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STRESS METABOLISM

Water stress, growth, and osmotic adjustment

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Many plant processes are affected by mild water stress, with cell growth probably the most sensitive. Except for turgor-mediated processes, the physicochemical basis for the transduction of small changes in water status into alterations in metabolism remains obscure. Turgor pressure is assigned a critical role in cell growth: the physical force needed to sustain enlargement. Simple physical models relating growth to turgor are conceptually useful in examining effects of water stress but can be misleading because metabolic and regulatory responses may be marked and vary temporally.

Osmotic adjustment has long been known as a means by which higher plants adapt to salinity, with much of the cell osmotica being ionic and accumulated from the medium. Though not generally recognized, osmotic adjustment also appears to be an important mechanism for adaptation to water-limiting conditions, even in mesophytic plants. In this case much of the osmotica might possibly be internally generated. Recent field data on seasonal and diurnal adjustment and vertical water-potential gradients in plant canopies are discussed relative to growth and water-potential components.

Introduction – a question of transduction

Rarely are terrestrial plants growing in a natural environment free from water stress for a period of more than a few days. Even in the humid tropics, the plant may undergo brief stress due to dynamic changes in the energy environment (Coster 1927). In the more arid areas, water deficit is a ubiquitous problem for most species. How plants are affected by water shortage and what mechanisms enable them nevertheless to survive or even thrive are problems of prime significance in plant physiology and with immediate practical implications.

This paper covers two distinct but related aspects of the physiology of plants under water-limiting conditions. The first part deals very briefly with the better known effects of mild to moderate water stress on physiological and metabolic processes, with particular attention to one of the most sensitive, growth by cell enlargement. That part also includes an examination of the possible physicochemical causes for these effects of water stress. The second part considers osmoregulation or osmotic adjustment as a mechanism for coping with water shortage and discusses recent field data illustrating this apparently widespread phenomenon and its importance to plant performance.

Having evolved in a medium of water, life of all forms, including plants, is inextricably dependent on water for function and survival. The essential roles of water in the plant are endless, ranging from being reactants, to serving as medium for the ionization of metabolites

and stabilization of biomembranes, to being the inflating agent in maintaining structure rigidity. In fact, any attempt to elaborate on the functions of water in the plant can only understate the case. Yet, in spite of all that is known about the functions of water, the physicochemical bases for most of the effects of deficiencies of water on plants are little understood. The difficulty lies in the fact that a removal of only 10 or 15% of the water held in the tissue at full turgor can markedly affect metabolism. In mesophytic crop plants, a water loss of 10–15% may correspond to a lowering of water potential (Ψ) of only about 0.6 MPa (6 bar). Thus, the challenge is not so much in knowing which plant processes require water, but in finding reactions in the plant that provide rational explanations of the sensitivity to such small changes in water status. The problem may be termed a problem of transduction: How are small changes in plant water status transduced physicochemically into changes in metabolism?

A loss of water from tissue has the following direct effects: (*a*) reduction in the chemical potential or activity of water; (*b*) concentration of macromolecules and of solutes of low molecular weights; (*c*) changes in spatial relations in membranes and organelles through the reduction in volume; and (*d*) reduction of hydrostatic pressure (ψ_p) inside the cells. For mild water stress, effect (*a*), a reduction in chemical activity, should be insignificant because lowering Ψ by 0.5 and 1 MPa corresponds respectively to reductions in chemical activity of water of only some 0.4% and 0.7%. Effect (*b*), the concentration of cell contents, may play a role, but probably only if the biochemical reactions involved are catalysed by allosteric enzymes extremely sensitive to small changes in the concentration of specific effectors. It is still harder to visualize a significant role for effect (*c*), the effect of volume changes, especially in view of the known nonstatic nature of the membranes and organelles and the volume and spatial fluctuations occurring independent of changes in tissue water status. In contrast, effect (*d*), a reduction in turgor (ψ_p), has been shown to affect directly crucial physiological processes, as will be elaborated on below. These effects, with reference to available data, are discussed more extensively elsewhere (Hsiao 1973). The conclusion drawn was that for mild to moderate water stress, effect (*a*) is least likely to be a mechanism of transduction, whereas effect (*d*) can be considered a proven mechanism.

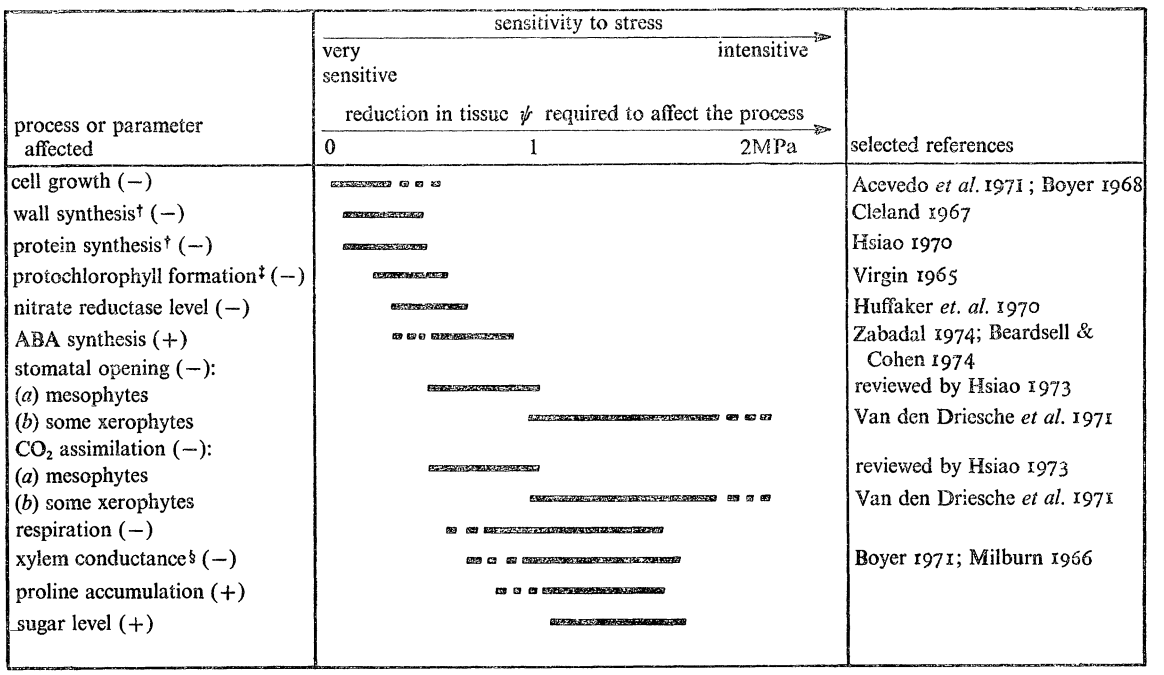
In addition to the effects mentioned, there are speculations on the possibility that a loss of cell water might somehow disturb water of hydration or a hypothetical special water structure adjacent to macromolecules which is said to be necessary for maintaining normal metabolism. These ideas seem too far-fetched, especially for the water stress levels normally encountered in mesophytes. The data bearing on this point, much of them indirect, have been evaluated by Hsiao (1973).

Changes effected by water stress

Regardless of the transduction mechanism, what are some of the plant processes readily affected by water stress? Indications are that almost any plant constituent and process can be altered if the water stress is severe enough and lasts long enough. In assessing the overall effect of stress on a plant and in attempting to understand the physical and metabolic mechanisms underlying the plant responses, their interactions, and causal relations, the time sequence of events is significant. In most cases the stress develops slowly relative to the speed of molecular events. Processes or responses occurring first (and therefore at the highest tissue Ψ) can be considered most sensitive to stress. And since all organisms are highly integrated in their physiology and metabolism, the primary effects of water stress should give rise to secondary

and tertiary effects. Knowing the time sequence of events provides insight into which are direct and which indirect, and which are likely to be the causes, and which the effects.

