THE INFLUENCE OF SEVERAL TRANSPIRATION SUPPRESSANTS ON TRANSPIRATION, PHOTOSYNTHESIS, AND WATER-USE EFFICIENCY OF COTTON LEAVES

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Summary

The influence of transpiration suppressants on transpiration and apparent photosynthesis of cotton leaves was investigated under moderate and high light intensity ($7 \cdot 5$ and $22 \cdot 5 \times 10^4$ erg cm^{-2} sec^{-1}) and low windspeed ($1 \cdot 5$ and $3 \cdot 1$ cm sec^{-1}) conditions. The substances used were Adol 52 (a formulation of cetyl alcohol), OED Green (a formulation of oxyethylene docosanol), S-600 (a plastic transplanting spray), and phenyl mercuric acetate.

All substances caused significant reductions in transpiration which were associated with marked increases in the diffusive resistance of the leaf to water vapour transfer due primarily to stomatal closure. All substances also caused reductions in photosynthesis which appeared to be due in part to increased resistance to CO_2 diffusion, and in part to metabolic inhibition of the photosynthetic process.

Phenyl mercuric acetate, at concentrations of 10^{-4} and 10^{-5}M, was the only substance which caused proportionately greater reductions in transpiration than in photosynthesis. This was achieved by stomatal closure without apparent effect on the photosynthetic apparatus. This caused a significant reduction in the transpiration ratio.

It is emphasized that while substances such as those tested can induce substantial reductions in transpiration, for maximum effectiveness the applied material should either develop a low permeability film over the entire leaf, or induce stomatal closure, without metabolically interfering with photosynthesis.

I. Introduction

For a number of years attention has been given to the possibility of suppressing transpiration from plants by reducing the permeability of the leaf surface to water vapour movement. One obvious means of achieving this is to manipulate the stomatal apparatus and many examples of phenomena or substances known to induce stomatal closure are reported in the literature (Heath 1959). An alternative procedure is to impose a low permeability film over the entire leaf, as has been the aim in nursery practice during planting out of seedlings (Miller, Nielson, and Bandemer 1937; Miller *et al.* 1950).

Interest in this problem has been stimulated recently by experiments concerned with suppression of evaporation from exposed water surfaces (Mansfield 1955, 1958) and, as a result, a considerable number of investigations have been conducted, using not only various formulations of cetyl alcohol (Roberts 1961) but also a range of plastic films (Gale 1961; Gale and Poljakoff-Mayber 1964), wax emulsions (Miller

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et al. 1950; Allen 1955), and dilute concentrations of metabolic inhibitors (Zelitch 1961; Smith and Buchholtz 1962; Stoddart and Miller 1962; Zelitch and Waggoner 1962*a*, 1962*b*). Most of these materials have been observed to reduce transpiration to various degrees, but the various modes of action are still not properly evaluated, even though there is good evidence that the inhibitor group acts primarily through inducing stomatal closure (Heath 1959; Zelitch and Waggoner 1962*a*, 1962*b*).

From the physiological viewpoint, it is important not only to understand the nature of transpiration suppression but also to investigate the effects of compounds, such as those given above, on photosynthesis and plant growth. Although transpiration suppression, if achieved without serious injury to treated plants, could in itself be of considerable practical value, the benefit would be greatly enhanced if suppression could be obtained without a proportional reduction in photosynthesis.

Since both transpiration and photosynthesis involve gaseous diffusion across the leaf–air interface it seems probable, from a superficial examination, that an increase in the resistance across this zone would affect both processes to a similar degree. However, more detailed examination reveals an important difference in the two diffusion pathways.

Transpiration involves the evaporation of water from sites primarily located in the walls of internal mesophyll cells, and its diffusion through two resistances, connected in series, to the free air outside the leaf. The first of these resistances is encountered by the diffusing vapour as it moves up to, and through, the stomatal pore, and in this paper it will be termed leaf resistance r_l. (Some evaporation takes place from the epidermal cell walls and the vapour diffuses directly through the leaf cuticle. The amount is very small, however, and this pathway will be neglected in this analysis.) The vapour then diffuses across the external boundary layer, which sheathes the leaf, finally reaching the free air beyond where convective transfer processes dominate gas transfer. This external resistance in the boundary layer will be termed r_a.

Transpiration can therefore be represented by an expression basically similar to Fick's law where the total flux, T, in terms such as g (or cm^3) cm^{-2} sec^{-1} can be written as

$$T = \Delta c/(r_a+r_l), \tag{1}$$

where Δc is the difference in water vapour concentration at the evaporating sites and in the free air [in g (or cm^3) cm^{-3}] and (r_a+r_l) is the total resistance to flow in sec cm^{-1}.

Photosynthesis also involves these internal and external resistances since CO_2, in entering the plant, must pass through the same pathway in the reverse direction. These resistances for CO_2 transfer, r'_a and r'_l, are related to r_a and r_l by the relationship

$$(r'_a+r'_l)/(r_a+r_l) = D/D', \tag{2}$$

where D and D' are, respectively, the diffusion coefficients of water vapour and CO_2 in air. Their approximate values are 0·24 and 0·14 cm^2 sec^{-1} so that the ratio of D/D' is about 1·7. However, the CO_2 must diffuse, not only to the mesophyll cell walls, but through the walls to the photosynthetic sites in the chloroplasts, and this liquid phase transfer involves an additional resistance, r'_m, connected in series to r'_a

and r'_l. Consequently, an expression for photosynthesis, P, can be written in a form similar to that for transpiration in equation (1) as follows

$$P = \Delta c'/(r'_a+r'_l+r'_m), \tag{3}$$

where $\Delta c'$ is the concentration difference between the photosynthesizing sites in the chloroplasts and the free air, and $(r'_a+r'_l+r'_m)$ is the total resistance to CO_2 transfer, all in the same units as used previously.

