EFFECTS OF SEVERAL OSMOTIC SUBSTRATES ON THE WATER RELATIONSHIPS OF TOMATO

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Summary

Osmotic substrates, comprising 5- and 10-atm concentrations of potassium nitrate, sodium chloride, mannitol, and sucrose were added to standard culture solutions in order to determine the effect on relative turgidity, DPD, osmotic potential, transpiration, and growth of tomato plants. ^{14}C-labelled mannitol and ^{36}Cl-labelled sodium chloride were incorporated into the high-concentration treatments to provide additional information on solute absorption.

After an initial loss of water content (fresh weight − dry weight) and associated wilting, recovery took place rapidly in all except the mannitol treatments and after 28 hr did not differ significantly from the control. At this time the first stage of the experiment was concluded and all plants were replaced in standard culture solutions.

Recovery of water content and turgor during the first stage was associated with a rapid increase in internal osmotic potential. This proceeded to such an extent that, by the end of stage 1, the internal potential in each treatment exceeded the substrate potential by approximately the same amount as the potential of the control exceeded that of the base nutrient solution. The increase in internal osmotic potential was achieved mainly by solute absorption except in the mannitol treatments in which dehydration appeared to account for almost half of the increase.

Absorption of ^{36}Cl from labelled sodium chloride was closely related to the increase in osmotic potential in the high-concentration sodium chloride treatment, the relative concentration of the isotope in the plant at the end of stage 1 compared with concentration in the substrate being approximately 1 : 1. In the mannitol treatment the relative concentration of ^{14}C was only 1 : 4, confirming that mannitol absorption alone could only account for a small proportion of the total increase.

Following the removal of the osmotic substrates an initially rapid and progressive decline in internal osmotic potential occurred, together with an associated reduction in ^{36}Cl and ^{14}C concentration in the tops of the plant. In both cases this decline could be satisfactorily accounted for by increased water content of the plants, leakage of solutes to the substrate being negligible.

The effect of the osmotic treatments on transpiration appeared to be closely related to changes in volume (expressed as water content), showing an initial severe reduction and subsequent recovery to values close to the control in all except the high-concentration mannitol treatment. Because of the short term nature of the experiment no significant changes in growth (measured as increase in dry weight) could be detected in stage 1. Following the removal of the osmotic substrates however, dry weight increase in the low-concentration sucrose and potassium nitrate treatments was more rapid than in the control and in the high-concentration mannitol treatment was significantly less.

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I. Introduction

There is now general agreement that the availability of soil water for plant growth decreases progressively as soil moisture stress increases (Richards and Wadleigh 1952) and that the first evidence of decreased growth occurs at quite low stress levels. Evidence to the contrary (Veihmeyer and Hendrickson 1950) is based mostly on field experimentation, the proper interpretation of which is rendered difficult because of the variation which exists between different experiments with respect to root distribution, hydraulic conductivity of the soil water, total depth of the root zone, and other experimental factors.

It has also been proposed by research workers at Riverside, California (Wadleigh and Ayers 1945; Wadleigh 1946) that the total water potential (total soil moisture stress) equals the sum of the soil moisture tension and the osmotic potential of the soil solution. It is normally assumed that this viewpoint requires that the plant acts as an ideal osmometer and that the solutes contributing to the osmotic potential are non-diffusible. It has been widely accepted (Magistad 1945; Bernstein and Hayward 1958), and is embodied in the concept of "physiological dryness" frequently applied to saline soils. It is supported by a considerable volume of experimental evidence indicating reduced water absorption, growth, and metabolism as the substrate concentration increases (Richards and Wadleigh 1952; Kramer 1956). However, it has been challenged by Walter (1955) on the basis that, if the solutes in the substrate or soil solution are freely diffusible, the osmotic potential of the soil solution is balanced by the intake of solutes. Supporting evidence is also available for this view (Eaton 1927, 1942) and, in many respects, it is to be expected from known facts concerning salt absorption (Gauch 1957; Robertson 1958).

More recently Philip (1958) and Bonner (1959) have suggested that the opposing viewpoints could be reconciled by envisaging, in soil–plant water systems, the development of a vapour gap and disruption of liquid phase continuity, at the soil–root interface. It is suggested that prior to the development of such a vapour gap the Walter view should hold, and that the effective soil moisture stress would not include an osmotic component, but subsequently the view of the Riverside workers would be valid and that the effective soil moisture stress would include an osmotic component. The Riverside workers have rejected this view (Bernstein, Gardner, and Richards 1959) on the basis that the observed effects are apparent in culture solution experiments in which no vapour gap can be envisaged and that rates of vapour transport are inadequate to supply the amounts of water required.

If the osmotic potential of the soil solution is a component of the total soil water potential, it must result in the development of a water potential in the plant, of equal magnitude to the sum of soil moisture tension and osmotic potential. Consequently, the measurement of the internal water relations of plants grown on osmotic substrates should provide a ready solution to the controversy. Since no data of this type have been discovered in the literature, a study involving these measurements was conducted with plants grown in culture solutions to which were added osmotically active organic and inorganic substrates to ascertain the nature of the plant response.

Sucrose and mannitol were selected as the organic substrates on the assumption that sucrose is absorbed by plant roots and is readily metabolized in the plant, whereas evidence suggests that mannitol is only slowly absorbed (van Overbeek 1942; Groenewegen and Mills 1960) and is not metabolized to any degree. Potassium nitrate and sodium chloride were selected as the inorganic substrates. Both are readily diffusible but it was thought that the known toxic effects of excess sodium chloride could result in a different order of response.

II. Experimental Details

(*a*) *Preparation of Material*

Tomato seedlings (cv. Grosse Lisse) sown in sand culture were transferred to standard water culture solutions after 3 weeks and grown to the five-leaf stage, when the average fresh weight of each plant was of the order of 25 g. The culture solution was prepared according to solution 1 of Hoagland and Arnon (1938).

The osmotic substrates were prepared with A.R. reagents to provide osmotic potentials of approximately 5 and 10 atm in the low-concentration and high-concentration treatments respectively. The concentrations appropriate to each solution were computed from data on freezing-point depressions and osmotic coefficients tabulated by Robinson and Stokes (1955) and Hodgman (1955). Because particular interest was associated with the sodium chloride and mannitol treatments, the former was labelled by the addition of 0·1 mc of ^{36}Cl as NaCl and the latter by the addition of 0·1 mc of ^{14}C-labelled D-mannitol with the isotopes at the 1 and 6 positions.

At the commencement of the experimental period, plants selected for uniformity were arranged into nine groups to provide a control in addition to low- and high-concentration treatments of KNO_3 (K_1, K_2), NaCl (N_1, N_2), mannitol (M_1, M_2), and sucrose (S_1, S_2). Each treatment group consisted of four replications each of 10 plants, and each set of 10 plants was arranged in standard 3 l. containers.

Imposition of the osmotic substrates involved the replacement of the standard culture solutions by the specific substrate treatments. This was achieved in minimum time by having pairs of standard and treatment containers and rapidly transferring each group of 10 plants. Removal of the osmotic substrates involved the reverse procedure.

The experimental period commenced at 10.00 a.m. on June 27, 1960, with the transfer of all plants to the substrate treatments. The plants were exposed to these substrates until 2.00 p.m. on June 28 and then returned to standard culture solutions. The experiment was then continued until 10.00 a.m. on July 1 so that the short-term effects of the exposure to osmotic substrates could be observed. Nine sampling occasions were spread through the experimental period.

(*b*) *Measurement Techniques*

(i) *Relative Turgidity.*—Relative turgidity was measured using the general technique of Weatherley (1950, 1951) except that (1) leaf disks of 0·8 cm diameter

were employed; (2) the period of floating was 4 hr at constant temperature; (3) during floating the disks were continuously illuminated by a 20-W fluorescent light mounted about 20 cm above the bench top.

(ii) *Water Potential.*—The water potential of the leaf tissue was measured as diffusion pressure deficit (DPD) using the vapour equilibration technique described by Slatyer (1958). The length of time allowed for each determination was 8 hr.

(iii) *Osmotic Potential.*—Measurements were made using the expressed sap method. Although sap expression may introduce some errors, it was thought (Crafts, Currier, and Stocking 1949) that these would be minor compared with those which could arise in the plasmolytic method. The osmotic potential of the leaf tissue sap was determined by (1) snap-freezing the fresh tissue to −20°C; (2) expressing the sap in a hydraulic press at 140 kg/cm^2 (2000 lb/in^2); (3) measuring freezing-point depression with standard cryoscopic equipment; (4) estimating equivalent osmotic potential using the procedure described by Crafts, Currier, and Stocking (loc. cit.).