Because of the importance in the interpretation of plant responses to stress, a comparison of the sensitivities to stress of some selected parameters was made earlier (Hsiao 1973) from an extensive review of the literature. With some updating, this comparison is presented as figure 1. Such a comparison is rather risky since it relies on data from diverse studies employing different species and methods, often involving inexact estimates of tissue water status. Thus some details in the figure may be questionable, although the overall patterns should be generally correct. The figure also does not include many well known changes that have appeared in the



†fast growing tissue
‡etiolated leaves
§should depend on xylem dimension

FIGURE 1. Generalized sensitivity to water stress of plant processes or parameters (based on Hsiao, 1973). Length of the horizontal lines represents the range of stress levels within which a process becomes first affected. Dashed lines signify deductions based on more tenuous data. The reduction in tissue Ψ is in comparison to Ψ of well-watered plants under mild evaporative demand. In the left column, (+) indicates that water stress causes an increase in the process or parameter and (−) signifies a decrease.

literature. One important reason is that there were no quantitative indicators of the severity of the water stress employed. Another reason for excluding some data is that the stress lasted too long before the altered parameters were measured. Under that condition the changes may be too far removed from the early effects of stress and be more a reflection of general modulation of metabolism under adversity.

Reductions in Ψ are used in figure 1 to describe the degree of water stress because Ψ is the indicator commonly given in such studies. All the same, although Ψ is all-important in the consideration of water transport, it is not necessarily a good indicator of water stress from the viewpoint of plant responses. As discussed below, ψ_p is perhaps a much better indicator, especially in dealing with turgor-dependent processes such as growth. Relative water content

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(RWC) also may be better, although a high noise-to-signal ratio restricts its effectiveness for indicating mild stress (Hsiao 1973). It should be noted that in using Ψ reduction as an indicator, one implicitly assumes a virtual absence of osmotic adjustment in response to the stress (see below).

Figure 1 depicts cell growth as the most sensitive process. Growth, defined here as irreversible cell enlargement, has long been recognized as being easily retarded by water stress. Recent data greatly strengthened and expanded on those early findings and focused attention again on this stress effect. In many cases any reduction in tissue Ψ reduced growth, completely stopping it when Ψ was lowered 0.3 or 0.4 MPa (Boyer 1968; Acevedo, Hsiao & Henderson 1971). The primary effect of stress on growth appears to be physical, as several lines of evidence indicate (Hsiao & Acevedo 1974). Turgor pressure is a prerequisite for cell enlargement; this fact is discussed in some detail later herein. Because growth is extremely sensitive to water stress, many of the alterations in metabolism associated with stress may be indirect results of the reduced growth.

Closely related to expansive growth is cell division. A review (Hsiao 1973) of recent data concluded that if stress is prolonged, cell division in some cases can be as sensitive to stress as is enlargement. This sensitivity is possibly an indirect effect of stress, restricting meristematic cells from enlarging to the size minimal for the commencement of division. Though division *per se* does not add to tissue size, it contributes to the number of enlarging cells in an organ and thus to the overall enlargement of the organ. Increments in total DNA in an organ are restricted by stress concurrent with the inhibition of cell division (Kirkham, Gardner & Gerloff 1972; Meyer & Boyer 1972), as may be expected.

Next in sensitivity to stress after cell growth are the synthesis of cell wall and of protein in rapidly growing tissue (figure 1). As inferred from changes in polyribosomes, the stress effect on protein synthesis occurs at about the time a difference in Ψ between the stressed and control tissue becomes detectable and within a fraction of an hour after the imposition of stress treatment (Hsiao 1970). Whether wall synthesis and protein synthesis are also sensitive to stress in fully grown tissue is not clear and remains to be investigated. Possibly the high sensitivity of both processes in rapidly growing tissue is connected with the extreme sensitivity of expansive growth to stress. Presumably, once growth is reduced, feedback controls may operate to minimize the accumulation of wall material and of protein, basic building blocks of the cell.

A reduction in tissue Ψ of a few tenths of MPa also reduces light-induced chlorophyll accumulation in etiolated or greening leaves (Alberte, Fiscus & Naylor 1975; Duysen & Freeman 1974). The reduction is apparently caused by an inhibition of protochlorophyll formation (figure 1), since the conversion of protochlorophyll to chlorophyll is rather insensitive to stress (Virgin 1965). In contrast to greening leaves with developing chloroplasts, in green leaves with more mature chloroplasts the formation of chlorophyll does not appear to be affected by mild stress (Alberte *et al.* 1975; Hsiao 1973).

A small water deficit also reduces levels of nitrate reductase (figure 1) and phenylalanine ammonia-lyase (Bardzik, Marsh & Havis 1971). Both of these enzymes have a short half-life *in vivo*. Bardzik *et al.* have pointed out that the reduced enzyme levels can be caused by suppressed synthesis under water stress while degradation continues. The high sensitivity of nitrate reductase level to mild stress observed in fully expanded leaves (Huffaker, Radin, Kleinkopf & Cox 1970) suggests the intriguing possibility that protein synthesis in non-growing tissue is also very susceptible to stress, as in rapidly growing tissue, and points to the need of further investigation.

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Levels of various other enzymes (Todd 1972), including some in the photosynthetic pathway (Hsiao 1973), are not easily reduced by mild stress. Levels of still other enzymes, such as the hydrolytic enzymes ribonuclease and amylase, have been observed to rise markedly with a moderate stress lasting many hours. The functional significance of those increases remains obscure (Hsiao 1973).

Abscisic acid (ABA) follows nitrate reductase in sensitivity to water stress (figure 1). The change in this case, however, is a dramatic increase under stress instead of the reductions observed for the more sensitive parameters listed in figure 1. The increase in tissue ABA content can be many-fold (Wright 1969) and arises apparently from *de novo* synthesis (Milborrow & Noddle 1970). The ABA level in stressed tissue returns readily to the prestress level after the plant again receives water (see, for example, Beardsell *et al.* 1974). Some other growth regulators are also affected by water stress (Hsiao 1973). Additional information on growth regulators and their postulated interactions and significance for plant adaptation to stress are discussed by Vaadia (this volume).

The general inhibition of stomata opening and photosynthesis by water stress has been long established. Much recent data, reviewed by Hsiao (1973), show that stomata commonly are not affected until leaf Ψ drops to a threshold level which differs among species and probably also depends on growing conditions and the past history of the plant with respect to water stress (Brown 1974). The extreme differences are found between some drought resistant species and mesophytic crop plants, as indicated in figure 1. In many cases, the inhibition of CO_2 assimilation by water stress sets in at about the same Ψ level as the threshold for the effect on stomata. Though these parallel behaviours of stomata and CO_2 assimilation are indicative of the importance of stomatal conductance for the transport of CO_2 into leaves for photosynthesis, they do not rule out the possibility of concurrent effects of stress on the biochemical mechanism of assimilation. In fact, considerable data have accumulated indicating inhibitory effects on the chloroplasts or its components. These effects, along with more details on photosynthesis as affected by stress, are elaborated on by Boyer (this volume).

The current intense interest in stress-induced ABA accumulation stems in part from the apparent role of ABA in stomatal modulation. The potency, rapidity, and ready reversibility of the action of ABA in causing stomatal closure would make it a good modulator (Raschke, this volume). However, there remains the question (Hsiao 1973) of whether ABA could accumulate rapidly enough and in sufficient quantity to account for the fast stomatal closure effected by stress in some plants. After a stressed plant is rewatered, stomatal opening often recovers only slowly (several days) in spite of a rapid and total recovery in leaf water status (Fischer, Hsiao & Hagan 1970). Earlier it was speculated that the basis for the after-effect of stress may be a persistence of the elevated level of ABA (Wright & Hiron 1969). Studies published in the last two years, however, cast serious doubts on that since, after rewatering, leaf ABA content returned to normal considerably before complete stomatal reopening (see, for example, Beardsell *et al.* 1974).