Few values for r'_m are available, but most evidence suggests that it is generally several times as high as (r_a+r_l) (Gaastra 1959, 1962). Because of this, and because r_a is usually relatively low under even moderate windspeed conditions (0·5–2·0 sec cm^{-1}) it is apparent that factors which influence r_l may cause (r_a+r_l) to increase several fold and hence cause a marked reduction in transpiration. The effect on $(r'_a+r'_l+r'_m)$, however, and hence on photosynthesis, could be comparatively small so that a significant improvement in efficiency of water use could be obtained.

Because of the potential significance of this phenomenon, it was decided to conduct a series of experiments to investigate the direct effects of various transpiration suppressants on both transpiration and photosynthesis first, and then to interpret these effects in terms of the various diffusion resistances known to occur in the water vapour and CO_2 pathways. The experiments were conducted on the leaves of young cotton plants in conjunction with comprehensive studies of transpiration (Slatyer and Bierhuizen 1964*a*) and photosynthesis (Bierhuizen and Slatyer 1964*a*) from which a considerable amount of basic material concerning diffusive resistances had been obtained. The experiments were preceded by preliminary investigations on whole plants (Slatyer and Bierhuizen 1964*b*).

II. Experimental

The experiments were conducted with a large number of cotton plants (cv. Pope) grown in earthenware pots in a heated, naturally lit, greenhouse. During the experimental period environmental conditions were fairly constant, maximum day temperature ranging from 35–40°C and daylength from 14–15 hr. At night minimum temperature was kept at 25°C with artificial heating. The plants were grown at a high level of nutrition and with frequent watering to minimize water stress. They were used for experimental purposes when they had reached a height of 35–40 cm. Measurements were made on one leaf which was selected for size and uniformity by using a standard leaf template of 100 cm^2. In this way the leaf material actually used for each experiment was very similar in geometry and seldom varied more than $\pm 5\%$ in leaf area. Even so leaf area was measured, by tracing the leaf outline and planimetering the enclosed area, and proportional adjustments made. In this paper leaf area therefore means the area of the leaf outline and *not* the total area of the upper and lower surfaces (i.e. 2 × outline area) as in some investigations.

When the plants had reached the desired growth stage, those required for experimental purposes were selected for uniformity and the transpiration suppressant treatments imposed. The following four substances, all of which had been used by other investigators, were used and concentrations were selected following preliminary

experiments which indicated lower limits below which no significant effects could be observed and upper limits above which serious injury was caused during the test period:

(1) *Adol 52*: This material was obtained from Archer Daniels Inc., U.S.A. The stock material consisted of 95% cetyl alcohol plus about 1·5% C_{14}-alcohols and 1·5% C_{18}-alcohols. The solid material was made up into a stable water emulsion and the actual concentrations used (weight of solids/volume of emulsion) were 1·25, 2·5, 12·5, and 25%.

(2) *OED Green*: This material was supplied through the courtesy of Nikken Chemicals Ltd., Japan. It consisted of a 55 : 45 mixture of $C_{22}H_{45}O(C_2H_4O)H$ and $C_{18}H_{37}O(C_2H_4O)H$ and was made up into a 10% water emulsion. Actual concentrations used (weight of solids/volume of emulsion) were 0·5, 1·0, 2·0, and 5·0%.

(3) *S-600*: This material was obtained from Synchemicals Ltd., London. (It is sold as VL. 600 by the B. F. Goodrich Co. in the U.S.A.) No specific details of its composition or concentration were available, but it is a stable colloidal water dispersion of a modified vinyl resin (Witty 1953). Actual concentrations of the stock emulsion used were 5, 10, 50, and 100% (v/v) dilutions.

(4) *Phenyl mercuric acetate*: This compound was dissolved in 70% alcohol and stirred into water at concentrations of 10^{-5}, 5×10^{-5}, 10^{-4}, 10^{-3}, and 10^{-2}M.

To all solutions and emulsions a spreader (Tween 20) was added at a dilution of 1 : 2000. The control plants were treated with distilled water plus spreader.

The treatments were imposed between 4 and 5 p.m. on day 0. Each plant was inverted and dipped in the appropriate treatment solution, removed, and allowed to drain until dripping ceased. (Spraying was used initially but it was found that the amount of material applied could vary by a factor of more than 2 and it was therefore discontinued.) Two series of experiments were then conducted, one being short and one long term. The short-term experiments involved two sets of observations on a group of plants receiving all treatments on days 1 and 3 after dipping. Measurements of transpiration, photosynthesis, and leaf and air temperature were made on the same leaf of each plant at two light intensities, 7·5 and $22·5\times10^4$ erg cm^{-2} sec^{-1} (corresponding to 2000 and 6000 f.c.), and at two windspeeds, 1·5 and 3·1 cm sec^{-1}. Other conditions, held constant, were bulk air temperature 35°C, bulk air relative vapour pressure 0·6, bulk CO_2 concentration 0·03%.

