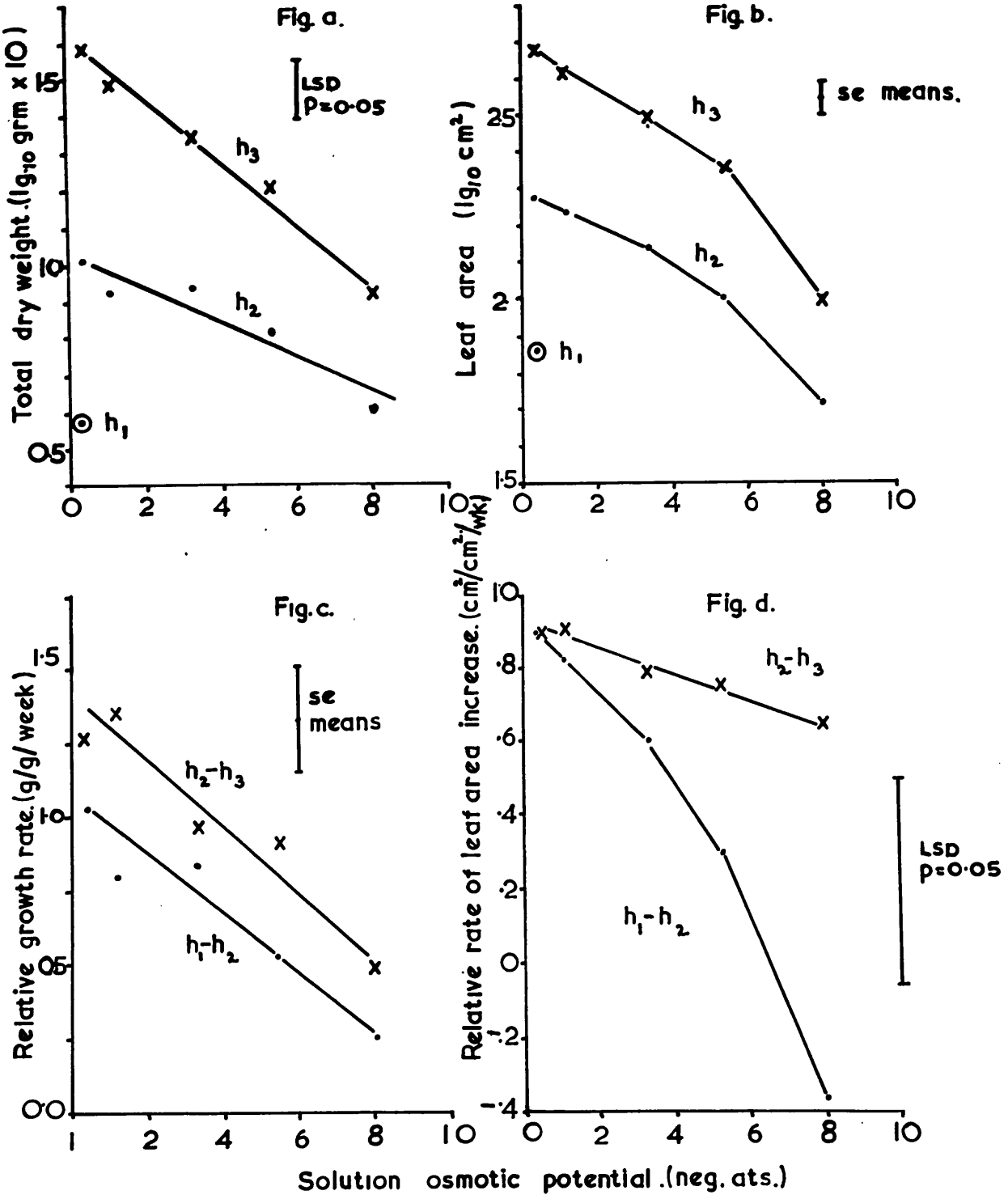


Fig. 30. The effects of solution osmotic potential and (a) the dry weight, (b) leaf area, (c) relative growth rate and (d) relative rate of leaf area increase of Zea.

shown by the Harvest X Treatment interaction. Severe reduction in dry weight accumulation was found with the increased water stress; the $\log_{10}$ dry weight decreased linearly with π sol.

The leaf, stem and root dry weights, figure 31, were depressed by decreasing π sol., although accumulation of dry material took place between each harvest, except for the leaves between 1 and 2, where a reduction in the leaf dry weight was observed at - 8 atm π sol.. Root growth was least affected by the decrease in π sol.

2. Leaf area

The leaf area, shown in figure 30 b and analysed in table 23, increased in each treatment except in the - 8 atm PEG solution between h_1 and h_2, when a fall was observed. Between h_2 and h_3 there was, however, an increase at this potential. No interaction between the treatments and harvest was found. The reduction in leaf area at - 8 atm between h_1 and h_2 was due to an increased rate of leaf senescence, without compensating growth of the younger leaves. The ratio of the mean weight of dead leaves per plant to mean total plant weight for each harvest and the form of the variance analysis has been summarized.

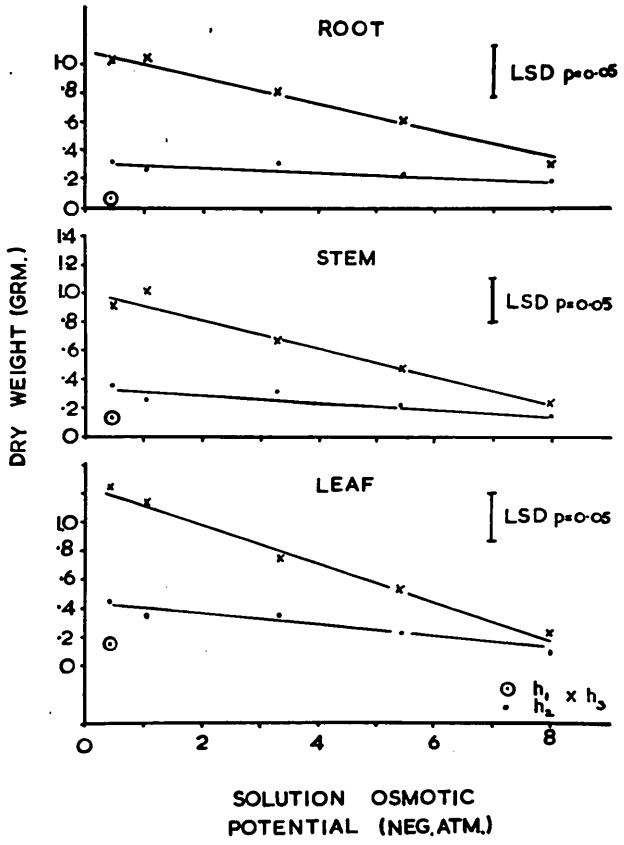


Fig.31 Change in dry weight distribution in Zea plants with decreasing solution potential.

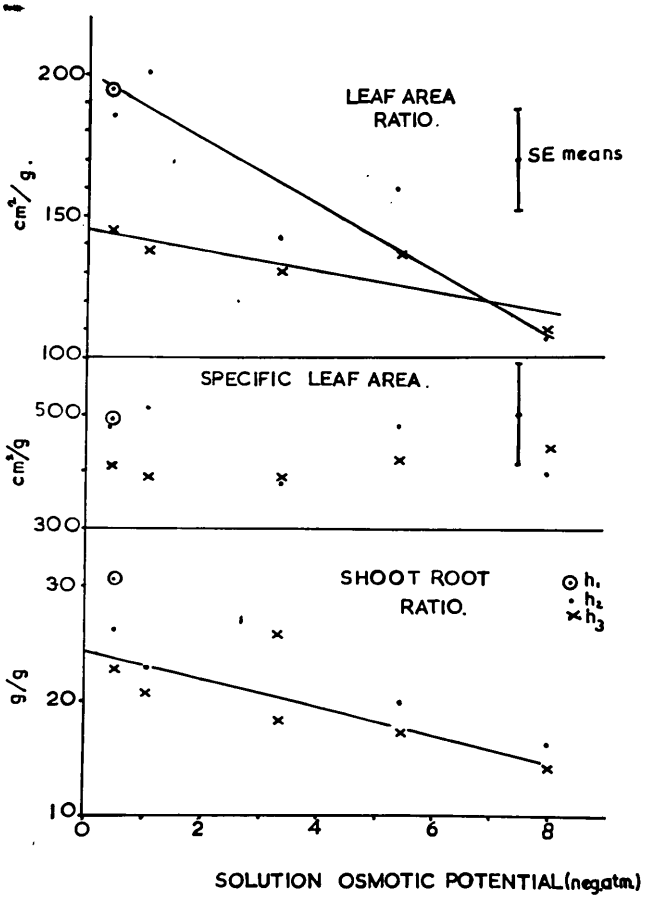


Fig.33. Change in the relative distribution of dry weight of shoot and root and leaf area.

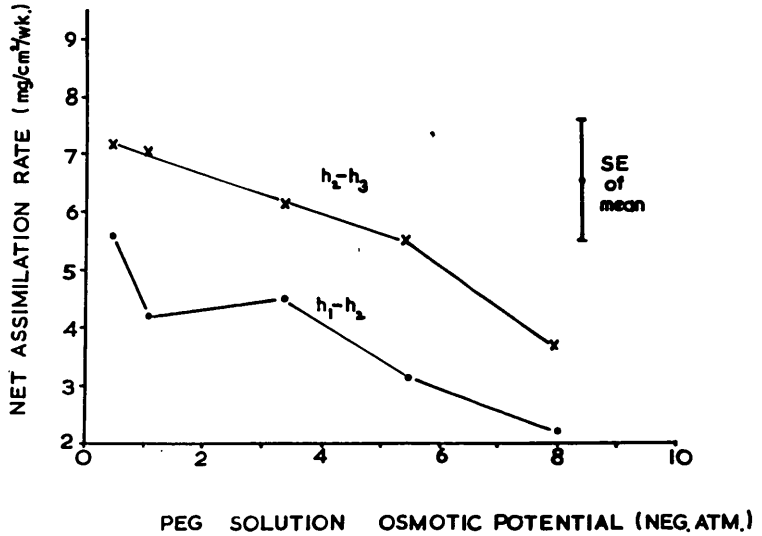


Fig. 32. Net assimilation rate of Zea with decreasing solution potential.

Table 25 Analysis of variance of growth process estimations and indices of form.

Source	df	R_W	R_L	E	SLA	LAR
Period	1	*	**	**	ns	*
Treatment	4	**	**	*	ns	*
P x T	4	ns	*	ns	ns	ns
Residual	10					
Total	19					

Significance at P = 0.05 (*), 0.01 (**), 0.001(***)

ns = not significant at P = 0.05

R_W was greater between h_2 and h_3 than between h_1 and h_2. Decreasing π_{sol} reduced R although at - 8 atm it was still positive.

3. Relative rate of leaf area increase.

R_L, which is shown in figure 30 d, was severely affected by treatment and in the period h_1 - h_2 was negative in the - 8 atm treatment. Between h_2 and h_3 R_L increased in the lower osmotic potential solutions and this suggests that the plant was able to compensate, during the period of growth, for the effects of water stress and partially recover the ability to expand the leaf area.

4. Net assimilation rate

The mean net assimilation rates for the two periods at each treatment are shown in figure 32. E was reduced by decreasing $\bar{\pi}_{sol}$ at each harvest but no significant interaction of harvest with treatment was found.

5. Leaf area ratio

The LAR is plotted in figure 33 for h_1, h_2 and h_3. The ratio declined from h_2 to h_3 for the control plants due to a more rapid accumulation of dry matter than of leaf area-production. With decreasing $\bar{\pi}_{sol}$ the LAR fell but no interaction with treatment was apparent. At h_2 the marked decrease in LAR with treatment was due to reduction of the leaf area, but at h_3 the LAR was less affected by treatment. The slight effect, which treatment had on the leaf area ratio at h_3, was due to an increase in the plant dry weight relative to the leaf area in the higher potential solutions, whilst in the lower potential solutions the increases in leaf area and dry weight were such that the ratio remained more or less constant. The mechanism by which plants under severe stress may increase the leaf area, will be discussed in a later section.

6. The specific leaf area

SLA was unaffected by treatment or harvest, as the results of the variance analysis, table 25, and figure 33

show.

7. The α index

From the R_W and R_L values for each period the ratio has been calculated of growth in dry weight to growth in leaf area for each treatment and is shown in table 26.

Table 26. The values of α for each treatment over two periods

α sol (neg.atm)	$h_1 - h_2$	$h_2 - h_3$
0.4	1.11	1.36
1.1	0.96	1.46
3.3	1.44	1.22
5.4	1.82	1.18
8.0	-0.74	0.76

The values of α greater than 1, in all but the - 8.0 atm treatment, suggest that the leaf area ratio has decreased over the period of study and that the plants were able to accumulate dry matter above that used in the expansion of the leaf area. The negative value of α in the - 8 atm treatnent during the first period is indicative of a decrease in the leaf area ratio between the harvests and of senescence. The rise in α at - 8 atm in the second period suggests that the LAR was increasing but that all

dry matter production was employed in the expansion of the leaf area.

It would be of interest to know if α could exceed 1 in the - 8 atm PEG solution, if a sufficiently long period were given. This would signify recovery from the stress condition.

8. Conclusions

The effects of water stress on the growth of Zea mays include a reduction in the existing leaf area at low water potentials due to senescence of the older leaves and a reduction in the rate of growth of the leaf area. It is apparent that the plant is capable of leaf area expansion even at root medium potentials which are about that of the leaf at zero turgor. However, the extension growth of the leaves, in short term experiments, stops at Π_{sol} values in which, over longer periods, leaf expansion may continue.

The net assimilation rate is also reduced by stress, but does not become zero when Π_{sol} is low enough to severely restrict growth of the leaf area. The reduction in both leaf area and net assimilation rate is reflected by the fall in the relative growth rate with stress.

The proportions of the plant alter with stress due to leaf senescence, the root shoot ratio declines and also the leaf area ratio but the specific leaf area remains constant

D. The Relationship between Leaf Water Deficit and the Water and Osmotic Potentials of Zea mays Leaves

During the course of measurements of plant water potential (ψ plant), the osmotic potential (π plant) and leaf water deficit (LWD) were measured. The importance of LWD (or its complement, relative turgidity) as an indicator of water stress has been shown by Weatherley and Slatyer (1957), provided the LWD:ψ plant relationship is known. In the present work only the ψ plant values have been given because they are regarded as of greater meaning in problems of growth and water uptake. The significance of the measurements of π plant and LWD, which may be regarded as an approximate measure of the cell volume, is that they can be used to calculate the turgor of the tissue. This may be of importance in the understanding of leaf growth and transpiration. In this section, therefore, the relation between ψ plant, π plant, turgor potential, P, and LWD will be examined and also the bearing which they have on growth.

I. Methods and Materials

The way in which ψ plant, π plant and LWD were determined was described in Chapter 1. The determinations of ψ plant and π plant were made on the same samples of

leaf, and LWD was determined on parallel samples. Three sets of measurements were made on plants subjected to water stress over varying periods of time and under different conditions.

A. Measurements were made on the plants grown in soil in experiment III, Section B. The soil was allowed to dry over a period of 4 days, and the range of ψ plant measured during the period was from about -2 to -11 atm.

B. Zea plants were grown in polyethylene glycol solutions (experiment II, Section C) for a period of about 2 days before the determinations were made.

C. Well watered plants were detached from their roots and allowed to wilt in the air over a period of about 4 hours.

II Results

a) Leaf water deficit and water potential

The relationship is shown in figure 34. ψ plant falls with increasing LWD from about - 2 atm at 2% LWD to - 11 atm at 40% LWD. The points fall within about $\pm$ 1 atm of the mean at any leaf water deficit, despite the diversity of materials and conditions. The plants grown in PEG solution and soil showed a close agreement although Macklon and Weatherley (1965) showed, for Ricinus, that

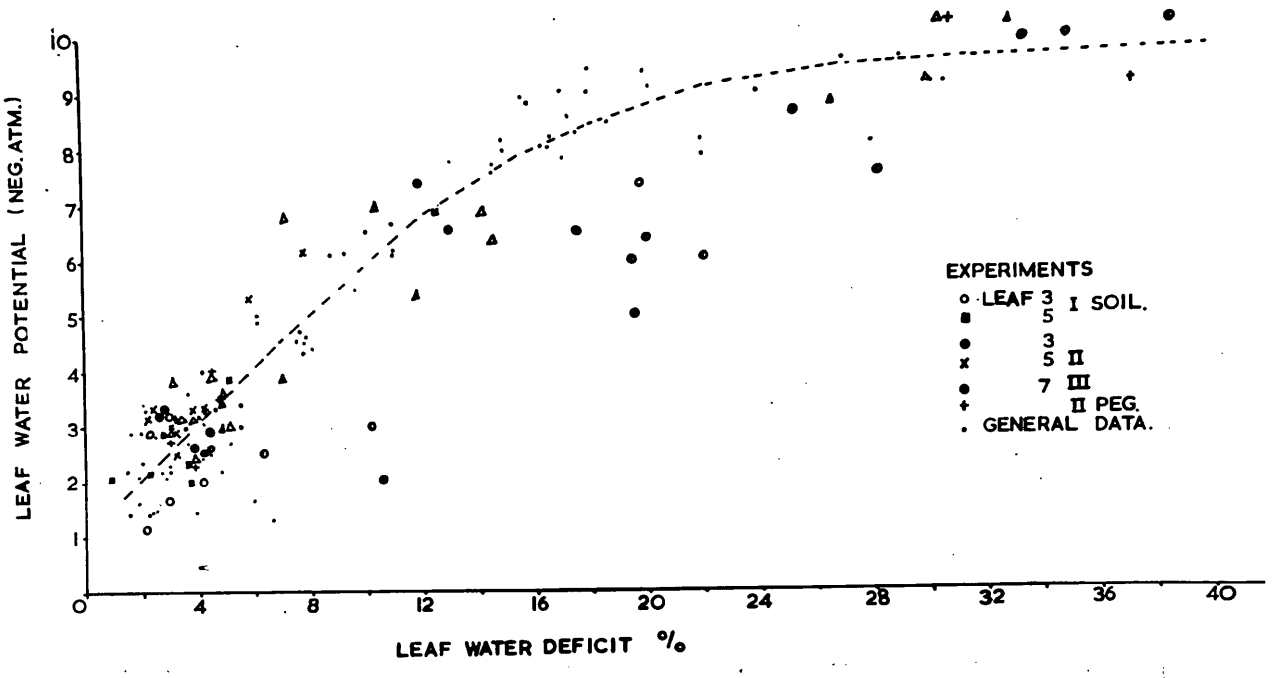


Fig. 34. Leaf water potential of Zea with increasing leaf water deficit.