Most effects of water stress are either totally metabolic or closely linked to metabolism. One effect is, however, strictly physical. Figure 1 shows that xylem conductance for water is reduced by moderate to severe stress. This effect apparently arises from cavitation of the water in the larger xylem elements under the excessive tension caused by low leaf Ψ (Milburn 1966), and seems to account for the persistent depression in leaf Ψ sometimes observed after plants under substantial stress are rewatered (Boyer 1971). At least some of the cavitated xylem elements

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refill when water is again provided (Milburn 1966). Presumably, given time, new xylem vessels would form (Torrey, Fosket & Hepler 1971) to replace the cavitated ones which did not refill.

Growth as related to turgor pressure

Because growth is so sensitive to water stress and constitutes a factor of prime concern in considering plant productivity, the remainder of this paper focuses on growth and the maintenance of turgor through osmoregulation. Considered first are the basic relations between growth and turgor pressure.

In his pioneer investigation of osmotic pressure, Pfeffer (1877) apparently recognized the importance of turgor for expansive growth and the role of solute accumulation in maintaining growth or turgor. He did not, however, give expansive growth much emphasis. An English translation (Ewart 1899, p. 141) of Pfeffer's classic book on plant physiology states:

'High turgidity ... is of subordinate importance only. In other cases, as for example in growing cells, the maintenance and regulation of turgor are of the utmost importance....'

In the process of cell expansion, the first step in wall yielding is often viewed as a relaxation of mechanical stress in the wall (Cleland 1971; Ray, Green & Cleland 1972; Yamamoto, Kawamura & Masuda 1974). The increase in cell volume, basic to growth, requires water uptake. In the simplest case, if the osmolarity of the cell does not change, the only way to create the Ψ gradient for uptake (assuming initial equilibrium between cell and surroundings) is through a decrease in the stress borne by the cell wall (stress relaxation). Turgor stress on the wall is prerequisite to stress relaxation. Water uptake, cell-wall yielding, and, consequently, cell enlargement follow stress relaxation. When cell enlargement is sustained, the processes that produced initial stress relaxation must be continued. For enlargement to be true growth, the increase in dimension must be irreversible. This requires biochemical modifications of the wall and, commonly, deposition of new wall material. In fact, biochemical events of stress relaxation and wall synthesis are probably not separable from the physical events of water uptake and wall yielding. Both models proposed by Cleland (1971) for cell wall extension envision a close interdependence and mingling of the physical and biochemical components.

Another model proposed recently (Hettiaratchi & O'Callaghan 1974) considers solute buildup as the initial step leading to a critical turgor pressure that stretches the cell wall and only after this expansion is new wall material synthesized.

Lockhart (1965*a*, *b*) considered cell expansive growth in terms of the rheology of the cell wall and of cell water uptake and provided an analysis that is conceptually useful for the quantification of cell growth. His analysis treated cell wall constituents as Bingham substances and growth strictly as a physical phenomenon of viscous flow and resulted in an equation expressing growth rate as being proportional to turgor pressure (ψ_p) above a threshold level:

$$\mathrm{d}V/V\mathrm{d}t = E_g(\psi_p - \psi_{p,th}). \quad (1)$$

Here, enlargement rate is given relative to the total cell volume V, and t denotes time, E_g is gross extensibility, and $\psi_{p,th}$ is the threshold or minimal turgor below which the wall does not yield. Equation (1) adequately describes the growth data of *Nitella* (Green *et al.* 1971), oat coleoptile (Cleland 1959), pea roots (Greacen & Oh 1972), and radish cotyledons (Kirkham *et al.* 1972). It may be invoked to explain the extreme dependence of growth rate on ψ_p. If $\psi_{p,th}$ is substantial, then the cell turgor may be only 0.2 or 0.3 MPa above the yield threshold.

It follows that a drop of a few tenths of MPa in tissue Ψ can reduce ψ_p to the threshold level and virtually stop growth. Values of $\psi_{p,th}$ have been found to be as high as 0.8 MPa in some studies (e.g. Boyer 1970).

The role of water uptake in growth becomes more explicit if equation (1) is combined with an equation for water uptake, as done by Lockhart (1965*a*). A slight modification of Lockhart's combined equation is

$$dV/Vdt = E_g C \frac{\Delta\Psi + \psi_p - \psi_{p,th}}{E_g + C}, \quad (2)$$

where C is the overall conductance of the cell for water and $\Delta\Psi$ is the difference in Ψ between the external medium and the cell interior. Equation (2) shows that, in addition to turgor and other parameters delineated in equation (1), Ψ of the external medium and cell conductance are also important in determining the growth rate. Equations (1) and (2), though expressed explicitly only in physical terms, implicitly incorporates the biochemical aspects of the cell wall in the parameters E_g and $\psi_{p,th}$. Those two parameters reflect the viscoeleastic properties of the wall, and hence any biochemical process that modifies those wall properties.

The seeming simplicity of equation (1) can be misleading. The temptation is to view the terms on the right hand side of the equation as independent variables. While this probably is true momentarily when ψ_p is suddenly perturbed, evidence indicates that, with time, both $\psi_{p,th}$ and E_g may shift in a manner that tends to keep growth rate within a narrow range. The most definitive data on this point are those of Green (1968) and Green, Erickson & Buggy (1971), who monitored ψ_p directly in *Nitella* internodal cells while following their growth minute by minute. For example, a sudden drop in ψ_p of about 0.1 MPa caused growth to stop. At the reduced turgor, however, growth resumed within minutes, and in 15 min recovered to the original rate. When ψ_p was again increased, there was a transitory phase of rapid growth which slowed gradually to the original steady-state rate. Apparently, during steady growth $\psi_{p,th}$ was only slightly lower than the actual ψ_p. Thus, any sudden and substantial decrease in turgor stopped growth. With time, however, $\psi_{p,th}$ decreased as a result of metabolic changes in the cell wall ('wall softening'). Growth commenced again when $\psi_p > \psi_{p,th}$. Similar shifts, but in the opposite direction, presumably explain the growth response when turgor was increased. The stability of the growth rate is maintained under small changes in ψ_p by shifting $\psi_{p,th}$ so that the difference $\psi_p - \psi_{p,th}$ is kept nearly constant (Green *et al.* 1971).

For higher plants, detailed or continuous monitoring of ψ_p is not yet possible. One clear indication of possible variations in $\psi_{p,th}$ or E_g as ψ_p is varied came from high-resolution recording of the growth of intact maize leaves while Ψ of the root medium was changed stepwise (Hsiao *et al.* 1970; Acevedo *et al.* 1971). Shifts in the growth rates resembled the patterns observed by Green with *Nitella* so much as to suggest a similar adjustment in $\psi_{p,th}$ and possibly E_g. A more recent and detailed growth study on excised rye coleoptile (Green & Cummins 1974) strengthened this conclusion. The rye data were reported on a basis of changes in ψ_p. The exact changes in ψ_p, however, are open to question since they were deduced from the Ψ values of the medium by assuming a constant tissue permeability to water (C in equation (2)) and a constant tissue ψ_s; these latter two parameters could change during the course of the experiment.

The gradual shifts in the other terms of equation (1) when ψ_p is altered underscore the importance of the time scale of measurements and experimentation. Short-term responses to changes in water status may be greatly modified on a long-term basis. In this context it is rather surprising that some data on growth of tissue in media of different Ψ, though measured only once

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to span a period of 1 or 2 days, actually yield plots conforming to equation (1) (Greacen & Oh 1972; Kirkham *et al.* 1972). If both E_g and $\psi_{p,th}$ change as ψ_p is altered, the use of equation (1) for growth quantification becomes more complicated. On the other hand, changes in $\psi_{p,th}$ and E_g following changes in ψ_p should be an important part of the regulatory mechanism that stabilizes growth.

Other than data on excised coleoptiles, for higher plants there is a dearth of data relating growth to a complete range of ψ_p with growth rate averaged over a time interval shorter than 1 day. Data of this nature are particularly lacking for intact green leaves growing in light.