In the long-term experiments observations were made on days 2, 4, 8, 12, 18, and 25 after dipping. In this case only six treatments were employed, apart from the control. These were S-600: 10 and 50%; OED: 1·0 and 5·0%; and phenyl mercuric acetate: 5×10^{-5} and 10^{-4}M. The same measurements were made, again using the same leaf on each occasion, but at one light intensity ($22·5\times10^4$ erg cm^{-2} sec^{-1}) and one windspeed (1·5 cm sec^{-1}) only. The same bulk conditions of temperature, humidity, and CO_2 concentrations were employed.

The experiments were conducted in conjunction with a large series of related investigations on transpiration and photosynthesis of cotton plants (Slatyer and

Bierhuizen 1964*a*; Bierhuizen and Slatyer 1964*a*, 1964*b*). Full details of the experimental techniques used for transpiration and photosynthesis measurements can be found in these papers, together with details of the calculations used to determine external and internal resistances to water vapour and CO_2 transfer. All photosynthesis data refer to apparent photosynthesis and therefore include respiration effects.

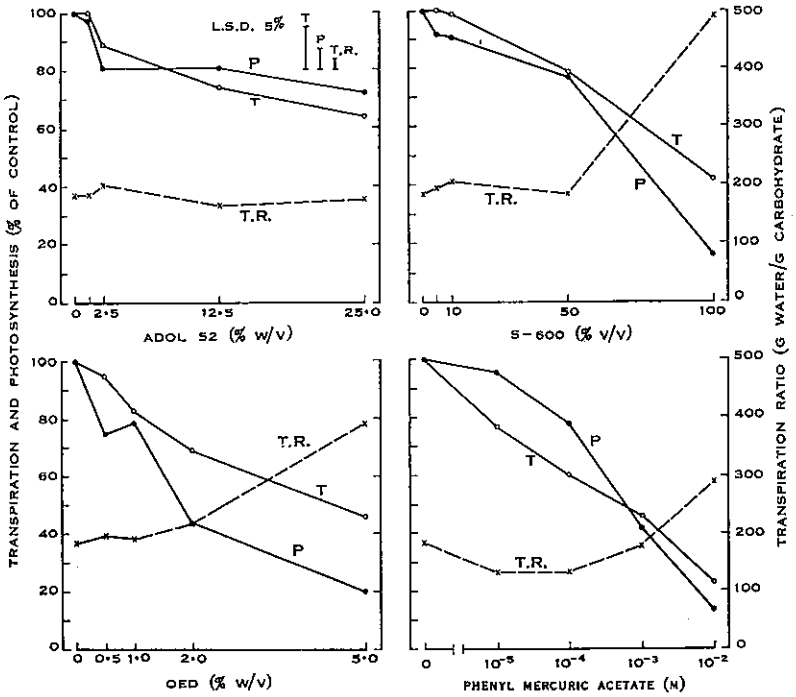

Fig. 1.—Transpiration (T), photosynthesis (P), and transpiration ratio ($T.R.$) of cotton leaves treated with various concentrations of four transpiration suppressants.

III. Results

(*a*) *Short-term Experiments*

In Figure 1 the transpiration and photosynthesis data are presented for all concentrations of the four transpiration suppressants investigated. The primary data were collected in terms of weight (in grams) of water and volume (in cm^3) of CO_2 per unit leaf area per unit time, but for ease of comparison are plotted as percentages of the untreated controls. Also included is the "transpiration ratio" calculated by dividing the transpiration rate by the rate of carbohydrate production, assuming that 44 g CO_2 is associated with 30 g carbohydrate in the plant. Differences required for significance at the 5% level were calculated from the variation found amongst the four nil-treatment controls. Converted to percentages, as used in Figure 1, differences in transpiration, to be significant at this level, had to exceed 15% and in photosynthesis had to exceed 7·5%. Differences in transpiration ratio had to exceed 20.

Because of space limitations and the fact that the other treatments provided similar results, the data given represent only the high light intensity ($22 \cdot 5 \times 10^4$ erg cm^{-2} sec^{-1}), high windspeed ($3 \cdot 1$ cm sec^{-1}) treatment and are the means of the observations made 1 and 3 days after treatment imposition. Under these conditions, the photosynthetic apparatus was light-saturated (Bierhuizen and Slatyer 1964*a*) so that photosynthesis was limited by the rate of diffusion of CO_2 to the chloroplasts. Also, stomatal resistance was minimal. The effects of any factors which influenced r_l, r'_l, or r'_m would therefore be expected to be most apparent.

Figure 1 shows that with Adol 52 photosynthesis and transpiration both decreased to a similar extent with increasing concentration, the transpiration ratio remaining virtually the same. With S-600, the trend was similar at low concentrations but at the highest concentration transpiration declined rapidly. However, the decrease in photosynthesis was even more pronounced, resulting in a marked increase in transpiration ratio. The same response pattern was observed with OED.

The effect of phenyl mercuric acetate on transpiration was also generally similar, but photosynthesis was less affected at concentrations below 10^{-3}M. This resulted in a significant decrease in the transpiration ratio at these concentrations.

The total and internal resistances to water vapour transfer, (r_a+r_l) and r_l, and to CO_2 transfer $(r'_a+r'_l+r'_m)$ and r'_m were calculated from the data of Figure 1 and are given in Table 1. Actual values of r_l were obtained from equation (1) using an r_a value of $1 \cdot 6$ sec cm^{-1} which was obtained by Slatyer and Bierhuizen (1964*a*) for a windspeed of $3 \cdot 1$ cm sec^{-1} using identical environmental conditions and the same plant material.

