

THE INFLUENCE OF PROGRESSIVE INCREASES IN TOTAL SOIL MOISTURE STRESS ON TRANSPIRATION, GROWTH, AND INTERNAL WATER RELATIONSHIPS OF PLANTS

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Summary

The responses of tomato (*Lycopersicon esculentum* (Mill.), privet (*Ligustrum lucidum* Ait.), and cotton (*Gossypium barbadense* L.) to conditions of increasing total soil moisture stress were measured in terms of vegetative growth, stem elongation, transpiration, leaf turgor, diffusion pressure deficit, and osmotic pressure.

The response pattern of each species showed a close relationship to water stress, and in each species growth (as total dry weight) did not continue beyond a stress value such that there was zero turgor pressure in the tissue of adult leaves. In privet and cotton, stem elongation also ceased at this value; in tomato it continued until higher stress levels developed. As permanent wilting of the leaves was also associated with the point of zero turgor pressure, these results indicate that the permanent wilting percentage of any one soil is determined by the osmotic characteristics of the plant under study rather than by any soil characteristic. Permanent wilting occurred at soil water contents of 11.8, 9.7, and 10.2 per cent. in tomato, privet, and cotton respectively, equivalent to total soil moisture stress values of 20, 38, and 48 atm. The soil water content at 15 atm stress was 12.2 per cent.

At high stress levels there was strong evidence of nocturnal absorption of water directly from the atmosphere, and its accumulation in the tissues of the shoots of two of the species. Although a diffusion gradient appeared to exist favouring movement of water out of the plant into the soil no evidence of such movement was obtained. This is attributed to the development of discontinuity in root-soil contact.

I. INTRODUCTION

There is now general agreement that soil water becomes progressively less available to plants as total soil moisture stress (TSMS) increases (Richards and Wadleigh 1952). Evidence to the contrary (Veihmeyer and Hendrickson 1950; Veihmeyer 1956) is based almost entirely on field experiments where availability is complicated by uneven root distribution, and the measurement of plant response cannot be conducted accurately or readily interpreted. Depending on the sensitivity of stomatal closure, the effectiveness of stomatal control, and on the actual rates involved, transpiration is generally less affected by low levels of soil moisture stress than is growth or metabolism, since transpiration is primarily a passive phenomenon controlled by atmospheric conditions and rate of movement of soil water to the absorbing surfaces of the roots.

The diffusion pressure deficit (DPD) (Meyer 1945) in the plant system can never fall to a value less than that of the TSMS unless there is direct absorption of water from the atmosphere by the plant leaves, since the absorption gradient from

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soil to plant is eliminated at the point of DPD-TSMS equilibrium. Thus increase in TSMS results in an associated increase in DPD in the plant, with consequent loss of turgor. It is this progressive decrease in turgor pressure, in the cells of the active tissues, which has direct and indirect effects on most plant processes.

Data from controlled experiments provide direct evidence of these effects. Reductions in rate of growth (Davis 1942; Wadleigh and Ayers 1945; Haynes 1948; Salter 1954; Gates 1955*a*, 1955*b*) and elongation (Furr and Reeve 1945; Wadleigh and Gauch 1948) have been observed to commence at low TSMS values and to become more pronounced with increasing TSMS. The effect of even small water deficits on stomatal closure (Magness, Degman, and Furr 1935; Oppenheimer and Elze 1941) is reflected in decreased rates of transpiration (Chung 1935, Martin 1940), and photosynthesis is affected through the general effects of soil moisture stress (Schneider and Childers 1941; Loustalot 1945), and through the direct effect of reduced tissue hydration (Walter 1929; Rabinowitch 1945).

In general, most studies have been concerned more with the stress value at which reductions first occurred, and with the rate of decrease with increasing stress, than with the stress value at which the particular process ceased. It has been generally assumed that growth ceases at a stress value close to that at the permanent wilting percentage (Veihmeyer and Hendrickson 1950). Slatyer (1957*a*) has suggested that permanent wilting occurs at a stress value such that there is zero turgor pressure in the leaf tissue, and that this stage would also represent a probable end-point for most of the assimilatory and developmental processes which are integrated in vegetative growth. This implies that the permanent wilting percentage is a value determined not by any particular soil characteristic but by the osmotic characteristics of the plants under study, and as a result could vary considerably from plant to plant.

The present experiment was designed to examine the response patterns shown by three different plant species to conditions of increasing soil moisture stress, and to interpret these patterns in terms of the energy relations of the plant-soil water system and the turgescence of the plant tissues. Particular attention was paid to the significance of the permanent wilting percentage as a valid indicator of the lower limit of soil water availability, and to the internal water relations of the species at very severe TSMS levels.

