

Effect of Soil Moisture and Phenylmercuric Acetate upon Stomatal Aperture, Transpiration, and Photosynthesis¹

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When sprayed on plant leaves, several compounds, notably phenylmercuric acetate (PMA), can induce stomatal closure (16, 17). Most of these investigations with PMA were carried out on detached leaves with petioles dipping in water; it was found that the stomatal closing markedly reduced transpiration, whereas photosynthesis was reduced to a lesser extent. In these experiments it was demonstrated that the relations between both transpiration and photosynthesis and stomatal opening agreed with the equations relating diffusion of water vapor and CO₂ to resistance calculated from stomatal dimensions (8, 17).

The possibility of reducing plant transpiration by chemical treatment, without materially reducing photosynthesis, is of practical importance in arid regions. In addition, this method of transpiration control offered a promising tool for the study of soil-water-plant interrelations. To serve both ends, we sprayed various plants with PMA, and observed stomatal opening, transpiration, and photosynthesis or growth. This was performed on young maize growing in soil in a controlled-environment chamber, as well as on tobacco growing in a greenhouse and sunflowers outdoors. These experiments, performed in the controlled-environment chamber are reported here; the other experiments are reported in the accompanying paper.

It was realized that entire plants, drawing their water from the soil, might respond differently to a spray of PMA than did detached leaves with petioles dipping in water. The prevailing opinion concerning transpiration (13) is that, within the entire range of available soil moisture, the main resistance to transpiration is in the gaseous phase, from the evaporating surfaces of the mesophyll cells, through the stomata to the ambient air; therefore, at a given diffusion pressure gradient from the soil to the air, the most decisive factor in controlling transpiration is the stomatal opening. If this be so, stomatal closing by chemical treatment should affect transpiration in the same way and magnitude observed in detached leaves. Further, this effect should be nearly independent of soil moisture per se. This similarity of

response might apply to photosynthesis as well.

These similarities, however, would not occur if changes in the soil moisture brought about changes in the resistances of the soil and the plant that would be of a comparable magnitude to the diffusion resistance in the gaseous phase. Under these latter circumstances, stomatal opening might not be the only major factor controlling transpiration.

The analysis of the interaction of stomatal opening and soil moisture in their effect on transpiration and photosynthesis is usually complicated by the fact that low soil moisture is generally associated with a closing of stomata; thus it is difficult to evaluate these factors independently. The control of stomatal opening by chemical treatment and the measurement of stomata provided a possibility of isolating the stomatal resistance at various levels of soil moisture.

Materials and Methods

The effect of PMA spray on the transpiration and photosynthesis of young maize plants was determined in a controlled-environment chamber, under 4 soil moisture levels.

Maize (*Zea mays* L. var. Connecticut 870) was grown in the greenhouse in pots containing 800 g (dry wt) of Cheshire sandy loam that had been screened through a 2 mm mesh. The moisture retention properties of this screened soil were determined (9, 10). The water content at the 15-bar tension was 6.5%. Also the pot capacity of the soil (analogous to the field capacity of natural soil) was determined by applying water on the top of a column of air-dry, screened soil, and measuring the average moisture content from the top to the wetting front after 24 hours; this moisture content was 18.0%.

One plant per pot was grown. A plastic lid was placed on the pot to minimize direct evaporation from the soil, and the plants emerged through a hole in the lid. Soil moisture was determined by weighing the entire pot, soil, and plant. The soil was kept moist by frequent watering to the pot capacity during the first 3 weeks of growth. The actual experiment was performed 24 to 30 days after planting, when the plants had 4 fully expanded leaves and the fifth leaf was beginning to unfold. By irrigating or withholding irrigation a few days preceding experimentation, the soil was brought within $\pm 0.8\%$ of 4 moisture contents: 24% (above pot capacity), 18% (pot

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capacity), 13% and, 10% (below pot capacity, but still above the 15-bar moisture which is considered to be the wilting point of the soil). The corresponding moisture tensions were: < 0.10, 0.25, 1.0, and 2.0 bars. Each morning 2 plants which had reached a predetermined soil moisture, and which had approximately the same leaf area were selected. The lowest leaf, which tended to shrivel at this stage, was removed. A control plant was sprayed with 0.05% Triton X 100 wetting agent; the other plant was sprayed with 90 μ M PMA in the 0.05% Triton X 100 solution. Immediately afterward the plants were placed in the controlled-environment chamber.