(iv) *Transpiration and Water Absorption.*—Transpiration was measured by weighing each container at each sampling occasion, and water absorption calculated by adjusting this value by the loss of water represented by the changing fresh weight of the plants. The containers were topped up with water at each sampling occasion.

(v) *Plant Weight.*—On each sampling occasion one plant was moved from each replication of each treatment and the tops and roots immediately placed inside polythene bags, weighed, and transferred to a constant-temperature room. The plants were then partitioned into laminae, petioles, stems, and roots. Although portions of each plant were utilized on the measurements described above, a record of all dry weights was maintained and fresh weight and dry weight for all treatments obtained.

(vi) *Water Content.*—It was desirable to measure changes in water volume so that the effect of solvent volume on osmotic potential and turgor pressure could be estimated. These data were obtained as fresh weight−dry weight from the determination described above and, in the text, use of the word "volume" implies water content determined in this manner.

(vii) *Isotope Concentration.*—On each sampling occasion the plants sampled from the treatments containing ^{14}C and ^{36}Cl were partitioned into leaves (laminae only) and stems (including petioles) and snap-frozen to −20°C. Also on each sampling occasion 10-ml aliquots were pipetted from the substrate solutions. The mannitol and chloride was removed from the thawed plant material by aqueous extraction, involving repeated boiling and filtration until a negligible amount of radioactive material remained in the residue. Aliquots from the partially evaporated and concentrated filtrate were then evaporated in planchets and counted in an end-window G.M. counter.

The results have been expressed as the concentration of the isotope per unit water in the plant (fresh weight−dry weight) relative to the concentration of the isotope in the substrate.

(c) *Weather Conditions*

The experiment was conducted in a heated greenhouse exposed to natural light. During the experimental period overcast conditions prevailed on each day, and the maximum temperatures from June 27 to July 1 were 27, 23, 27, 26, and 25°C respectively. The minimum temperatures did not fall below 15°C, the basal temperature for the greenhouse. Relative humidity ranged between 60–80% over the period.

III. Results

In Figures 1, 2, and 5, plant responses to the various osmotic substrates are shown in terms of relative turgidity, water potential (DPD), water content, osmotic potential, transpiration, fresh weight, and dry weight. In each diagram the responses to the inorganic solutes are shown on the left side and to the organic solutes on the right side; also the sampling occasions are indicated on the linear time scale across the bottom of each diagram. Where applicable differences required for significance at the 1 and 5% levels are shown. In every case the osmotic treatments were imposed at the beginning of the experimental period depicted and were removed immediately after sample 4. This initial phase of the experiment is subsequently referred to as stage 1 and the remainder of the experimental period as stage 2.

A photographic record of the progressive changes in appearance is shown in Plates 1 and 2. Following the imposition of the osmotic substrates, the plants in all the high concentrations wilted severely (Plate 1, samples 0 and 1). Slight visible wilting also occured in the low-concentration treatments, but when sample 1 was taken 75 min after the beginning of the experiment, apparent recovery in these treatments had occurred in all except S_1. Subsequent visible recovery of all treatments except M_2 proceeded rapidly (Plate 1, samples 2 and 3) and appeared complete 28 hr after the commencement of the experimental period. At this time sample 4 was taken and the osmotic substrates removed, even though M_2 remained severely wilted. Subsequent recovery in M_2 was gradual (Plate 2, samples 5–7) but appeared complete at sample 7, 48 hr later.

These visual responses at first appear to be contradicted by the results of the relative turgidity and DPD determinations (Figs. 1*A* and 1*B*), since, after the initial loss of turgor, these quantities did not show significant recovery during stage one in any treatments, and in M_2 continued to deteriorate. However, while the DPD's were held at approximately the level of the external osmotic substrates, it can be seen from Figure 1*C* that complete recovery in water content occurred in the potassium nitrate, sodium chloride, and sucrose treatments, and this was presumably due to solute absorption and a consequent increase in internal osmotic potential and hence in volume and turgor pressure. In the mannitol treatments the recovery in volume was incomplete by sample 4, suggesting much reduced solute entry.

The lack of recovery in relative turgidity, while partial or complete recovery occurred in water content, likewise appears attributable to solute uptake, since this would result in an increase in internal osmotic potential and an associated increase in water content, even though the DPD of the tissue remained at the level of the

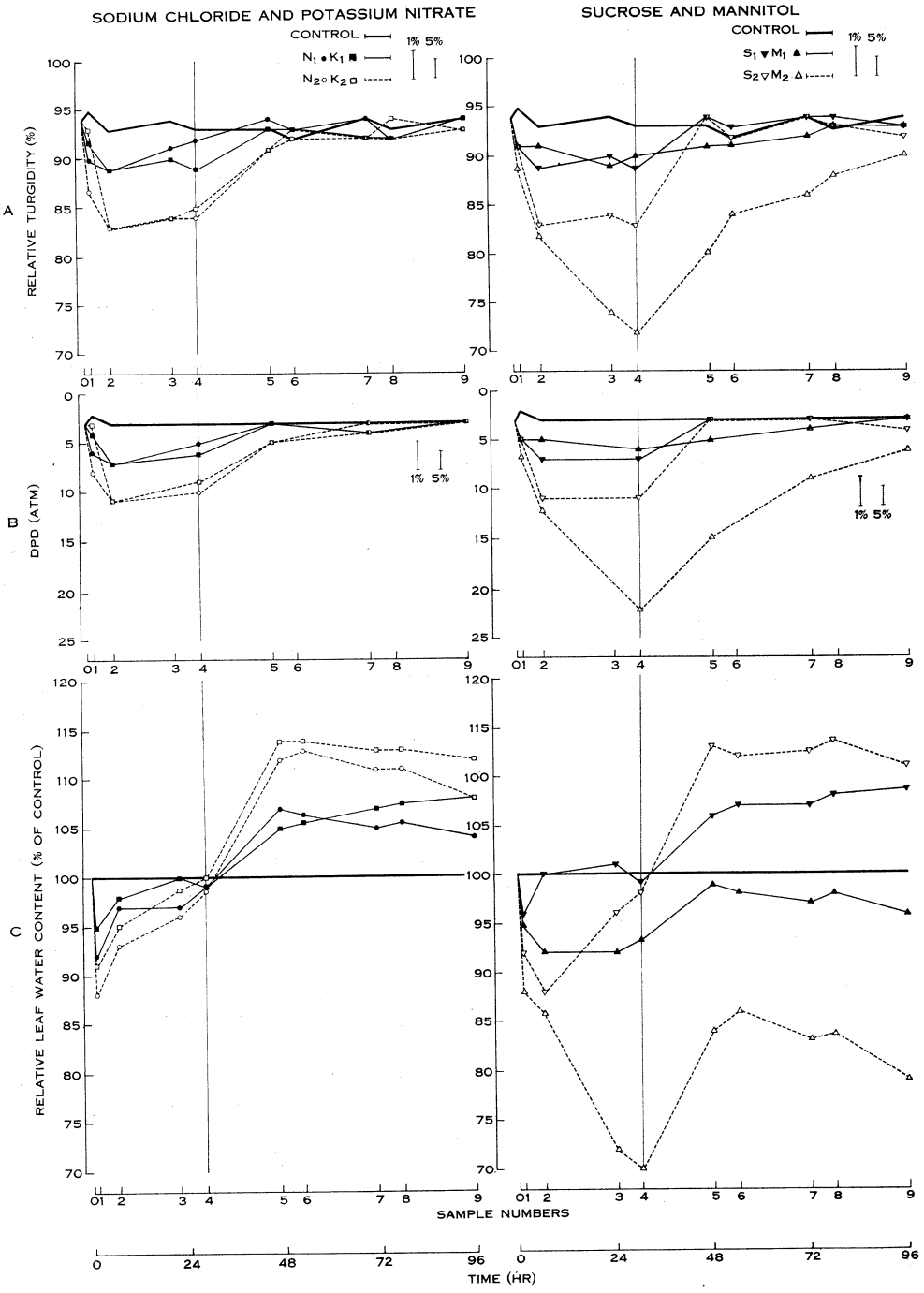

Fig. 1.—Changes during the experimental period in: *A*, leaf relative turgidity; *B*, leaf diffusion pressure deficit (DPD); and *C*, relative leaf water content expressed as fresh weight minus dry weight of treatment leaf tissue relative to that of control.

external substrates. Thus at sample 4, although some treatments had regained their original volume, the fact that all plants still had a DPD equivalent to that of the external solution resulted in the expansion of leaf disks when floated on water. The relative turgidity so measured presumably reflected the additional expansion which occurred before the DPD of the disks was reduced to that of water.