II. EXPERIMENTAL METHODS

(a) General

Seeds of cotton (*Gossypium barbadense* L. var. Pima S1) and tomato (*Lycopersicon esculentum* Mill. var. Marglobe) and year-old plants of privet (*Ligustrum lucidum* Ait.) were planted in prepared, fertilized soil in steel cans 8 in. in diameter and 8 in. high. Each can contained about 4.5 kg of soil and weighed about 5.5 kg when the soil was at field capacity. The cans were set out in a heated greenhouse in early winter and were grown until the plants were well established and the roots thoroughly penetrated the soil mass. Re-watering to field capacity took place when wilting of the lower leaves was evident at sunrise. This watering regime was utilized to pre-condition the plants to water stress. Each can was then given a final watering to bring the whole soil mass to a value slightly above field capacity. To prevent

direct evaporation losses, the top of each can was then covered with oilcloth and the gap around the stem was filled with cotton wool to minimize losses through this channel. The efficacy of these seals was such that on each of four control cans the total losses by direct evaporation during the final experimental period was less than 15 g. In order to minimize heating of the cans by direct sunlight, and to minimize movement of soil water in response to thermal gradients, the cans were placed on a bench which had raised sides, and the oilcloth on top of each can was itself covered with aluminium foil. The plants were then allowed to dry out the soil without further additions of water. Harvests were made 3 days before the last watering, on the day after the last watering, and at intervals of several days thereafter depending on the condition of the plants. At each harvest determinations were made of fresh and dry weight of the plant parts, stem length, leaf turgor, DPD and osmotic pressure of the leaves, and soil water contents. In order to keep the plants continuously in a vegetative phase all floral organs were removed as soon as they appeared. In addition, lateral growth in tomato was removed as soon as it appeared and the plants were trained to a single stem. All physical measurements which varied with temperature were corrected to 20°C, the basal temperature of the heated greenhouse.

(b) *Plants*

All harvests were made before sunrise on the sampling day. This procedure was adopted so that the plants had overnight to regain turgor, and to ensure comparative values from one harvest to the next. Eight replications were made of each of the rates or attributes.

At each harvest, measurements were made of the fresh and dry weights of the stems and leaves, dry weights of the roots, and of stem length. From these data total dry weights and stem lengths were used to obtain a picture of growth.

From the fresh material, 1.0-cm diameter leaf disks were punched from standard active adult leaves for determination of relative turgidity and DPD. The remainder of the leaves were wrapped in aluminium foil and frozen at -20°C for osmotic pressure determinations. After each of these determinations the appropriate fresh and dry weights were incorporated into the total dry weights for each plant. Relative turgidity was measured using Weatherley's (1950) technique. DPD was measured using a modification of the vapour equilibration method of Arcichovskij and Arcichovskaja (1931) described separately by Slatyer (1957*b*). Osmotic pressure determinations were made by cryoscopy on sap expressed from the thawed leaves using a hydraulic press at 15,000 lb/in² pressure. The agreement between replications in these determinations was very good, and at no harvest did the standard error of the mean values exceed ± 0.7 per cent. in the relative turgidity determinations, ± 2.3 atm in the DPD determinations, or ± 1.4 atm in the osmotic pressure determinations.

Transpiration was measured by daily weighings of all cans. The differences were corrected for the changing fresh weight of the plants, so that the data obtained represented net transfer of water from soil to atmosphere rather than total water loss from plant and soil.

(c) Soils

The soil used in this experiment was a sandy clay loam belonging to the White Store series (Perkins *et al.* 1924) of the Intrazonal Planosol group. It was selected for this experiment on the basis of prior soil moisture retention determinations which indicated that it had a fairly high soil moisture content below the value at 15 atm tension.

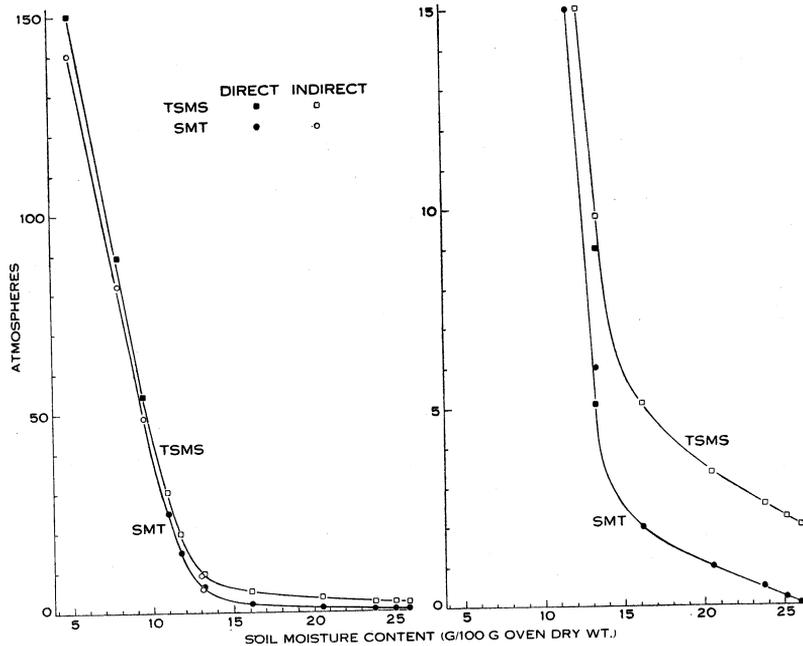


Fig. 1.—Soil water characteristic curves for the soil used in the experiment. The diagram on the right is a large-scale ($10\times$) version of part of the curve shown in the diagram on the left. The outside curve in each diagram represents the soil water content/total soil moisture stress (TSMS) relationship, the inside curve the soil water content/soil moisture tension (SMT) relationship. The key in the diagram indicates which of the points on the curves represent direct, and which indirect, determinations.

The soil was prepared by sieving three times through a $\frac{3}{8}$ in. sieve to provide as thorough mixing as possible. A complete fertilizer mixture was added during the final sieving.