The transparent chamber, 40 \times 35 \times 50 cm, was illuminated from above by spotlights that were immersed in a transparent tank and cooled by circulating water (9, 17). The flux density of radiation < 3 μ in wave length was 1.5 cal. cm⁻² min⁻¹ at the top of the chamber and 1.1 cal. cm⁻² min⁻¹ 30 cm below the top; this was the zone where most of the leaves were found. Without opening the chamber, the pots and plants could be weighed within \pm 0.02 g by means of balances placed below the chamber. Flap openings on the sides of the chamber, which could be sealed with adhesive tape, facilitated the manipulation inside the chamber; when the plants were not being weighed, and the flaps were sealed, the chamber was essentially air tight. Replenishment of CO₂ was provided by blowing about 6 liters min⁻¹ of air through the chamber. This air was moistened by bubbling it through water; also, the floor of the chamber was flooded. Inside the chamber a small fan provided turbulence and outside a large fan blew on the walls of the chamber and cooled it. A dry-wet bulb psychrometer was placed inside the chamber. Evaporation was measured from a blotting paper with thermocouples embedded in it. It was found that the apparent length of path of water vapor diffusion, L, ranged between 0.07 and 0.27 cm, depending on the position of the evaporator. It was also found that the temperature of the evaporating surface ranged between \pm 2° from the air temperature, depending on its position.

After the plants were placed in the dark chamber, the lights were gradually turned on; the maximum light intensity was applied for 2 hours. The temperature in the chamber was 30° \pm 1°, and the relative humidity 87% \pm 1%. During these 2 hours the pots were weighed at half-hour intervals. Three hours after the plants had been sprayed, which was 1.5 hours after the start of the full light period, radioactive NaHCO₃ was pipetted into an excess of H₂SO₄ in the chamber; thus 0.2 μ moles of labeled CO₂ were introduced into the chamber. For 4 minutes no external air was blown in; the air was then swept out by renewing the blowing in of external air. The flaps were opened and silicone rubber impressions were made of the upper and lower surfaces in the middle of the fourth leaf (16). At the same time the permeability of the leaf was measured by means of a porometer. This porometer is a modification of

that described by Alvim at the International Symposium on the Methodology of Plant Eco-Physiology, Montpellier, 1962. In this porometer, pressure was built up in a rigid air reservoir; the air was then released through a flexible tube to a cup made of 2 soft rubber rings, which were clamped on the leaf by means of a pair of clinical tongs with a toothed lock; the rate of pressure drop, observed on a pressure gauge during a known interval indicated the air permeability through the leaf area enclosed in the cup, and is presumably a function of stomatal aperture. For a given reservoir volume, cup area, and time interval, the permeability is proportional to log (p_0/p_1), p_0 and p_1 being the initial and final pressures. Throughout the experiment, pressure drop was observed during a 10-second interval, with a reservoir of 100 cm³ and a cup area of 0.28 cm². Moreover, p_0 was always 200 mm Hg. The results were expressed as "permeability index" = log (p_0/p_1).

After removing the impressions, the plants were taken from the chamber; the fully expanded leaves were cut, and the partly-rolled fifth leaf was also cut at the point where the leaf edges overlapped. The excised leaves were quickly weighed and their area measured; they were then immersed in boiling water for a few minutes and ground in water. Aliquots of the homogenate were dried and their radioactivity measured. This radioactivity, expressed as counts per cm² of leaf surface per minute served as a comparative index of photosynthesis rate. Transpiration rate obtained from the weighings of the pots in the chamber was expressed in absolute units, mg water per cm² of leaf surface per hour. Cellulose acetate replicas of the rubber impressions were observed under microscope, and the average width of 25 stomata was recorded for both the upper and lower surfaces.

An attempt was made to perform the same experiment at 43% air relative humidity. Even plants growing in relatively moist soil (18%), however, wilted severely within a few minutes after the lights were turned on, and the attempt was discontinued.

Since only 2 plants could be examined each day, the experiment was carried out for approximately 3 months. Maize was planted every 2 to 3 days to ensure a steady supply of suitable plants at the required soil moisture contents, which were randomized throughout the experimentation period. In all, 6 pairs were observed at each of the 4 soil moistures; the entire experiment was subjected to a factorial analysis of variance with 2 PMA treatments (control and PMA), 4 soil moistures, and 6 replications.

Theory

Both transpiration and photosynthesis can realistically be conceived as steady state processes, the former of water from soil, through the plant to the air, the latter of CO₂ from the air to the chloroplasts. As such they may be considered as catenary processes, where the 2 substances move through a series of

resistances according to potential or concentration gradients, very much in analogy to Ohm's law (4, 6). Since transpiration is primarily a physical process it is easier to analyze than photosynthesis, whose metabolic factors are difficult to evaluate. Therefore, transpiration will be dealt with more extensively, with inferences concerning photosynthesis wherever possible.