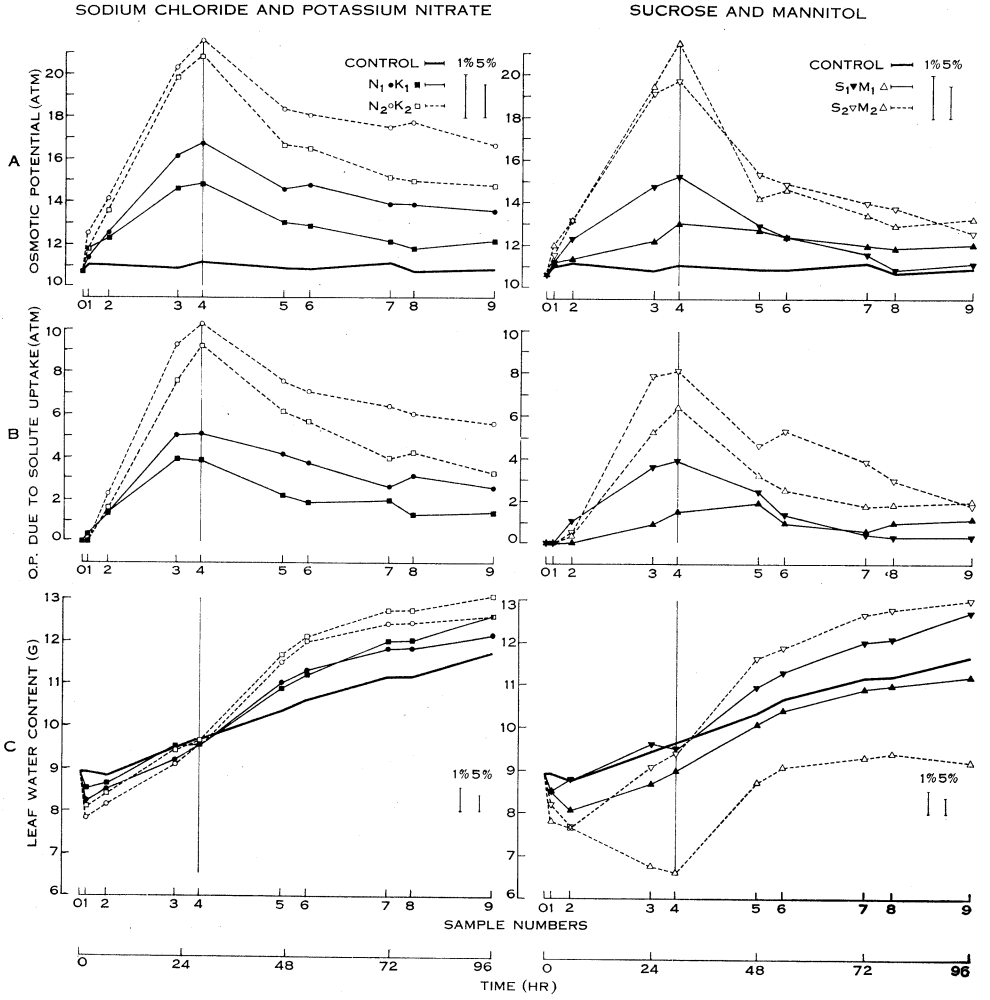

Fig. 2.—Changes during the experimental period in: *A*, total osmotic potential of expressed leaf sap; *B*, total osmotic potential minus osmotic potential due to decreased water content; *C*, leaf water content, expressed as fresh weight minus dry weight.

The response patterns of the plants in stage 2 supports this explanation. Except in M_2, increase in leaf water content relative to that of the control is seen to be only slightly greater than the initial decrease during stage 1. At the same time relative turgidity and DPD values returned to the control level. In the potassium nitrate, sodium chloride, and sucrose treatments this resulted in an increase of

water content above that of the control, but in M_1 the increase served to bring the water content back to the control level. In M_2, no compensatory increase in volume occurred even though the recovery pattern showed a close relationship between DPD and relative turgidity. Recovery in M_2 continued throughout the experimental period and appeared visibly complete, but the slow response would appear to indicate tissue injury during stage 1 and complicates data interpretation.

The data of Figure 2 support the hypothesis of solute uptake invoked to explain the data of Figure 1, and indicate substantial increases in internal osmotic potentials

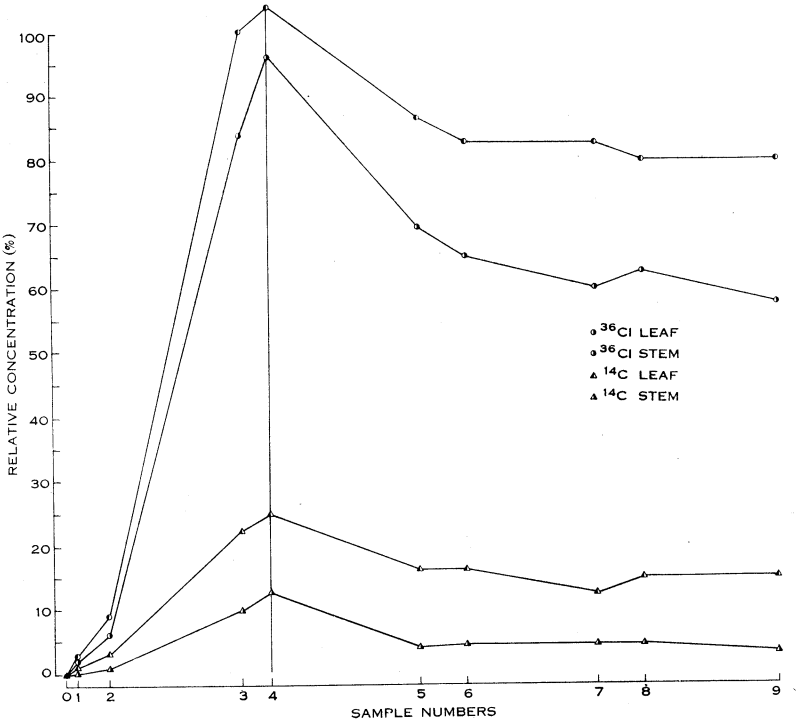

Fig. 3.—Concentration of ^{14}C and ^{36}Cl in the leaves (laminae only) and stems (including petioles) of the high-concentration mannitol and sodium chloride treatments, expressed as a percentage of the isotope concentration in the substrate. Values for samples 5–9 inclusive are given relative to the substrate concentration at sample 4.

during stage 1. From Figure 2*A* it is apparent that the osmotic potentials increased rapidly in all treatments as soon as the substrates were imposed and, except in M_1, reached levels which exceeded the control by approximately the same amount as those of the imposed substrate treatments exceeded the base culture solutions.

An increase in osmotic potential can follow the absorption of osmotically active solutes from the substrate, metabolic changes in materials already in the plant, or reduction of internal plant water content. In order to evaluate the contribution of the latter factor, Figure 2*B* has been constructed by subtracting the osmotic

potential which can be attributed to changes in internal water content (measured as leaf fresh weight—leaf dry weight) from the total osmotic potential. This procedure indicates that the marked reduction in water content in M_1 and M_2 was responsible for about 30 and 40% respectively of the total increase observed. In the other treatments the contribution due to reduced water content was insignificant, since these values were the same as the control by the end of stage 1.

The initial marked increase in internal osmotic potential during stage 1 (due to factors other than dehydration) in the sodium chloride, potassium nitrate, and sucrose treatments, compared with the relatively small increase in the mannitol treatments, suggests that there was greater absorption of osmotically active solutes in the former treatments. In Figure 3, data from the isotopically labelled N_2 and M_2 treatments supports this impression and provides the interesting result that in the case of N_2, the concentration of ^{36}Cl in the leaves (laminae only) and stems (including petioles) was approximately equal to the concentration in the substrate by the time sample 4 was taken. In the case of M_2, uptake was much slower and at sample 4 the concentration of ^{14}C in the leaves of the plant was only 25·4% of the substrate concentration. The final concentration in the stems was also much lower (8·3% of substrate concentration) than that of ^{36}Cl. In both the chloride and mannitol treatments initial concentrations developed relatively more rapidly in the leaves than the stems.