Estimates of $(r'_a+r'_l+r'_m)$ were obtained using equation (3) and assuming that $\Delta c'$ was equal to $0 \cdot 03\%$ CO_2 so that $(r'_a+r'_l+r'_m) = \Delta c'/P$. Then, r'_m can be found by subtraction, using equation (2) to calculate $(r'_a+r'_l)$. It should be emphasized that the validity of this approach to provide estimates of r'_m, which are in fact estimates of liquid-phase resistance to diffusion of CO_2, depends on the chloroplast concentration of CO_2 being effectively zero and on the validity of equation (2). The first assumption appears to be reasonable in the control treatments which were light-saturated at the CO_2 level used, but when the photosynthetic reaction is partially inhibited by the transpiration suppressants it is probable that the concentration of CO_2 at the chloroplasts is significantly greater than zero. If this is so r'_m is overestimated but it still gives an indication of the effective resistance which can be used to explain observed reductions in rate of photosynthesis. The second assumption, involving equation (2), will introduce errors if the "film"-type compounds are differently permeable to CO_2 and water vapour. However, at the concentrations used, transpiration suppression did not approach the stage associated with stomatal closure and it seems probable that the effective pore size of the films was relatively large and differential permeability effects relatively small.

Figure 2 is of value in explaining the results, and the specific effects of the various transpiration suppressants, of Figure 1. It can be seen from Figure 2 that Adol 52 has only a small effect on r_l and r'_m and the absence of marked changes in the ratio of the total resistances $(r'_a+r'_l+r'_m) : (r_a+r_l)$ is a reflection of the relative

constancy of the transpiration ratio. In the case of S-600 a similar pattern is observed except at the highest concentration where a steep increase in r_l is associated with a steeper increase in r'_m, and a consequent increase in the transpiration ratio. In OED both r_l and r'_m increase through the range of concentrations. With phenyl mercuric acetate the explanation of the previously observed reduction in the transpiration ratio is seen to be due to r'_m remaining at a steady value at concentrations up to 10^{-4}M, while a progressive increase occurred in r_l. Subsequently r'_m increased more rapidly than r_l, the final value being of a similar order to that observed with OED.

TABLE 1

SUMMARY OF RESISTANCES IN COTTON LEAVES TO DIFFUSION OF WATER VAPOUR AND CARBON DIOXIDE

Treatment	Concentration	Resistances to Water Vapour Movement		Resistances to Carbon Dioxide Movement	
		(r_a+r_l)	r_l	$(r'_a+r'_l+r'_m)$	r'_m
Adol 52	Nil	2·3	0·7	11·3	7·4
	5% (w/v)	2·4	0·8	11·6	7·5
	10% (w/v)	2·6	1·0	11·8	7·4
	50% (w/v)	2·4	0·8	12·4	8·3
	100% (w/v)	3·1	1·5	15·6	10·3
S-600	Nil	2·3	0·7	11·4	7·5
	5% (v/v)	2·4	0·8	11·5	7·4
	10% (v/v)	2·3	0·7	12·8	7·9
	50% (v/v)	2·6	1·0	12·9	8·5
	100% (v/v)	12·1	10·5	59·6	39·6
OED	Nil	2·6	1·0	12·1	7·6
	5% (w/v)	2·9	1·3	14·9	10·0
	10% (w/v)	3·4	1·8	15·3	9·5
	20% (w/v)	5·5	3·9	21·0	11·4
	50% (w/v)	9·6	8·0	50·4	33·8
Phenyl mercuric acetate	Nil	2·2	0·6	10·0	6·3
	10^{-5}M	3·8	2·2	12·3	5·8
	10^{-4}M	5·3	3·7	14·6	5·6
	10^{-3}M	9·7	8·1	22·6	6·1
	10^{-2}M	21·5	19·9	70·8	34·2

As mentioned above, it is difficult to obtain, from estimates of r'_m, a definite indication as to whether the observed inhibitions of photosynthesis were due to a real increase in r'_m and so act physically or are only apparent, not directly affecting CO_2 diffusion, and hence act metabolically. Accordingly, additional data on r'_m are presented in Figure 3 from the experiments at a light intensity of $7{\cdot}5 \times 10^4$ erg cm^{-2} sec^{-1} in which the photosynthetic apparatus was not light-saturated; in other words, when the rate of diffusion of CO_2 to the chloroplasts was presumably not limiting the rate of photosynthesis. On this assumption it may be proposed that, should the

transpiration suppressants act by directly increasing r'_m, this increase should be delayed and reduced at the lower light intensity where CO_2 diffusion was not directly limiting. If, on the other hand, the mode of action is by metabolic inhibition, it is more probable that the reduction in photosynthesis, and hence the proportional change in r'_m, could be expected to be similar at both low and high light intensities.