At each harvest soil water content was determined by taking four cores each of $\frac{3}{4}$ in. diameter from each of eight cans. Two cores were taken near the stem and two near the edge of the can. The cores were divided into a top and bottom portion and the eight subportions were oven dried separately to obtain a gravimetric value for soil moisture content. At no stage was there any significant difference (at $P=0.01$) between the top and the bottom parts of each core or between the cores taken near the stem and those taken near the edge of the cans. This substantiated the visual indications of thorough root penetration.

Soil moisture tension values were obtained from separate undisturbed cores also taken from the cans at each harvest. Values up to 600 cm tension were obtained with a suction-plate apparatus (Richards 1942) and from 1 to 25 atm with pressure membrane equipment (Richards 1947). As it was desired to examine the reactions of the plant to total soil moisture stress rather than to soil moisture tension alone, the soil moisture tension values were adjusted to include osmotic effects. This was done in the manner suggested by Richards and Weaver (1944) who measured the osmotic pressure of the saturation extract and computed the osmotic pressure values applicable to any soil moisture content.

In addition TSMS was measured directly on soil cores by equilibrating them in vapour of known vapour pressure using a modification of the method of Thomas (1921) and Edlefsen (1934). The modification involved the use of micro-desiccators and very accurate temperature control, both of which improved the precision of the determinations. The direct determinations made in this manner were in good agreement with the indirect estimates which combined soil moisture tension and osmotic pressure determinations. In Figure 1 data for soil moisture tension and TSMS are plotted against soil moisture content. Soil moisture tension levels in excess of 25 atm were computed by adjusting the direct TSMS measurements for osmotic pressure.

Each point on these curves was made up from at least 24 separate determinations, and at 200 cm, 15 atm, and 150 atm 64 determinations were made. The standard error of the mean value of any one set of determinations did not exceed ± 0.16 per cent. soil moisture content.

On each sampling day, the soil moisture content was obtained from gravimetric determinations on the cores as described above, the mean value for any one harvest being obtained from 32 subsamples. TSMS and soil moisture tension values equivalent to the soil moisture contents were obtained from the subsequently derived calibration curves.

There was also a high order of accuracy in these determinations, the standard error of the mean at any one sampling occasion not exceeding ± 0.15 per cent. for any species. This variation was equivalent to not more than ± 3.2 atm TSMS even at high stress values.

(d) *Atmospheric Conditions*

A white atmometer and thermohygrograph were installed in the greenhouse on the same bench as the cans. This enabled estimates of evaporation, temperature, and relative humidity to be obtained. During the sampling period the daily maximum temperatures did not fluctuate beyond the range of 86–97°F, except for a cloudy period at the end of the experiment when temperatures fell to 80°F. On sunny days the greenhouse was opened as soon as the temperature approached 90°F. The minimum temperatures were held to the basal temperature of the heated greenhouse 65°F. Evaporation from the white atmometer ranged from 16–24 c.c. per day. Supplemental light was provided to give a 15-hr day to all plants.

III. RESULTS

Primary data from the three crops is presented in Figure 2. The first harvest shown on the diagrams was that made on the morning after the final watering. The date of the first harvest of privet was 2 days after tomato, and of cotton was 3 days after privet. This was done in order to avoid, where possible, two harvests on the one day, which presented considerable handling difficulty. Because the climatic conditions did not vary much over the experimental period the variation introduced by this procedure was slight.

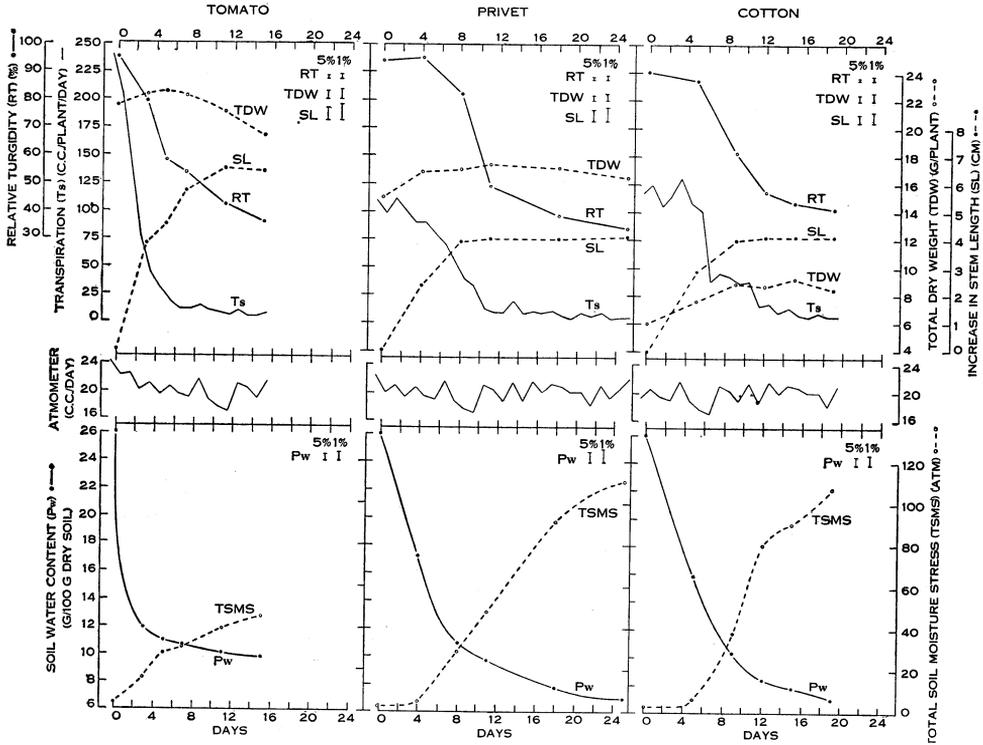


Fig. 2.—Growth (TDW), increase in stem length (SL), relative turgidity (RT), and transpiration (Ts) of tomato, privet, and cotton, in relation to soil water content (Pw) and total soil moisture stress (TSMS). Daily atometer evaporation is shown in the body of the diagram. When applicable, minimum significant differences at 5 per cent. and 1 per cent. levels are indicated.