Consider a plant growing in the soil and transpiring. Water moves from the soil to the air through a path, the various segments of which have various resistances and gradients. These gradients may be expressed in terms of water potential or of vapor concentration in equilibrium with a given potential. The former is commonly applied to describe the water energy status of the soil (moisture tension) or the plant (diffusion pressure deficit), whereas the latter is used when dealing with vapor diffusion (Fick's first law). The theoretical treatment will be carried out mainly in terms of vapor concentration gradients for 3 reasons: A. simplicity; B. within the range encountered in the present experiment, water potential and the corresponding vapor concentration are almost linearly related; C. the most important resistance to the water and CO₂ flux is in the gaseous phase, where flux is proportional to concentration gradients. It should be borne in mind, however, that when dealing with the liquid phase in the soil-plant-air system, the term vapor concentration does not imply the presence of any vapor, but it describes the energy status of the liquid phase by means of the vapor concentration which would be in equilibrium with the water potential at any point in this phase. For interpretation, vapor concentration may be reconverted to the corresponding water potential.

For the transpiration process,

$$T = [(\Delta C)_s + (\Delta C)_p + (\Delta C)_a] (R_s + R_p + R_a), \quad I$$

where T is transpiration, grams of water per cm² of leaf surface per second; ΔC is the difference in vapor pressure along a segment of the water path, reduced to units of vapor concentration, grams per cm³; R is the resistance to water movement of the segments, in seconds per cm. The subscripts affixed to ΔC and R , designate the segments of the water path: s , the soil; p , the plant; these segments are those in which water moves in the liquid phase; a , the air; in this segment water moves in the gaseous phase, and it is divided into 2 sub-segments: st , the substomatal cavities and the stomata; ex , the external air outside the stomata.

Assuming transpiration to be a steady-state process, it would equal the water flux through any segment of the water path; therefore,

$$T = \Sigma(\Delta C)/\Sigma R = (\Delta C)_s/R_s = (\Delta C)_p/R_p = (\Delta C)_a/R_a. \quad II$$

In the gaseous phase, the movement of water follows Fick's first law of diffusion,

$$T = D(\Delta C)_a/(S + L) \quad III$$

where D , cm²sec⁻¹, is the coefficient of diffusion of water vapor in the air; S and L , cm, are the apparent path lengths of diffusion in the stoma and in the external air. By definition,

$$S = DR_{st}; L = DR_{ex}; R_a = R_{st} + R_{ex} = (L + S)/D. \quad IV$$

L may be estimated from the evaporation from liquid water or a wet surface, under a given turbulence (the higher the turbulence, the lower L) and when the temperatures of the wet surface and the ambient air are known.

S may be calculated from stomatal dimensions, using Penman and Schofield's relation (8),

$$S = l/(\pi nab) + 1/[2n(ab)^{1/2}] \quad V$$

where n is the number of stomata per cm² of leaf surface; l is the depth, a the width and b the length of the stomata, in cm. For a given species, with assumed constant n , l and b , S is a function of a alone.

Equation (I) may be inverted and S isolated,

$$1/T = (R_s + R_p + L/D)/\Sigma(\Delta C) + [S/D\Sigma(\Delta C)] = K_1 + K_2S \quad VI$$

where K_1 and K_2 are constants. This linear relation between the reciprocal of transpiration and S can be conveniently analyzed through correlation and regression.

The overall resistance from the soil to the air may be calculated from equation II. Assuming isothermal conditions in the soil-plant-air system, the overall vapor concentration gradient is

$$\Sigma(\Delta C) = [(RH)_{soil} - (RH)_{air}]/100] C_{sat} \quad VII$$

where $(RH)_{soil}$ and $(RH)_{air}$ are the relative humidities of the soil air and the external air, respectively, in percent, and C_{sat} is the vapor saturation concentration at the given temperature.

The relative humidity of the soil air is expressed by

$$\log (RH)_{soil} = 2 - (0.095\psi)/\theta \quad VIII$$

where ψ is the soil moisture tension in bars and θ is the absolute temperature in K°. Obviously, within the range of available moisture, the soil air is practically saturated, for even at the wilting point (approximately 15 bars) the relative humidity of the soil air is 98.9%; thus the overall gradient is essentially the saturation deficit of the external air.

The combined resistance of the soil and the plant, R_{s+p} , can be obtained from equation (VI),

$$R_{s+p} = K_1\Sigma(\Delta C) - L/D. \quad IX$$

The vapor concentration at the evaporating surfaces of the mesophyll cells lining the substomatal cavities, C_{ev} , is

$$C_{ev} = C_{soil} - (R_{s+p}/\Sigma R)(C_{soil} - C_{air}) \quad X$$

C_{soil} and C_{air} being the vapor concentrations of the soil air and the external air.