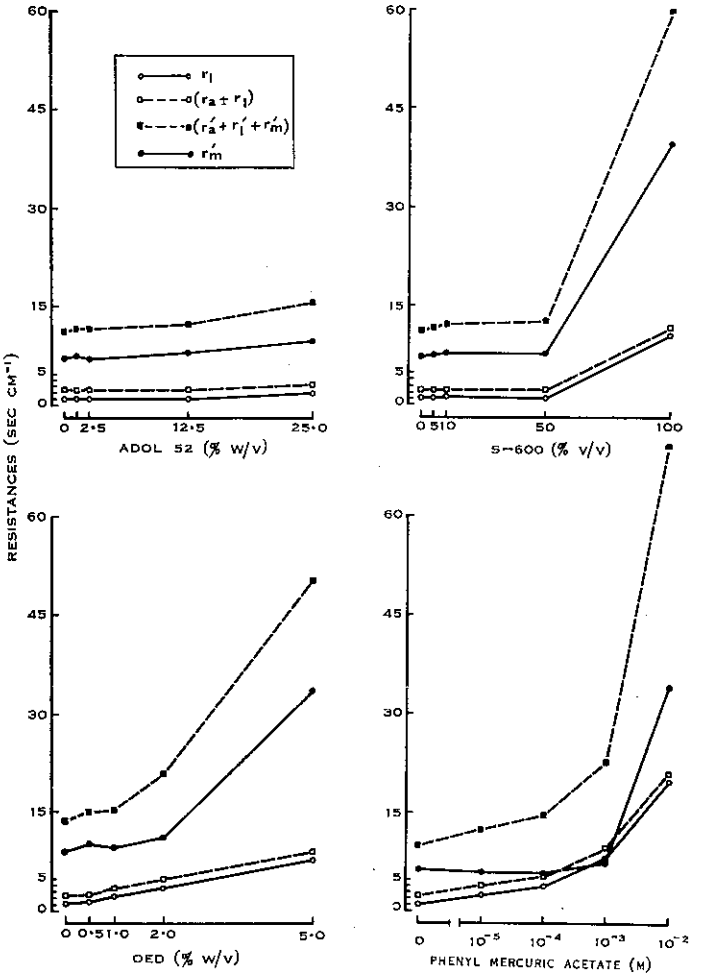

Fig. 2.—Total (r_a+r_l) and internal (r_l) diffusion resistances to water vapour transport and total $(r'_a+r'_l+r'_m)$ and internal (r'_m) diffusion resistances to CO_2 transport in cotton leaves treated with various concentrations of four transpiration suppressants.

From Figure 3 it can be seen that the ratio of $r'_{m(7\cdot5)}$ to $r'_{m(22\cdot5)}$ is relatively constant in all treatments. Although it was not possible to calculate meaningful values for least significant difference, all values lay within a range of ratios of 1·8–2·1 so that any direct effects of the treatments on r'_m appear to be small.

(b) Long-term Experiments

In Figure 4 data for transpiration, photosynthesis, and transpiration ratio are plotted against time for the long-term experiments which continued for 25 days following treatment imposition. The experiments were conducted at a windspeed of $1 \cdot 5$ cm sec^{-1} and a light intensity of $22 \cdot 5 \times 10^4$ erg cm^{-2} sec^{-1}. As in Figure 1, differences required for significances at the 5% level were calculated from the variation found among nil-treatment control plants. To be significant at this level differences in transpiration had to exceed $0 \cdot 15$ g 100 cm^{-2} hr^{-1}; in photosynthesis $1 \cdot 2$ mg 100 cm^{-2} hr^{-1}; and in transpiration ratio 14.

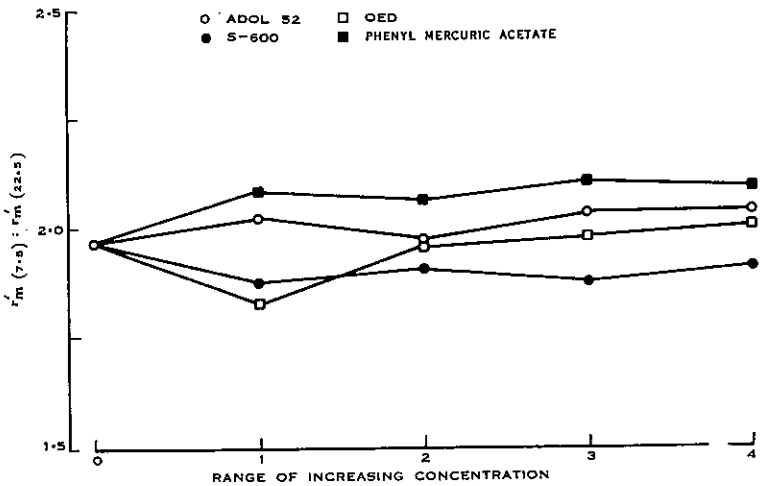

Fig. 3.—Influence of concentration of transpiration suppressants on the ratio of r'_m at $7 \cdot 5 \times 10^4$ erg cm^{-2} sec^{-1} $[r'_{m(7 \cdot 5)}]$ to the value at $22 \cdot 5 \times 10^4$ erg cm^{-2} sec^{-1} $[r'_{m(22 \cdot 5)}]$. The numbers 0–4 refer to increasing concentrations of transpiration suppressants in the ranges used for Figures 1 and 2.

The initial responses are very similar to those reported earlier but it is most interesting to observe the subsequent trends in the treatment and control plants. In the control, both transpiration and photosynthesis declined throughout the experimental period, the final values of transpiration being about one-half, and those of photosynthesis less than 40%, of the original. In consequence the transpiration ratio increased significantly. By comparison transpiration of all of the high-concentration treatments remained fairly steady during the experimental period and photosynthesis only declined slightly, resulting in a relatively small increase in transpiration ratio. In the low-concentration treatments the general response was intermediate, photosynthesis declining at about the same rate as the control, but transpiration less rapidly.

The actual values of the transpiration ratio are also of interest. The high-concentration OED treatment was significantly higher than the control throughout the experimental period and at both phenyl mercuric acetate concentrations were significantly lower, the differences being a similar order to those observed in Figure 1.