The tomato plants were by far the largest at the beginning of the experimental period. As a result, transpiration per plant was initially much higher, and rapid soil moisture depletion caused an early onset of water stress in these plants. The first evidence of water stress in tomato was a sharp drop in transpiration after 2 days, and an associated progressive decrease in relative turgidity. Water extraction continued so rapidly that on day 3 a soil moisture stress value of 15 atm was observed. At this time relative turgidity had fallen to 78 per cent. and transpiration was at about one-third of the maximum rate. No significant increase in dry weight was observed after this time. Transpiration continued to decrease rapidly until, at a

TSMS of about 30 atm, it reached a level of less than 10 g/plant/day, at which it remained fairly stable to the end of the experimental period. This rate was about 5 per cent. of the maximum rate. Leaf turgor levels continued to fall, but stem elongation continued until approximately 38 atm soil moisture stress existed. The last harvest was made when the lower leaves were dying, and when the first dead patches were appearing on the leaves used for water relations purposes. On this occasion the soil moisture stress was 45 atm, and the reduction in soil water content below the 15 atm value was 2.4 per cent. The first visible signs of wilting were observed

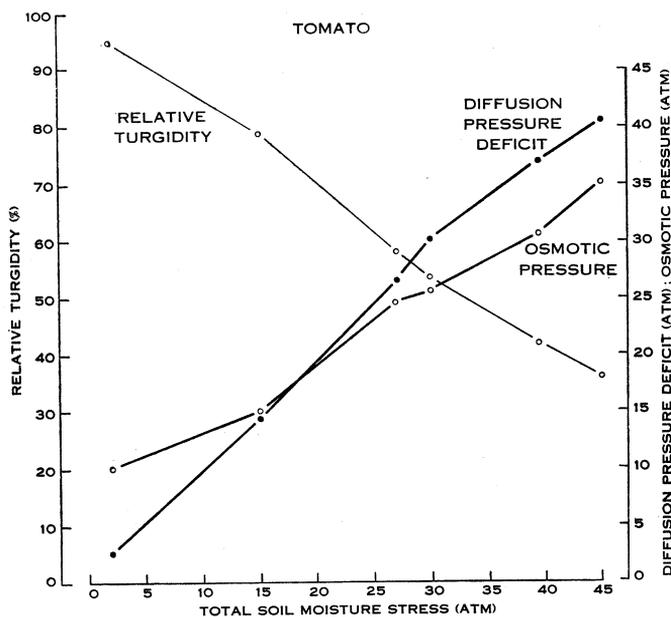


Fig. 3.—Diffusion pressure deficit, osmotic pressure, and relative turgidity of adult leaves of tomato in relation to increasing total soil moisture stress.

on day 2 when diurnal wilting around midday was apparent. At dawn on day 4, the lower leaves appeared completely wilted and this appearance was regarded as providing an approximate indication of the first permanent wilting percentage of Furr and Reeve (1945). At this stage the soil moisture stress was 20 atm and the relative turgidity in the upper leaves was 70 per cent. Stem elongation, frequently a good indicator of the first permanent wilting percentage, continued beyond this point although at a reduced rate, and transpiration was at a level of about 15–20 g/day. At dawn on day 7 the apical leaves appeared severely wilted and this was considered to indicate the approximate ultimate permanent wilting percentage. By then some of the lower leaves were dying, and total dry weight was decreasing. The soil moisture stress at this stage was 32 atm.

Evidence concerning the physical response of the tomato to increasing stress is provided in Figure 3 where the relative turgidity, DPD, and osmotic pressure of the leaf tissue is plotted against TSMS. Although the sample leaves used for these deter-

minations were in the upper part of the plants, their relative turgidity/DPD relationships resembled those of the lower leaves rather than of the apical leaves, as the majority of the leaves wilted at about the same TSMS, and only the apical leaves exhibited delayed wilting. This also applied in cotton and privet, except that in privet the previous season's leaves were so rigid as to not exhibit any signs of wilting. In this case only the current season's growth could be used as an indicator.

From Figure 3, it is evident that the DPD values at dawn were generally in good agreement with the TSMS, indicating that turgor recovery normally proceeded overnight to a stage equal to DPD-TSMS equilibrium. As TSMS increased further it could be expected that, because of slower recovery of turgor, this equilibrium value would not be reached by dawn, so that the DPD would remain in excess of the TSMS. Examination of Figure 3, however, shows that on the last two harvests the DPD values were actually lower than the TSMS. This could have arisen from direct nocturnal absorption of water vapour from the atmosphere by the leaves of the plants, and the possibility of this occurring is considered in the discussion.

The changes in osmotic pressure which followed the onset of stress were slightly more than could be expected as a result of simple concentration of the cell sap following tissue dehydration. This is probably a result of breakdown of some organic complexes to osmotically active substances which frequently occurs as stress is imposed (Meyer 1956). An interesting feature of the osmotic pressure curve was that the osmotic pressure became less than the DPD at high stress levels. On the day of the last harvest this difference was 5 atm. If the classical osmotic theory holds, the osmotic pressure should never be less than the DPD unless other sources of negative pressure are being developed. The possibility of this occurring will also be considered in the discussion.