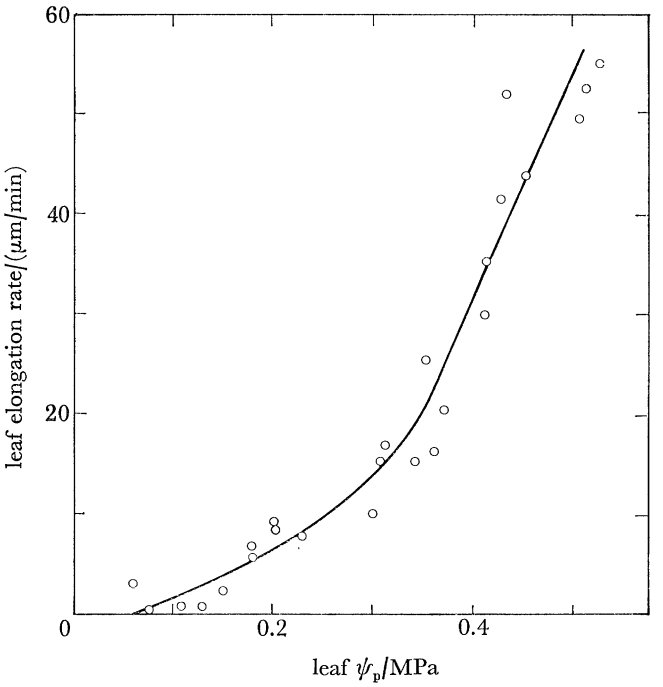

FIGURE 2. Relation between growth rate and turgor pressure of sorghum leaves. Plants were grown in a sandy loam soil in pots in a growth chamber with 400 μE m^{-2} s^{-1} (400–700 nm) for the light period. Soil water potential was maintained around −0.03 MPa until the seventh leaf appeared, then allowed to dry. Growth rate of the seventh leaves of plants at different stages of drying was measured with a transducer (Hsiao *et al.* 1970) and after a steady rate was observed the leaf was excised and its expanding part measured for water potential on a thermocouple psychrometer (Richards & Ogata 1958) isopiestically. Afterwards, the psychrometer cup containing the tissue was frozen and thawed and measured again to obtain solute potential. ψ_p was calculated as the difference between Ψ and ψ_s.

Therefore we conducted an experiment with *Sorghum bicolor* (L.) Moench. Well-watered plants, rooted in a restricted volume of soil, were allowed to deplete the soil moisture. Growth rates spanning intervals of 20–30 min and turgor were measured periodically during depletion periods of 24–36 h. It should be noted that using this experimental approach, a more severe stress also involves a longer stress period. The results (figure 2) show a relation between growth and ψ_p, consisting of both a linear and a nonlinear portion. The first part of the response, when ψ_p was still fairly high, is linear. Extrapolating that straight line to zero growth would give a threshold turgor of around 0.25 MPa. As ψ_p was reduced to below 0.35 MPa, however, the relation departed from linearity. With further reductions in turgor, $\psi_{p,th}$ appeared to decrease, becoming almost zero when growth stopped. Although shifts in $\psi_{p,th}$ are apparent, we cannot exclude the possibility that the extensibility also changed in the nonlinear portion of the curve.

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It is possible that the apparent changes in figure 2 in $\psi_{p,th}$ (and possibly E_g) are artefactual and arise because of the totally physical basis of the conceptual analysis leading to the formulation of equation (1). The departure in behaviour of biological systems from this physical model is likely to depend on the regulatory responses of cells to environmental changes. These responses seem to be of a biochemical nature (Evans 1974) and takes time to develop. As a result, relatively fast responses in growth to fast changes in turgor pressure probably will conform to equation (1), while more prolonged water stress will be transduced as changes in cell metabolism which will affect both E_g and $\psi_{p,th}$ of the equation. Lockhart (1965*a*) and more recently Grenetz & List (1973) have expanded on equation (1) by including changes in ψ_s associated with the cell expansion process. The resulting equations improved the description of the cell growth process; their complexity, in so far as the number of parameters required to be known, however, restricts their present applicability.

Osmoregulation

In view of the critical roles of turgor in growth and other crucial plant processes, the means by which a plant keeps turgor when water is limiting deserve close attention. When tissue Ψ is lowered, to maintain substantial turgor, the other components of tissue water potential, ψ_s and ψ_m (solute potential and matric potential, respectively), must be lowered accordingly. This is easily seen from the equation relating Ψ to its components,

$$\Psi = \psi_p + \psi_s + \psi_m.$$

Clearly, the more negative Ψ is, the more negative $\psi_s + \psi_m$ must be to keep ψ_p constant. Both ψ_s and ψ_m become more negative with water loss. For the crop species studied (Wiebe 1966; Boyer 1967), however, the magnitude of ψ_m remains insignificant until the tissue is badly dehydrated, e.g. to 40% relative water content. So, with mild or moderate water stress, if turgor is to be maintained in spite of low tissue Ψ, alterations must occur in ψ_s. ψ_s can be lowered in two ways. One is by dehydration, thus concentrating the existing tissue solutes. The other is to raise the solute content per cell, either by uptake or by internal production of osmotically active substances. A lowering of ψ_s of more than 0.2 or 0.3 MPa through dehydration should reduce ψ_p to nearly zero for many crop species (figure 3). In contrast, the accumulation of solutes under conditions of decreasing Ψ would result in the maintenance of turgor and cell volume as well as any turgor-mediated process. This paper refers to the phenomenon of solute build-up as *osmoregulation* or *osmotic adjustment*, and an effort is made to differentiate it from the lowering of ψ_s due to dehydration.

As mentioned, Pfeffer (1877) had already fully recognized the importance of solutes in maintaining turgor. Yet, the principle of osmotic adjustment as a mechanism for adaptation when water is limiting has yet to meet general acceptance in the literature on water relations. A part of the problem appears to stem from the failure of many investigators to realize the importance of turgor in plant processes. Another reason is that many of the older studies on ψ_s did not make a clear distinction between dehydration and osmotic adjustment as means of increasing solute concentration in the tissue. A corollary of this is that there had been a dearth of good data bearing on this point. Recent developments, supplemented by some early data scattered in the literature, however, appear to be well on the way toward establishing osmotic adjustment as one of the important mechanisms of plants in coping with conditions of water

shortage. The case is particularly strengthened if one considers it in the context of the well-known osmoregulation processes in lower organisms and the established role of osmotic adjustment in the adaptation of higher plants to saline media.

In lower aquatic plants

A striking example of osmoregulation is found in *Ochormonas malhamensis*, a unicellular alga. Kauss (1969, 1973) showed that this organism accomplishes osmoregulation by synthesis of an unusual organic compound, α-galactosyl glycerol, which apparently serves as a specific osmoticum. Increases in α-galactosyl glycerol accounted for more than 80% of the drop in cell ψ_s initiated by a sudden lowering of medium ψ_s. Full osmotic adjustment judged by volume recovery occurred within 30 min, but the initial response in terms of increased synthesis was detected 2 min after the external ψ_s was decreased (Kauss 1969).