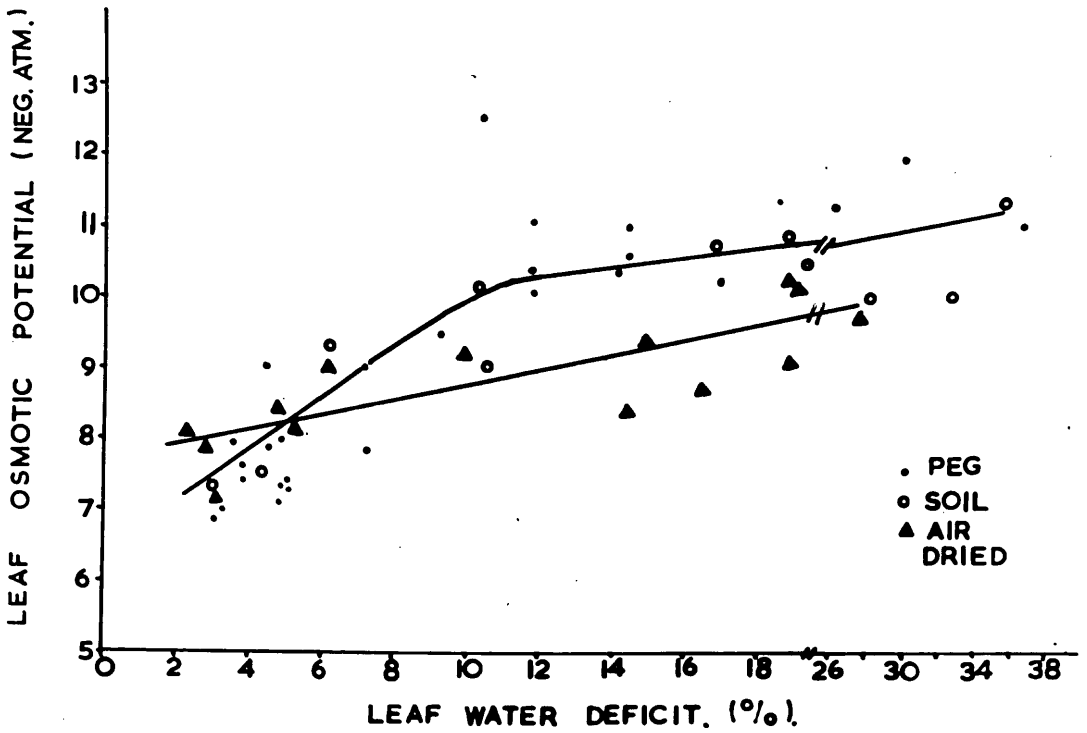


Fig. 35. Leaf osmotic potential of Zea with increasing leaf water deficit.

the ψplant:LWD relationship was different for soil and solution grown plants.

The most obvious discrepancy is shown by the data for soil experiment III when compared to the rest of the data. In III the LWD changed from about 2 to 10% before ψplant fell, so that, at a given LWD in this range, ψ plant was higher in plants from experiment III than in the other plants. It was only at a LWD of about 35 % that ψplant and LWD values agreed for all experiments. The discrepancy between the data for experiment III and the other data may be explained by assuming that a large volume of the tissue exists external to the cell, cytoplasm or vacuole, and that the water content of this extra vacuolar tissue changes without influencing the cell or vacuole. In the case of experiment III the plants were very large compared to the other experiments, with thicker walls and larger vessels. The cell wall may act either as a source of water for the cell during equilibration in the osmometer chambers with consequent increase in the cell or vacuolar wall potential, or the cell water content remains constant and the wall water content fluctuates. Gaff and Carr (1961) have shown that such a buffering action by the cell wall is possible.

Obviously for plants of uniform size, the ψplant: LWD relationship is a close one and LWD could be used as an indicator of the plant water potential, although changes in the tissue due to maturation or uptake of materials into the cell, may alter the relationship.

b) Leaf water deficit and osmotic potential.

The data are given in figure 35. The data agreed for plants grown in soil and solution culture, but the air dried plants differed in their LWD: ψ plant relationship. At the minimum leaf water deficit of about 3% the plants in soil experiment III, PEG experiment II and the air dried plants showed π plant values of about -7 to -8 atm. With increasing LWD the air dried plants π plant values did not fall as markedly as those of the plants grown in soil and PEG solutions. The discrepancy was about 1.5 atm above a LWD of 10%. πplant was linearly related to the $\log_{10}$ LWD.

The reduction in π plant with decreasing water content may be due to concentration of the existing materials in the cell or to production of osmotically active materials within the cell. If the decrease in πplant is due to concentration of the cell contents, the product of osmotic potential and cell volume should remain constant, but if increase in osmotic materials takes place then πplant

x volume should rise. Weatherley (1965) equated volume with the relative turgidity or relative water content, i.e. 100 - LWD. For the present experiments π plant has been calculated and the results are shown in figure 36. For the air dried plants the π plant x volume remains constant except at high leaf water deficits, indicating that over short periods of water deficiency the cell osmotic content remains more or less the same. In the soil grown plants the product is constant with only a very slight fall, indicating that in the plants there was no significant increase of osmotically active substances in the cell by uptake from the soil or from hydrolysis of insoluble carbohydrates. Slatyer (1961) indicated that the osmotic potential of the sap of plants grown in nutrient solutions decreased in proportion to the potential of the nutrient solution. Janes (1966) showed an increase in the "soluble solids" of bean and pepper plants after periods of water stress where this was applied in the form of polyethylene glycols.

Eaton and Ergle (1948) observed that, under stress, cotton plants suffered a decrease of starch and increase in hexose sugars. The evidence for changes in the materials of the cell and, therefore, potentially, for increase in osmotically active materials, has been reviewed by

Stocker (1960).

The plants grown in PEG solutions exhibit a very marked increase in π x V; although the data are scattered a large increase is indicated in the cell osmotic materials. A possible source of this increased solute is either uptake of ions or PEG from the solution or the production of osmotically active substances by the cell. However, production of osmotically active materials, e.g. conversion of starches to sugars, by the cell under conditions of water stress must be excluded as the plants in soil or dried in the air show no such increase in materials. It is not clear if PEG is taken up by plants. Janes (1966) suggests that they are not, but Lagerwerff and Eagle (1961) concluded that PEG's can enter the plant, but not necessarily the cells.

The effect of water stress on the plants is, therefore, dehydration of the cell and a rise in the cell sap concentration. If diffusible materials are readily available at the root then they may be absorbed into the cell with a consequent lowering of π plant.

c) Leaf water deficit and turgor

In figure 36 the turgor potential, P, and ψ plant and π plant from which it is derived, are given as functions

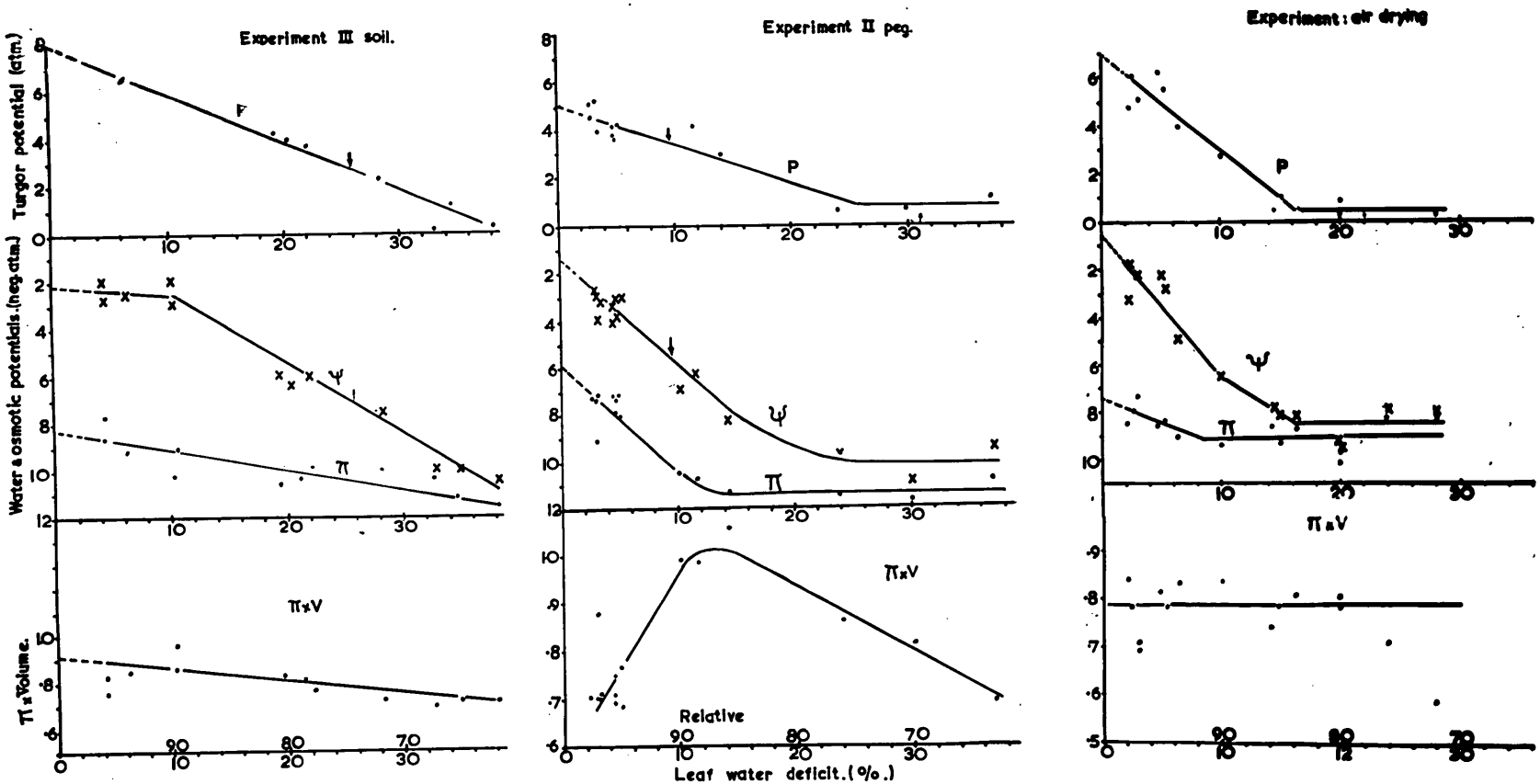


Fig. 36. The leaf water potential, osmotic potential, turgor and the osmotic concentration of Zea mays leaves in relation to leaf water deficit.

↓ denotes the cessation of leaf extension.

of water deficit. The LWD ψ plant and π plant relationships have been considered and only the turgor pressure will be discussed.

P fell with increasing LWD in each experiment, from a maximum of about 6 atm to 0.5 atm. Possibly absorption of the cell wall water could have led to over estimation of ψ plant and therefore P would have been positive. The relationship between P and LWD was approximately linear down to P of about 0.5 atm, P remaining at that level with increasing LWD. Minimum turgor was reached at about 32% LWD in soil grown plants 24% in plants from culture solutions and 14% in air dried plants. The differences in the P: LWD relationships between the experiments are probably due to the contribution of the cell wall in estimations of LWD and the variation in the cell wall's elasticity. This has been discussed previously.

The maximum estimated values of P were small compared with those mentioned in the literature Gardner and Ehlig (1965) showed for pepper and sunflower maximum P was about 10 bars, and 11 or 12 for cotton and trefoil. Ruf, Eckert and Gifford (1963) gave maximum values of P as 13 bars for Agropyron sp. and about 6.3 bars for Solanum. Janes (1966) indicates that P would be about 8 atm for beans..

How significant is maintenance of turgor to growth? In Sections B and C the extension growth of the first leaf of Zea plants, in soil experiment III, and PEG experiment II, was determined in relation to the plant water potential. Figure 36 shows the level of turgor at which extension growth ceased and this proved to be the same in both experiments, about 3 or 3.5 atm.

It is generally assumed that extension growth stops at zero turgor, (Slatyer, 1957), but obviously in these experiments this not so. Gardner and Ehlig (1965) have concluded that marked changes take place in the elastic properties of the cell when turgor drops below a given turgor, rather than at zero turgor. Interestingly the turgor at which visible wilting occured in their material, was about 2 or 3 bars. The growth of a cell is not only affected by turgor, but also by the capacity to synthesize materials involved in the production of the cell wall etc. Bonner, Bandurski and Millerd (1953) and Ordin (1960) have shown that auxin influences the uptake of water and presumably the extension properties of the cell wall. In short term experiments metabolism of cell wall materials may not be of prime importance in growth and probably an growth is dependent upon turgor. However, the maintence

of turgor by changes in the osmotic constituents of the cell may be achieved at the expense of the cell organization and lead to effects on growth even though turgor would seem to be adequate. Certainly the effect of water deficit on subcellular organization has been shown by Schnepf (1961) to produce severe damage to cell structure.

Chapter 4. The Growth and Transpiration of Lolium perenne in Soil and Polyethylene Glycol Solutions

A. INTRODUCTION

Growth and water uptake of Lolium were compared in soil and polyethylene glycol solutions. The introductory remarks to the chapter on the response of Zea mays to reduced water potentials in the medium and plant apply to the studies on Lolium. Whereas large Zea plants were grown in very limited volumes of soil in order to reduce the effects of soil water heterogeneity. in the experiments to be described this was treated as an experimental variable and transpiration and growth related to changing soil water distribution. In addition estimates of the density of rooting were made because, as Gardner (1964) has shown, soil water depletion proceeds more rapidly where the rooting density is greater.

The experiments to be described allow the changes in soil water potential, ψ, in 30 or 60 cm deep soil columns to be related to the plant water potential (ψ plant) transpiration and growth during the course of a drying cycle.

B. Growth of Lolium in soils.

I. Methods and Materials.

The most suitable containers for growing the plants were sections of plastic drainpipe (Marley Co.,Ltd.) 7.7 cm diameter (3") and 30 cm long. These enabled soil columns of uniform diameter to be used without the problems of handling large volumes of soil. The tubes, as the containers will be called, were filled with sieved Silwood soil equivalent to 1.6 kg of dry soil, i.e. 1.8 kg at 14% water content. Packing was performed by hand, by filling and tamping which gave uniform bulk densities of soil. For the larger, 60 cm columns, two tubes were joined by adhesive tape. Each tube was filled separately and joined after disturbing the soil at the junction to ensure a uniform profile.

Lolium perenne was a suitable species to grow in the soil columns because it germinated and grew easily and,whilst remaining short, provided a large leaf area. Some disadvantages were the difficulty of measuring stomatal aperture and estimating leaf area and rooting density.

The seeds were sown on the soil surface, covered with sand and when the plants showed two leaves, thinned

to 15 seedlings per pot. Some roots grew out of the bottom of the columns and before the start of the experiments these were removed. Roots also accumulated at the walls of the tubes. Although the pattern of water uptake could have been modified by this root distribution, leading to lower τ values at the soil surface than in the centre of the column, such an effect was not observed.

The distribution of the soil water content was estimated by dividing the soil columns, which were extruded from the tubes into 5 cm thick horizons. The bottom horizon was usually about 3 cm thick. The fresh weight and dry weight (dried at 105°C.) were determined and the water content calculated. Matric potentials were derived from this data and the moisture characteristic. 4 pots per treatment were sufficient to estimate the water content to within about $\pm$ 1.5% of soil dry weight, provided the rates of loss from the tubes were uniform. The values of τ could be estimated to about $\pm$ 2 atm in the drier part of the moisture characteristic and $\pm$ 0.5 atm in the wetter.

Before the experiments the soil was watered to about saturation and allowed to drain for 12 hours to "pot capacity" as it has been called, about 14% at the top and 18 to 20% water content by weight at the bottom

of the column.