In privet the smaller leaf area of the plants resulted in less rapid transpiration than in tomato, and hence slower soil moisture depletion. The first sign of stress was a cessation of significant increases in total dry weight on day 4, when 5 atm soil moisture stress existed. No significant decrease in leaf turgor was evident at this stage, and reduction in transpiration rate was only just commencing. Subsequently a progressive and steady decrease in turgor and transpiration occurred. Stem elongation proceeded at a reduced rate until it ceased when the relative turgidity was 82 per cent. and the soil moisture stress 29 atm. First evidence of permanent wilting occurred at 49 per cent. relative turgidity, a much lower value than in tomato, and ultimate wilting occurred soon afterwards at a relative turgidity of 46 per cent. The appropriate TSMS values were 48 and 60 atm.

Transpiration declined to a low and fairly stable level of about 5-8 g/plant/day at about the time first wilting was observed, and continued at this rate until the end of the experiment. The final harvest was made on day 25 when, as in tomato, dead patches appeared on the sample leaves. At this last harvest the relative turgidity was 34 per cent. and the TSMS 110 atm. This value represented a decrease of 5.3 per cent. soil moisture content below the value observed at 15 atm TSMS.

In Figure 4, the response of privet in terms of leaf turgor, DPD, and osmotic pressure is shown in relation to TSMS. The DPD at zero turgor pressure was 27 atm,

and the relative turgidity 83 per cent. Stem elongation ceased at an almost identical value. The association of the state of zero turgor pressure with first permanent wilting, evident in tomato, was not applicable in the case of privet, and appeared to be due primarily to the rigid leaf characteristics of privet, which tended to defer the appearance of wilting until high stress values obtained.

The responses of privet in terms of DPD and osmotic pressure were of particular interest. As in tomato, there was good agreement between the dawn values of TSMS and DPD in the early stages of dehydration. Beyond about 50 atm stress,

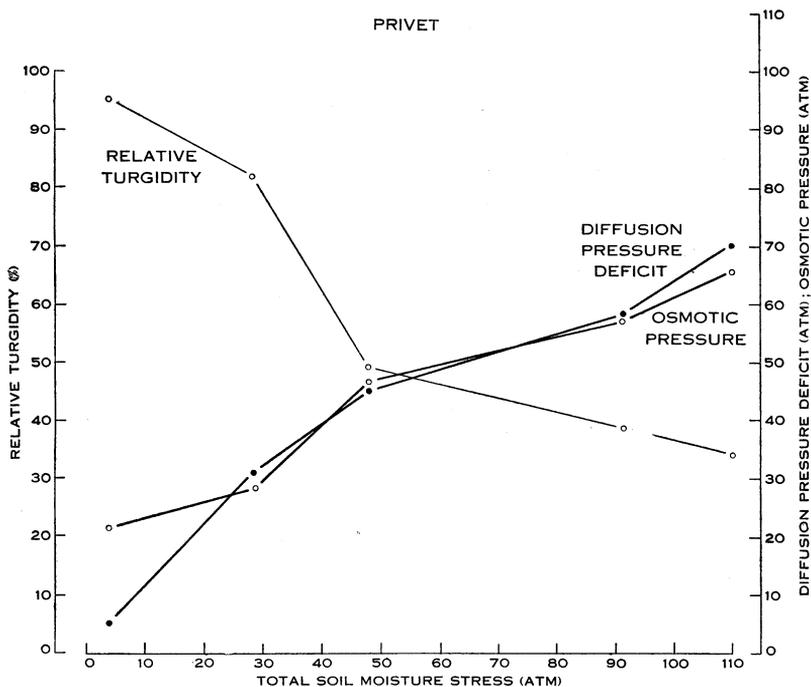


Fig. 4.—Diffusion pressure deficit, osmotic pressure, and relative turgidity of adult leaves of privet in relation to increasing total soil moisture stress.

however, the DPD values fell below those of TSMS and the divergences became progressively more marked so that, at the last harvest, the DPD was 70 atm and the soil moisture stress 110 atm. This provided further strong evidence for the occurrence of direct absorption from the atmosphere. The response of osmotic pressure was similar to that in tomato, and again the change in osmotic pressure with increasing stress was only slightly more than would be anticipated due to increase in cell sap concentration due to dehydration alone. Whereas in tomato the osmotic pressure fell below the DPD at high stress levels, in privet there was good agreement of DPD and osmotic pressure at DPD's greater than those at zero turgor pressure.

In cotton the first evident response to stress was a reduction in relative turgidity. Transpiration showed little change until 10 atm stress, when it decreased in the space of 2 days from a value of the order of 100 g/plant/day to 35 g/plant/day.

A marked reduction in rate of stem elongation occurred at the same time, which corresponded to a relative turgidity of 83 per cent. Increase in dry matter and elongation continued until relative turgidity had fallen to 62 per cent. and TSMS was 38 atm. This corresponded closely with first appearance of permanent wilting.

Transpiration rate continued to fall after this stage until it reached a fairly steady rate of 6–8 g/plant/day at about 60 atm TSMS. At the conclusion of the experiment, relative turgidity was 41 per cent. and TSMS 107 atm. This represented a reduction of 5.2 per cent. soil moisture content below the percentage at 15 atm TSMS.