After the removal of the osmotic substrates the osmotic potential values declined, but only in the case of S_1 was the control level reached by the end of the experiment. Decline in osmotic potential could have been due to metabolism of absorbed solutes, increase in volume of water in the plant, or loss of solutes to the substrate. In Figure 2*C*, the primary data for leaf water content is plotted for the different treatments. After the removal of the substrates it is apparent that there was a marked increase in water content in all treatments, and that the relative increase was most marked between samples 4 and 5. These results indicate a similar pattern to that of the decline in osmotic potential and suggest that volume was the primary factor involved. In Figure 4 data for water content, osmotic potential, and isotope concentration during stage 2 are plotted for all treatments relative to the values at sample 4. This confirms the close relationship between the three quantities, but indicates a general tendency for change in water content to exceed the change in osmotic potential.

During stage 2 it is of interest to note from Figure 3 that the concentration of ^{36}Cl in the stems dropped by approximately 40% and the concentration in the leaves by about 25%. This evidence of increased mobility of the material in the stems was more apparent in the mannitol treatments in which the stem concentration dropped to about one-quarter of its value at sample 4. In the leaves the ^{14}C concentration declined by about 40%. In both cases the reduction in concentration in the leaves would appear, from Figure 4, to be primarily due to the increase in the volume of water in the plant, but the marked reduction in stem figures suggests that migration of the ^{36}Cl and ^{14}C from the stems was also operative.

Although it was thought unlikely, the possibility of leakage back to the standard substrates was checked by determining the ^{14}C and ^{36}Cl concentrations in the sub-

strates on the sampling occasions 5–9. Leakage of ^{14}C was found to be negligible, (the counts not differing significantly, at $P = 0{\cdot}05$, from background). In the case of ^{36}Cl, leakage increased progressively but was so small that the total amount of chloride represented would not have reduced the osmotic potential in the leaves of the plant by more than 0·2 atm.

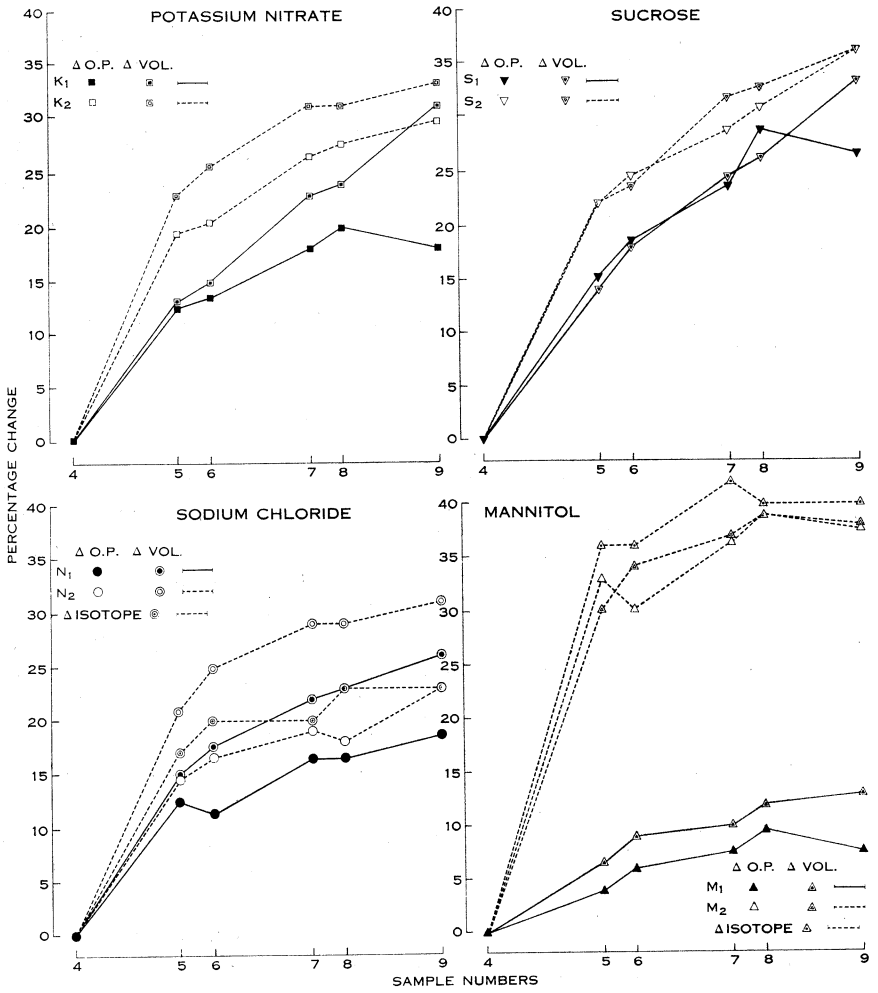

Fig. 4.—Changes in internal osmotic potential (Δ O.P.), isotope concentration (Δ isotope), and leaf water content (Δ volume) during stage 2, relative to values at sample 4.

The influence of the osmotic treatments on subsequent plant activity was difficult to determine during such a short experimental period but in Figure 5 data on transpiration and growth (measured as increase in dry weight) are presented.

The transpiration data reflect the marked reduction in water absorption at the beginning of the experiment and the rapid increase in water absorption as the plants regained water content and turgor. In all the low-concentration treatments

except M_1, the transpiration values were close to those of the control by sample 4. In all the high-concentration treatments except M_2, rapid recovery occurred during stage 1 but the values were still significantly lower than the control at sample 4.

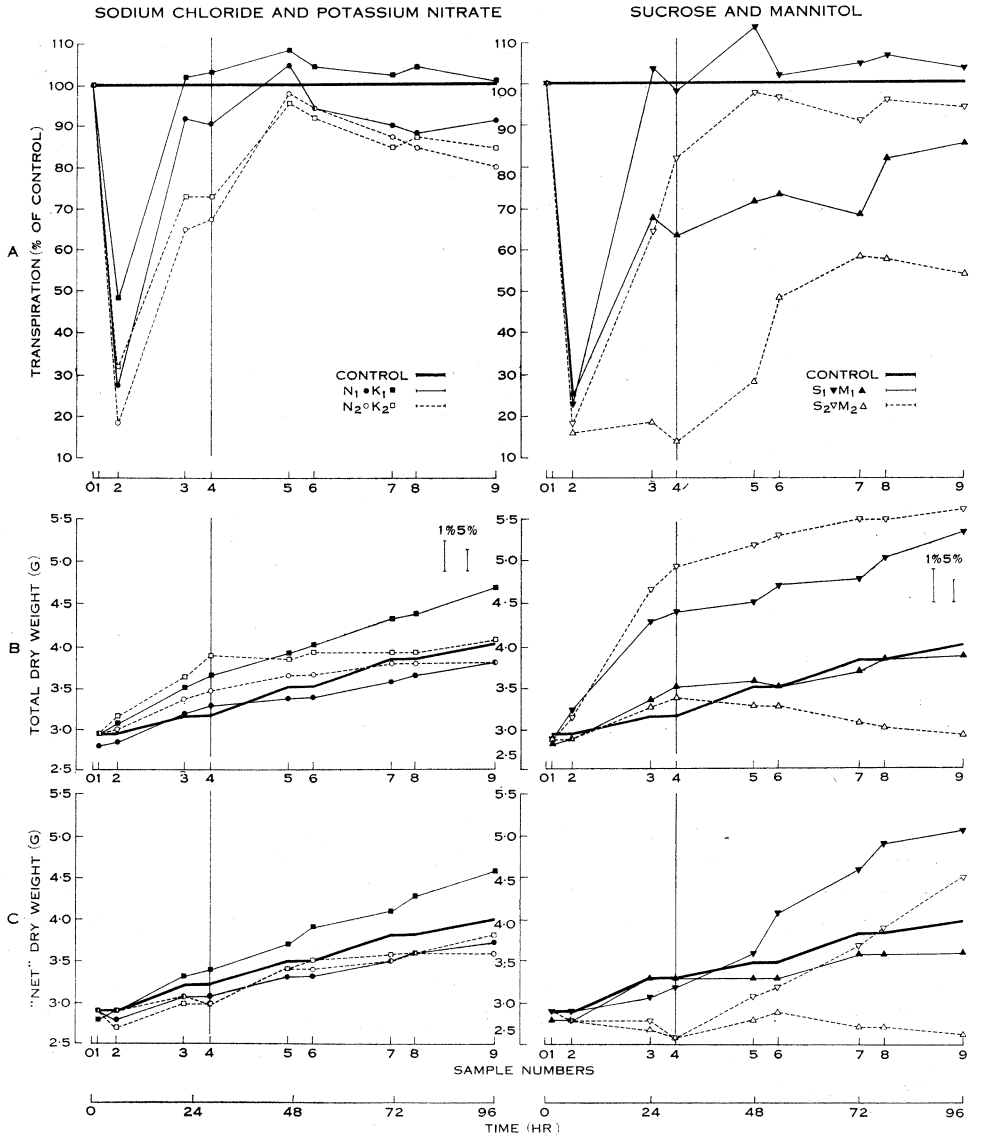

Fig. 5.—Changes during the experimental period in: *A*, transpiration, expressed as percentage of the control; *B*, total dry weight per plant; *C* "net" dry weight (for explanation see text, p. 530).