2 days after this period of drainage, loss from the soil by percolation was only 3% of the daily transpiration loss. Root lengths were estimated by sectioning the soil horizons, washing the roots from the soil using a fine sieve and water jet. and taking subsamples of measured lengths of roots and determining the dry weight of these. In some experiments the roots were sorted into two main classes, those of diameter greater than 0.02 cm which were relatively unbranched, and the remaining highly branched roots generally much smaller than 0.02 cm diameter. The 6 subsamples, each of 50 cm of large root or 100 cm of small root, were dried at 80°C. and the total lengths of the roots per horizon estimated from the total dry weights and the length weight ratios. The root density per unit volume of soil was calculated from the volume of each horizon. Considerable variation between individual tubes was found, both in the total root per horizon and in the proportions of large and small roots. Sampling for roots at each harvest was time consuming and the differences between harvests in such short term experiments were negligable, therefore the root distribution was found only for the first and last harvests.

Transpiration was measured by weighing the tubes on a balance accurate to about ± 0.1 grm. 4 replicates of each treatment were used and the standard error of the mean was between 5 and 10%. Loss of water from the soil surface was reduced by sealing the tops of the tubes with a layer of wax. After applying the wax the soil was watered by slow applications through holes which could be plugged. The most obvious effect of this treatment, observed in a preliminary experiment, was to slow down root growth, probably because of unfavourable aeration.

Leaf extension growth was taken as a measure of the plant response to treatment. The length of four of the youngest leaves per tube was measured from the ligule of the next oldest leaf. The leaves were about 1 or 2 cm long at the start of the experiments and new leaves were measured when they were about 8 or 10 cm long; this was done to eliminate the effects of leaf maturation on growth. The tubes used were the same on which transpiration was determined.

Root extension growth was measured in one experiment. Plants were grown in clear acrylic plastic tubes of the same dimensions as those previously described. Light

was excluded by covering in black paper. The extension of the roots was measured over a period by marking the position of the roots on the surface of the tube. Only the main roots were measured as the lateral roots were found to be of limited growth. The root tips were frequently obscured by the soil and considerable variation was found between the roots within a tube.

When the tubes were harvested the top growth was divided from the roots and divided into stem, leaf and dead leaf material and the dry weight determined. The leaf area was estimated by taking 4 subsamples of the fresh leaves, each of 10 pieces of leaf, 5 cm long. From the area to weight ratio the total living leaf area was calculated.

Samples for the determination of ψ plant, LWD and stomatal infiltration, were taken from the mature leaves at 10.00 or 14.00 hours.

II. The Experiments

Three experiments will be described.

Experiment I. July 1965. Plants were grown in the tubes in a cool greenhouse until the longest roots penetrated the base of the soil columns. 20 tubes were maintained at "pot capacity" and 20 allowed to dry by

normal transpiration. Soil water distribution and root content was sampled at harvests taken during the experiment. Transpiration was measured over the morning, afternoon and evening periods and overnight throughout the experiment. The conditions during the experiment were cool and humidity was high. The plants were relatively small; the leaf area index was about 1.5 and the rate of water loss was about 0.2 $cm^3/cm^2/day$, which is low for pot grown plants.

Experiment II. April 1966. 80 tubes of plants were grown until a substantial cover was developed and the roots had thoroughly permeated the whole soil mass. One set of 50 plants was allowed to dry the soil whilst another 30 were maintained at the initial water content by replacing the daily transpirational losses. Repeated samplings for soil water content in the controls was unnecessary and experiment samples were taken at every other harvest. Root growth was determined from an initial and final harvest. The experiment continued for three weeks. In this experiment recovery of the plant leaf extension growth and transpiration after a period of 5 days wilting was determined following the resaturation of the entire soil column or after applying half of the daily transpiration loss either to the top or bottom of

the soil. The transpirational losses of the fully re-watered plants were taken as the controls. The bottom watering should have enabled the effects of a supply of water from the soil (e.g. by capillary rise from a water table) to be compared to the effects of top watering (e.g. from light rainfall) on the recovery of droughted plants. If root resistance to the flow of water was high then growth and transpiration would be reduced in the bottom water plants.

During the experiment the conditions were hot and dry and together with a leaf area index of about 10. induced high rates of soil water depletion.

Experiment III. July 1966. In order to study the effects of a deeper rooting system on the water balance, Lolium plants were grown in 60 cm long columns. 9 columns were used for controls and 15 tubes were observed over a drying cycle. 3 tubes were taken for sampling at each harvest and divided into 12 horizons, a to l. Leaf length and plant were determined every other day during the drying period and transpiration daily. The conditions were hot and dry. The leaf area index was about 8.

III. Results

The data have been presented in chronological order in the experiments, with the true scale retained in order that comparison may be made of the processes and events taking place within a drying cycle. The results of the experiments will be considered together and the figures of each experiment assembled.

a) Soil water content and water potential.

Experiment I. The data are shown in figure 37 (a) of the soil water content per horizon a to f on a dry weight basis, for the harvests of the dry treatment. The root length per unit volume of soil in the different horizons is also shown in the figure. In figure 37 (b) the soil water potential, τ, derived from the water content data, is shown as a function of time from the start of the experiment for each horizon.

The water content decreased from the top to the bottom of the soil column at each harvest and as the columns dried the upper horizons were depleted of water before the lower. This corresponds to the density of rooting in that the upper horizons were more densely occupied by roots than the lower.

The soil water potential fell in the surface layers

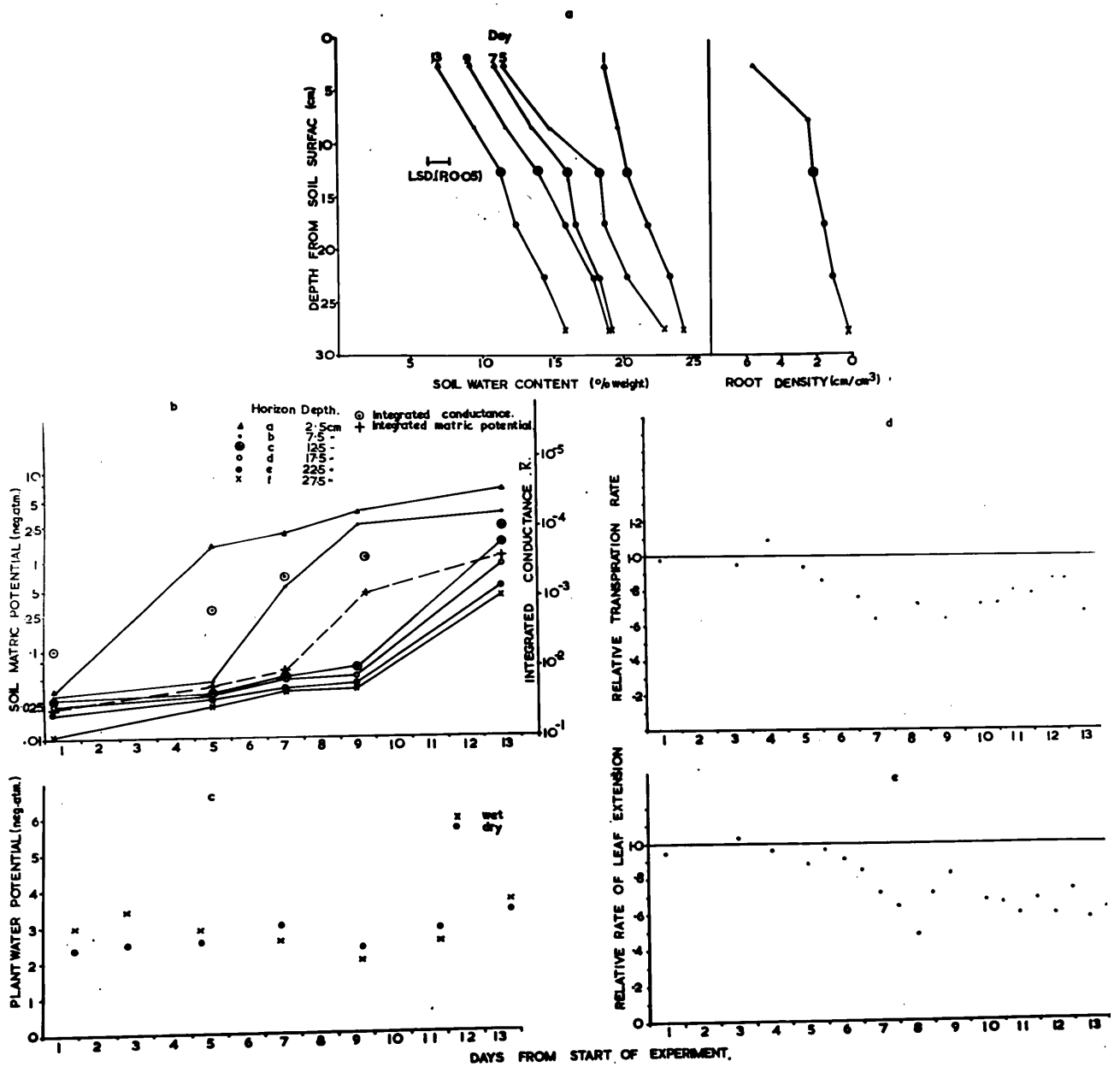


Fig.37. Experiment I Lolium a) Soil water content in the dry treatment and root density per horizon, b) soil water potential and conductance, c) plant water potential, d) relative transpiration and e) relative leaf elongation during the experiment.

of the soil as water was removed and was then reduced throughout the column. The surface was dried from -0.03 atm on day 1 to -3.5 atm on day 9 whilst the lower layers of the soil fell from -0.01 atm to -0.1 atm in this time. From the ninth to thirteenth days the surface horizon fell to -7 atm while the lowest horizon reached a potential of -0.4 atm. Obviously with such heterogeneity of water potential in the column it is difficult to define a single value of Υ to which growth and transpiration may be related.

Taylor (1952) devised an approach to the integration of soil water potential by weighting the measured potentials by a depth factor. Gardner (1964) has emphasised that the importance of the water potential at a given level in the soil depends upon the effective rooting density of the plant, on the depth and also upon the capillary conductivity of the soil. An equation relating Υ_i, the potential in the i^{th} layer of the soil to capillary conductivity, k_i, rooting density, L_i (cm/cm^3) and to the depth Z_i below the soil surface, was derived. The integrated soil water potential $\bar{\Upsilon}$ is given by

$$\bar{\Upsilon} = \frac{(\Sigma\, k_i L_i \quad (\Upsilon_i + Z_i)}{\Sigma\, k_i L_i}$$

$\Sigma k_i L_i$ is defined as the integrated capillary conductivity, $\bar{K}$.

In figure 37 (b) the derived $\bar{\psi}$ and $\bar{K}$ estimates have been given, calculated using the values of L_i shown and k_i from chapter 2. $\bar{\psi}$ fell slightly with soil drying from day 1 to 7 when a sharp decrease was observed.

In general $\bar{\psi}$ corresponded to ψ of horizon c. in the centre of the column but the agreement was not exact. The values of $\bar{K}$ fell progressively throughout the course of the drying cycle from about 10^{-2} to 10^{-4} cm^2/day.

Experiment II. The measured moisture content data have been transformed to water potentials which are shown in figure 38 (a). At the initial harvest ψ was about -0.2 atm in the uppermost horizon and -0.1 atm in the lowest. Drying was most rapid in the upper horizons and proceeded down the column of soil with progressive water loss. At the third harvest, day 7, horizon a was at about -1.5 atm and f at -0.15 atm. Between days 7 and 10 the lower zones of the soil dried rapidly and ψ fell, in horizon f, to -2 atm when a was reduced to -6 atm. With further drying ψ in each horizon approached -14 atm although even with severe drying the lowest horizons were at a higher water potential than the upper.

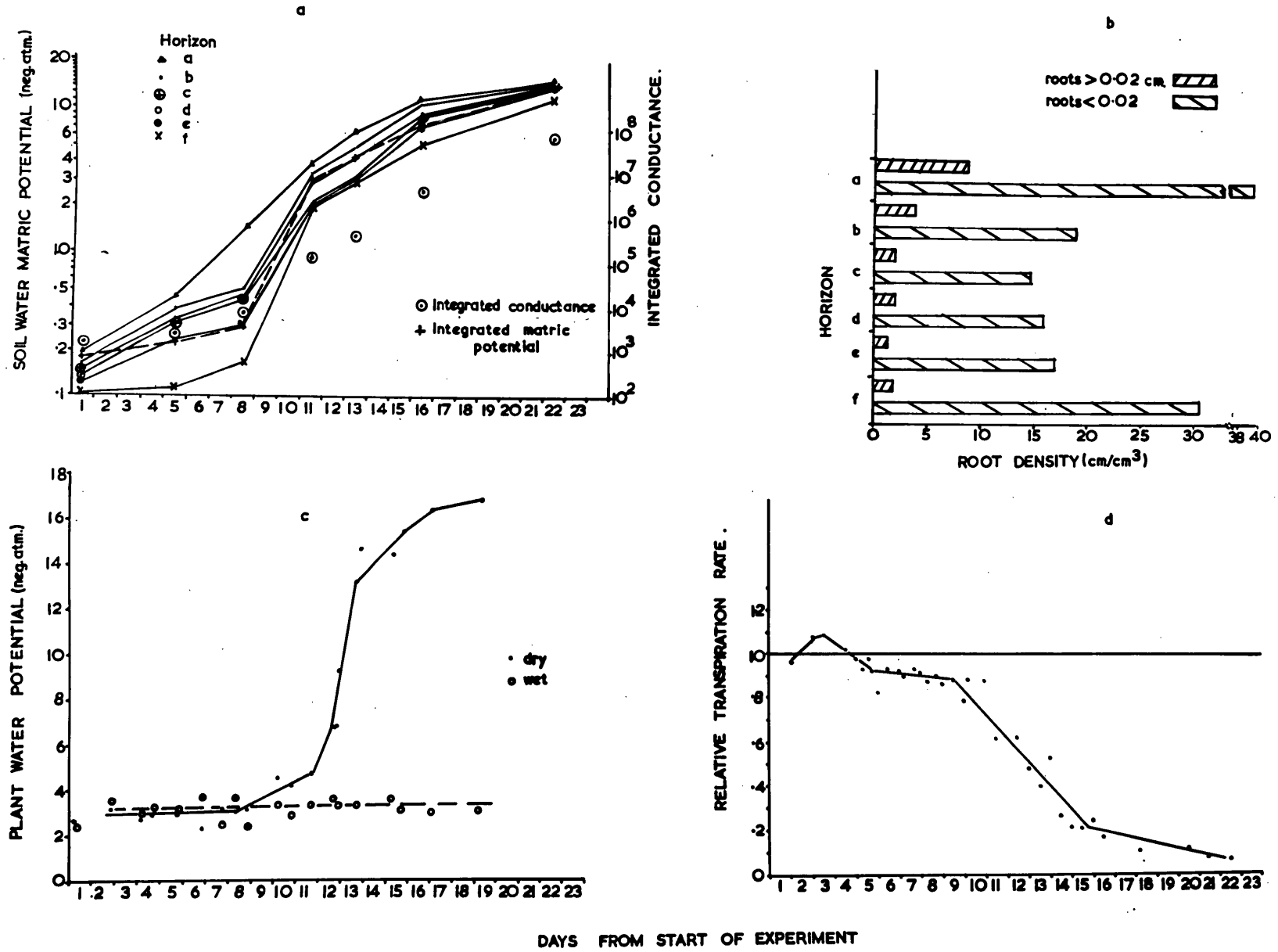


Fig. 38. Experiment II Lolium. (a) Soil water potential and conductance. (b) Root distribution; (c) Plant water potential; (d) Relative transpiration rate during the experiment.