In Figure 5, values of the diffusive resistances r_l and r'_m are plotted from the data of Figure 4. It is at once apparent that the marked reduction in transpiration rate

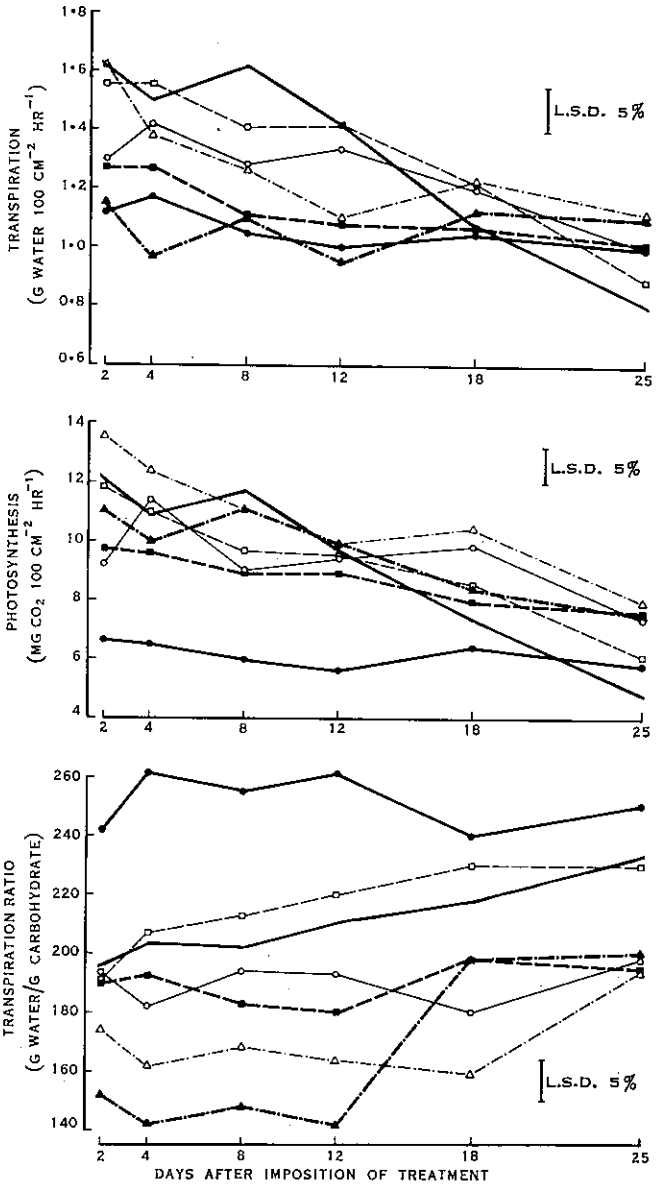

Fig. 4.—Effect of three transpiration suppressants on transpiration, photosynthesis, and transpiration ratio of cotton leaves over a 25-day period. —— Nil (control); ○ OED, 1%; ● OED, 2%; □ S-600, 10%; ■ S-600, 50%; △ phenyl mercuric acetate, 5×10^{-5}M; ▲ phenyl mercuric acetate, 10^{-4}M.

of the control, and the related increase in the transpiration ratio, is due to a steep increase in r_l in the latter part of the experimental period, r'_m increasing to a relatively small degree.

In the initial part of the period there was little change in r_l, the high-concentration treatments giving, in general, values 2–3 times as high as the control and the low-concentration treatments, 1–2 times. Subsequently r_l increased in most treatments