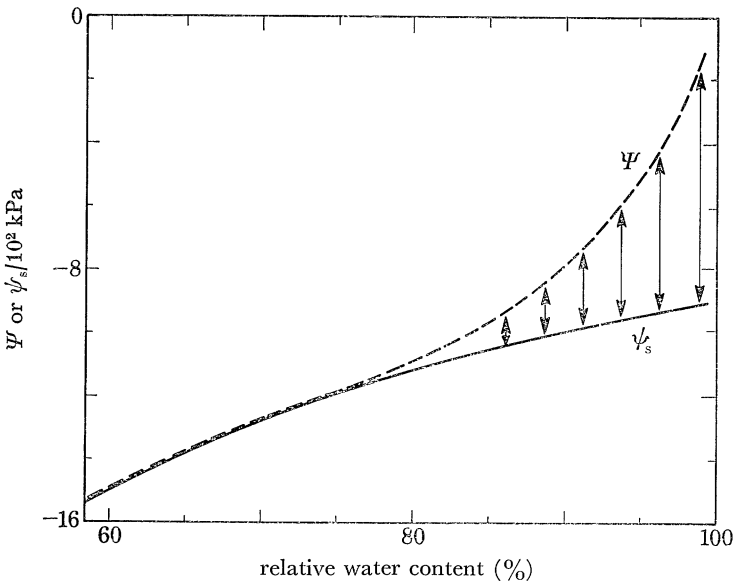

FIGURE 3. Generalized relation among water potential (Ψ), solute potential (ψ_s), and relative water content for leaves of herbaceous crop plants. Length of arrows indicates the magnitude of positive pressure potential (ψ_p) which accounts for the difference between Ψ and ψ_s.

The synthesis of a compound unique for osmoregulation would require a specialized metabolic pathway. It is possibly more common for organisms to osmoregulate through the accumulation of common metabolites. Hiller & Greenway (1968) observed that when *Chlorella pyrenoidosa* was placed on a medium of low ψ_s (−1 MPa), the sucrose content of cells rose whereas starch and other polysaccharides declined after a short period (15 min). By using ^{14}C-labelled glucose, they demonstrated that the reduced starch content was due to the increase in sucrose, which would presumably result in lowering the internal ψ_s. They attributed to this osmotic adjustment the partial recovery in respiration observed previously (Greenway & Hiller 1967).

In the examples given above, the solutes were generated internally. If the external medium is well supplied with absorbable solutes, however, solute uptake provides another means of osmoregulation. A notable example of osmoregulation via solute transport is that of the giant-celled marine alga, *Valonia*. Clearly documented for this organism is not only osmoregulation

but also a modulation of ion pump by ψ_p. Gutknecht (1968), expanding on some early observation by others, showed that K^+ influx was greatly accelerated when turgor was reduced from 100 kPa to zero by lowering the hydrostatic pressure through a microcapillary inserted in the cell. When turgor was again raised to 0.1 MPa, the accelerated K^+ influx was much reduced. The change in ion flux did not result from mechanical damage and was accompanied by corresponding changes in membrane potential (Zimmerman & Steudle 1974).

Another example is provided by work with two marine yeasts of differing salt tolerance (Norkrans & Kylin 1969). After being placed in concentrated NaCl, *Saccharomyces cerevisiae* shrank more and recovered more slowly than did *Debaryomyces hansenii*, the more salt-tolerant of the two yeasts. When K^+ and Na^+ fluxes were followed with tracers, the capacity for osmoregulation was found to be higher in *D. hansenii* in terms of both K^+ uptake and Na^+ extrusion. Since the increased accumulation of K^+ (and some Na^+) and the corresponding anions did not account for more than 50% of the solute potential changes, it was suggested that the cells were able to produce other osmotic agents to achieve the total recovery observed. The nature of those substances was not investigated.

The examples of lower aquatic plants are illustrative of osmoregulatory mechanisms which have been well delineated. A comprehensive review of osmoregulatory mechanisms, covering various lower and higher plant species, can be found in Cram (1975). The refinement achieved in some organisms, discussed above, is rather astonishing. On the other hand, only with such highly evolved mechanisms can organisms with such large ratios of surface to volume gain adaptive advantage in a fresh-water or marine environment of fluctuating osmolarity, where bursting due to excessive turgor and dehydration and loss of volume and form could be an ever-present problem.

Osmotic adjustment in higher plants in response to salinity

With higher plants, osmotic adjustment in saline media is a well-documented phenomenon. When placed in a medium containing large amounts of ionic solutes, and hence of low ψ_s, many plants adjust their internal ψ_s at least partly by the uptake and accumulation of these solutes (Bernstein & Hayward 1958; Bernstein 1961, 1963; Slatyer 1961; Meiri & Poljakoff-Mayber 1969). In some cases the adjustment was total (see, for example, Bernstein 1963; Gale, Kohl & Hagan 1967; Slatyer 1961) in that the lowering of ψ_s of the external solution was paralleled by a lowering in internal ψ_s of the same magnitude. In other cases, osmotic adjustment was only partial (see, for example, Janes 1966). The osmotica responsible for the change in tissue ψ_s obviously will depend on the composition of the external solution. Cl^-, when present in large amounts in the medium, seems to serve as an important osmoticum for this process (Slatyer 1961). On the other hand, there is indication that K^+ uptake can have a predominant role in osmoregulation, although Na^+ and Cl^- were the major ions responsible for the lowering of external ψ_s (Bernstein 1963). Despite the full osmotic adjustment in some cases, most plants, except for some halophytes (Greenway 1968), grew less under saline conditions. Presumably, with full adjustment, turgor is maintained. Explanation for growth reduction may lie partly in toxicity of the ions taken up in large amounts in achieving the adjustment, or may lie simply in ionic imbalance (Bernstein 1963). Another reason may be the energy expended in osmoregulation and ion uptake (Jennings 1968).

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Osmotic adjustment in higher plants under water stress

In contrast to the well established case for salinity, osmotic adjustment as an important process for coping with drought has yet to gain wide acceptance. A part of the difficulty undoubtedly lies in the fact that the phenomenon is apparently very limited in extent in many cases and therefore hard to document. Perhaps the more important reason, however, is the failure of many investigators to realize the importance of adequate turgor for certain critical plant processes, as already mentioned. For example, in several review articles concerned with water stress, the crucial role of turgor in expansive growth was hardly mentioned or completely overlooked (Crafts 1968; Gates 1968; Stocker 1960; Walter & Stadelmann 1974). This is particularly surprising when one considers how well the role of turgor has been elaborated on in other reviews (Cleland 1971; Kramer 1959; Vaadia, Raney & Hagan 1961; Slatyer 1967; Hsiao 1973). Still another reason is the failure in many cases to separate the effect of dehydration, thus preventing any firm conclusion as to whether there actually was an increase in solute content of the tissue and hence maintenance of turgor.

The general idea behind osmotic adjustment as a means of coping with water stress has been touched upon intermittently, and often vaguely (Crafts, Currier & Stocking 1949, p. 101; Iljin 1957; Stocker 1960) since Pfeffer's time. Two kinds of early evidence may be taken as indicative of osmotic adjustment, though often not so interpreted. On one hand there is the well-known effect of sugars accumulating in plants under water stress (Clements 1937; Eaton & Ergle 1948; Iljin 1957). On the other hand, numerous measurements showed that ψ_s was lowered by water stress and that drought resistance might be correlated with low tissue ψ_s under drought (Maximov 1929, p. 271; Iljin 1957; Walter & Stadelmann 1974). An interpretation of the latter kind of data illustrates the confusion in this field. In conjunction with his hydrature concept, Walter (1955) has emphasized the supposedly general detrimental effects of low ψ_s on the 'protoplasm'. He (Walter & Stadelmann 1974) apparently interpreted the correlation between drought resistance and low ψ_s found within certain plant groups to mean that the more resistant a plant (in a particular group) is to drought, the more likely it is to survive in spite of a low tissue ψ_s. Our interpretation, of course, is that low ψ_s is beneficial under drought and that the less resistant species were not able to lower their ψ_s by osmotic adjustment.

More recent data show definite osmotic adjustment under conditions of water shortage. In solution culture and under limited light, shoots of several crop species osmotically adjusted only slowly and to a limited extent under water stress imposed by the addition of polyethylene glycol of high molecular mass to the culture (Janes 1966; Ruf, Eckert & Gifford 1967; Lawlor 1969). The situation with the alga *Nitella* appears to be similar (Green 1968). There was perhaps an adjustment of 0.1 MPa in ψ_p in 10 h after ψ_p was lowered 0.3 MPa by water stress. In comparison, osmotic adjustment via ion uptake in saline media proceeded much faster and more completely for *Nitella* (Cram 1975).