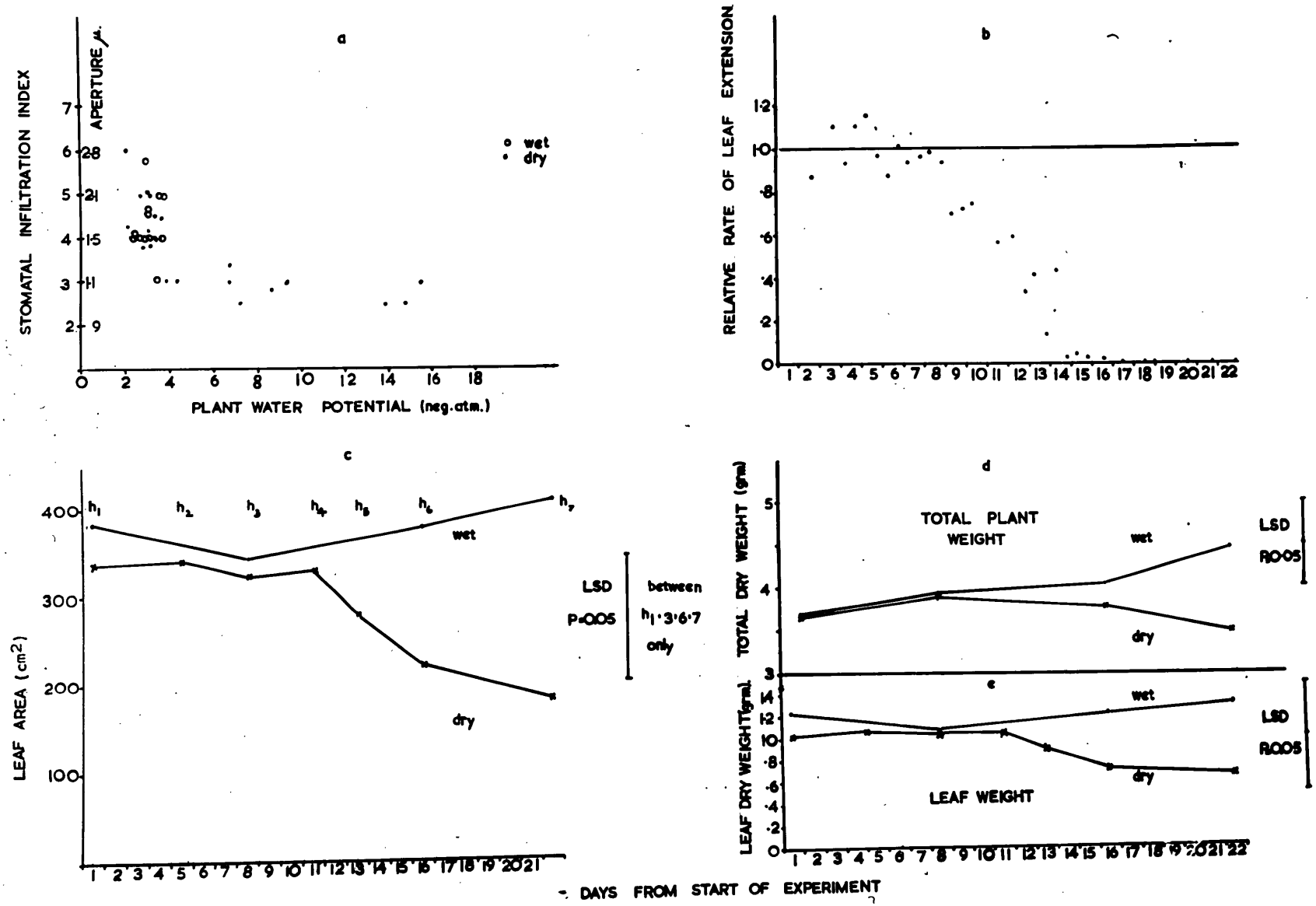


Fig.39. Experiment II Lolium. a) Stomatal infiltration as a function of plant water potential, b) relative leaf extension rate, c) leaf area, d) total plant weight in response to a drying cycle.

The values of $\bar{\tau}$ declined with drying, and from the first to the seventh day fell from -0.2 atm to -0.3 atm and then to -3 atm by the tenth day. $\bar{\tau}$ did not correspond to the values of τ in any particular horizon. The calculated $\bar{K}$ values fell from 10^{-2} cm/day on the first day to 10^{-3} cm/day on the seventh and finally to 10^{-7} cm/day on the twenty-first.

Experiment III. The greater length of the soil columns led to considerable differences in the water potential between the top and bottom of the soil columns. In figure 40 (a) τ for each harvest is shown. Only the data for the first and each alternate horizon are presented, in order to clarify the figure. The initial harvest, made after the columns had been allowed to drain for 24 hours, showed that horizon a was at -0.3 atm whilst 1 was about -0.01 atm. As the soil water was depleted by transpiration the upper horizons lost water before the lower and τ fell progressively with time from the top to bottom of the soil column. The $\bar{\tau}$ value calculated, fell with soil water depletion and reflected conditions about 40 cm from the soil surface. The differences between the top and bottom of the soil column were preserved even on day 13 when the top of the column was at about -18 atm and the

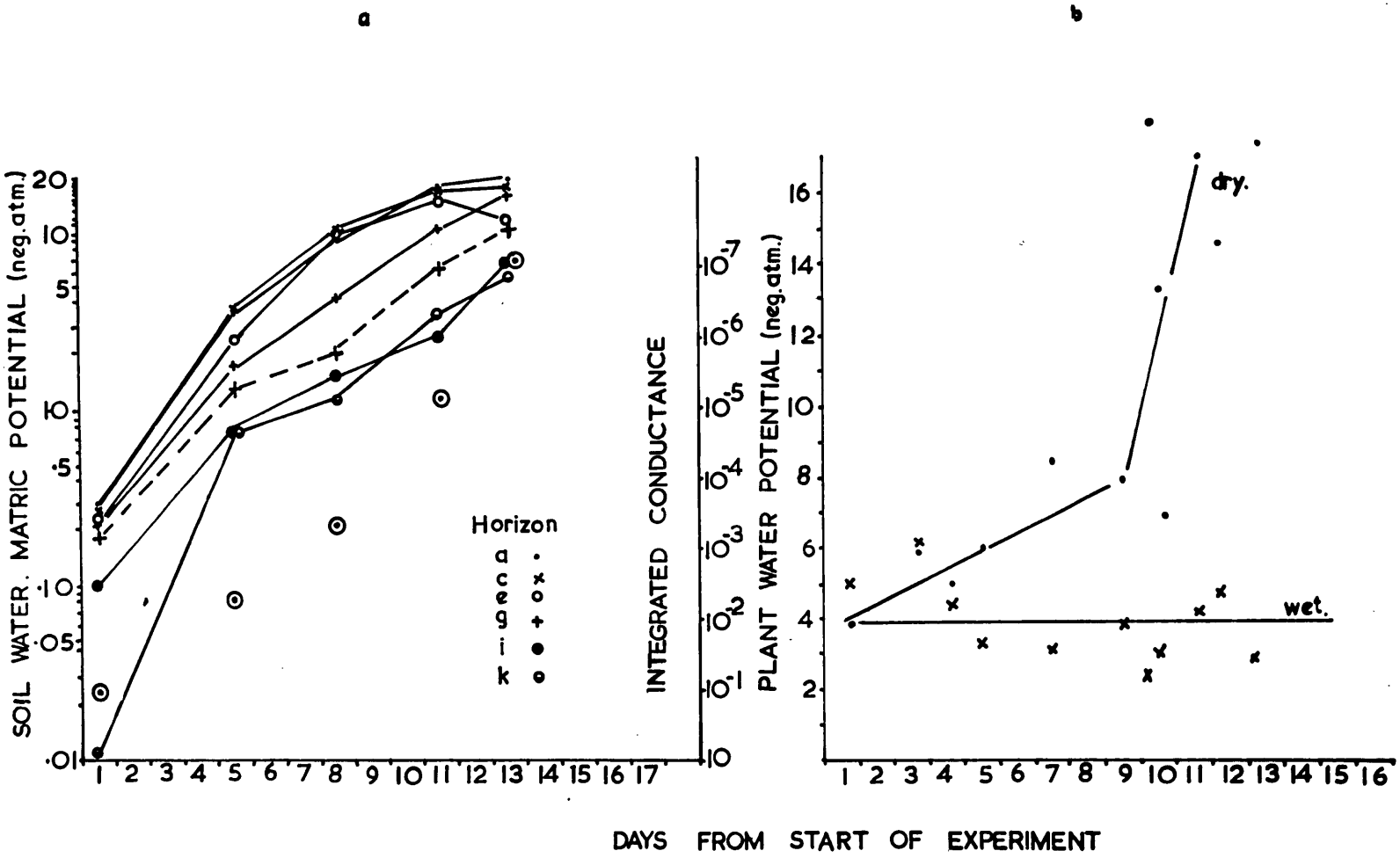


Fig. 40. Experiment III Lolium. (a) Soil water potential for each second horizon and integrated potential and conductance; (b) the plant water potential during the experiment.

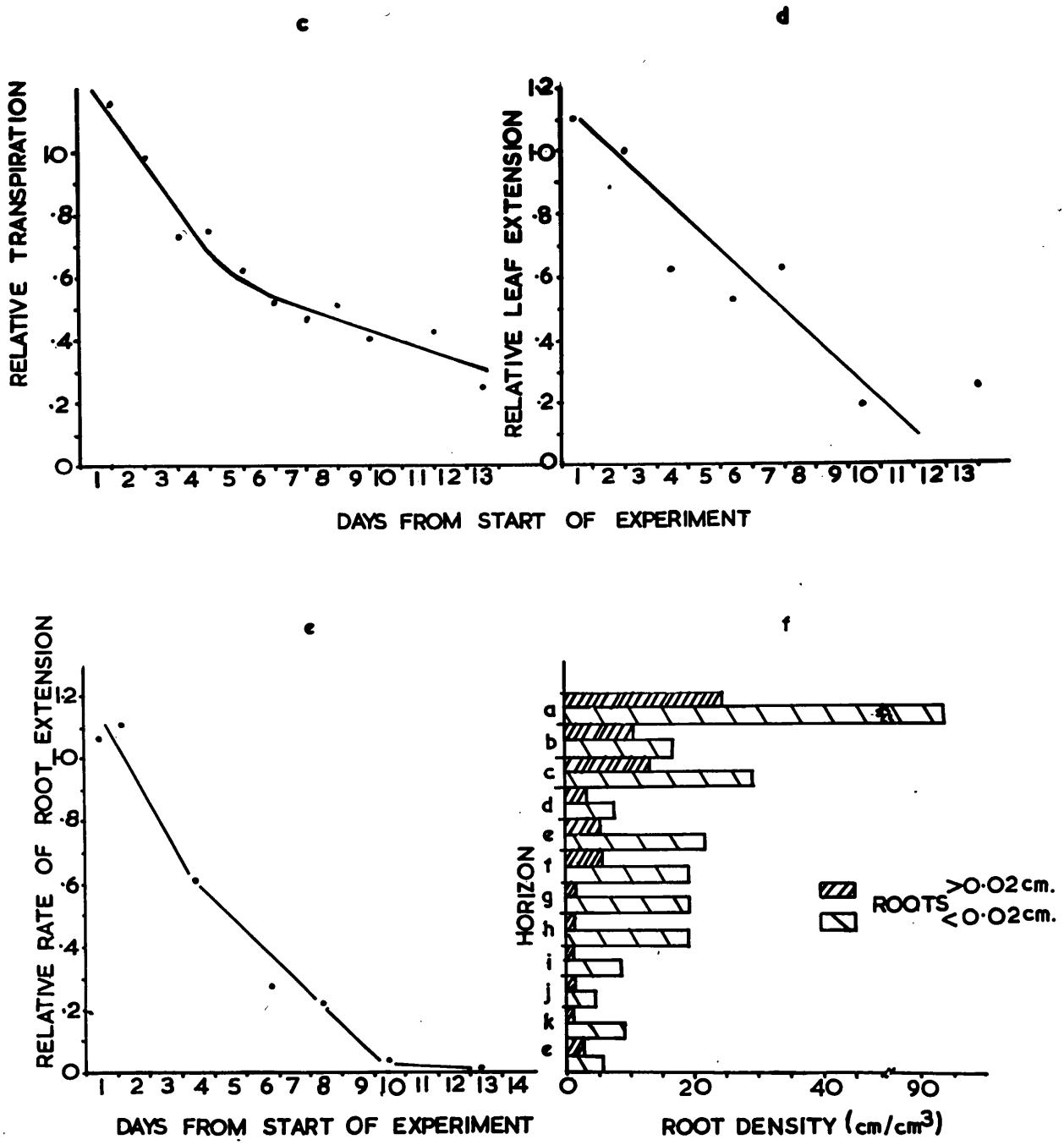


Fig. 40 cont. (c) relative transpiration; (d) relative leaf extension and (e) relative root extension rates during the experiment; (f) root density per horizon.

lower horizons at about -5 atm. $\bar{K}$ fell from about 1×10^{-1} cm/day on the first day to 10^{-7} cm/day on the 24th.

The root distribution in the soil for experiment I has been shown. That for experiment II, divided into large and small diameter roots, is given in figure 38 (b) and that for experiment III in figure 40 (f).

In experiment III the root density was very high in the a horizon compared to the other experiments due to accumulation of roots on the soil surface below the wax. In II the root density was high throughout the soil column, with a concentration in the bottom horizon which was due to the effects of restricted root growth. In III the root density decreased with the depth of the column, both for large and small roots. The distribution of roots in relation to the depletion of soil water suggests that uptake depends not only on the density of rooting but upon the length or resistance of the roots to water transport and also upon the depth of the soil horizon. The latter point will be referred to later.

b) Leaf water potential

In chapter 3 the values of ψ plant were found to depend not only upon τ but upon the transpiration rate.

In the present experiments no attempt was made to study the changes in ψ which could have arisen from variability in transpiration or as a result of interaction between transpiration and τ.

Experiment I. ψ plant was sampled every other day, usually during the afternoon and the results are shown in figure 37 (c). The water potential of the control and drying plants remained at about -3 atm throughout the course of the experiment and did not change with decreasing τ as a comparison of figure 37 (b)and (c) will show.

Experiment II. Samples for the determination of ψ plant were made at intervals during the course of the experiment and on several days in both the morning and afternoon. ψ plant (figure 38 (c)) remained at about -3 atm in both drying and control pots during the first eight days of the experiment and decreased to about -4 atm on the tenth and eleventh days. On the twelfth day ψ plant fell to about -8 atm and on days 14 to 19 decreased further whilst the control plants remained more or less constant. Comparison of ψ plant to τ in figure 38 (a) shows that ψ plant remained at the level of the controls whilst part of the root system was in soil above -0.5 atm, that is when τ was above -1 atm. When τ fell below -1 atm

the plants lost water and were wilted on day 13 when τ was about -7 atm in the a horizon and -3 atm in the f horizon. When ψplant was was about -17 atm water was still removed from the soil and τ continued to decline.

Experiment III. The changes in ψplant during the drying cycle are shown in figure 40 (b). ψplant remained at -4 atm in the experiment during the first 4 days and fell on the fifth to -6 atm and to -8 atm on the 7th day. Despite rather variable results on day 10, it may be seen that from the seventh day ψ plant declined to values of -17 atm in the dry treatment. Comparison with the soil water potential data for this experiment shows that ψplant fell from the level of the controls when τ fell below -1 atm and the top of the soil column was at -4 atm, although the bottom was at about -0.5 atm. Progressive decrease in τ was accompanied by a decline in ψ plant.

The experiments agree in major aspects. ψ plant was not affected, measurably, by reduction in τ until the entire root zone was below about -0.5 atm. In experiment I no change in ψplant was observed during the entire course of the experiment as the bottom of the root zone was above this potential. When τ fell below -0.8 atm ψplant was reduced and, because of the rapid changes in τ associated

with small decreases in soil water content within this region, fell rapidly to the level of wilting.

c) Transpiration.

In each experiment transpiration from the plants subjected to drying was compared to the control plants.

Experiment I. The mean relative transpiration rate per pot for the wet and dry treatments has been plotted as a function of time in figure 37 (d). The reduction in transpiration began in the dry treatment on the fifth or sixth day. The reduction in transpiration was not accompanied by changes in ψ plant or leaf water deficit. The transpiration rate fell when $\overline{\Upsilon}$ was below -0.2 atm, despite the fact that the lower horizons of the soil columns were still at a potential of about -0.1 atm.

Experiment II. The rate of transpiration of the dry treatment plants has been expressed relative to the control plants in figure 38 (d). Three phases may be distinguished. During the first 4 days the dry treatment plants tended to transpire more than the control plants, but the increase was not significant at P = 0.05. From the fifth to ninth day they showed reduced transpiration, about 10% decrease, which was significant after the eighth day. From day 9 a more rapid decline in transpiration was observed until that of the dry treatment

plants was only 20% of the control on the fifteenth day. After this stage a further slow reduction in the water loss was observed.

The initial increase in transpiration was found in other experiments and was possibly a response to improved aeration as the air filled pore space of the soil increased. The subsequent 10% reduction in transpiration occured when τ was below -0.3 atm in the upper half of the soil column but whilst the lower zones were above this potential. When τ in the entire root zone fell below -0.4 atm the transpiration was severely reduced and the final phase, after day 11, corresponds to the value of τ when the whole rooting zone was between -5 and -18 atm. The initial rise and fall in transpiration between the first and ninth day was not accompanied by measurable changes in ψ plant. but in the period of marked reduction in transpiration. ψ plant declined from -3 atm on days 10 and 11 until at the lowest ψ plant values of about -18 atm. transpiration was only 10% of the controls.

Experiment III. The relative transpiration, measured over 24 hours, is shown in figure 40 (c). For the first 2 days the transpiration was unaffected by soil drying but declined from 80% on the third day to 20% on

the thirteenth. The initial fall in transpiration took place as ψ descended below -0.4 atm although the bottom of the soil column was at -0.1 atm and the surface at -1 atm. The transpiration rate declined more or less regularly with decreasing ψ, without the phases which were observed in experiment II. In the longer columns the rapid change in water content and therefore ψ, are less marked than in smaller columns. As an example in 30 cm columns ψ changed from -0.3 to -3.0 atm in three days whilst in the larger columns the change took nine days and even after this time the basal horizons were still above -2 atm. The relative transpiration rates were similar in both experiments II and III at equivalent values of ψ; II tended to be slightly higher than III at a given water potential. The relative transpiration declined by 20% and 50% when ψ fell from more than -0.1 atm to -1 atm and -3 atm respectively. Comparison with the PEG data in section C shows that relative transpiration was reduced to 50% only when the osmotic potential of the solution was -9 atm. The possible reasons for this discrepancy will be discussed later.

The relation of transpiration to ψplant is similar to that found in the previous experiments. Transpiration fell by 10% before measurable changes in ψplant took place. As ψ plant declined from the level of

the controls transpiration was further reduced, and fell to 20% of the contro.. rate at -18 atm.

d) Stomatal activity.