The lowest values recorded, approximately 20% of the control, may well be associated with complete stomatal closure since this low value was maintained, in the M_2 treatment, until the removal of the osmotic substrates. After sample 4, transpiration

values in all treatments increased further, reaching levels significantly higher (at $P = 0{\cdot}05$) than the control in K_1 and S_1. In these treatments transpiration values did not fall below the control for the remainder of the experiment, but in all other treatments final values were below the control and the transpiration of the high-concentration treatments declined slightly towards the end of the experiment.

The dry weight data of Figure 5*B* indicate that, during stage 1, increase in dry weight in some treatments was much more rapid than the control and in no treatment was slower than the control. However, this result was undoubtedly influenced by the fact that stage 1 was the period of rapid absorption of osmotically active solutes and the increases cannot be regarded entirely as due to photosynthetically induced dry weight increase. Subsequently the rate of increase appeared similar to the control in all treatments except K_1 and S_1, in which it was more rapid, and in M_2, in which it was markedly depressed.

In order to give an indication of photosynthetically induced dry weight increase Figure 5*C* has been included. This diagram has been constructed by subtracting, from the data of Figure 5*B*, amounts equivalent to the weight of treatment solute which is represented by the observed increase in osmotic potential. (Data for this calculation were based on Figure 2*B* and excluded dehydration as a factor causing increased osmotic potential.) This procedure involves the assumption that only the added solute, in each treatment, contributed to the increase in osmotic pressure. Although an oversimplification, this is of value in that it indicates that "net" growth rates were significantly depressed by the osmotic substrate treatments in N_2, M_2, and S_2. The extent of the apparent depression in S_2 and M_2 is much greater than anticipated and would appear to provide good evidence that only part of the observed increase in osmotic potential in these treatments was due to absorption of sucrose and mannitol respectively. In stage 2 the data of Figure 5*C* are in good agreement with those of Figure 5*B*, although they suggest that subsequent growth in both sucrose treatments was significantly more rapid than the control.

All treatments were observed for 1 month beyond the end of the experimental period. At this time the general pattern of growth depicted in Figure 5 was still generally valid, although both sodium chloride and mannitol treatments were smaller than the control and the potassium nitrate and sucrose treatments did not appear significantly different. In N_2 and M_2 the leaves which were on the plants at the end of the experimental period did not fully regain the colour and vigour of the control, and the new leaves were smaller and the growth rate slower. Radioautographs of all leaves at this time indicated some migration of chloride and mannitol into the sixth and seventh leaves but virtually no evidence of radioactivity in subsequent leaf development.

IV. Discussion

At the present time there is general agreement in the literature that the exposure of plants to substrate solutions or soils containing soluble salts (or other osmotically active solutes) in excess of those required for normal growth usually results in decreased water absorption, disturbed nutrient uptake and metabolism, and reduced growth. It is also generally agreed that when soluble salts are the osmotically active agents the effects on plants are of two main types, due in part to the direct osmotic

effects of increased soil or substrate water stress and in part to specific toxic effects of individual ions (Magistad 1945; Hayward and Wadleigh 1949; Bernstein and Hayward 1958).

Evidence for the influence of direct osmotic effects may be found in the numerous experiments conducted with iso-osmotic concentrations of different mineral salts and organic solutes in which the degree of inhibition of growth has effectively been the same regardless of the solute employed (Eaton 1941; Long 1943; Magistad *et al.* 1943; Gauch and Wadleigh 1944; Hayward and Spurr 1944*a*, 1944*b*). It is also supported by those studies concerned with the influence of increasing total soil moisture stress on plant growth in which the effect has been the same regardless of whether the total stress was composed mainly of soil moisture tension or mainly of an osmotic potential in the soil solution (Ayers, Wadleigh, and Magistad 1943; Wadleigh and Ayers 1945; Wadleigh, Gauch, and Magistad 1946).

In order for the osmotic potential of the soil or substrate solution to contribute to the water stress around the roots in the same manner as soil moisture tension, it would appear to be necessary for the plant to act as an ideal osmometer so that osmotically active solutes in, or added to, the substrate remain outside the plant–water system and induce an additional internal water stress of equal magnitude to their own osmotic potential. If, on the other hand, the solutes are diffusible into the plant–water system it would seem that a water stress of equal magnitude could still be induced, but that the degree to which it simulated soil water tension would vary with the amount of solute absorbed and the degree to which the internal osmotic potential was consequently increased.

It is apparent that in the strictest sense the ideal osmometer concept is not valid since mineral nutrients must be absorbed by the plant. However, Bernstein and Hayward (1958) have suggested that a layer or layers of plant cells may exist in the root defining the limits of the free space. These cells would transmit water into the plant water system along normal DPD gradients but would restrict the absorption of certain ions, thereby building up, in the outer layers, a concentration which would prevent further diffusion of a particular ion into the plant and may even promote outward diffusion to the root medium if the concentration became high enough. The existence of a barrier such as this, which can offer high resistance to the passage of ions across the symplast of the plant roots, has also been indicated by the work of Scott Russell and Shorrocks (1959) and could probably effectively control ion and solute absorption. However, it would seem that such a barrier would still have to prevent the absorption of solutes, other than those required for normal growth and metabolism, to effectively simulate soil water tension effects in the induction of internal water stress.

If such a barrier exists and is differentially permeable as far as osmotic solutes are concerned, one would envisage that the water relations of plants, exposed to substrates in the same manner as in the present experiment, would respond as shown in Figure 6*A*. If, however, no such obstacle exists and the solutes are freely diffusible into the plant, but not freely diffusible back to the substrate, one would expect the water relationships to respond as shown in Figure 6*B*.*

* The terms "diffusible" and "non-diffusible" are used in this paper for convenience and are not intended to imply that diffusion is the only, or primary, factor involved in solute entry.

From the results presented earlier it is apparent that the response pattern of the plants to potassium nitrate, sodium chloride, and sucrose followed closely that suggested in Figure 6*B*. Mannitol, on the other hand, caused a response pattern intermediate between the two type situations as might be expected from its reduced