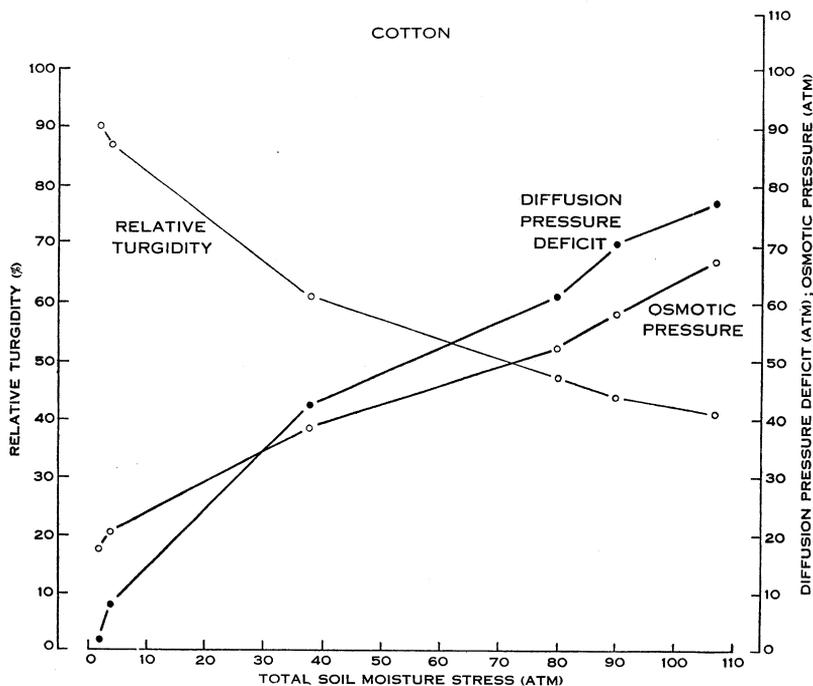


Fig. 5.—Diffusion pressure deficit, osmotic pressure, and relative turgidity of adult leaves of cotton in relation to increasing total soil moisture stress.

From Figure 5 it can be seen that the DPD at zero turgor pressure, 34 atm, corresponded to a relative turgidity of 66 per cent. This is in good agreement with the cessation of growth and elongation, and with the appearance of permanent wilting. Ultimate wilting occurred 1 day after first permanent wilting at a TSMS of 50 atm.

The responses of cotton in terms of DPD and osmotic pressure show characteristics of both the privet and tomato patterns. The strong divergence of DPD and TSMS at high stress levels observed in privet was again evident, suggesting, as before, direct vapour absorption by the leaves. The change in osmotic pressure was again only slightly more than could be expected from the direct effect of dehydration on sap concentration. Also of interest was the point observed earlier in tomato, that the osmotic pressure fell below the DPD at DPD's higher than those

at zero turgor pressure. The fact that good agreement occurred in privet would appear to be evidence for the validity of the measurement techniques and it would seem that negative pressure from sources other than of a direct osmotic nature was contributing to the DPD at high tensions in the other two species.

IV. DISCUSSION

Water stress has a marked effect on all plant processes and it is to be expected that this would be more pronounced on active processes which have their expression in elongation or vegetative growth than on predominantly passive processes such as transpiration. As soil moisture stress increases, turgescence must decrease, and the degree of turgidity which can be maintained by a plant is limited by the TSMS, as the absorption gradient is eliminated at the point of DPD-TSMS equilibrium. Thus, nocturnal recovery in turgor by a plant ceases when a state of DPD-TSMS equilibrium exists in the soil-plant system, unless additional water is absorbed directly from the atmosphere by aerial plant organs.

The progressive decrease in plant turgor which accompanies increase in soil moisture stress usually causes a progressive decrease in growth and elongation, as demonstrated by the detailed studies of Davis (1942), Furr and Reeve (1945), Scofield (1945), Wadleigh and Ayers (1945), Haynes (1948), Wadleigh and Gauch (1948), Bernstein and Pearson (1954), and Gates (1955*a*, 1955*b*). Decrease in transpiration is also to be expected because of the effect of turgescence on stomatal closure, and because of the slower rates of soil water movement to the absorbing surfaces of the roots. Growth (as increase in total dry weight) and elongation will finally be completely inhibited by decreasing hydration, and it is logical to assume that in any one tissue growth will cease at or before a DPD exists such that there is zero turgor pressure in the cells of the tissue concerned. Such a degree of dehydration should be reflected in a permanently wilted appearance of the leaves, and Slatyer (1957*a*) has suggested that this point corresponds to the point at which the permanent wilting percentage occurs.

In the results presented above this hypothesis appears valid. In tomato and cotton, increases in dry matter ceased approximately at the same DPD as that at zero turgor pressure in typical adult leaves. In privet, cessation occurred at much lower stress values. This may have been due partly to sampling error and partly to the fact that the relative turgidity at zero turgor pressure was highest in privet. This would mean that the relative cell volume at full turgor would be lowest in this species, so a slight reduction in turgidity would cause a proportionately greater reduction in cell volume and turgor pressure than in the other crops, which may in turn have caused inhibition of growth at lower stress values.

Cessation of elongation in privet and cotton occurred at stress values close to those obtaining at zero turgor pressure in the adult leaves. In tomato, elongation continued until very severe stress existed, and until the plant as a whole was losing dry weight. A similar result was reported for tomato by Wilson (1948). It is probable that in young elongating tissue, most of the cells are not completely vacuolated and osmotic theory is not strictly valid. Kerr and Anderson (1944) considered that imbibitional forces in developing cotton bolls enabled development to proceed at

water deficits which stopped the growth of other tissues, and a similar situation possibly existed in the apical tissue of tomato.