A most substantial adjustment appears to occur in roots, at least in young seedlings. Greacen & Oh (1972) grew radicals of 3–5 day old pea seedlings for 2 days in soil ranging in Ψ from -0.28 to -0.83 MPa of approximately the same mechanical strength. They found that the estimated ψ_p of the roots remained constant through osmotic adjustment effected by reductions of ψ_s of as much as 0.7 MPa. Growth rate of the roots was practically unaffected by the variation in soil Ψ, presumably because of the full adjustment. Limited data on the Ψ and ψ_s of roots of a maize crop growing in the field (Acevedo 1975) are consistent with the notion that roots

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have a high capacity to adjust osmotically under water stress. The possible significance of this adjustment to the shift in ratio of roots to shoots under drought conditions has been discussed (Hsiao & Acevedo 1974).

Germinating seeds or very young seedlings appear to have a greater capacity for osmotic adjustment when water is limiting. Meyer & Boyer (1972) measured growth, Ψ, and ψ_s in hypocotyls of soybean seedlings. As the water in the medium depleted, the hypocotyls underwent osmotic adjustment in such a way that turgor remained almost constant until growth stopped completely. The drop in ψ_s was about 0.6 MPa, and Ψ changed from -0.23 (with full growth) to -0.9 MPa (when growth became 10 % of the maximum). The ultimate source of solutes for adjustment was apparently the cotyledons; their removal prevented the adjustment in ψ_s and greatly increased the sensitivity of hypocotyl growth to reductions in Ψ. Growth in a 3 h period of the hypocotyl under applied pressure indicated a steep drop in growth with small increments in pressure. This was interpreted as a lack of substantial osmotic adjustment, the cause of which was presumably that the time interval employed (3 h) was too short to permit much solute buildup.

Pattern of behaviour in the field

The limited osmotic adjustment in the shoot in response to water stress mentioned above may have been a phenomenon peculiar to the indoor growing conditions in those earlier studies. Recent experiments indicate that substantial osmotic adjustment in the field may be common even among mesophytic crop plants.

Some early work indirectly suggested substantial osmotic adjustment in the field. Walter & Stadelmann (1974) cited extensive early data for some species showing more than a threefold decrease in ψ_s from the wet to the dry season. As pointed out, it is possible that most of this change was due to dehydration and not to solute accumulation.

Seasonal osmotic adjustment

A halophyte, *Atriplex confertifolia*, was recently shown to adjust osmotically to drought on a seasonal basis (Moore, Breckle & Caldwell 1972). Its ψ_s dropped from -5 MPa in the spring to -20 MPa (!) in midsummer. A part of the decrease in ψ_s, however, could be attributed to breakage of vascular hairs that behaved like salt glands. Interestingly, another halophyte growing at the same site, *Eurotia lanata*, changed little in ψ_s throughout the season. Moore *et al.* (1972) suggested that osmotic adjustment was one of the factors enabling *A. confertifolia* to maintain a higher rate of photosynthesis and dry-matter accumulation than *E. lanata* late in summer. Also contrasting are early results of Slatyer (1960) on a xerophytic acacia. The high osmolarity in the phyllodes of that species did not change noticeably with season or with soil water status, indicating that a high solute content was normal and not the result of osmotic adjustments.

As for crop species, two recent examples can be given of definite seasonal osmotic adjustment in the field in response to water stress. Goode & Higgs (1973) reported that unirrigated apple trees decreased their leaf ψ_s by 0.5 MPa from July to September. The corresponding decrease in minimum daily leaf Ψ was from -1.9 to -2.5 MPa. Since the reduction in ψ_s roughly matched the reduction in Ψ, turgor pressure was maintained through the season. Those workers speculated that this compensatory effect of ψ_s reduction on the decreases in Ψ must benefit

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turgor-mediated processes of cell enlargement and stomatal opening. Unfortunately, they did not measure either of the latter to determine the effects. A striking example for annual crops is osmotic adjustment in a sorghum crop drawing only moisture stored in the soil at the beginning of the season (Fereres, Acevedo, Henderson & Hsiao 1976). Figure 4 shows the seasonal trends in Ψ and ψ_s, measured between noon and 3 p.m., in the most recently matured leaves of the unirrigated sorghum. Leaf Ψ declined gradually from about -1 MPa at the time of panicle initiation to about -1.6 MPa at maturity. This was matched by parallel changes in leaf ψ_s, decreasing from about -1.4 to -2 MPa during the same period. Consequently ψ_p was kept more or less constant throughout the season.

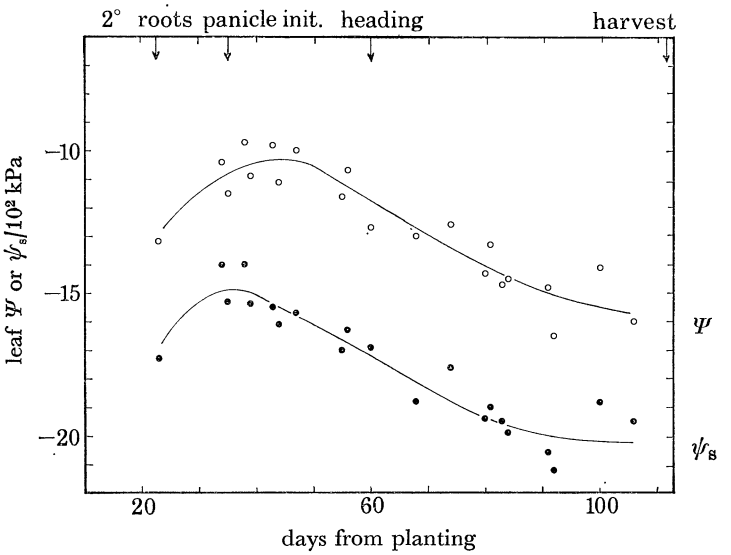

FIGURE 4. Seasonal trends of Ψ and ψ_s in unirrigated sorghum. The crop was planted on 17 May 1974 in a deep alluvial soil (Yolo clay loam) fully wet at planting and received no irrigation and virtually no rain throughout the season. Ψ and ψ_s were measured as in figure 3 on leaves sampled between noon and 3 p.m. Each point is the average of 3–8 measurements. (From Fereres, Acevedo, Henderson, & Hsiao 1976.)

The validity of comparing leaf Ψ and ψ_s early in the season with those near maturity may be questioned. Presumably, as well as osmotic adjustment, morphological and physiological changes associated with ontogeny could also account for the seasonal trends in leaf Ψ and ψ_s. The effects of ontogenetic changes on Ψ and ψ_s were apparent in the frequently irrigated control (figure 5). With ample soil water, leaf Ψ and ψ_s also declined with time after panicle initiation, though the decline was considerably less than in the unirrigated treatment. Goode & Higgs (1973) also reported leaf ψ_s becoming more negative in irrigated apple trees as the season progressed. As a result of the greater solute accumulation, leaf ψ_p in sorghum was maintained at the same level in the unirrigated as in the irrigated crop (figure 5). Overall, the seasonal trends in ψ_s may be taken as indicative of osmotic adjustment in response to mild water stress arising from two sources: soil water depletion; and increases in plant liquid-flow resistance due to age and to increased height.

The possible significance of the observed turgor maintenance on growth and plant performance in general deserves elaboration. Growth of the same sorghum variety in a growth chamber showed a reduction of 75 % with a reduction of ψ_p of 0.2 MPa from the well-water condition (figure 2). With the maintenance of turgor in the field, growth rates of the

unirrigated treatment were inhibited only 10–35 % below those of the control during the daily periods of maximum stress (Fereres, unpublished). Stomatal opening was virtually identical for the irrigated and unirrigated plants throughout most of the season (Fereres, unpublished), suggesting that CO_2 assimilation per unit area of green leaf may be similar for the two treatments.