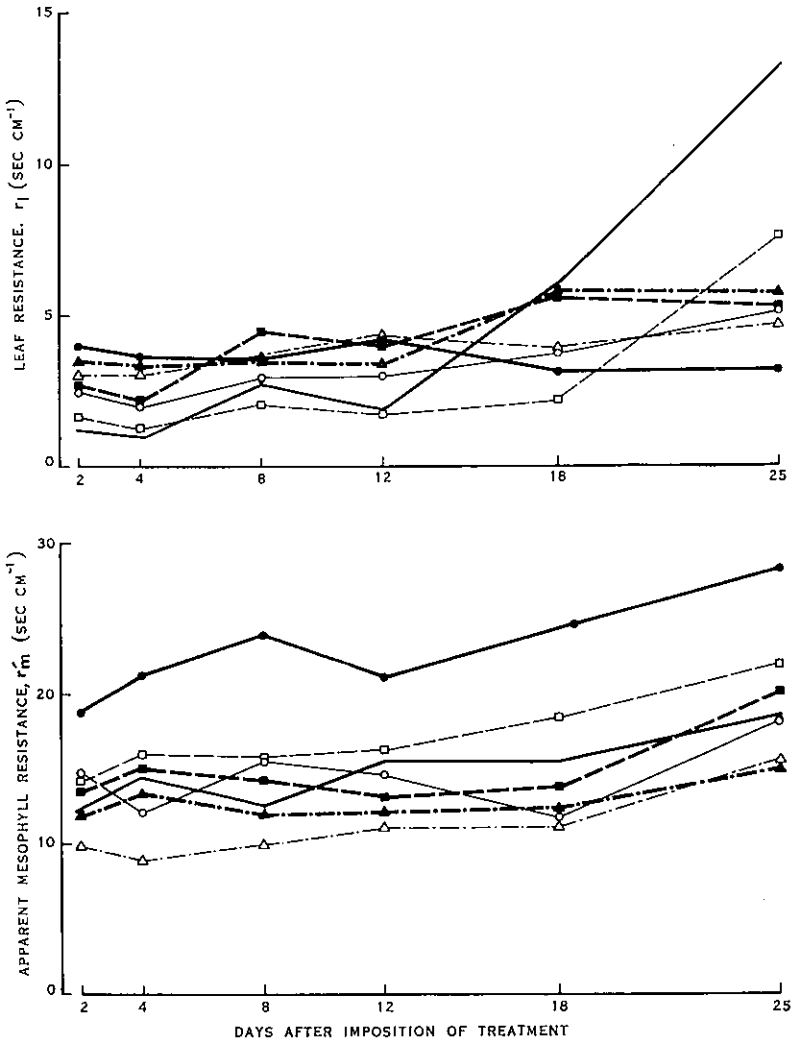

Fig. 5.—Effect of three transpiration suppressants on specific diffusive resistances to water vapour movement (r_l) and CO_2 movement (r'_m) in cotton leaves over a 25-day period. —— Nil (control); ○ OED, 1%; ● OED, 2%; □ S-600, 10%; ■ S-600, 50%; △ phenyl mercuric acetate, 5×10^{-5}M; ▲ phenyl mercuric acetate, 10^{-4}M.

but in no case as rapidly as in the control. By comparison, treatment values of r'_m showed a similar trend to those of the control over the whole period, and the actual values of r'_m did not differ markedly from those of the control except in the 20% OED treatment.

(c) *Effect of Transpiration Suppressants on Leaf Temperature*

Reduction in transpiration inevitably results in a greater proportion of the incident radiation which reaches the leaf being dissipated by processes other than latent heat transfer. Since the heat storage capacity of the leaf is small, and the proportion of energy used in photosynthesis is also small, the primary source of energy dissipation is by transfer of sensible heat from leaf to air.

While it is not the purpose of this paper to consider the nature of the sensible heat transfer process, it is of interest to examine the effect of transpiration suppression on leaf temperature, since most plants have fairly specific temperature thresholds and injury could be expected to occur if these were exceeded.

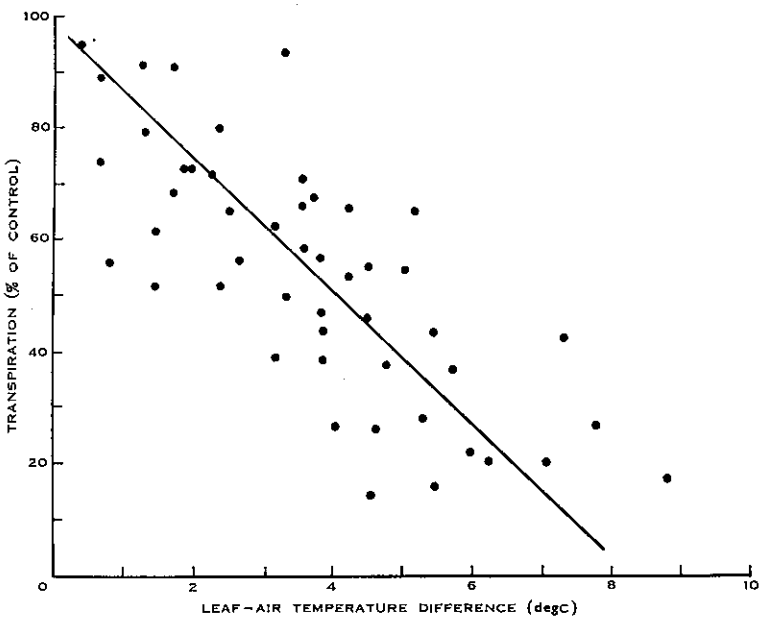

Fig. 6.—Effect of transpiration suppression on leaf–air temperature difference.

Accordingly, in Figure 6, the difference between leaf temperature and bulk air temperature, measured at the leaf chamber air inlet, is plotted against the percentage reduction in transpiration for all treatments used in both the short-term and long-term experiments.

The data, although providing a degree of scatter, give an approximate estimate of the order of leaf temperature change expected. With the windspeeds (and hence r_a values) used it can be seen that a reduction of 50% in transpiration is associated with an increase of leaf temperature over air temperature of about 4 degC, and that complete inhibition would be expected to give a difference of 8–9 degC.

IV. Discussion

Two main conclusions appear to stand out from the results, firstly that all the transpiration suppressants used appeared to cause significant reductions in transpiration; secondly that, except in the case of low concentrations of phenyl mercuric acetate, no treatments significantly improved water-use efficiency by reducing the transpiration ratio. These results are in good agreement with those obtained for whole plants by Slatyer and Bierhuizen (1964*b*).

However, it is appropriate to comment that the reductions in transpiration in the case of Adol 52, OED, and S-600 were achieved only at rather higher concentrations than those used elsewhere. Witty (1953), for example, recommends the use of S-600, as an antidesiccant, at equivalent concentrations of 10% and the manufacturers of OED claim significant reductions in transpiration at equivalent concentrations of less than 1%. By comparison results with phenyl mercuric acetate provided good agreement with the experiments of Zelitch and Waggoner (1962*a*, 1962*b*) at the concentrations used by these workers.