Because transpiration is primarily a passive phenomenon it does not necessarily cease at any particular stress level. It could be expected, however, that the transpiration rate would be reduced at low stress levels because of stomatal closure and reduced rates of soil water movement in unsaturated soils. Such reductions have been observed, for instance, by Chung (1935), Martin (1940), and Slatyer (1956*a*). Subsequently there seems to be no reason why transpiration should not continue until, or beyond, the death of the plant, limited only by the energy available for evaporation, the resistance to water movement into, through, and out of the plant, and by the rate of flow of soil water to the roots.

Although Veihmeyer and Hendrickson (1950, 1955) have claimed that transpiration is unaffected by TSMS until the permanent wilting percentage is reached when, in field soils, they consider that it virtually ceases, there is no valid physical or physiological reason why this should necessarily be so (Slatyer 1957*a*), and in general investigations of plants in their natural habitats (Alway 1913; Burr 1914; Batchelor and Reed 1923) and in containers (Briggs and Shantz 1912; Hendrickson and Veihmeyer 1945) have demonstrated continued absorption of soil water to values well below the permanent wilting percentage. In the present study these general contentions were borne out, reductions in transpiration rate first occurring at low stress values, but continued transpiration being observed, although at reduced rates, until the conclusion of the experiment, when the soil water content was 9.8, 6.9, and 7.0 per cent. for tomato, privet, and cotton respectively. This represented a decrease of 2.4, 5.3, and 5.2 per cent. respectively below the value at 15 atm TSMS.

The fact that privet and cotton continued to extract soil water at TSMS values beyond those which caused the death of tomato, appeared to be due to the DPD/relative turgidity characteristics of the different plants. Death no doubt ensues once a certain degree of dehydration has been reached, and the DPD appropriate to this critical value is probably reflected in the TSMS level at the death point. Marked differences in the DPD/relative turgidity characteristics of different plants exist (Weatherley and Slatyer 1957) and it is evident from Figures 3-5 that at similar stress values, relative turgidity was much lower in tomato than in the other species.

The significance of the permanent wilting percentage in studies of plant and soil water relations must be reduced when it is recognized that the permanent wilting percentage is not a soil constant as suggested by Briggs and Shantz (1912) and Veihmeyer and Hendrickson (1927, 1950) but is a value dependent primarily on the osmotic characteristics of the plants under study. Slatyer (1957*a*) proposed that permanent wilting occurred when the DPD reached a value such that there was zero turgor pressure in the leaves of the plant under examination and that the permanent wilting percentage was consequently at the TSMS value which corresponded to this DPD. This would be reflected in wilting of the leaves and the approximate cessation of growth.

In the present study the DPD at zero turgor pressure in the sampled leaves was 17, 27, and 34 atm in tomato, privet, and cotton respectively. Beyond this degree of stress there was no further growth or elongation except in the case of tomato, where elongation continued until severe stress existed. This meant that the concept of permanent wilting being associated with growth cessation was valid for any one species, but it also demonstrated that no one TSMS value, and hence no single soil constant, could be ascribed to the stage of permanent wilting of all species, as the wilting of any plant is a function of its turgor pressure, which at any one DPD or TSMS level is determined by its osmotic pressure. The order of variation between the three species examined in the present study, with respect to osmotic pressure, DPD, TSMS, and soil water content at first permanent wilting, and at the conclusion of the experiment, is shown in Table 1.

TABLE 1

OSMOTIC PRESSURE, DIFFUSION PRESSURE DEFICIT (DPD), TOTAL SOIL MOISTURE STRESS (TSMS), AND SOIL WATER CONTENT AT FIRST PERMANENT WILTING PERCENTAGE AND AT THE CONCLUSION OF THE EXPERIMENT

Plant	First Permanent Wilting Percentage*				Conclusion of Experiment			
	Osmotic Pressure (atm)	DPD (atm)	TSMS (atm)	Soil Water Content (%)	Osmotic Pressure (atm)	DPD (atm)	TSMS (atm)	Soil Water Content (%)
Tomato	18	19	20	11.8	35	41	45	9.8
Privet	47	45	48	9.7	66	70	110	6.9
Cotton	38	43	38	10.2	67	77	107	7.0

*Using the terminology of Furr and Reeve (1945). For comparative purposes the soil water content at 15 atm TSMS was 12.2 per cent.

Errors can be introduced in any wilting experiment because of the difficulties in recognition of wilting in rigid leaves. In tomato and cotton the estimated TSMS at which first permanent wilting occurred bore a close relationship to the DPD which obtained at zero turgor pressure (tomato, 20 : 17; cotton, 38 : 34). In privet, on the other hand, wilting was not evident at dawn until 48 atm TSMS existed (cf. DPD of 27 atm at zero turgor) because of the rigid characteristics of the leaf tissue. The soil water content at the first permanent wilting percentage was 11.8, 9.7, and 10.2 per cent. for tomato, privet, and cotton respectively. The last two values differed markedly from the soil water content at 15 atm TSMS, the value usually ascribed to the first permanent wilting percentage (Richards and Weaver 1944; Veihmeyer and Hendrickson 1950), which was 12.2 per cent.