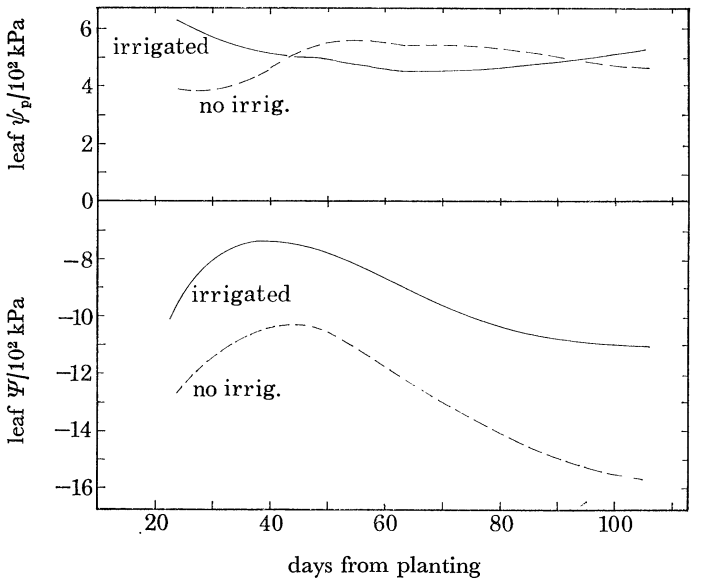

FIGURE 5. Comparison of the seasonal trends of Ψ and ψ_p in field grown sorghum. Data from the same experiment as depicted in figure 4. The irrigated treatment received weekly irrigations and was sampled for Ψ and ψ_s at the same time as the unirrigated crop. (From Fereres *et al.* 1976.)

Diurnal changes in water-potential components and growth

Midday depression of the Ψ of leaves or shoots of exposed plants is probably ubiquitous on sunny days. One may expect this depression in water status to slow growth during the hot part of the day. Indeed, detailed records of diurnal growth obtained in the tropics in the early part of this century (Coster 1927) showed that the growth of many species slowed when the sun shone, temperature increased, and relative humidity declined, but recovered toward evening as relative humidity rose. This inhibition during the day was attributed to water deficit since it was not observed on rainy days and could be ameliorated by excising certain parts of the plant to reduce transpiration and improve the water status of the remaining growing tissue. Similar growth-temperature-humidity patterns have been observed in temperate zones, for example, in maize (Loomis 1934).

More recently, Boyer (1968) deduced from the quantitative relation between leaf Ψ and leaf enlargement of sunflower that most growth should occur only at night, when the Ψ gradient from the soil to the leaf is minimum. He confirmed this deduction by measurements of day and night leaf growth in the greenhouse. In Davis, California, virtually all summer days are hot, rainless, and cloudless, whereas the nights are cool. With the high evaporation demand during the daytime, we (Acevedo, Fereres, Henderson & Hsiao 1976) recently found that leaf Ψ undergoes pronounced diurnal oscillation (figure 6) with a minimum around noon and a large amplitude (e.g. 1 MPa). At first glance, results of the previous studies would lead to the conclusion that growth should be related directly to leaf Ψ. Particularly, since an earlier growth-chamber

study (Acevedo *et al.* 1971) found elongation of maize leaves to stop at a leaf Ψ of about -0.7 MPa, one might expect growth to drop to zero by late morning in the field at Davis when this low value is attained. Instead, measured growth (figure 7) actually increased as the morning progressed despite the fall in leaf Ψ. Further, growth at noon was substantial although leaf Ψ had fallen to -1.2 MPa. The explanation for this apparently contradictory behaviour

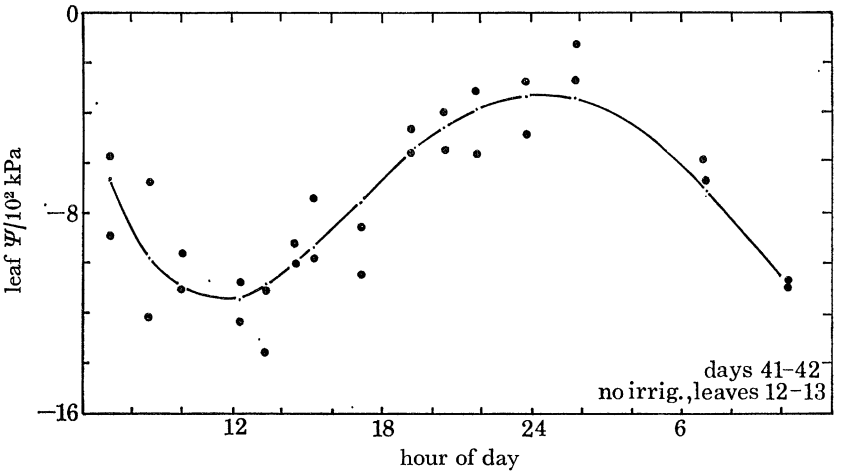

FIGURE 6. Diurnal pattern of Ψ in expanding leaves of an unirrigated maize crop 41–42 days after planting. Soil type and water supply were the same as described in figure 4. Data points represent individual measurements. The curve was computed by least square fitting of fourth degree polynomial to the data points. (From Acevedo *et al.* 1976.)

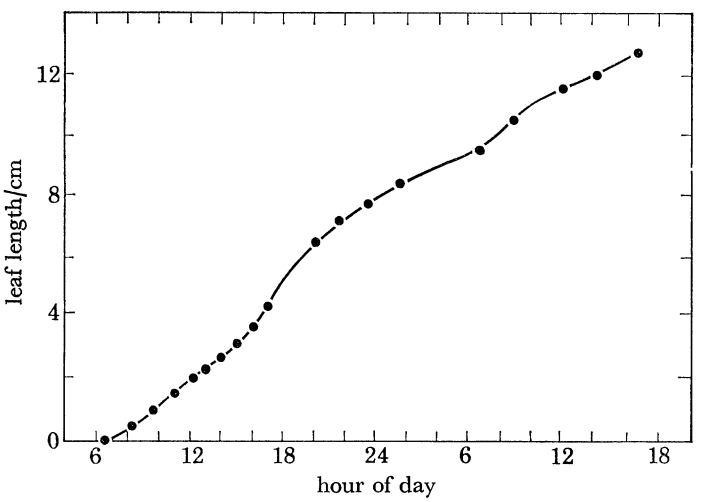

FIGURE 7. Diurnal course of elongation of leaf number 13 in unirrigated maize. Measurements were taken concurrently with and from the same plot as the Ψ measurements depicted in figure 6. (From Acevedo *et al.* 1976.)

appears to lie in the diurnal pattern of ψ_s. Figure 8 shows that leaf ψ_s not only oscillated diurnally as did leaf Ψ, but also lagged behind in that the minimum for ψ_s was attained around 2 h after the Ψ minimum at noon. The oscillation in ψ_s permitted the maintenance of substantial turgor (figure 8) despite the rapid decline in Ψ in the morning. This presumably accounts for the partly sustained growth during that period. The lag or phase shift in ψ_s behind Ψ had

the effect of raising ψ_p earlier in the afternoon than would be permitted by the recovery in Ψ (figure 8). The resulting enhancement in turgor is apparently responsible for the accelerated growth during that time (figure 7).

The increase in osmolarity in the morning and at noon, correspondng to the fall in ψ_s, must be largely due to solute accumulation. It if were due mainly to dehydration, ψ_p could not be maintained even partially. Even a small loss of water would reduce ψ_p to nearly zero, whereas a much greater water loss is needed to lower ψ_s substantially (see figure 3). A more direct indication of solute buildup as the main cause for the lowering in ψ_s is the change in the moisture-release curve (Ψ against relative water content) of the leaves between early morning and noon. These data have been presented by Acevedo *et al.* (1976), along with some indications that soluble sugars may account for a part of the oscillation in osmolarity.