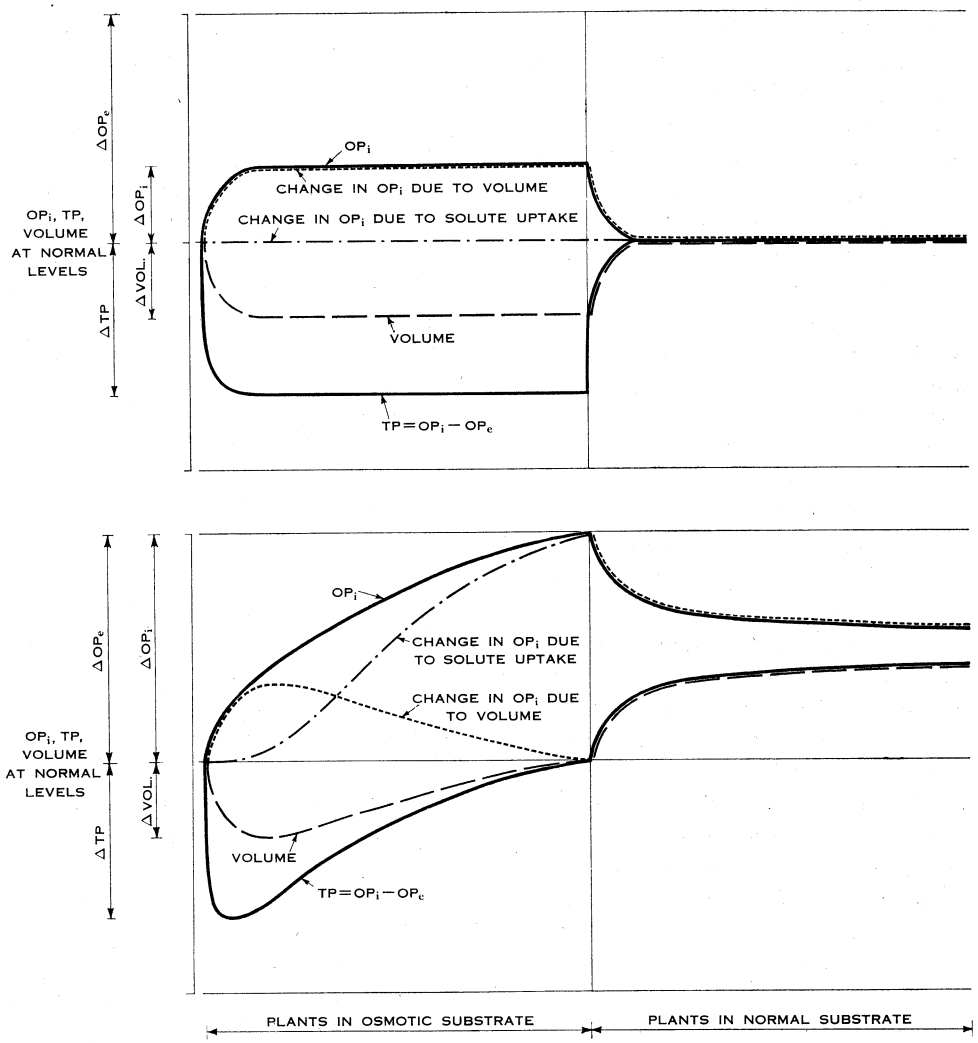

Fig. 6.—Diagrammatic representation of expected changes in internal water relations of plants exposed to a non-diffusible osmotic substrate (*A*), or exposed to an osmotic substrate in which solutes are freely diffusible into the plant but non-diffusible from plant to substrate (*B*). TP and OP_i represent the turgor pressure and osmotic potential, respectively, and OP_e represents the increase in substrate osmotic potential induced by the addition of osmotically active solutes.

diffusibility. In both cases it is apparent that the plant DPD, represented by the difference between the internal osmotic potential and turgor pressure, was maintained at approximately the level of the substrate osmotic potential. However, the

response patterns of relative turgidity, water content, turgor pressure, and internal osmotic potential differed in several important respects and it is consequently of value to discuss these relationships in more detail.

(a) Significance of Water Content and DPD Changes

Of particular interest is the fact that, during stage 1, visual recovery of turgor occurred in the potassium nitrate, sodium chloride, and sucrose treatments without compensating recovery in DPD. It appears that the type situation of Figure 6*B* provides a valid explanation of this phenomenon and suggests a close, but expected, relationship between tissue water volume and turgor pressure. Thus, initially, turgor pressure and volume decreased, from the direct osmotic effects of the imposed substrates, until the DPD approximately equalled the external osmotic potential. This was associated with severe wilting of plants in the high-concentration treatments. Subsequently, although the DPD remained at this level, solute entry proceeded and internal osmotic potential, turgor pressure, and volume increased until the latter two quantities regained their original values. At this point it is apparent that the normal appearance of the treatment plants was to be expected, even though the tissue was not turgid; the reduction in turgor pressure below the value at full turgor being equivalent in magnitude to the imposed external osmotic potential.

In the case of non-diffusible substrates, it is suggested in Figure 6*A* that the initial response should be similar to that just described, but the absence of solute entry should preclude any increase in internal osmotic potential apart from that due to reduced volume. Thus, turgor pressure and volume would remain below the original levels and a direct osmotic stress would be imposed on the plants.

It appears that the mannitol treatments responded in a manner between the two type situations in that, although some solute uptake occurred, there was no significant recovery in volume by either M_1 or M_2 during stage 1. In M_2, not only volume, but also relative turgidity continued to decline steeply, and DPD continued to increase. This was unexpected since it was anticipated that DPD should not increase to a value much beyond the substrate osmotic potential and that any absorption of mannitol would result in an increase in volume. Consequently, it appears the continued decline in the internal water balance was due to toxic side effects of the high concentration of mannitol. The subsequent response pattern of the plants in the M_2 treatment supports this view. Even so, it is of interest to note the close relationship between DPD and internal osmotic potential in M_2 as leaf volume and relative turgidity levels continued to decrease; the DPD values at samples 2 and 4 being 14 and 22 atm and the osmotic potential values 13·2 and 21·0 atm respectively. This provides evidence that negative wall pressure was not of significance at stress levels greater than those prevailing when the DPD first rose to the level of the internal osmotic potential.

In all cases other than M_2, DPD increased to values of approximately 7 atm in the low-concentration treatments and 12 atm in the high-concentration treatments and were maintained at these levels throughout stage 1. When tissue segments are floated in aqueous solutions containing diffusible solutes, it is normally assumed that the tissue DPD equilibrates with the osmotic potential of the solution. However,

Weatherley (1955), in a study of sucrose uptake by floating leaf disks, found a higher DPD in the disks than in the surrounding solution and concluded that the sucrose was absorbed actively and that water entered the tissue passively along a diffusion pressure gradient. It is possible that a similar mechanism operated in the present instance, although the DPD in the control plants averaged 3 atm and it could be argued that the imposition of the osmotic substrates merely served to increase the treatment DPD's by similar amounts.

(b) Characteristics of the Changes in Internal Osmotic Potential

From Figures 2 and 3 it is apparent that internal osmotic potentials increased rapidly in all treatments as soon as the osmotic substrates were imposed. Initially, this appeared to be due primarily to the direct effect of dehydration on the concentration of the tissue fluids since the data of Figure 3 indicate that little ^{36}Cl and ^{14}C was absorbed in the first hour. Subsequently, however, solute entry proceeded rapidly, and it is apparent that (from Fig. 2*B*) by the end of stage 1 virtually all of the increase in osmotic potential in the non-mannitol treatments could be attributed to absorption of osmotically active solutes from the substrate or to metabolic changes within the plant.

Volume recovery and increase in osmotic potential was most rapid in the sodium chloride and potassium nitrate treatments and probably reflected the more rapid uptake of these inorganic solutes than of sucrose. This is in accord with other studies (Beck 1927) even though Weatherley (1955) has shown that sucrose uptake by leaf tissue can result in a virtual doubling of the dry weight within 24 hr. In the case of sodium chloride it is apparent from Figure 3 that the increase in osmotic potential was almost entirely due to uptake of chloride and probably also of sodium ions. This is in agreement with other studies (Eaton 1942; Black 1956) in which chloride concentrations in the plant have reached values as high or higher than in the substrate. No evidence is available to indicate to what extent absorption of KNO_3 or of potassium or nitrate ions was responsible for the osmotic potentials developed in the K_1 and K_2 treatments, but, because of its high molecular weight, indirect evidence from Figure 5*C* indicates that, in the case of sucrose, only part of the increase in osmotic potential was due to sucrose uptake and the remainder was presumably due either to stimulated uptake of inorganic solutes, polysaccharide breakdown, or conversion of the absorbed sucrose to hexoses.

Compared with the other osmotic substrates, the relative contribution of absorbed solutes to the total internal osmotic potential was much smaller in the mannitol treatments. From Figures 2*A* and 2*B* it can be seen that about 40% of the M_2 value at sample 4 could be attributed to the direct effect of reduced water content and in M_1 the figure was approximately 30%. However, the ^{14}C absorption data indicate that the concentration of ^{14}C in the M_2 plants at sample 4 was only 25% of the substrate concentration. This suggests that a significant proportion of the observed osmotic potential may have been due to absorption of other solutes from the substrate, or to breakdown of an absorbed polymer, as suggested by Thimann, Loos, and Samuel (1960). The data of Figure 5*C*, interpreted previously as in the case of sucrose, support this view.

It is of interest that these authors found little evidence of mannitol uptake by potato tissue disks, except in the free space. It is possible that the relatively substantial uptake observed in M_2 may have been associated, to some extent, with tissue injury.

At the end of stage 1 it is of interest to examine the differentials between the osmotic potentials in the leaf tissue of the treatment plants and in the various substrates, in comparison with the differential between the osmotic potential in the control plants and in the base nutrient solution. These data are listed in Table 1 and demonstrate that in all except the low-concentration mannitol treatment the differential was maintained in all treatments within 1 atm of the control value.