Assuming that the soil air is essentially saturated, and that the system is under isothermal conditions, the relative humidity of the air at the evaporating surfaces is

$$(RH)_{\text{ev}} = 100 - (R_{s+p}/\Sigma R)[100 - (RH)_{\text{air}}].$$

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From this relative humidity, the corresponding water tension at the evaporating surfaces is calculated using relation VIII.

This theoretical treatment assumes isothermal conditions, in which the energy required for moving and evaporating water is derived entirely from an external source. Any temperature gradients present in the soil-plant-air system will modify these relations.

The effect of PMA on stomatal opening provides, at a given environmental condition a wide range of stomatal widths. This range makes possible a statistical evaluation of the function $1/T = K_1 + K_2S$; through this relatively simple relation, it is possible to estimate the rate of transpiration to be expected at any stomatal width, of which S is a function. From this estimate, the resistances and potential drops in the soil-plant-air system can be calculated, and the energy status of water at selected points along this system can be estimated.

If the soil-plant resistance is small in comparison with the overall resistance, then the vapor concentration at the evaporating surfaces of the mesophyll cells would be near saturation. If, however, the soil-plant resistance constitutes an appreciable portion of the overall resistance to water movement, then the air over the evaporating surfaces will be unsaturated to some degree, and the corresponding water tension at these surfaces may be quite high.

The same theoretical consideration elaborated upon for transpiration, is valid for photosynthesis;

that is, the rate of photosynthesis may be evaluated as a function of stomatal opening (through the intermediate function S), in the form of $1/P = K'_1 + K'_2S$ (where P is the rate of photosynthesis). However it should be recognized that although the path of CO_2 from the outside air to the site of the acceptors on the chloroplasts is much shorter than the path of water from the soil to the air, it is a much more complex path, since the rate is governed not only by concentration gradients and resistances to diffusion, but also by metabolic and enzymic processes which are difficult to evaluate.

Results

Stomatal Opening. Stomatal opening was studied both by direct microscopic measurement of width, and by permeability to air from the porometer (table I). Although there were, on the average, 1.25 times as many stomata on the lower surface as there were on the upper one, the width of the stomata on the upper and lower surfaces was averaged.

As was expected, the average stomatal width decreased with decreasing soil moisture, and at the lowest moisture, 10%, it was too narrow to be reliably resolved. Within each soil moisture where stomatal width could be measured—i.e. 24%, 18%, and 13%—PMA caused a clear decrease of stomatal width. This decrease was more pronounced at the higher soil moistures, but even at 13% moisture it was highly significant.

The permeability index of the leaves decreased with decreasing soil moisture, and within each soil moisture, PMA lowered the index. This difference, caused by PMA, was significant even at the 10% soil moisture. When the average stomatal widths at 24%, 18%, and 13% soil moistures were related to the corresponding permeability indexes, a significant linear correlation was disclosed, figure 1. It

Table I
Effect of 90 μM PMA on Stomatal Width, Leaf Permeability, Transpiration,
and Photosynthesis of Young Maize

Soil moisture, %	10		13		18		24	
	Control	PMA	Control	PMA	Control	PMA	Control	PMA
Average stomatal width, μ	1.63 *	0.92 *	2.95 *	1.39 *	3.60 *	1.38 *
Permeability index, $\log(p_o/p_t)\dagger$	0.042**	0.027**	0.142*	0.078*	0.359*	0.109*	0.497*	0.113*
Transpiration, $\text{mg cm}^{-2} \text{hr}^{-1}$	4.04 **	3.63 **	5.82 **	4.68 **	8.14 *	6.46 *	10.11 *	7.44 *
Photosynthesis, $\text{counts cm}^{-2} \text{min}^{-1}$	128***	132***	374***	355***	474**	435**	641**	572**

* Statistical significance of difference between control and PMA: $0.01 > p$.

** Statistical significance of difference between control and PMA: $0.05 > p > 0.01$.

*** Statistical significance of difference between control and PMA: not significant.

† Porometer volume, 100 cm^3 ; cup area, 0.28 cm^2 ; interval, 10 seconds.