The estimates of wilting were made by accepting a strongly wilted appearance at dawn as the wilting index. Such an index could introduce errors if severe water deficits had been developed on the preceding day, and the overnight period had not

been of sufficient duration to enable DPD-TSMS equilibrium to be established by sunrise. Reference to Figures 3-5 demonstrates that at the stress values which obtained at first permanent wilting percentage, there was DPD-TSMS equilibrium at dawn, and so the acceptance of a dawn appearance as an indication of the first permanent wilting percentage seemed to be valid. At ultimate wilting there was evidence in privet and cotton that the DPD values at dawn were less than the TSMS, and the use of the index in this connection is not as sound.

The responses of the plants to increasing soil moisture stress, in terms of DPD, osmotic pressure, and relative turgidity of the leaves, are of particular interest. As soil moisture stress increased, the changes in relative turgidity were reflected by opposite changes in DPD. The relationship between these responses is of considerable practical significance as the degree of turgidity at any one stress value is indicative of the physiological activity of the plant. Increase in osmotic pressure as soil moisture stress increased was only slightly more than was to be expected as a direct result of dehydration on solute concentration. It would appear that in each species the onset of stress was too rapid for the significant development of other sources of osmotic pressure by the breakdown of organic complexes to osmotically active substances, as was suggested by Ahrns (1924) and Spoehr and Milner (1939).

As dehydration proceeds beyond the state such that there is zero turgor pressure in the cells of a tissue, Meyer (1956) suggested that the water in the cell passes into a state of tension and the cell walls are subject to a strong centripetally directed pull due to the strong adhesion between the protoplast and the cell wall. This causes the development of negative turgor pressure and Thoday (1921) and Engmann (1934) have observed inward folding of cell walls as a result of such pressure during severe wilting. It would seem that the magnitude of the negative pressure which can develop in this manner must be limited by the degree of adhesion between the protoplast and the cell walls. Buhmann (1935) found values up to 10 atm for some species and reported similar results of earlier workers. In the present experiment little evidence of this effect was noted in privet, even under extreme stress, but in tomato the DPD exceeded the osmotic pressure by up to 5 atm, and in cotton the differences were as high as 10 atm.

As mentioned earlier, it is to be expected that as TSMS increases DPD must also increase, as the DPD cannot fall to a value lower than the TSMS unless an absorption gradient develops from atmosphere to plant and water is directly absorbed by the shoots. Even if absorption occurred in this manner, water should not accumulate in the plant, for the gradient should exist not only from atmosphere to plant, but from atmosphere to plant to soil, and movement ("negative transport") should continue into the medium surrounding the roots (Breazeale, McGeorge, and Breazeale 1950, 1951; Breazeale and McGeorge 1953*a*, 1953*b*; Haines 1952, 1953; Slatyer 1956*b*). For these reasons it was surprising to find, in the present experiment, evidence of water accumulation in the plants against a plant-soil diffusion gradient. In Figures 3-5 it can be seen that in privet and cotton there was a marked reduction in the DPD below the TSMS as TSMS exceeded a level of about 50 atm, and that in tomato, reductions were evident at TSMS levels of 30 atm and over. Although the reductions

in DPD were considerably less in tomato than in the other species, the amounts of water needed to cause the observed reductions were of the same general order in all species due to the much greater total weight of the tomato plants.

As it was not anticipated that evidence of absorption would be found, night and morning weights of cans were not taken to confirm that absorption definitely took place. However, there seems no doubt that the reductions were real, for TSMS was measured to within ± 3 atm and the validity of the DPD determinations was demonstrated by the good agreement between TSMS and DPD at low stress values, and the parallelism between the DPD and osmotic pressure curves at high stress values. Furthermore, by utilizing greenhouse temperature and humidity records it was computed that on each of the nights preceding harvests the amount of water in the atmosphere directly over each plant, which would have been yielded as dew, was always greater than the amount needed to cause the observed reductions in DPD in the plants.

A possible explanation for these results is that at low stress values, negative transport occurred, water being absorbed by the top of the plant and moving through the plant, along a gradient of increasing DPD, into the soil. As stress increased, suberization and death of roots, together with tissue and soil shrinkage, caused a progressive decrease in continuity in the water system between soil and root, so that the diffusion paths were progressively eliminated, and water tended to accumulate more and more in the plant. This hypothesis is supported by the evidence that transpiration decreased with increasing stress, until it reached a low and stable level at high stress values. These values corresponded very closely to those at which evidence of accumulation first appeared and the same explanation of discontinuity in the root-soil system could be applied to both phenomena. It also offers an explanation as to why, in separate experiments, Breazeale *et al.* (loc. cit.) were able to demonstrate negative transport of simulated dew through plants and into moist soil, but Stone, Shachori, and Stanley (1956) were unable to demonstrate negative transport into very dry soil, even though the tops of their plants regained turgor.

This has an interesting practical implication, as it suggests a mechanism whereby, under natural conditions, plants in dry soil could use atmospheric water for re-saturation of tissues without losing this water to the soil. Although absorption could theoretically occur whenever a favourable gradient existed there would be no real physiological benefit unless positive turgor pressure could be re-established in the active tissues. Thus the DPD of the water in the atmosphere would have to be low enough to reduce the plant DPD to a level below that at zero turgor pressure. If this were to occur, it is possible that sufficient photosynthesis could proceed in early morning, before transpiration re-established severe water deficits, to enable the plant to balance its respiratory losses. In this case a plant could conceivably persist for extended periods without further re-charge of soil water.

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