The stomatal infiltration index was estimated in each experiment but only the data for experiment II are presented in figure 39 (d) where the infiltration index is plotted as a function of ψ plant. When ψ plant was about -3 atm, its maximum level, the index fluctuated probably due to the stomatal light response, but with decreasing ψ plant the infiltration index was reduced and the fluctuations ceased, due, it is believed, to the overriding influence of ψ plant on the stomatal aperture. This correlates with the observation that the main reduction in transpiration was associated with the initial decrease in ψ plant.

The lack of response of ψ plant to decreasing τ over a considerable range has been referred to although transpiration was reduced by quite small changes in the soil. From the stomatal response to decreasing ψ plant it is suggested that small changes in ψ plant induced reduction of the stomatal aperture and transpiration which operates against any further reduction in ψ plant. However as $\bar{K}$ falls below 10^{-4} cm/day and τ below about -0.4 atm the rate of water supply to the plant is restricted and stomatal

closure cannot compensate for the imbalance between uptake and loss and therefore ψ plant falls.

c) Leaf extension growth.

Experiment I. The rates of growth of the youngest leaves in the dry treatments are shown relative to the wet treatment in figure 37 (e). The leaf extension of the dry treatment plants was reduced, compared to the controls, after the fifth day and significantly so on day 8 and from day 10 on, (an analysis of variance was performed on the original data). The growth reduction was associated with a fall in τ from above -0.1 atm to about -0.2 atm, although horizon a was at about -2.5 atm and the bottom of the column at -0.05 atm. Further reduction in τ was accompanied by a progressive reduction in growth, although no changes in ψ plant were observed.

Experiment II. The relative rates of leaf extension are shown in figure 39 (b). During the first 8 days it was unaffected by decreasing τ but from day 9 to 15 the extension rate was progressively reduced and after this time no extension took place. Comparison with the changes in τ over this period (figure 38 (a)) shows that whilst τ was above -0,3 atm no growth reduction was observed. Below that level on the seventh day, to -3 atm on the

tenth day, growth fell to 20% and further decrease in τ from -3 to -7 atm reduced growth to zero.

Growth was reduced before ψ_s plant fell below the level of the controls on day 10. After this time growth and ψ_s plant fell rapidly and growth ceased when ψ_s plant was about -15 atm.

Experiment III. Reduction in the rate of leaf extension was observed (figure 40 (d)) and, in agreement with the previously described results, it first occured at about -0.4 atm τ and fell to 60% of the control level on day 4 when τ was lower than -0.8 atm. Growth was reduced to 20% with τ at -10 atm but with the lower horizons at -5 atm. A reduction in ψ_s plant below that of the controls reduced leaf extension but at -18 atm some growth was observed.

f) Root extension.

In experiment III the growth rates of the main roots were measured in both control and dry treatment plants and the relative rate is shown in figure 40 (e). With decreasing τ from -0.2 atm to -0.6 atm, reduction in growth was measured and as τ fell below -5 atm the root extension rate became zero, despite the fact that the lower roots were in wetter soil. Decreasing ψ_s plant was associated

with a reduction to 60% of the control growth rate on the fifth day. At ψ_p plant of -14 atm growth ceased.

Both leaf and root extension growth were reduced progressively by decreasing ψ_p plant and stopped at about -16 atm. Leaf growth did not stop in experiment III at these low values of ψ_p plant, possibly because the measurements were made over a 48 hour period. During the night ψ_p plant may have been somewhat raised by uptake of water from the wetter soil in the lower root zones, enabling growth to take place.

g) Leaf area.

In experiments I and III no significant changes were detected in the leaf areas of the dry treatment plants although a reduction was apparent in II. Low replication and the short growth period reduced the efficiency of the experiments.

Experiment II. The leaf area at each harvest is shown in figure 39 (c). The LSD refers only to the comparison at harvests 1, 3, 6 and 7. The control plants did not increase in leaf area during the period, suggesting that the plants had attained the "ceiling leaf area". The dry treatment plants leaf areas remained constant during the first eleven days but a considerable reduction

took place after that time when $\bar{\psi}$ had fallen below about -3 atm and ψ plant was reduced from the control level. The reduced leaf areas suggest that leaves were dying but the dead leaf weights were not separated from those of the bases in this experiment. In a similar experiment in 30 cm long tubes it was shown that the reduction in leaf area was due to an increase in the rate of leaf senescence. In table 27 the ratio of dead leaf to living leaf and the dry weights of stem and leaves are shown. Harvest 1 was taken at the start of the experiment, harvest 2 at the time when wilting was observed in the morning after a drying cycle (which was completed in 6 days) and harvest 3 seven days after harvest 2 at which the plants were restored to full soil water supply.

Table 27 Dead and living leaf weight and their ratio

Harvest		1	2	3
Control	living leaf	1.21	1.22	1.57
	dead leaf	0.22	0.31	0.55
	Ratio $\frac{\text{dead}}{\text{living}}$	0.16	0.26	0.35
Droughted	living		0.95	1.28
	dead	"	0.44	0.59
	Ratio		0.46	0.46

Living leaf. SE of means (5 replicates) $\pm$ 0.14 grm.
Dead leaf. SE of " " " " $\pm$ 0.17 grm.

From the data, in which no significance may be attached to the harvest x treatment interactions for living or dead leaf, it may be seen that a decrease in τ and ψplant to wilting causes a considerable rise in the dead leaves due to increased rates of senescence.

h) Dry weight

The data for mean total dry weight per treatment at various harvests during the drying cycle in experiment II, are shown in figure 39 (d). The differences were not significant but a trend is apparent towards a reduction in the dry weight with decreasing τ and ψplant. In common with the leaf area the total dry weight and leaf weight were reduced when τ and ψplant were reduced below -3 atm -6 atm respectively.

No calculations of relative growth rate, relative rate of leaf area increase, leaf area ratio or net assimilation rate have been presented as all were negative in the dry treatment due to loss of leaves below about -3 atm τ and -6 atm ψplant.

IV. Recovery from stress

Although the main burden of the studies has been on the effects of decreasing water potentials during a progressive drought, the plant response to a restoration

of high water potentials in the leaf and soil is of interest. In experiment II on the eighteenth day, after the plants had been wilted for 5 days, the droughted plants were rewatered. The extension rates of the youngest leaves were compared for the plants growing in soil columns returned entirely to saturation and for plants given half the daily transpiration demand either at the top or at the bottom of the soil column. The results are given in figure 41 where the growth rates are expressed relative to the rate of growth of the original control plants in the experiment. The fully watered plants showed very marked increase in the extension rates, up to 4 times those of the controls. The maximum increased growth rate was observed about 2 days after the plants were returned to high soil water potentials, although recovery was observed in less than 1 day. The increased extension growth continued for about one week. In the plants supplied with only limited amounts of water, leaf extension growth was restricted and fluctuations were observed due to the imbalance between the rate of water supply and loss, which led to fluctuating conditions within the plant. No consistent differences between the top or bottom watering were observed.

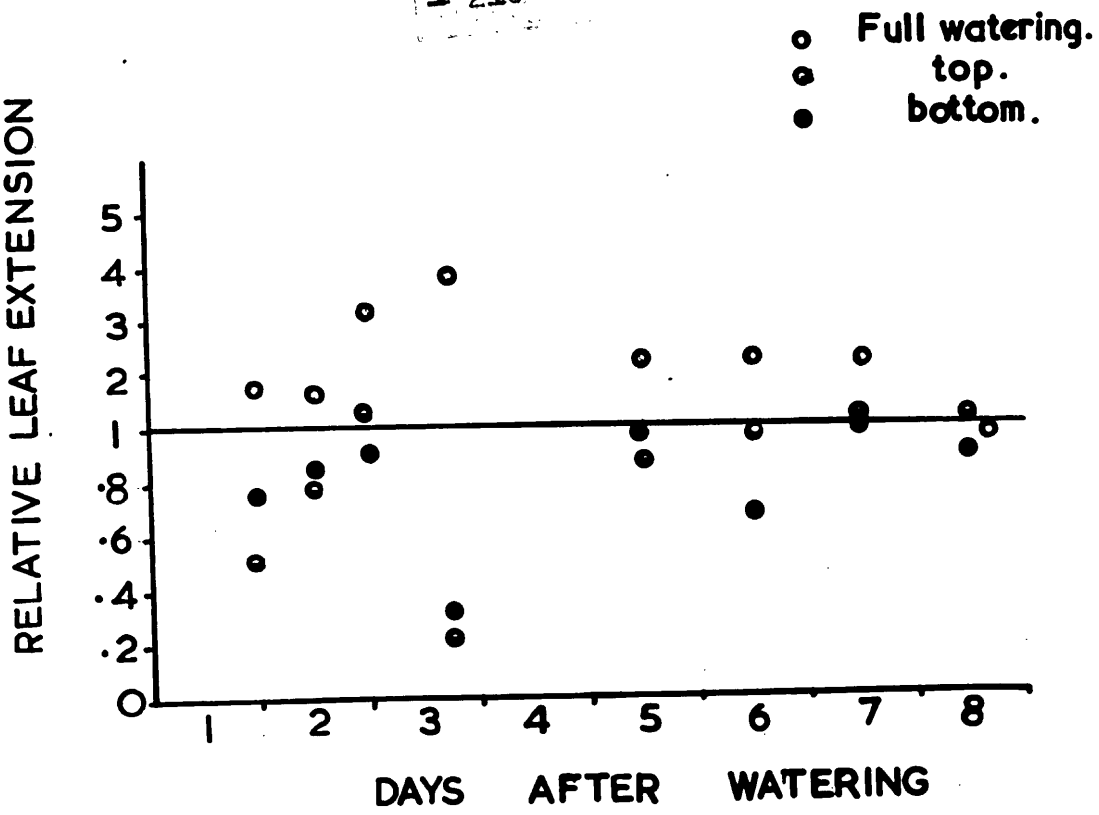


Fig. 41. Relative rate of leaf extension of Lolium upon rewatering after stress.

The transpiration rate per unit area of leaf of the rewatered plants rose within three days to the control levels but not above it.

C. Growth and Transpiration of Lolium perenne in Polyethylene Glycol Solutions

I. Materials and Methods

The Lolium perenne plants were grown in plastic boxes 13 x 13 x 8 cm deep painted black. The plants were germinated on rafts made of expanded polystyrene and nylon net, which floated on the surface of the solution. 50 seedlings were selected for uniformity from an initially higher population and supplied with 600 mls of Rothamsted solution which was replaced every 4 days. Growth was luxuriant. The plants were subjected to reduced water potentials during the experiment with the same solutions used in the Zea studies; details of preparation and the measurement of the osmotic potential are given in chapter 3.

The solutions were replaced at weekly intervals and the daily transpiration losses were replaced by an equal volume of nutrient solution in each treatment and the differences, arising from different rates of transpiration,

were made up with distilled water. Leaf extension growth was measured on 6 of the youngest leaves in each box, from the ligule of the second oldest leaf. Harvests were taken and the leaf area and dry weight of the principle parts were determined in the way described in section B. Transpiration was measured by pot weighing. For this purpose the surface of the container and raft were covered with wax after raising the level of solution in the container to within 1 cm of the top and pouring the molten wax around the stem bases. Solutions were added via an inlet hole. This effectively prevented evaporation from the box surface. The measurement of ψplant, stomatal infiltration index and leaf water deficits have been described.

II. The Experiments

Two experiments were performed, one to study the response of ψplant, transpiration and leaf extension growth to decreasing water potential at the root surface, and the other longer term experiment, to determine the response of growth in leaf area and dry weight to stress.

Experiment I. 15 boxes of Lolium plants were grown for 6 weeks in the greenhouse and at the start of the experiment the solutions were changed to -1.1, -3.3

-5.4 and -13 atm PEG solutions and controls. Each treatment was replicated hree times. Transpiration and leaf extension were measured for 4 days after the application of the solutions over periods of the day. The ψ plant values were determined after 13 hours in the solutions, during which period the plants had been four hours in the light and nine hours in darkness. ψ plant was again determined after 26 and 56 hours in the solutions.

Experiment II. 40 boxes of plants were grown as described. for 4 weeks from sowing. At the start of the experiment the boxes were selected for uniformity and allocated at random to a particular harvest, treatment and replicate. There were three harvests and the treatments were -0.4, -1.1 -3.3 -5.4 and -8.0 atm, each replicated three times. The initial harvest consisted of three boxes. The nutrient solution was replaced at the first harvest and renewed after 1 week, at harvest 2. Harvest 3 was taken after a further week. During the experiment ψ plant was determined in order that the relationship shown in the first experiment could be substantiated. In addition the transpiration was determined during the process of maintaining the solutions at the required concentration.

III. Results

Where possible the results of the two experiments will be considered together.

a) The effects of solution osmotic potential and leaf water potential.

In figure 42 (a) the results of determinations of ψ plant are shown as a function of the solution osmotic potential, π sol, for the various periods of determination. In the culture solution ψ plant was about -4 atm and did not differ between the time of determination or experiment. At -1.1 atm ψ plant remained unchanged but with further decrease in π sol fell to about -6.5 atm and -8 atm in the -3.3 and -5.4 atm solutions respectively. In the -8 atm solution ψ plant was lower than the solution potential and in the -13 atm solution was about or slightly higher than the solution. The two experiments are essentially in agreement.

b) Transpiration.

The effects of π sol on the rate of transpiration per unit leaf area, relative to the control plants is shown in figure 42 (b) for both experiments. Transpiration declined linearly with decreasing π sol. A reduction from -0.4 to -1.1 atm caused a fall in transpiration of about 5%, at -13 atm the reduction was about 70% Some

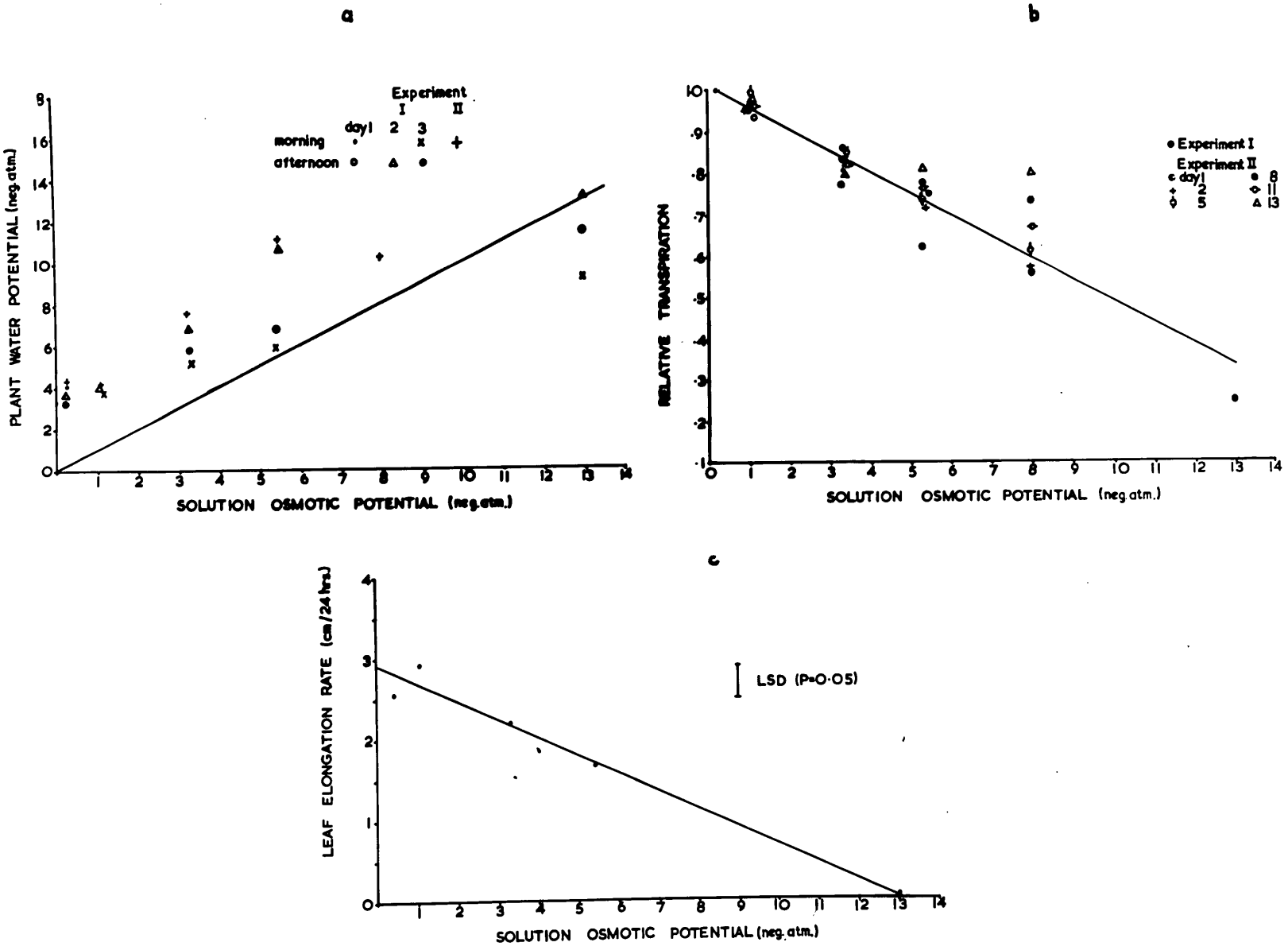


Fig. 42 a) Plant water potentials of Lolium b) relative transpiration rates c) the rate of leaf extension in relation to osmotic potential of the medium

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differences between days was observed and in experiment II the relative transpiration rate was somewhat higher at a given water potential after 13 days growth in the solutions although the effect was not significant.