TABLE 1

OSMOTIC POTENTIALS OBSERVED IN ALL TREATMENTS AT SAMPLE 4 COMPARED WITH SUBSTRATE POTENTIALS

Substrate	Low-concentration Treatments				High-concentration Treatments			
	Level	O.P. of Substrate (atm)	O.P. of Expressed Sap (atm)	Difference (atm)	Level	O.P. of Substrate (atm)	O.P. of Expressed Sap (atm)	Difference (atm)
Potassium nitrate	K_1	5·7	14·9	9·2	K_2	10·7	20·8	10·1
Sodium chloride	N_1	5·7	16·7	11·0	N_2	10·7	21·6	10·9
Mannitol	M_1	5·7	13·0	7·3	M_2	10·7	21·0	10·3
Sucrose	S_1	5·7	15·2	9·5	S_2	10·7	19·7	9·0
Control		0·7	10·9	10·2		0·7	10·9	10·2

Although this has previously been demonstrated by Eaton (1942), and was to be expected if the response pattern conformed to the "freely diffusible" model, it provides strong evidence of the degree to which absorption of osmotically active solutes can lead to the re-establishment of turgor pressure and tissue volume at normal levels.

In both mannitol treatments it was expected that the differentials would be less than with the freely diffusible substrates. Although this applied in M_1 it was not apparent in M_2 presumably because of the physiological damage to the plants in this treatment. Thus the M_2 differential can be regarded, not as representative of an undamaged plant in a stable plant–substrate water balance, but rather as representative of a plant in which the water balance was progressively deteriorating as long as it was exposed to an osmotic substrate. To this extent it appears to have been fortuitous that the differential at sample 4 was so similar to those of the other treatments.

Following the removal of the osmotic substrates the mannitol treatments responded almost as anticipated from Figure 6*A* and those in the potassium nitrate, sodium chloride, and sucrose treatments as anticipated from Figure 6*B*. In all cases a marked recovery occurred in relative turgidity and DPD, these quantities reaching levels not significantly different to the control within 24 hr (except in the case of M_2 where recovery was still incomplete at the end of the experimental period). Associated with these responses were substantial increases in volume (as water content) and reciprocal changes in internal osmotic potential.

The decline in osmotic potential and isotope concentration during stage 2 (Figs. 2, 3, and 4) could have been caused, apart from increases in volume and consequent dilution, by either leakage of osmotically active solutes back to the base nutrient solutions or by their metabolic incorporation. However, leakage of ^{14}C and ^{36}Cl was found to be almost negligible in the high-concentration mannitol and sodium chloride treatments and the work of Long (1943) in a similar experimental sequence suggests that leakage in the potassium nitrate and sucrose treatments would also have been very small.

Some idea of possible metabolic effects can be gained from Figure 4 in which a general tendency for volume changes to exceed changes in osmotic potential can be seen. This suggests that renewed growth of the plants in stage 2 was possibly associated with renewed mineral uptake from the culture solution and accumulation of osmotically active photosynthetic products. Such processes would be expected to contribute to both tissue expansion and osmotic potential and would tend to cause a trend of the type observed. The fact that this effect was most marked in the potassium nitrate and sucrose treatments, in which most rapid growth occurred, would support this contention as does the evidence that decline in isotope concentration in M_2 and N_2 was related to volume more closely than was osmotic potential.

The metabolic incorporation of the osmotic substrate solutes into non-osmotic compounds would be expected to result in reduced osmotic potential relative to volume except in the case of sucrose, since the normal fresh weight/dry weight ratio is of the same order as the water volume/sucrose ratio expected at the prevailing levels of osmotic potential. It is doubtful if significant incorporation of this type occurred in the non-sucrose treatments and the data of Figure 4 would, in summary, appear to provide satisfactory evidence of an effectively reciprocal relationship between changes in osmotic potential and volume in stage 2.

(*c*) *Relative Turgidity and Water Content Relationships*

The relative turgidity changes were associated, in M_1 and M_2, with water content and turgor pressure recovery towards the level of the control plants whereas in other treatments water content, which was already at the control level, increased with a proportional increase in turgor pressure as would be expected from Figure 6*B*. In all treatments except M_2 it is of interest to observe that the relative turgidity levels remained fairly constant during stage 1. This suggests that the reduction in volume during the initial period of reduced turgor measured, in the case of tissue disks, by the amount of water uptake during the course of the relative turgidity

determination, was equivalent to the increase in volume which occurred when disks taken at sample 4 were floated on water. In turn, this implies that the volume of the leaf tissue as a whole should have responded similarly when the plants were returned to base culture solution. This is generally borne out in the leaf water content data of Figure 1*C*, although the increase in water content in stage 2, relative to the control, was slightly greater than the initial decrease in stage 1 and the generally more rapid growth in the control plants compared with most treatments would, if anything, tend to enlarge this difference.

It was thought that a too-short period of floating during the determinations could have contributed to the apparent constancy of relative turgidity during stage 1 if water uptake and tissue expansion became slower as tissue volume regained its original value. Data relevant to this point were available from samples 1, 4, 5, 7, and 9 in which a 24-hr period of floating was adopted in addition to the standard 4-hr period. These determinations revealed that, in the present experiment, although there was a tendency for more water uptake, after the standard 4-hr period, in the high-concentration treatments compared with the low, the slopes of the different uptake curves in each treatment, from the various sampling occasions, did not differ significantly after the standard 4-hr period. Leakage of solutes from the floating leaf disks to the surrounding water was also investigated as a possible contributing factor by measuring the electrical conductivity of the water before and after floating and the dry weight of dissolved solids after the water had been evaporated. Although some leakage occurred the quantities were not sufficient to significantly affect the relative turgidity values obtained.

(*d*) *Transpiration and Growth*

The data of Figure 5 on transpiration and growth warrant comment on several points. It is of interest that reduction of water absorption was closely related to turgor pressure and water content and once recovery of these quantities commenced water absorption rapidly returned to values close to normal. This is similar to the results of Renner (1912) and Montfort (1920) but is at variance with findings by some other workers (Eaton 1941; Hayward and Spurr 1944*b*) in long-term experiments where continued reduction of water absorption was observed as long as the osmotic treatments remained. In such cases Hayward and Long (1941) have noted damage to the absorbing zone of the roots and it is probable that a similar effect would have been observed in the present study had the treatments been prolonged. The present results provide good support for transpirational control of absorption, since the degree of stomatal closure could be exected to be generally proportional to the turgor pressure in the leaf tissue. In stage 2 of the experiment transpiration in excess of the control was observed in K_1 and S_1 treatments. A similar response was observed by Rybin (1923) but in this instance it appeared to be due simply to the enhanced growth and greater plant size in these treatments. By comparison, transpiration in all the high-concentration treatments declined towards the end of the experiment. Although this possibly indicated toxic side effects, the tendency to relatively slower growth and smaller plants in these treatments provides a simpler explanation.

The data on dry weight changes in Figure 5*B* are difficult to interpret during stage 1 because of the considerable quantities of osmotic solutes absorbed. However, from the data of Figure 5*C* it appears doubtful if significant reductions occurred. During stage 2, when no further uptake of this type was possible, increase in dry weight appeared slower in all the high-concentration treatments relative to the low, confirming the results of other studies (Bernstein and Hayward 1958). From Figure 5*B* two treatments (K_1 and S_1) appeared to have more rapid rates of increase than the control due probably to the fact that during stage 1 these substrates provided low concentrations of solutes which were also plant nutrients. From Figure 5*C* it appears that S_2 also increased more rapidly than the control in stage 2. Since this treatment was characterized by a rapid reduction in osmotic potential it is probable that some of the absorbed sucrose was metabolized by the plants. Only in M_2 did dry weight decline in stage 2.

(*e*) *Concluding Remarks*

In conclusion it seems appropriate to emphasize that the plant responses to the potassium nitrate, sodium chloride, and sucrose treatments closely paralleled those to be expected if the added solutes were assumed to be freely diffusible into the plant. The responses to mannitol, while similar in some respects to those of a model based on non-diffusibility, differed to the extent that some mannitol was absorbed and the plants, to this degree, responded in an intermediate manner. These results suggest that Walter's (1955) premise, that if the substrate solutes are diffusible the osmotic potential is balanced by solute entry, is valid to the extent that turgor pressure and tissue volume returned to their original values after an initial period of adjustment. However, it is apparent that DPD did not return to normal and instead was maintained at the level of the imposed substrate osmotic potential. Hence the plants were not turgid and the turgor pressure appeared to be depressed below its value at full turgor by an amount equal in magnitude to the osmotic potential of the substrate. Thus, although the osmotic effect of diffusible substrates appears to be real in the sense that a water stress is induced in the plant, it is not strictly analagous to the effect of soil water tension since the osmotic potential and turgor pressure levels are displaced.