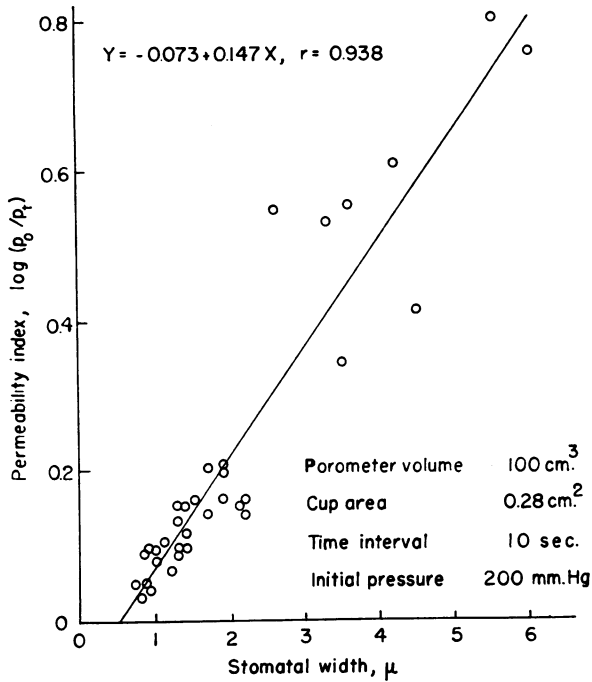


FIG. 1. The relation between stomatal width and leaf permeability, measured with a porometer, of young maize leaves.

should be noted, however, that this linearity may be only apparent; the theory of viscous flow predicts that permeability should be a function of a higher degree of the stomatal width (5). Since the regression line intersects the X-axis to the right of the origin, the function may actually curve toward the origin, especially where stomatal width is below the limit of microscopic resolution. For this reason, the linear regression was not extrapolated to estimate the stomatal width from the permeability index at 10% soil moisture. Nevertheless, at this soil moisture, the lower permeability of the leaves sprayed with PMA indicates a smaller stomatal width, although the absolute dimensions could not be directly observed.

Transpiration and Photosynthesis. Transpiration was affected by both soil moisture and PMA; it was reduced at lower soil moistures, and within each moisture level, it was reduced by PMA (table I). The relative reduction in transpiration due to PMA decreased with soil moisture, but even at 10% soil moisture it was significant.

Photosynthesis, as reflected by incorporation of labeled CO_2 , was markedly reduced at the lower soil moistures; in fact, photosynthesis was more adversely affected by lower soil moisture than transpiration; whereas transpiration at 10% soil moisture was about 2/5 of that at 24% soil moisture, the corresponding ratio for photosynthesis was about 1/5.

On the other hand, PMA reduced photosynthesis less than transpiration. Photosynthesis was signifi-

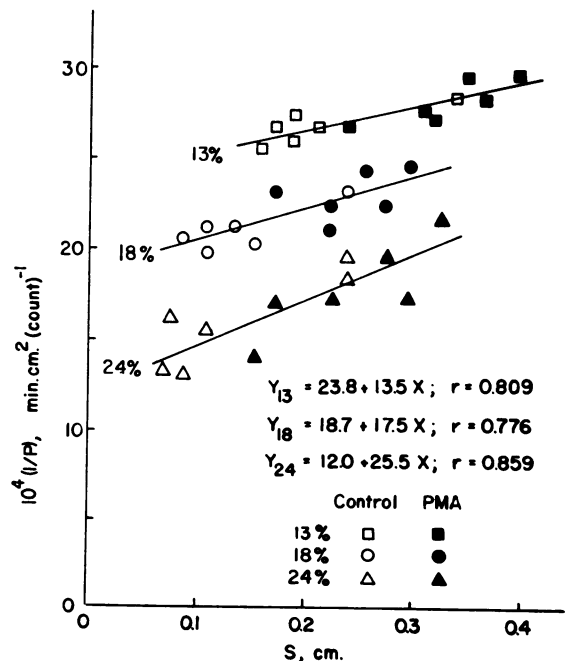
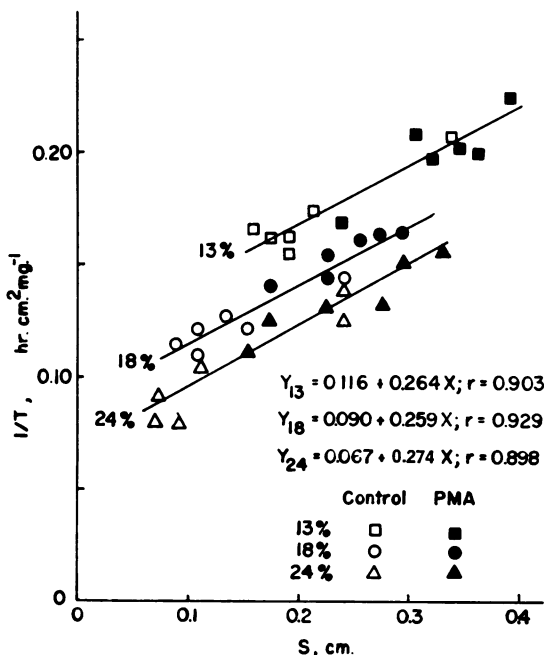


FIG. 2 (left). The reciprocal of transpiration, $1/T$, as a function of apparent length of diffusion path in stomata, S , at 3 soil moisture levels.