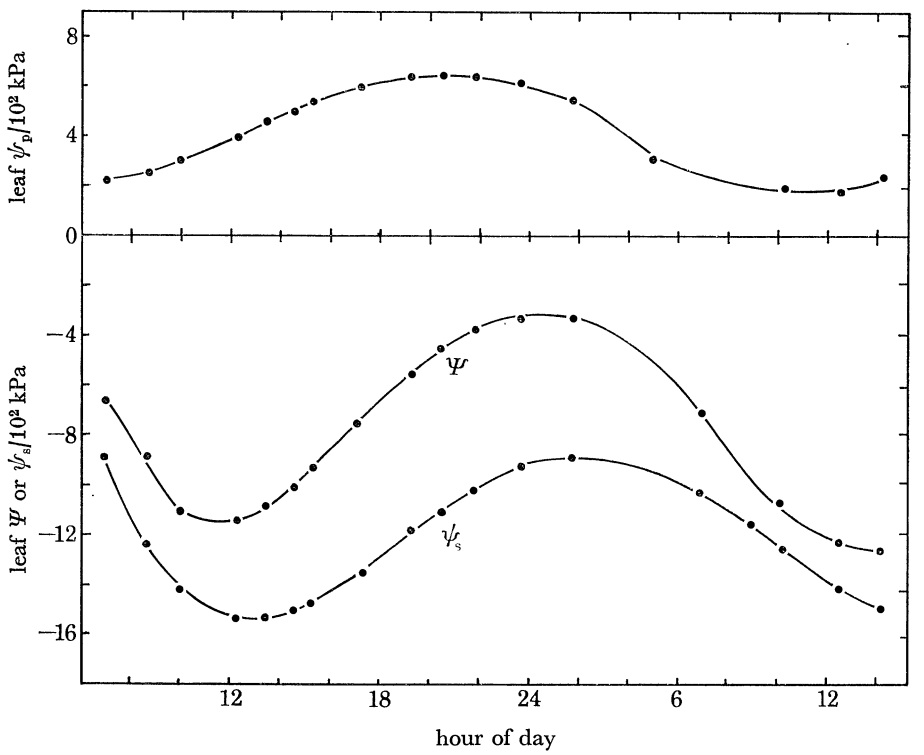

FIGURE 8. Diurnal patterns of Ψ, ψ_s and ψ_p in expanding leaves of unirrigated maize. The curve for Ψ is the same as that given in figure 6. The curve for ψ_s was computed by least square fitting of fourth degree polynomial to the ψ_s values, measured on the same samples taken to measure Ψ. The curve for ψ_p was calculated as the difference between the Ψ and ψ_s curves. Dots on the curves represent sampling times. Duplicate samples were measured at each time. From Acevedo *et al.* (1976). Days 41–42; leaves 12–13.

In addition to leaf water status, temperature was also oscillating diurnally. Parts of the growth pattern appeared to be dictated by temperature. The effect of temperature on growth is well known (Leopold & Kriedemann 1975). Watts (1972) determined the elongation-temperature relationship for maize leaves and obtained diurnal growth curves in the field (Watts 1974) similar to figure 7, though without detailed data on Ψ and ψ_s. Further elaboration on temperature-water status interaction is found in our original paper (Acevedo *et al.* 1976), the source of the data in figures 6–8, and in work of Kleinendorst & Brouwer (1972) and Watts (1974).

Suffice it to say that the slow growth at night and in the very early morning is probably due to low temperature, and that growth and temperature were linearly related in that period. The production of cold-sensitive crops (such as maize) in Davis and areas of similar climate must owe much of its success to diurnal osmotic adjustment. Since growth at night is apparently restricted by the cool temperature, total growth would be drastically curtailed had it not been for the day growth made possible by osmotic adjustment.

Vertical water potential gradients in plants

Within a plant canopy, differences in Ψ among the various leaves are determined largely by differences in their rates of transpiration and their upstream resistances to liquid-water transport. Generally, the higher leaves receive more radiation and hence transpire more. Additionally, they are also farthest away from the roots and hence are separated from the source of water by a larger total resistance. It follows that the higher leaves usually have a more negative Ψ than the lower leaves, as reported for various studies (Turner & Begg 1973; Hellkvist, Richards & Jarvis 1974).

As mentioned and shown in figure 1, there is a threshold leaf Ψ below which stomata begin to close in response to the water deficits. It turns out that the threshold Ψ value for stomata closure for the upper leaves may be lower (more negative) than for the lower leaves, at least in recent data of several groups now being prepared for publications.† This is gratifying since the higher leaves would otherwise receive most of the radiation but would not be able to assimilate CO_2 effectively because of stress-induced stomatal closure.

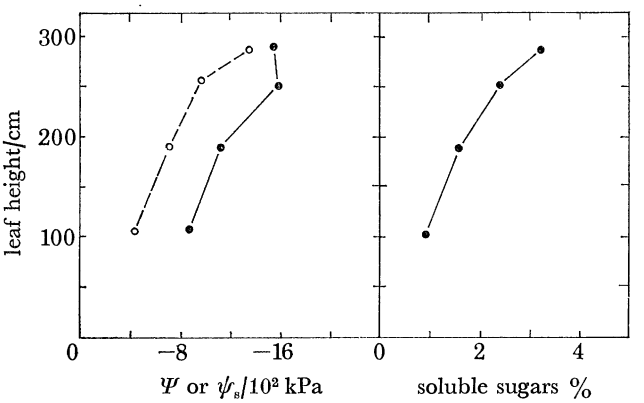

FIGURE 9. Vertical profiles of Ψ (○), ψ_s (●), and total soluble sugars at 11 h in leaves of an irrigated maize crop 90 days after planting. Data represent the means of two determinations, one from each of two plants. Ψ and ψ_s were measured as described in figure 2. Parts of the same leaves taken for Ψ determination were analysed for soluble sugars, which is given as glucose equivalent. (Unpublished data of E. Acevedo.)

What enables the leaves on top to withstand the lower Ψ and yet not close their stomata? The answer again appears to lie in turgor maintenance through osmotic adjustment. Figure 9 shows an example of vertical profiles of Ψ and ψ_s within a maize canopy (Acevedo, unpublished). It is seen that ψ_s became more negative going up the plant in such a way as to sustain some ψ_p even in the top leaves, which were 0.8–1.0 MPa lower in Ψ than leaves near the bottom. Soluble sugars in the leaves followed a vertical trend similar to that of ψ_s. The differences in sugar concentrations between the heights were insufficient, however, to account for the vertical gradient in ψ_s.

† See Jordan, W. R., Brown, K. W. & Thomas, J. C. 1975 *Pl. Physiol.* **56**. (In the press.)

Concluding remarks

We have presented ample evidence that plant reactions to water stress cannot be interpreted as simple responses to changes in Ψ, with ψ_s remaining relatively constant and with ψ_p changing in phase with Ψ. Certainly osmotic adjustment acts in many cases where plants undergo water stress. Since the mechanisms of the changes in ψ_s are largely unknown, primary stress responses may well be interrelated with processes such as photosynthesis and carbohydrate utilization.

It is essential, then, to look again at the processes such as those listed in figure 1 and critically evaluate their relationships to other indicators of plant water status in addition to Ψ. In many cases, as suggested in this paper, parameters other than Ψ, especially ψ_p, may be of more direct concern.

In spite of the strong theoretical and experimental evidence of a close relationship between growth and ψ_p, simple expressions stating this relation have limited applicability, particularly when environmental parameters do not remain constant. Expansive growth depends not only on ψ_p but also on biochemical processes which affect cell wall properties. Therefore it is not surprising that, as in the case of salinity, plants subjected to mild water stress may exhibit significant reduction on growth while turgor apparently is maintained. There are yet no definitive explanations for this behavior, but the lag in adjustment early in the morning, the extreme sensitivity of growth rate to small decreases in turgor near its maximum value and metabolic responses to mild stress may be partly the answers. Also, with procedures now used, errors in estimating ψ_p generally are large, and this contributes uncertainty. In any case, the causes of growth responses under water stress probably will not be understood until the sequence of physiological events developing as water stress sets in is better known.

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