Decreasing ψ plant in experiment I was associated with reduction in the rate of transpiration, as the data in figure 43 (a) show, for the period 56 hours after the application of PEG solutions. ψ plant and transpiration were measured over the same period. Transpiration fell with decreasing ψ plant from about 6 mg/dm^2/hr and -3.5 or -4 atm to 1 mg/dm^2/hr at -10.5 atm.

c) Stomatal aperture.

The infiltration index determinations are shown in figure 43 (b) in relation to ψ plant where the two were determined before and after the application of the PEG solutions. The stomatal aperture was reduced by decreasing leaf water potential, at about -4 atm the stomatal aperture depended upon the stomatal light response amongst other factors, but with decreasing ψ plant the stomatal aperture was reduced and at -6 atm the infiltration index was more or less minimal, no further reduction taking place with decreasing ψ plant.

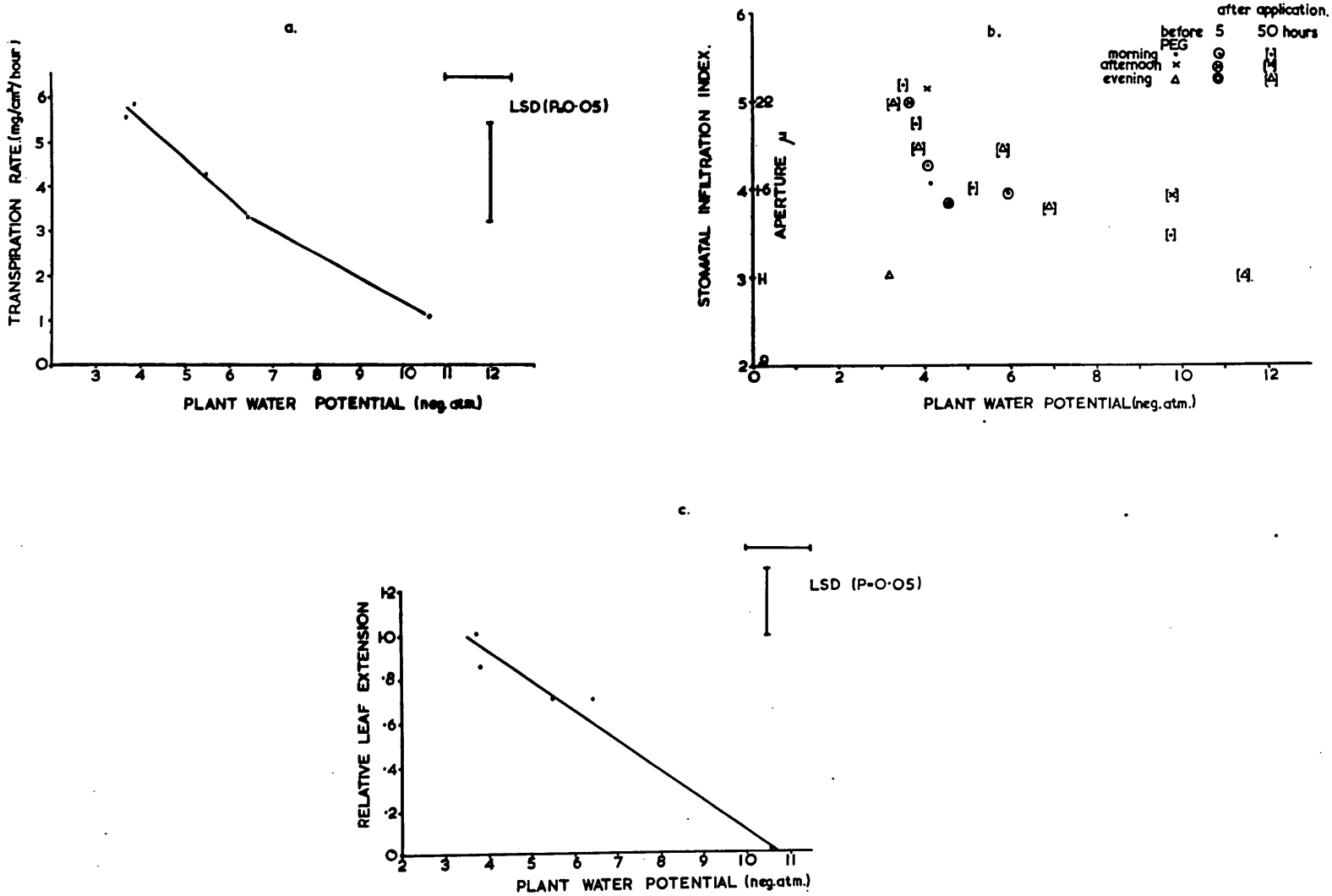


Fig. 43. (a) Transpiration rate; (b) stomatal infiltration index and (c) relative rate of leaf extension of Lolium in relation to plant water potential.

d) Leaf extension growth.

The rates of leaf extension in experiment I were reduced by increasing stress in the rooting medium, as figure 42 (c) shows. No significant effects were observed of the length of time that the plants were subjected to stress. Maximum growth occured at or above -1.1 atm π sol. Below this, further reduction in the potential of the solution was associated with decreased extension of the leaves. Growth ceased at -13 atm. The leaf extension in relation to ψ plant is shown in figure 43 (c) where both were determined over the same period. Maximum extension was observed at about -3.5 to -4.0 and growth ceased at -10.5 atm.

e) Leaf area and dry weight

The data for leaf area, dry weight, leaf area ratio and specific leaf area were subjected to analysis of variance after transformation to logarithms and the form of the analysis is shown in table 28, together with the levels of significance of the components of the analysis. The analyses were performed on the data for harvests 2 and 3 only, or, as in the case of relative growth rate etc., on the data for the periods between harvests 2 and 3. The relative growth rate, relative rate of leaf area

increase and net assimilation rate were calculated for each replicate and the statistics applied to these data.

Table 28 Analysis of variance and significance levels of growth.

Source	degrees of freedom	Dry weight	Leaf area	R_W	R_L	E	LAR	SPLA
Harvest (or period)	1	***	**	***	**	**	ns	ns
Treatment	4	***	**	*	*	*	ns	ns
Harvest x Treatment	4	ns	ns	ns	ns	ns	ns	ns
Residual	20							
Total	29							

Significance at P = 0.05, 0.01. 0.001 indicated by *, **, ***, respectively

ns = not significant at P = 0.05

Table 29 Growth indices under conditions of decreasing medium water potential

sol (neg.atm)	Dry weight (g) ≠	Leaf area (cm^2) ≠	Relative growth rate g/g/week §	Relative rate of leaf area increase $cm^2/cm^2/week$ §	Net assimilation rate $mg/dm^2/week$ §	Leaf area ratio cm^2/g. ≠	Specific leaf area cm^2/g. ≠
0.3	3.91	591	1.03	0.89	5.7	152	335
1.1	4.03	598	0.99	0.96	5.8	153	331
3.3	3.30	506	0.94	0.76	5.2	154	349
5.4	2.91	427	0.89	0.69	5.1	148	335
8.0	2.33	333	0.74	0.56	4.8	145	334
LSD P = 0.05	0.42	13.8	0.16	0.19	0.99	-	-

≠ means of harvests 2 and 3

§ means of periods harvest 1 to 2 and 2 to 3

* means of h

The results of the analyses show that no significant Harvest x Treatment interactions were observed and therefore the means of harvest 2 and 3 and those of the two periods of growth have been given in table 29. It should be noted that leaf area and total dry weight increased between each harvest but that relative growth rate, relative rate of leaf area increase and net assimilation rate were all greater between harvests 1 and 2 than between harvests 2 and 3. All the growth characteristics were reduced by decreasing solution water potential except leaf area ratio and the specific leaf area.

The apparent similarity of the plant response to treatment over the two weeks of growth in PEG solutions, despite very different meteorological conditions, is striking and will be discussed in another section.

The α index was calculated (see chapter 3). During the first period α was greater than 1 in all the treatments (table 30), suggesting that the plants were able to accumulate material in excess of that required for leaf area expansion in about the same proportions with all treatments. In the second period however α was only just 1 in all the treatments, indicating that the dry matter production was only just able to maintain leaf area expansion.

Table 30 Values of κ for the two harvest periods

π sol. (neg.atm.)	$h_1 - h_2$	$h_2 - h_3$
0.4	1.29	0.97
1.1	0.96	1.21
3.3	1.20	1.27
5.4	1.47	0.93
8.0	1.59	0.96

The greater values at -5.4 and -8.0 atm in the first period suggest that leaf area expansion was more seriously affected by water stress than assimilation but the data from the second period indicate that the plants had attained an equilibrium with respect to the balance between leaf area and dry weight growth in all solutions.

D. Leaf Water Potential, Osmotic Potential and Leaf Water Deficit

I. Introduction

During the course of measurements of ψ plant in experiments with Lolium plants grown in soils and solutions, the osmotic potential of the leaf, π plant, was also determined in many cases, as well as leaf water deficit. As in the section dealing with the water relations of

Zea mays (chapter 3 section D.) LWD will be considered in relation to the ψ plant and π plant values and also to the turgor potential of the tissue, P.

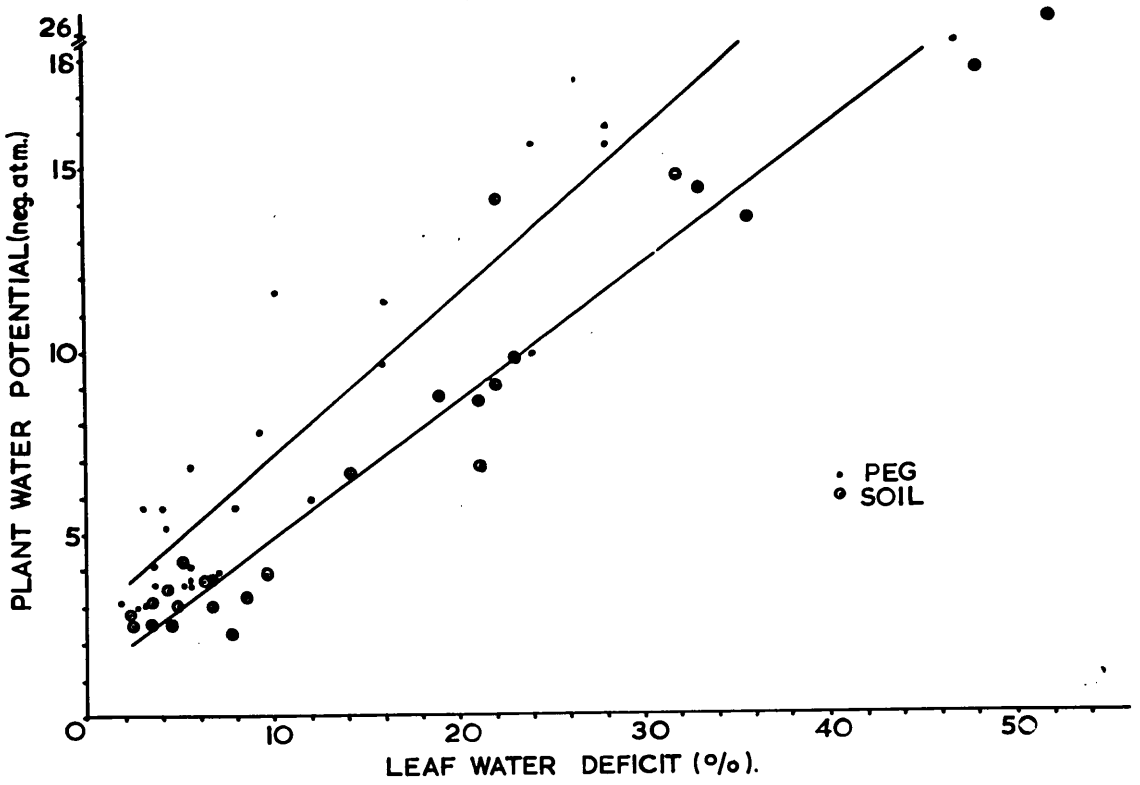
II. Results

a) Leaf water potential and leaf water deficit.

The data in figure 44 (a) were collected from PEG and soil grown plants and show the relation between the LWD and ψ plant. At the minimum LWD, about 2%, ψ plant was -3 atm. With increasing LWD ψ plant fell and at 50% LWD was about -28 atm, at this level death of the tissue was observed. The relation between ψ plant and LWD was linear over the range from 0 to 35% LWD and beyond although the scarcity of data does not allow rigorous conclusions to be drawn. The relationship between ψ plant and LWD was not the same for plants grown in soil and solutions but ψ plant was lower, at a given LWD, in the culture solution grown plants.

b) Osmotic potential and leaf water deficit (figure 44 (b).

At the minimum leaf water deficit of 2%, π plant was about -12 atm; as LWD rose so did π plant and at 30% was about -20 atm and at 50% -26 atm. The relationship was apparently linear and no distinction could be drawn



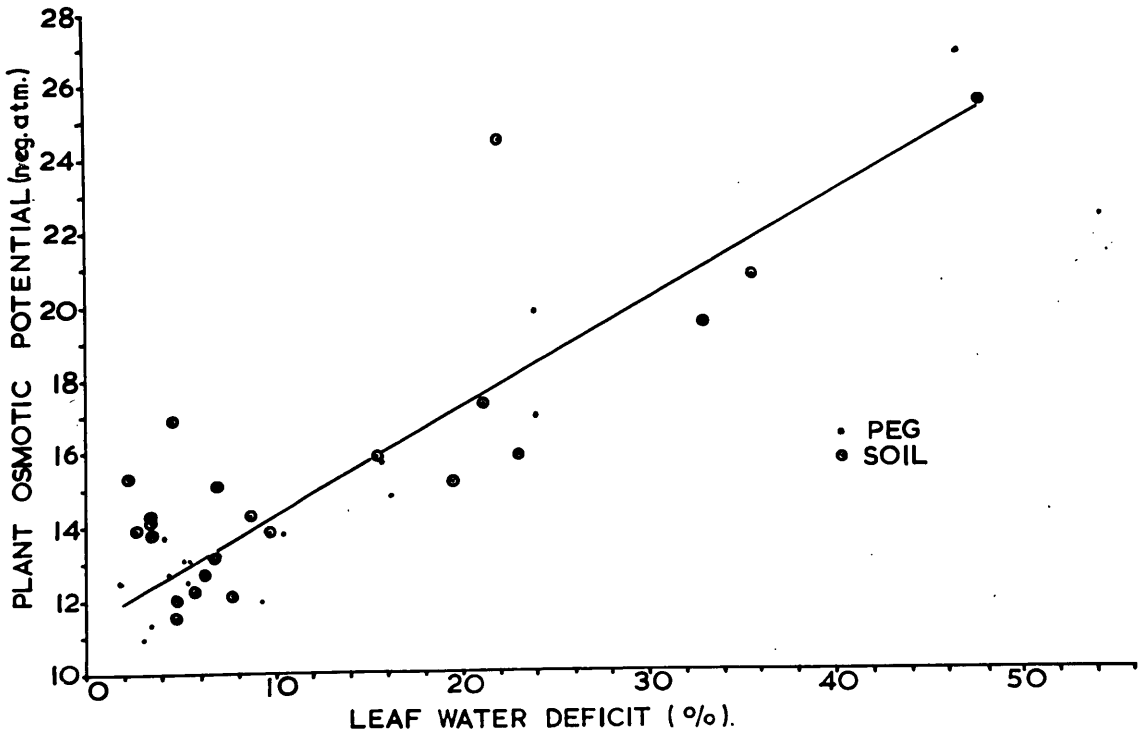


Fig. 44. The water potentials and osmotic potentials of Lolium leaves in relation to leaf water deficit.

between the soil and solution grown plants with respect to the osmotic potential at a given LWD.

c) Turgor potential.

The data for the soil experiment II and PEG experiment I have been examined more closely and the turgor potential calculated. The turgor potential is shown in figure 45 in relation to LWD. (The relative turgidity is also given i.e. 100 - LWD).

P was maximum at the highest water content, about 9 atm at 2% LWD for the PEG grown plants and about 12 atm for those grown in soil. P decreased more or less linearly with falling water content and at 20% LWD was about 8 atm in the soil and 3 atm in the PEG grown plants. P did not fall to zero in any determination and the lowest values of P were about 2 to 4 atm.

The leaf elongation of the soil grown plants ceased at 6 atm P and at 3.5 atm in those grown in PEG solutions. These values correspond to leaf water deficits of 30% and 18% respectively. The point of zero extension growth for both experiments is indicated in figure 45. by the arrows.