FIG. 3 (right). The reciprocal of photosynthesis, $1/P$, as a function of apparent length of diffusion path in stomata, S , at 3 soil moisture levels.

cantly reduced only at 24 % and 18 % soil moistures; in drier soil this reduction became negligible, and in fact at 10 % soil moisture PMA slightly increased photosynthesis.

The reciprocals of transpiration, $1/T$, and of photosynthesis, $1/P$, for each plant are presented in figures 2 and 3 as functions of the apparent length of diffusion path through the stomata, S . The S was calculated from the observed average stomatal width. For each soil moisture there are 12 points, corresponding to 6 control and 6 treated plants.

Linear correlations and regressions were calculated for 3 soil moistures. For each soil moisture there exists a statistically significant correlation, and the linear regressions are significantly different for each soil moisture. In both the $1/T$ and $1/P$ functions the regression line is higher as the soil is drier. Within each regression, the points belonging to the PMA-treated plants tend to be distributed at the upper end of the line. The linearity of these functions is in agreement with the theory that $1/T = K_1 + K_2S$ and $1/P = K'_1 + K'_2S$; however, they are not single-valued functions of S , since the parameters K_1 , K'_1 and K'_2 vary with soil moisture. The slopes of the $1/T$ lines are practically parallel, whereas in the $1/P$ lines the slope decreases with soil moisture.

The linear regressions of figure 2 and 3 were transformed into the curvilinear functions which

show the relation between stomatal width and the rates of transpiration and photosynthesis (fig 4 and 5). Thus at a given stomatal width, the rates of both transpiration and photosynthesis are lower for the drier soils. The shape of these curves is in agreement with many previous works, notably those of Stalfelt (15). At a given soil moisture, the increase in transpiration and photosynthesis caused by an increase in stomatal width is more pronounced at small stomatal openings.

At a given stomatal aperture, the reduction in transpiration and photosynthesis associated with the drier soil indicates that as the soil dries there is an increase in the soil-plant resistance to water movement, and a corresponding increase in the resistance to movement of CO_2 from the surface of the mesophyll cells to the chloroplasts.

Transpiration Resistance. Table II presents the following calculated values for the transpiration process: overall resistance, plant resistance, saturation deficit at the evaporating surfaces of the substomatal cavities, and the water tension corresponding to this deficit. These values, calculated for 3 soil moistures and 4 stomatal widths, are based on the following simplifying assumptions; the temperature, 30° , is uniform throughout the soil-plant-air system; the soil air is saturated and the chamber air is at 87 % relative humidity; the diffusion coefficient D

Table II

Calculated Values for Overall and Soil-plant Resistance to Water Movement, Saturation Deficit, and Moisture Tension at Mesophyll Evaporating Surfaces of Young Maize, at 3 Soil moisture Levels, 4 Stomatal Widths, and 2 Conditions of Turbulence

	L , cm	Soil moisture, %	Stomatal width, μ			
			1.0	1.6	3.0	5.0
Overall resistance, sec cm^{-1}	...	13	2.91	2.53
		18	2.61	2.22	1.92	1.76
		24	2.08	1.69	1.38	1.22
Soil + plant resistance, sec cm^{-1}	0.15	13		1.17		
		18		0.87		
		24		0.34		
resistance, sec cm^{-1}	0.20	13		0.98		
		18		0.68		
		24		0.15		
Saturation deficit, %	0.15	13	5.4	6.2
		18	4.2	4.9	5.8	6.2
		24	2.1	2.6	3.1	3.5
%	0.20	13	4.6	5.3
		18	3.1	3.7	4.2	4.6
		24	0.9	1.1	1.4	1.6
Tension, bars	0.15	13	76	88
		18	59	70	82	90
		24	29	36	44	50
bars	0.20	13	65	75
		18	44	52	60	66
		24	13	16	19	22

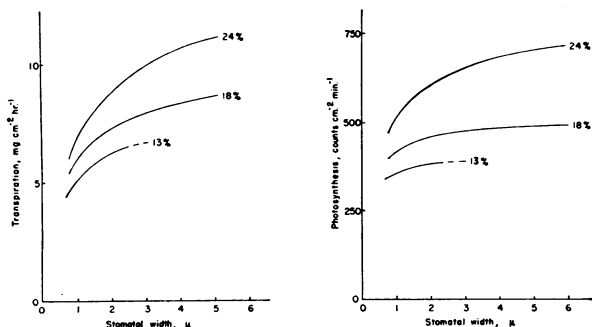


FIG. 4 (left). Transpiration as a function of stomatal width, at 3 soil moisture levels.