It seems that the present results are of value in interpreting the extensive literature dealing with the effects of osmotic substrates on plant growth since the *absolute* reduction in turgor pressure below the internal osmotic potential appears to be equal in magnitude to the imposed substrate potential, regardless of whether this is due to diffusible or non-diffusible solutes, and it therefore appears logical to expect that growth inhibition should increase with increase in substrate concentration. However, the *relative* reduction in turgor pressure decreases proportionally with the amount of solute absorbed and hence with the increase in internal osmotic potential. Thus, when diffusible solutes are involved, it seems that the analogy between water tension effects and osmotic effects becomes less valid as substrate concentration is increased and to this extent the attribution of growth inhibition and other plant responses entirely to direct osmotic effects should be undertaken with caution.

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VI. References

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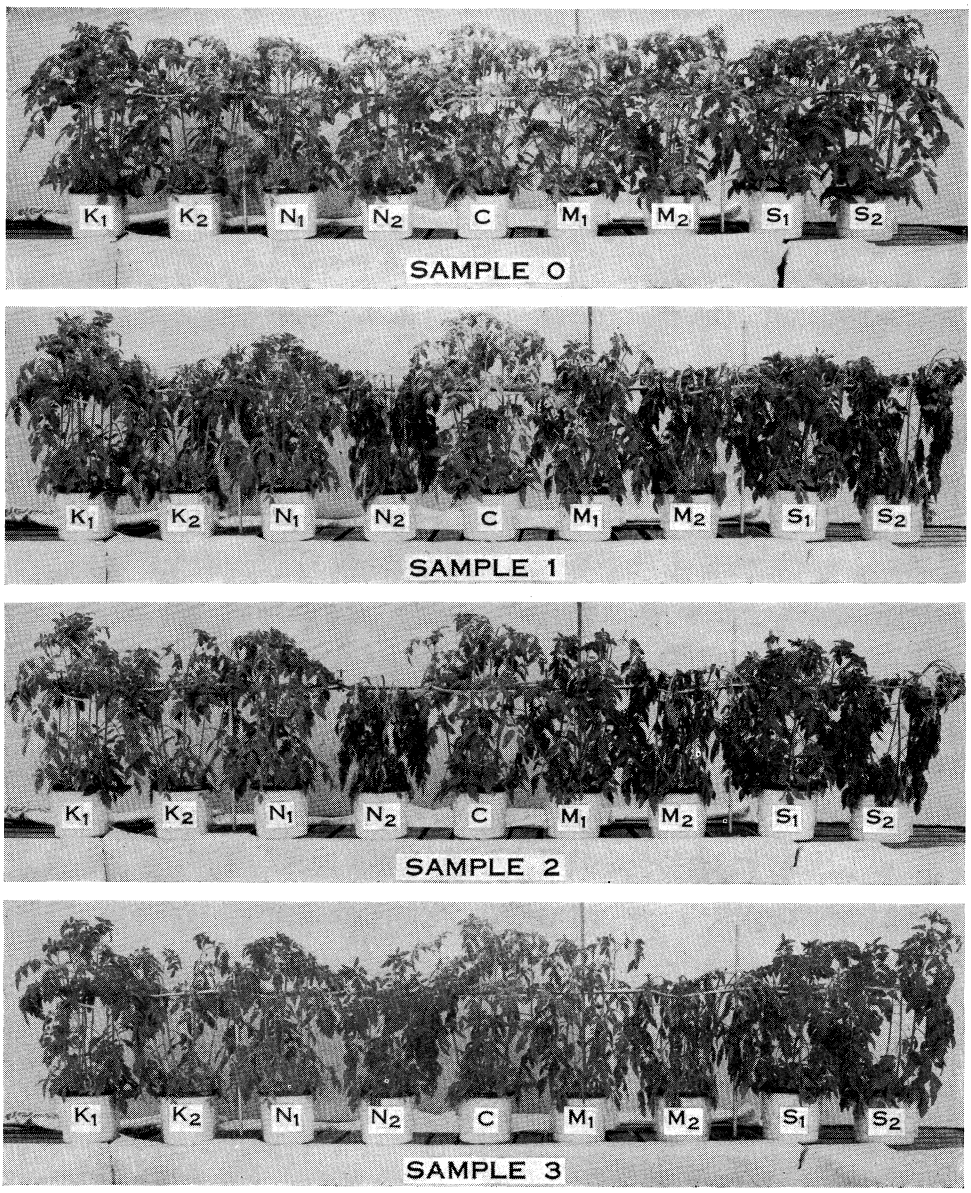
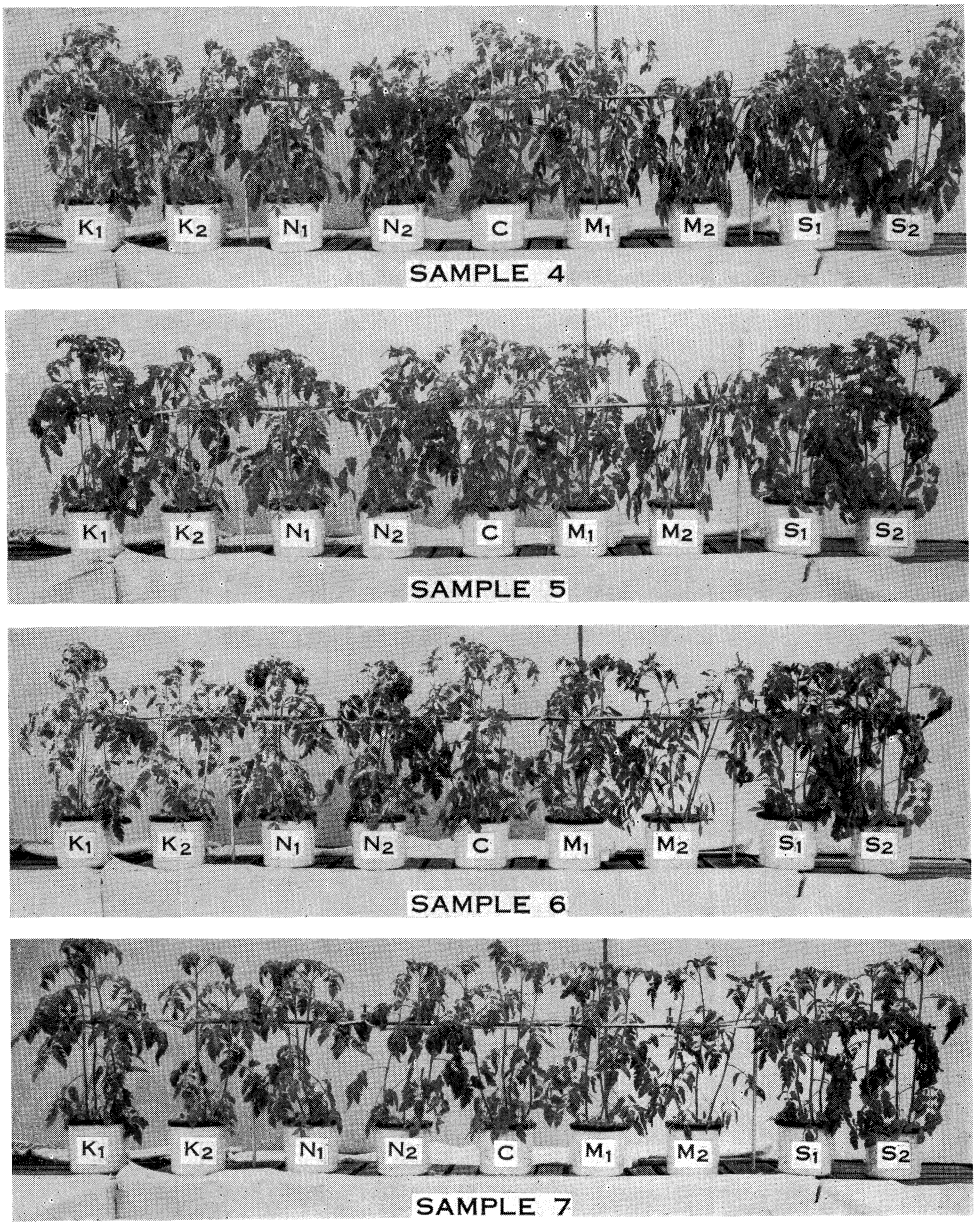
PLANT–WATER RELATIONS IN OSMOTIC SUBSTRATES



Visual appearance of tomato plants at sample numbers 0–3. Treatments are indicated on the containers, using the same key as in the text. C, control.

PLANT–WATER RELATIONS IN OSMOTIC SUBSTRATES



Visual appearance of tomato plants at sample numbers 4–7. Treatments are indicated on the containers, using the same key as in the text. C, control.