The effects of decreasing tissue volume on the osmotic potential have been examined and the product of

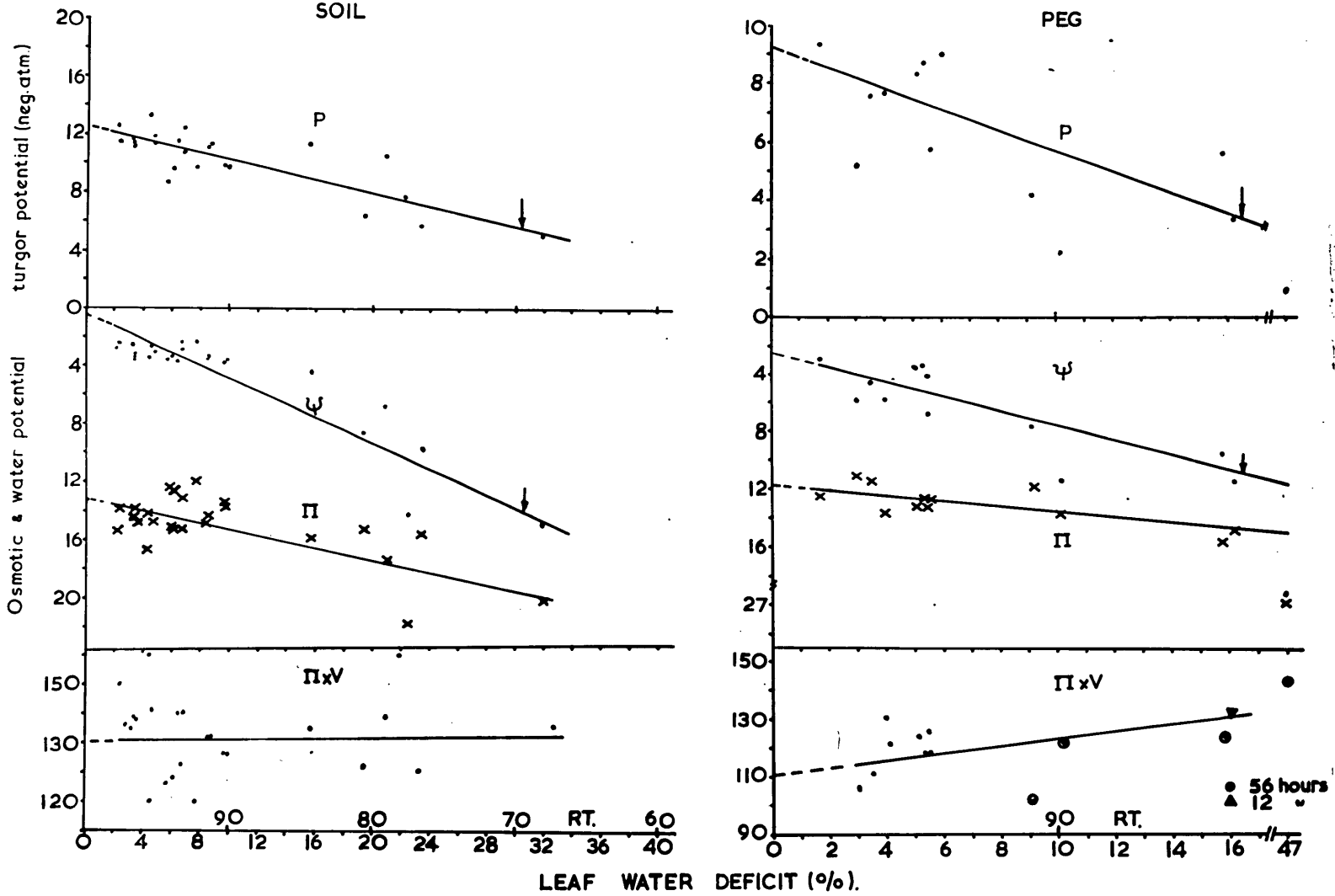


Fig. 45. Leaf water and osmotic potentials and turgor of Lolium leaves with leaf water deficit.

100 - LWD x π plant plotted in figure 45 (a) and (b). In the soil grown plants the product was more or less constant, showing that concentration of the cell contents was the sole factor governing osmotic potential. In PEG however, the increase in the product with decreasing water content suggests that some other factor than the concentration of the cell sap was responsible for the decrease in π plant. The effect was shown after 12 hours growth in the solutions of PEG showing that the change in the cell contents was rapid. The reasons for the decrease in cell osmotic potential has been considered in the case of Zea.

Chapter 5. Discussion.

The results presented in the preceeding sections on the water relations of Zea mays and Lolium perenne may be examined in two stages, firstly the processes by which water stress, (i.e. reduction in plant water potential) develops in the plant in relation to atmospheric and soil conditions, and secondly the influence of plant and turgor on growth processes. There is considerable interplay between different parts of the system and attempts will be made to show how the most important aspects interact.

I. Plant Water Balance

The water content and plant water potential depend on the rate of loss, i.e. transpiration, which is governed by the energy available for evaporation of water from the leaf and on the conditions regulating the uptake, namely the resistances to the supply of water at the site of evaporation and on the potential of the water supply (Gardner,1965; Slatyer and Gardner,1965). Following these authors, the rate of transpiration T (vol/unit area/unit time), is described by

$$T = \frac{e_{leaf} - e_{air}}{R_s + R_a} \qquad (1)$$

where e_{leaf} and e_{air} are the vapour pressure at the

evaporating surface and in the ambient air, and R_s and R_a the resistances to vapour diffusion offered by the stomata and the boundary layer of the leaf.

The rate at which water is supplied to the evaporating surfaces, Q, is given by

$$Q = \frac{\Upsilon \text{soil} - \Psi \text{plant}}{R \text{ soil} + R \text{ plant}} \qquad (2)$$

where Υ soil and Ψ plant are the water potentials in the soil and the leaf respectively and R soil and R plant are the resistances to water movement offered by the soil and plant.

In the work which has been described the energy requirements for transpiration have been ignored and transpiration, both actual and potential, has been measured. Potential transpiration has been estimated from plants kept under adequate water supply in the soil. However it is not possible to define the phase "adequate water supply" and this may be a hindrance in comparison of experiments performed on different occasions or under different conditions. Control plants have been kept at water potentials greater than -0.1 atm in soil and -0.3 atm in solutions, but the effects of capillary conductivity on water movement, even at much higher soil water potentials, and the influence of aeration

on the resistance of the root, should not be ignored.

Under these conditions where the plant water content is constant, Q equals T, stomatal resistance is at a minimum (given optimal light and carbon dioxide levels) and ψ plant remains constant. When T increases or ψ soil falls because of depletion of the soil moisture content, or R soil or R plant increase, Q lags behind T and the plant water content, ψ plant and turgor potential fall. The fall in ψ plant results in increased uptake and may also cause decreased transpiration as a result of stomatal closure. Q and T ultimately achieve a new balance, unless conditions are constantly changing, in which the water content and water potential of the plant may be lower (Van den Honert, 1948; Rawlins, 1963). Stocker (1960) presents much evidence supporting the view that most of the plants assimilatory functions are at an optimum at the highest water contents, that is under lowest stress. Although ψ plant does not necessarily control growth directly, the turgor and osmotic characteristics of the cell are closely related to ψ plant and it is in these that the controlling factors in growth are to be sought. In addition the effects of reduced water movement on nutrient uptake, (Hylmö, 1953) should not be ignored, nor the effects of stomatal response on carbon dioxide absorption, Moss (1963).

a) Factors affecting the plant water potential

Re-arranging equation (2) the controlling influences on ψ plant may be written

$$\psi \text{ plant} = Q\,(R \text{ soil} + R \text{ plant}) + \tau \text{soil} \quad (3)$$

and the effects of transpiration rate, soil and plant resistances and soil water potential on ψ plant will be considered. In the case of plants transpiring in soil, the form of the moisture characteristic for the sandy soil used in the experiments shows that small changes in soil water content below 10% by weight, results in large decreases in τ soil. The soil water conductivity also declines by several orders of magnitude from 10^{-2} to 10^{-6} cm/day between -0.2 and -5.0 atm respectively. The soil however does not dry uniformly but where root distribution is not restricted, drying begins near the surface and progressively deeper horizons are depleted during a drying cycle, as in the Lolium experiments in deep soil columns. The resulting soil water distribution leads to a gradient of potential down the profile and water at high potential is available to a decreasing part of the total root surface. In the Zea experiments the soil water depletion proceeded very uniformly as the root density was high throughout the pot and the plants were large in relation to the size of containers,

but in the Lolium experiments considerable differences in the distribution of soil water potential arose. In addition the fall in transpiration rates observed when only the upper 10 cm of soil were depleted of water, suggests that the resistance in the roots was sufficiently large (Wind, 1955) to induce changes in the uptake rate and some stomatal closure.

The change with depletion of soil water in the total resistance R soil + R plant could be calculated for the Lolium experiment through equation (2). The rate of transpiration per unit area of leaf was taken as equal to Q, as it would be except in the case of a plant undergoing dessication. The calculated resistances are therefore appropriate to the supply of water to unit area of leaf. This enabled different experiments to be compared independently of the type of growing conditions, pot surface etc., and leaf area index which has little significance for plants grown in containers. However the use of leaf area as a basis for determining Q is complicated by variable leaf distributions and exposure, which may lead to variations in the rates of transpiration from different leaves. In this calculation it was not clear which value of Ψ soil should be used since it varies with depth.

It was decided that the integrated soil water potential (described in Chapter 3) should be employed. The data for experiment II were used and the calculated values of R soil + R plant are given in table 31 for the duration of the experiment. The resistance of the plant alone was calculated from the data for plants grown in polyethylene glycol solutions. The plant resistances for both Lolium and Zea are shown in table 32 and 33 respectively. No calculations were made of R soil + R plant for Zea as the apparent inversion of the potential gradient, which will be considered later, precluded this treatment.

The R plant values of Zea in -0.3 atm solution were about 25 atm day/cm on the basis of leaf area. Although there was some variability between periods, no trend with time was apparent. The R plant values of Lolium were lower, about 15 atm day/cm, but because of the variable conditions under which the experiments were performed this may be regarded only as an indication of a difference in the behavior of species. In both species R plant remained more or less constant between -0.3 and -1.1 atm in PEG solutions, but fell somewhat with further decrease in π soil.

Table 31 Transpiration and water potential data dna calculated soil and plant total resistance for Iolium

Days from start of experiment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	19	20
Wet: Q (g/cm² leaf/day)	.107	.110	.074	.086	.052	.096	.105	.134	.140	.112	.114	.124	.06	.106	.085	.100	.06
Soil	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	.2
Plant	3.5	3.0	3.0	3.2	3.6	2.5	3.0	3.0	3.0	3.2	3.5	3.2	3.2	3.5	3.0	3.5	3.5
Soil − Plant	3.3	2.8	2.8	2.9	3.4	2.2	2.8	2.8	2.8	3.0	3.3	3.0	3.0	3.3	2.8	2.3	3.3
R (atm day / cm)	30.9	25.5	38.0	34.2	65.0	22.8	26.6	21.0	20.0	26.8	23.5	24.1	50.0	31.1	33.0	33.0	55.0
Dry: Q	.116	.113	.071	.077	.048	.089	.098	.130	.131	.069	.061	.084	.019	.030	.024	.01	.009
Soil	0.2	0.2	0.21	0.24	0.27	0.30	0.40	1.0	2.2	3.3	4.0	5.0	6.0	7.0	8.0	12.0	14.0
Plant	3.5	3.0	3.3	2.5	3.1	3.1	3.0	3.5	4.5	5.8	8.0	14.5	15.0	15.0	16.5	17.5	17.5
Soil − Plant	3.2	2.8	3.1	2.3	2.8	2.8	2.6	2.5	2.3	7.5	4.0	9.5	9.0	8.0	8.5	5.5	3.5
R	27.6	24.8	44.0	29.9	58.1	31.5	26.6	19.3	17.6	21.7	65.8	113	484	266	360	550	370

Table 32 Transpiration and water potentials in plant and solutions and calculated resistance of Lolium

Days after application of PEG

	1					3				
Φ (cm^2Leaf/day)	0.254	0.225	0.270	0.200	0.160	0.234	0.206	0.186	0.164	0.146
π sol	0.3	1.1	3.3	5.4	8.0	0.3	1.1	3.3	5.4	8.0
Ψ plant	3.6	3.7	5.1	5.9	9.6	4.0	4.3	5.5	6.9	8.5
$\Psi - \pi$	3.3	2.6	1.8	0.5	1.6	3.7	3.2	2.2	1.5	0.5
R plant (atm day/cm)	13.0	11.6	6.7	2.5	10.0	15.9	15.4	11.8	9.2	2.2

	5				10				
	0.260	0.260	0.186	0.225	0.295	0.290	0.240	0.200	0.187
π sol	0.3	1.1	3.3	5.4	0.3	1.1	3.3	5.4	8.0
Ψ plant	4.3	5.0	7.8	11.6	3.5	5.4	5.0	6.5	9.0
$\Psi - \pi$	4.0	3.9	4.5	2.2	3.2	4.3	1.7	1.1	1.0
R plant (atm day/cm)	16.5	15.0	24.2	10.0	10.8	14.9	7.1	5.5	5.4

Table 33 Transpiration and water potentials in plant and solutions and calculated resistances of *Zea* plants

Days after application of PEG

Zea experiment II

					III									
	1				1					8				
q	0.102	0.102	0.076	0.047	0.445	0.384	0.335	0.274	0.182	0.124	0.101	0.107	0.091	0.136
sol	0.3	1.1	3.3	7.0	0.3	1.1	3.3	5.4	8.0	0.3	1.1	3.3	5.4	8.0
plant	2.9	3.6	5.1	7.2	3.5	3.5	4.0	5.6	8.4	3.0	3.7	5.5	6.7	10.0
	2.6	2.5	1.8	0.2	3.2	2.4	0.7	0.2	0.4	2.7	2.6	2.2	1.3	2.0
R plant	25.5	24.6	23.6	4.3	7.2	6.3	2.1	0.7	2.2	21.8	25.8	20.6	14.3	14.7

					II					13				
					0.0[illegible]7	0.083	0.097	0.081	0.123	0.116	0.101	0.0725	0.098	0.130
					0.3	1.1	3.3	5.4	8.0	0.3	1.1	3.3	5.4	8.0
					3.5	4.0	5.0	7.5	9.0	3.5	4.0	5.0	7.5	9.0
					3.2	2.9	1.7	2.1	1.0	3.2	2.9	1.7	2.1	1.0
					33.0	35.5	17.2	26.0	8.2	27.6	28.6	23.5	21.7	7.7

A change in the resistance of root systems with increasing tension in the xylem has been shown by Mees and Weatherley (1957), Brouwer (1954) and Hylmö (1958) and also for intact plants by Macklon and Weatherley (1965). Kuiper (1963) suggests that increased "porosity" of the cell membranes results from a stress in the cell. From the experiments it is not possible to gain any idea of the sites of the major resistances in the plant. Kramer (1938, 1959) showed that roots offered the major resistance to flow, but Rawlins (1963) drew attention to the importance of the resistance in the leaf. The xylem resistance is generally regarded as small and due to viscous flow in the vessels, whereas in roots and leaves a more complex situation exists with flow partially dependent upon the changing properties of living cells. (Jensen and Taylor, 1961; Jensen, Taylor and Wiebe, 1961).

In Lolium growing in soil the calculated total resistance R soil + R plant, though variable, showed no trend from its mean of about 30 atm day/cm during the first 10 days of the experiment. During this time the integrated soil water potential fell from above -0.1 to -3 atm although the lower roots were in soil at ψ above

-0.5 atm. When the entire root system was in soil below -0.5 atm R soil + R plant rose rapidly. The total resistance when the plants were wilted and the soil was at about -15 atm, was about 500 atm day/cm. Because of the difficulty of obtaining values of integrated conductance and soil water potential it is hardly possible to relate the change in R soil + R plant to a critical value of $\tilde{\Gamma}$ or k; if the integrated values are taken they are -3 or -4 atm and 10^{-4} cm/day but if the value of the lowest horizon ($\tilde{\Gamma}$) is taken the changes in R soil + R plant take place at about -0.5 and 10^{-3} cm/day respectively. Gardner and Ehlig (1962 suggest that the critical values are about -0.6 atm $\tilde{\Gamma}$ and 5×10^{-3} cm/day k for plants growing in shallow soil.

If the resistance of the Lolium plants growing in soil is assumed to be similar to those grown in solutions then the resistance of the soil and plants would have been about equal in wet soil i.e. about 15 atm day/cm. However it is impossible to say if the soil grown plants are comparable to those grown in solutions. The uniform values of R soil + R plant over the first 10 days of the drying cycle suggest that although capillary conductivity was falling and R soil was presumably increasing, R plant

must have fallen (as in the PEG solutions) sufficiently to offset the rise in R soil. Below -0.5 atm τ soil the major resistance in the system is in the soil, changes in R plant is unable to compensate for the increase in R soil and the total resistance increases rapidly.

b) Gradients of water potential in the soil

Because of the very low capillary conductivity especially in semi dry soils, the water removed from the soil around the root is not replaced readily and this leads to a lower water potential at the root surface than in the bulk of the soil. The magnitude of the gradients of water potential expected to arise at the root surface has been expressed by Gardner (1960), through the equation

$$\tau_{rs} = \frac{q}{4\pi k}\left(\ln \frac{4Dt}{a^2} - \gamma\right) + \tau_{soil}$$

where q is the rate of water uptake per unit length of root ($cm^3/cm/day$) τ_{rs} and τ_{soil} the soil water potential at the root surface and in the bulk of the soil respectively; k the capillary conductivity and D the diffusibility (Chapter 2, D derived from $D = \frac{B}{\alpha^2}$); t the time (days); a the root radius (cm) and γ Euler's constant 0.5777

The formula was used to calculate τ_{rs} for given values of τ_{soil} in Zea experiment III. The rooting characteristics were measured by washing out the roots for the soil at the end of the experiment, separating the roots to two size classes, shown in table 34, and obtaining estimates of the length from the total dry weight and weights of measured lengths of root. Diameters were measured microscopically on subsamples.