FIG. 5 (right). Photosynthesis as a function of stomatal width, at 3 soil moisture levels.

= 0.26 cm² g⁻¹ at 30°; and since L was found to vary considerably within the space of the chamber, 2 values of L were used in the calculations: 0.15 and 0.20 cm. For the 13% soil moisture, the above values were calculated for the 2 smaller stomatal widths only, since no stomata wider than 2μ were observed at this moisture.

At any given stomatal width there was a steady increase in the overall resistance to water movement as the soil became drier; but since the resistance in the gaseous phase is assumed to be constant at a given stomatal opening, this increase in resistance must be attributed solely to the soil-plant segment of the water path.

At high soil moisture, small stomatal width and low turbulence, the soil-plant resistance constitutes a relatively minor part of the overall resistance (7% at soil moisture = 24%, stomatal width = 1μ, and L = 0.20 cm); at low soil moisture, wide stomata and high turbulence it may constitute up to 45% of the over-all resistance. Under such conditions the saturation deficit of the air over the evaporating surfaces of the mesophyll cells may reach 7% (93% relative humidity). While this deficit may not seem very high, it should be noted that it corresponds to a water tension of over 80 bars. The possible occurrence of such high water tension at the evaporating surfaces is quite remarkable in view of the fact that even the plants at 13% soil moisture were not wilted. Some measurements of maize leaf sap, using refractometric, conductimetric, and psychrometric methods (2, 12), indicated that even in severely wilted plants the osmotic pressure of leaf sap was about 20 bars, and lower in turgid plants. Only when soil moisture was high, stomata almost closed and turbulence low, did the tension at the evaporating surfaces approach such low values.

Photosynthesis Resistance. The observations of photosynthesis indicate that as the soil dries, the resistance to the diffusion of CO₂ from the mesophyll cell surfaces to the chloroplasts markedly increases, which could not be attributed to stomatal closure (fig 3 and 5; table I). Since only comparative

values were obtained by radioactivity, these resistances could not be calculated in absolute terms. It appears that the effect of soil moisture on the ratio of plant resistance to the overall resistance is even greater in photosynthesis than in transpiration, whereas the effect of stomatal opening on the overall resistance to CO₂ movement is relatively less pronounced than on water movement.

Discussion and Conclusions

In earlier experiments, carried out with detached leaves (17), the closing of stomata by PMA reduced transpiration more than photosynthesis, and this verified the hypothesis that stomatal resistance constitutes a larger part of the overall resistance in transpiration than in photosynthesis; the resistance to water movement exists largely in the gaseous phase (the liquid phase resistance being negligible), whereas the CO₂ diffusion proceeds through this same gaseous resistance, as well as an additional resistance in the mesophyll.

The results of the present experiment indicate that in intact plants growing in the soil, too, the relative importance of stomatal resistance is greater in controlling transpiration than photosynthesis. The results also indicated, however, that both transpiration and photosynthesis were not single-valued functions of stomatal opening, but changed with soil moisture well above the wilting point. At any given stomatal opening, as the soil dried, the rates of transpiration and photosynthesis decreased.

The reduction in transpiration cannot be attributed to any appreciable change in the overall water potential gradient from soil to air. This gradient in the dry soil system is less than 1% lower than in the wet, whereas the reduction in transpiration exceeded 30%. That the overall gradient is essentially the same in the 3 soil moisture levels is borne out by the fact that the slope K_2 of the $1/T$ function, which according to equation VI is inversely proportional to vapor concentration drop, remained practically unchanged (fig 2). As the reduction in transpiration takes place at a constant gaseous resistance, it must be reasoned that the decrease in soil moisture was associated with an increase of the soil-plant resistance. Following Gradman and van den Honert's concept of water movement as a catenary process, this resistance appears to be great enough to influence water flux. This also means that as the soil dries, a considerable tension develops at the evaporating surfaces of the mesophyll cells, and a significant saturation deficit appears in the air in the substomatal cavities.

These findings contradict the present concept on the relative magnitude of resistances and gradients along the path of transpiration. This concept (13) states that the soil and plant resistances are negligible in comparison to the gaseous resistance, and therefore it is this master resistance which controls,

mainly through stomatal opening, the rate of transpiration.