The mode of action of the suppressants on transpiration through an effect on r_l (Fig. 2) is almost certainly due to stomatal resistance, since cuticular resistance has been shown to be so high (32 sec cm^{-1}, cf. Slatyer and Bierhuizen 1964*a*). An increased cuticle resistance would only be of significance when the stomatal resistance reached a similar order of magnitude. The apparent degree of stomatal closure can be seen from Figure 2 to be only partial, even in the highest concentration of phenyl mercuric acetate where r_l reached a value of about 20 sec cm^{-1}. With S-600 and OED the values did not exceed 10 sec cm^{-1}. Even so this was still 10 times as high as the value for open stomata. It is also possible that some reduction in transpiration could be achieved by increasing the albedo of the leaf. However, net radiation, measured above the highest S-600 treatment (which was the most effective for this purpose) gave values only 5% lower than the control. There was no significant difference in the net radiation received by the other treatments, compared with the control, even at the highest concentrations used.

For maximum effectiveness a transpiration suppressant should operate on r_l alone so that r'_m remains unchanged and transpiration is reduced proportionally more than photosynthesis. In the control treatments of the present experiments the values of r_l averaged 1·0–1·5 sec cm^{-1} and r'_m 8–12 sec cm^{-1} so that with r_a at 1·5 sec cm^{-1}, trebling of r_l could be expected to cause a 50% reduction in transpiration and, if r'_m remained unchanged, only a 20% reduction in photosynthesis. In the case of the 10^{-4}M phenyl mercuric acetate treatments this readjustment was almost achieved and the transpiration ratio was reduced by 25% indicating stomatal aperture alone was affected. In all the other treatments, however, increases in r_l were linked with increases in r'_m and in those cases photosynthesis was reduced, proportionally, more than transpiration, causing an increase in the transpiration ratio.

Since phenyl mercuric acetate tends to act as a metabolic inhibitor, it was anticipated that at the low concentrations stomatal closure would be induced and that at the high concentrations there would be direct inhibition of photosynthesis. The

evidence presented supports this view. By comparison the primary mode of action of the other substances was expected to be through the formation of a relatively impermeable film over the entire leaf. In the case of OED and Adol 52 it was also thought that a monomolecular film may have spread over the internal substomatal surfaces.

Both of these phenomena may have occurred but the fact remained that the apparent value of r'_m also was affected and, rather than a direct increase being caused by film formation over the mesophyll surfaces, the primary effect appeared to be on metabolic inhibition of photosynthesis itself. However, it should be mentioned again that the procedure for calculating r'_m involves possible errors as outlined previously. Conclusions based on assumed values of r'_m should therefore be interpreted cautiously.

It is unlikely that other factors, such as increased leaf temperature, would have metabolically suppressed photosynthesis, since the highest temperatures measured did not exceed 42°C and cotton is frequently grown in areas with daily maximum temperatures exceeding 45°C without injury (Eaton 1955). In consequence it appears that these compounds produced undesirable side effects and it would seem that, when more other substances are being examined, emphasis should be placed either on the ability to develop and maintain relatively impermeable films on plant leaves or to induce stomatal closure, without seriously inhibiting the metabolic effectiveness of the photosynthetic apparatus.

The long-term experiments provided the interesting result that effective reductions in transpiration could be maintained for 25 days, even though reductions in photosynthesis were also maintained, and the transpiration ratio did not change greatly. Also of interest was the progressive decline, particularly in transpiration, of the control at a more rapid rate than in the treated plants so that at the end of the test period lowest transpiration was observed in the control. The most probable explanation for this phenomenon is that in the control, total growth of the plants was unimpeded and a substantial amount of new leaf growth occurred during the test period. Associated with this was probably a considerable degree of solute translocation to the new tissue with consequent senescence of the test leaves. By comparison, in the treated plants, total growth was suppressed along with photosynthesis and less new leaf material developed. In consequence less translocation would be expected and the test leaves could be expected to remain relatively more active than in the controls. Good supporting evidence for these conclusions, obtained with whole plants, has been obtained by Slatyer and Bierhuizen (1964*b*).

The observed increases in leaf temperature due to transpiration suppression, although substantial, were almost certainly without deleterious effects on cotton growth. However, it is probable that growth of some species could be impaired by increases of 5–10 degC, should leaf temperatures exceed physiological threshold levels.

The work of Raschke (1956, 1960) and Takechi and Haseba (1962) enable some estimates of expected temperature change to be made, assuming different degrees of transpiration suppression. Taking an extreme case of 80% suppression under radiation levels of $1 \cdot 0$ cal cm^{-2} min^{-1}, it can be assumed that about $0 \cdot 8$ cal cm^{-2} min^{-1} will have to be dissipated by sensible heat transfer processes. Assuming also that

the heat transfer coefficient is 0·06 cal cm^{-2} min^{-1} $degC^{-1}$ for both leaf surfaces (appropriate for a windspeed of about 1 m sec^{-1} and a leaf width of about 10 cm) the leaf–air temperature difference would need to be approximately 13 degC to dissipate this amount of energy. Under most outdoor conditions effective windspeed would exceed 1 m sec^{-1} and radiation levels of the order quoted are unusual, but this estimate provides an example of the care which should be exercised if heat-sensitive plants are under investigation.

In conclusion it may be emphasized that, although only one transpiration suppressant produced a significant improvement in water-use efficiency, all substances tested caused marked reductions in transpiration rate, without apparent long-term ill effects on the plants used. While considerable scope still exists, therefore, for a compound acting more specifically on r_l and, hence causing a real and pronounced improvement in water-use efficiency, the present results indicate that transpiration suppression is already possible and may have far-reaching practical implications if carried out in an appropriate biological and economic situation and with an understanding of the physical and biological phenomenon involved.

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