Table 34 Rooting characteristics of Zea plants

Root Class Diameter (cm)	>0.1	<0.1	Total
Length (cm	1130	14,000	15,130
Mean diameter (cm)	0.2	0.04	0.1
Root density (cm/cm^3 soil)	0.30	3.8	4.1

Table 35 Calculation of root surface water potential

Transpiration rate	τ_{soil} (neg.atm)	k(cm/day)	$D(cm^2/day)$	$4\pi k$ (g/cm^2 day)	τ_{rs} (cm. water)
252 g/pot/day	300	2×10^{-3}	5.50	25.14×10^{-3}	0.0166 304.7
187	4000	5×10^{-6}	0.63	62.85×10^{-6}	0.0123 4,985
66	7000	7×10^{-7}	0.11	85.5×10^{-7}	0.00436 8,580
42	10000	4×10^{-7}	0.06	50×10^{-7}	0.00277 12,540
41	14000*	1×10^{-7}	0.03	12.6×10^{-7}	0.00272 18,210

* by extrapolation

ψ_{rs} was calculated for the situation that uptake of water proceeded through all the roots at an equal rate. Time was taken as 1 day, since the change in $\psi_{rs} - \psi_s$ is very insensitive to changes in t after 1 day. k and D were derived from Chapter 2 and where they had not been determined, as at the lower potentials, they were estimated by extrapolation. The results are given in table 35. They show that the gradient of water potential between the root surface and bulk of the soil is likely to be small when the soil water is at high potential and that the gradient increases as the soil becomes drier. Gardner (1960) calculated ψ_{rs} for a soil of similar texture and assumed values of q. At -10 atm ψ soil and equivalent values of q the values of ψ_{rs} from his calculations and from the present work were about -13 atm. It should be noted that the calculated ψ_{rs} values at -14 atm are lower than the lowest ψ plant values determined. In this situation it would be impossible for water to move into the root.

c) Potential gradients between the soil and the leaf

In the Lolium experiments ψ plant, even in the drying soils was always lower than the lowest values of ψ soil in that part of the soil volume exploited by the roots and Lolium and Zea both showed values of ψ plant

equal to or lower than the potential of PEG solutions, except that Zea in a -13 atm solution exhibited a higher ψ plant. In soil however, Zea showed an inversion of the gradient at potentials below some -6 atm τ soil, with ψ plant higher than τ soil, although the plants were transpiring. The possible causes of the inversion will now be considered. One explanation may be that the potentials were incorrectly measured; the errors of measuring ψ plant and τ soil have been considered in Chapters 1 and 2 respectively and they are not believed to be in error by more than about $\pm$ 0.5 atm (ψ plant) or $\pm$ 1 atm τ soil. However if transpiration took place from a particular site in the leaf, e.g. the leaf tip, the measured values of ψ plant could be in error for this site. Alternatively soil water potential might have been measured at unrepresentative sites in the soil. However in the Lolium experiments where considerable heterogeneity of soil water conditions existed, ψ plant was always lower than the lowest values of τ (found in the uppermost horizons). This suggests that soil water heterogeneity is an unlikely explanation for the negative gradients found with Zea. In addition in the Zea experiments under consideration, experiment III in soil, the

soil volume was small, the density of rooting high, and measurements made at top and bottom of the can showed that ζ was nearly uniform throughout the soil.

One possible explanation which should be considered in relation to the contradictory water potential measurements, is that of the effects of temperature gradients. Cowan (1965) has emphasised that if temperature gradients exist in the system, water potentials are inadequate to explain the movement of water because heat and water transport may be coupled, although he comments that the efficiency of the process is poor. In the experiments temperature conditions were not controlled and especially under the lights, temperature gradients of some magnitude may have arisen. However in this case the expected tendency would be to reduce flow from the roots to the leaves since the temperature gradient probably operates in the reverse direction to normal transpiration. It is possible that transpiration from the plant was occuring without corresponding uptake from the soil. Transpiration was about 15 g per pot during the period when transpiration was measured, in the driest treatment. The water content of the plants decreased by about 10%; the fresh weight of the plants was about 180 g of which 150 g was water. A 10% fall in water content could therefore account for

the observed transpiration.

It is, however, still necessary to explain how the plant water potential could be higher than the soil water potential at the start of the daily transpiration period since it might be expected that the plants should attain equilibrium with the soil. It could be that during the preceeding night the plants were able to absorb water from the air, particularly in Zea experiment I where the plants were placed in a humid atmosphere. Slatyer (1957) advanced this explanation to account for negative gradients in his experiments. An active uptake from the soil could also be involved in the increase of ψ plant above τ soil. In PEG solutions the gradient of potential was inverted only when the plants at high ψ plant were placed directly in PEG solutions and prevented from transpiring overnight. When plants were placed in PEG solutions, allowed to transpire and then placed in the dark no increase in ψ plant above τ soil was observed. Barrs (1966) has, however, shown that plants in PEG solutions of -6 atm could take water from the medium. He concluded that a metabolic link with uptake was involved. Obviously the explanations given to account for the reversed potential gradients between

the medium and the plant are without experimental foundation and a serious examination of this problem should be undertaken bearing in mind the points raised in the discussion.

d) The interrelation between plant water potential and transpiration

As Gardner and Slayter (1965 and Rawlins (1963) point out ψ plant over the normal range will have no measurable effect on the vapour pressure gradient between the leaf and the air, and if transpiration declines with ψ plant it might be through a change in the stomatal of some other resistance, although Shimshi (1963) and Rawlins (1963) have presented evidence that non-stomatal control of transpiration may be important. The determinations on Zea and Lolium suggest that as ψ plant falls the stomatal aperture is reduced and so is the transpiration rate. Similar results have been described by Ehlig and Gardner (1964). As ψ plant fell from about -3 to -4 atm the transpiration rate fell in a similar manner in both species, until at -7 atm transpiration was reduced by 50% and with a decrease in ψ plant to -10 atm transpiration was 10% of the control rate. Closure of the stomata was nearly complete by about -7 atm in both species and the further reduction in transpiration probably occured as a result

of the final small degree of closure, because as Bange (1953) showed, under condition of still air stomata may not control transpiration until almost closed.

e) Conclusions on water movement and water balance

The description of water movement from the soil through the plant to the air implied by equations 1 and 2 provides a framework which is inadequate for the interpretation of the results obtained when it is accepted that R plant is not a constant and that the model breaks down in the case of Zea growing in soils at low potentials where inversion of the potential gradient has been found.

II. Growth in relation to water balance

a) Growth and leaf water potential

Growth is likely to be most closely related to cell functions, including plant, turgor and osmotic potentials and only indirectly to transpiration or soil water potential.

The growth data for Lolium and Zea have been summarized in table 36, where various aspects have been expressed, for reduced water potentials, relative to the values at maximum water potential.

Table 36 Growth indications of Zea and Lolium in relation to solution and plant water potentials.

π sol (neg atm)		0.3	1.1	3.3	5.4	*(7.0)8.0	13.0	
		Lolium						
π plant (neg atm)	Z	3.5	4.0	5.0	7.0	8.0	10	- - -
	L	3.0	4.0	5.0	7.0	9	13	12 14 16
π plant (neg atm)	Z	7.5	8.5	9.5	11	11	11	
	L	12.0	13	14	14.5	15	16	
P (atm)	Z	4.0	4.0	3.5	3.0	3.0	1.0	
	L	10.0	9	9	7	6	3	
Transpiration	Z	100	90	75	50	30	10	- - -
	L	100	90	75	60	45	35	30 25 20
Leaf extension	Z	(100 (100	100 50	75 2	10 0	0 0	0 (PEG) 0 (Soil)	
	L	100	90	80	55	40	0.0	0.0
Leaf area	Z	100	83	65	47	20	-	harvest 3
	L	100	100.1	85	72	55	-	harvest 3
Dry weight	Z	100	81	58	40	20	-	" 3
	L	100	101	84	75	60	-	" 3
Relative Growth rate	Z	100	108	76	72	40	-	
	L	100	96	91	87	72	-	
Net assimilation rate	Z	(100 (100	98.5 75.0	86.0 80.0	77.5 55.0	52.0 25.5	second week in solution first " " "	
	L	100	102	91	90	77		

Leaf extension growth was at a maximum at ψ plant values of about -3 or -4 atm, in both Zea and Lolium extension was reduced to 50% at -4 atm in Zea and Lolium and ceased at -6 atm and -11 atm in Zea and Lolium respectively for plants grown in PEG solutions. Root extension growth in Lolium (see Chapter 4 figure 40 (e)) was related to ψ plant in a similar manner to leaf extension.

In soils reduction of extension growth was observed before ψ plant was found to change measureably. Zea, for example, showed reduced leaf growth when τ soil fell from -0.1 to -0.3 atm. Lolium leaves were reduced in extension before $\bar{\tau}$ or τ of the top horizon fell below -1 atm. Similar effects of small reductions in τ soil have been shown by Jarvis and Jarvis (1962) on the leaf extension of several species and by Rutter and Sands (1958) on leaf length and height of pine trees. In PEG solutions extension of both Lolium and Zea was reduced only when τ soil fell below - 1 atm. Gingrich and Russel (1957) showed that root growth of Zea was restricted by both osmotic and matric stress. The latter affected growth below -0.3 atm but the former stress not until about -1 atm, below which the response to stress was parallel.

It seems that extension is very sensitive to stress in soils, but the response of extension in solutions is less sensitive, suggesting that absorption of materials into the cell could maintain high turgor although ψ plant fell.

Other aspects of growth besides leaf and root extension were reduced by falling plant water potentials, turgor or osmotic potential, although it is difficult to relate growth processes to a critical value where measurement were made over a period of hours. Leaf area, dry weight and net assimilation rate were reduced, and it is particularly interesting that they were much more restricted at a given level of ψ plant (or related cell potential) for Zea than for Lolium; in fact Zea was about twice as sensitive to stress. Leaf area was reduced by leaf senescence in Zea with the sudden application of stress by PEG solutions, whereas Lolium was unaffected. However leaf senescence of Lolium plants, subjected to stress when growing in soils, increased rapidly. Probably age and physiological status of the plants greatly modifies the response to stress (see Gates; 1957). In Zea the leaf area apparently recovered after a period of severe stress and the relative rate of

leaf area increase rose with the length of time the stress treatment was applied, suggesting that recovery was taking place. In Lolium no changes in response were observed over the period of stress. Dry weight changes in the plant accompanied decreased ψ plant. Zea total dry weight was more severly reduced by a given level of stress than Lolium, although even in the severest stress treatment both species were able to maintain positive values of the relative growth rate and this was much less affected by low water potentials than leaf area or leaf extension growth.

The reduction in relative growth rate was caused, at least partially, by a decreased net assimilation rate with falling potential. E was reduced more in Zea than in Lolium, and in Zea there was also a trend towards a more severe reduction in the first week of stress than in the second, again indicating a capacity of the plants to recover from the stress conditions. Large differences in E were observed between the periods of growth due it is thought to meteorological conditions. Unfortunately the experiments were not performed at the same time, although the first weeks growth in PEG solutions of Lolium coincided with the second weeks growth of Zea. During this time, the reduction in E caused by

a -8 atm solution, was about 50% in Zea and 22% in Lolium.

From consideration of the resistances offered by the leaf to the exchange of water vapour and carbon dioxide with the air, it is generally accepted (Moss, 1963) that carbondioxide flux is governed by a relatively high mesophyll resistance in addition to that of the stomata and air which affect transpiration (equation 1). Therefore carbondioxide flux should be less reduced by stomatal closure than transpiration. Although the value of E is not a very accurate indicator of carbon dioxide movement, incorporating as it does the respiration of the tissue, it is of interest to compare the effects of increasing stress on transpiration and assimilation. From table 36, transpiration is seen to be more severely reduced by decreasing ψ_{plant} than E, suggesting the total resistance of the carbondioxide pathway increases less than that of the transpiration pathway.

It is important to note that leaf extension and E are not affected to the same extent by stress; the leaf extension of Zea ceases when E is reduced by slightly less than half, similarly in Lolium E is much less affected than leaf extension.

The other factors which might have affected the relative growth rate, the leaf area ratios, changed in Zea with time. Initially it fell with increased stress, but this was a temporary response for after 2 weeks the leaf area ratio was similar in all treatments. The leaf area ratio of Lolium was unaffected by stress. In neither species was the specific leaf area altered by reduced water potentials, but changes in the ratios of the principle parts accompanied leaf senescence in Zea at the lower potentials, although Lolium was not affected.

Although the growth characteristics described refer to solution grown plants, the relative growth rate, leaf area ratio and net assimilation rates of Lolium plants in soil also declined when subjected to stress below -1 atm. This was a result of an increased rate of leaf senescence compared to the solution grown plants.

Much of the evidence in the literature supports the observations. Gates (1955; 1957) showed that short periods of soil water stress reduced the relative growth rates and net assimilation rates. Schneider and Childers (1941), Ashton (1956) and Brix (1962) have all shown decreased rates of photosynthesis and increased respiration as a result of moisture stress.

In the preceeding studies ψ plant has been regarded as the most important potential, as it undoubtedly is in the movement of water through the plant. It is made up, however, of osmotic and turgor potential components which may have different and distinct effects on processes of growth. For the two species examined the relation between ψ plant, π plant and P is very different and it is also affected by the cultural conditions and age of the plants. In addition the cell characteristics show a variable relationship to leaf water deficit. It is therefore desirable to examine the relations of growth, stomatal behaviour etc. to these cellular components of ψ plant.

b) Growth, turgor and osmotic potentials

π plant was much higher in Zea than Lolium at minimum leaf water deficit, some -7 compared to -11 atm. Turgor was maximum at about 5 atm in Zea and 11 atm in Lolium and when it fell to the minimum levels of 0.5 atm in Zea and 3 atm in Lolium ψ plant was about -10 and -20 atm respectively. It must be recognized that estimates of P are subject to the errors of determining both ψ and π and it may be due to these errors that P is always positive. π plant is overestimated possibly due

to loss of water or dilute cell sap during freezing and thawing. For Zea, π plant was shown to be subject to only small errors but in Lolium was underestimated by about 2 atm.

The errors could, therefore, account for the observation that P never became zero, and should be taken into account when the relation between P and growth is considered.

In table 36 the approximate levels of the turgor and osmotic potentials have been included. It will be seen that leaf extension growth is maximum when P is also at a maximum and ceases when P is about 3 atm in both species, although ψ plant is very different in the two cases. The fact that extension growth ceased when P was greater than zero may be due to the error in estimating P or possibly to changes in the elastic properties of the cell walls such as Gardner and Ehlig (1965) have discussed. It may be that a minimum turgor is required to keep the cell walls extended; once P falls below this, further reduction in P has no effect on growth because the wall has attained a minimum "size" e.g. closest packing of the cellulose micellae (Frey Wyssling, 1952), although ψ could still decrease if plasmolysis occurred.

The changes in stomatal aperture cannot be attributed to either change in ψ plant or P from the data presented. However from table 32 it will be noticed that over a two week period in solutions, transpiration rates at -8 atm were about the same as the control, suggesting that stomatal opening had occurred. This is probably related to the observation that the osmotic potential of the tissue in Zea fell by more than could be accounted for by change in the water content. The decrease in potential may be explained as a result of uptake, by the plant, of osmotically active substances from the medium. Such a process would enable P to be maintained whilst ψ plant was lowered. The changes in leaf area and transpiration of Zea with the length of time that the plants were subjected to stress suggests that turgor is the major factor controlling leaf area growth and stomatal aperture, (Bernstein, 1961). If this is so then it would be of interest to compare the response of extension growth and assimilation to different levels of P, ψ plant and π plant which could be attained by supplying the plant with ions or low molecular weight PEG solutions.

The net assimilation rate was reduced by decreasing

P and π plant (table 36) but Lolium was less affected than Zea; at about 5 atm P, E was reduced by about 30% in Lolium and at 3 atm P by 50% in Zea.

The comparison of E and leaf extension serve to show that the relation of assimilation of carbon dioxide to water potential is very different from that of leaf (and cell) extension. The latter process is more sensitive to stress than the former and depends on turgor whereas assimilation seems to depend on the change in osmotic potential of the tissue. From table 36 it may be seen that a reduction in π plant of about 60% in Zea and 30% in Lolium is associated with a reduction in E of about 50% and 30% respectively.

The experiments which were performed allowed no comparisons to be made between species with respect to the components of the net assimilation rate, that is photosynthesis or respiration. It would be of the greatest interest to relate these to the cell characteristics which have been discussed.

From the complexity of cell organization and metabolic activities it is suspected that factors other than osmotic potential would directly affect assimilation. It is however essential that the effects of turgor should be separated from the osmotic potential changes in the cell suffering water stress.

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