Since the increase of soil-plant resistance, as the soil dries, diminishes the relative weight of the stomatal resistance to water movement, the effect of PMA is expected to decrease at low soil moisture. This tendency is partly offset by the fact that at small stomatal widths, such as encountered at low soil moistures, even a small decrease in width has a relatively large effect on transpiration, because of the steep slope of the transpiration/stomatal width function in this range (fig 4).

Whether the findings of the present experiment are results of special experimental conditions, or are more general, is not certain, although evidences of significant vapor saturation deficits in substomatal cavities have been reported (7, 14). It should be noted that the reduction of transpiration occurred in turgid, potted plants, above the wilting range of the soil. Within the soil moisture range investigated, a very marked reduction in the unsaturated capillary conductivity of the soil may be expected (3). In general, the effect of capillary conductivity is of minor importance on plants growing in the field, as long as the soil does not reach the lower range of available moisture; the plant roots are relatively widely spaced, and as the soil around them dries, small growth is sufficient to bring them to moist soil where capillary conductivity is high enough to ensure a steady water supply for transpiration. The situation is different when the roots are confined in a small pot; the soil is so densely permeated by roots that it dries uniformly, and no untapped soil remains. Thus before the soil dries to the wilting point, the decrease in capillary conductivity may markedly curtail the supply of water to the plant. Simultaneously, the resistance between leaf tissue and air must increase to balance the water loss to air with the reduced water absorption from the soil; otherwise the plant wilts. The increased resistance is generally believed to be caused by stomatal closure; however, the present experiment indicates that an additional important resistance, independent of stomatal width, may appear when the soil dries; this resistance is probably located at the cytoplasmic membranes or the cell walls lining the substomatal cavities.

The mechanism of this adjustment of resistance can only be conjectured. Although changes of permeability to various compounds are characteristic of cell membranes, they are not confined to biological systems. Boon-Long (1) found that evaporation from a collodion membrane separating water from air was reduced by the presence of sucrose in solution more than could be accounted for by the lowering of the vapor pressure by the solute. Hence, although the solution had an osmotic pressure of about 25 bars, the tension at the evaporating surface of the membrane was possibly a few hundred bars. One possible explanation is concentration, or even precipitation of the solute at the surface, since it cannot diffuse back rapidly into the main body of the

solution; another possibility is the clogging of the submicroscopic pores by the large solute molecules, resulting in lower membrane permeability. In a plant the membrane resistance may change as the result of the above mentioned, as well as other biological processes.

In view of what is found in nonliving membranes, a tension of 80 bars at the evaporating surface of the mesophyll cells, whose sap has an osmotic pressure of less than 15 is not surprising. Obviously, this tension is not transmitted into the interior of the cells, since a herbaceous plant is not likely to withstand a tension over 60 bars without collapse.

The membrane resistance in Boon-Long's experiment increased with increasing evaporation. Accordingly, the plant resistance might increase with wider stomata. In the function $1/T = K_1 + K_2S$, the plant resistance was included in the term K_1 and therefore considered independent of S . Although K_2S may include not only the positive linear dependence of stomatal resistance on S , but also a smaller negative dependence of plant resistance on S , this was not supported by the results of the experiment.

Photosynthesis, too, is governed by resistance in the plant, which increases as the soil moisture decreases. At low soil moisture this resistance is so high, that stomatal width ceases to affect photosynthesis significantly.

The slope K'_2 in the $1/P$ function, unlike its transpiration counterpart, decreases as the soil dries. Since, according to equation (VI) this slope is inversely proportional to the overall CO_2 concentration gradient, it must be assumed that this gradient was greater in the drier soil. This may be attributed to the fact that photosynthesis of the plants growing in 24 % soil moisture was almost twice as rapid as that in 13 %; as a result, the air in the chamber may have been depleted of CO_2 more about the well-watered plants, resulting in a smaller overall gradient of CO_2 concentration.

Summary

The effect of phenylmercuric acetate on stomatal opening, transpiration, and photosynthesis in young corn plants was studied at various soil moistures. Stomatal closure induced by phenylmercuric acetate significantly reduced transpiration, while it reduced photosynthesis considerably less. In addition, decreased soil moistures, even within available range, caused a further reduction in both transpiration and photosynthesis which could not be attributed to stomatal closure. From the analysis of the resistance to water movement it seems that as the soil dries, a considerable resistance to water movement develops at the evaporating surfaces of the mesophyll cells. The water tension at these surfaces may reach 80 bars without the plants wilting. Similarly, the mesophyll resistance to CO_2 movement increases as soil moisture decreases.

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