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Part I: Potential Usefulness of Antitranspirants for Increasing Water Use Efficiency in Plants

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PART I

TECHNICAL COMPLETION REPORT

on

POTENTIAL USEFULNESS OF ANTITRANSPIRANTS FOR INCREASING WATER USE EFFICIENCY IN PLANTS

W-174

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WATER RESOURCES CENTER
UNIVERSITY OF CALIFORNIA

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ABSTRACT

Antitranspirants conserve water and maintain favorable plant water balances by reducing stomatal apertures, by forming a thin film over the leaves, or by reflecting excessive radiation. Under normal conditions, reductions in both transpiration and photosynthesis are to be expected, but reduction in growth does not always occur, and need not always be disadvantageous when it does. Antitranspirants do not raise leaf temperature excessively, and are not likely to interfere greatly with mineral nutrition. They are likely to be most effective in reducing transpiration when other factors (boundary layer and stomatal resistances) are not large. The effectiveness of an antitranspirant also depends on plant factors such as stomatal distribution and amount of new foliar growth, and on spray factors such as degree of coverage, concentration and amount of spray, and phytotoxicity.

Investigations on possible uses for antitranspirants included experiments on: 1) reducing irrigation frequency and growth of highway oleanders; 2) reducing water requirement of turf grass; 3) growth, yield and water use of an annual field crop; 4) increasing survival of transplants; 5) increasing vase life of cut flowers and reducing water loss from bedding plants for shipment; 6) prolonging life of cut Christmas trees; 7) correcting plant disorders associated with water balance, e.g., lettuce tip burn, bean blossom drop, prune cracking, and cherry cracking; 8) increasing water potential and fruit growth of orchard trees, including olives, peaches and apricots.

INTRODUCTION

Objectives

1. Evaluation of new antitranspirant materials (e.g., films, reflectants and metabolic inhibitors) for phytotoxicity and their effect on transpiration, relative turgidity, leaf temperature, net photosynthesis and growth.

2. Develop techniques for applying antitranspirants and for evaluating the completeness of their coverage on plant leaves, their stability and specific effects on stomatal movement.

3. Investigate the potential usefulness of various combinations of antitranspirant materials as a means of increasing the water-use efficiency of plants under various environmental conditions.

4. Study special uses of antitranspirants in agriculture, ornamental horticulture, and watershed management.

The first three objectives will be covered in Part I which deals with Basic Investigations, and the fourth objective in Part II which deals with Applied Investigations. The basic and preliminary work enables greater efficiency in carrying out the applied experiments in the greenhouse and field. The applied experiments often bring up new problems which require investigation at the laboratory level.

Relevance

Antitranspirants are chemicals that reduce water loss from plants when applied to their foliage. They are used to 1) save water by reducing transpiration; 2) maintain a favorable plant water balance by reducing lag between water loss and uptake; and 3) possibly act as a physical barrier against injury by pests, salt spray and smog.

To accomodate an expanding population faced with diminishing resources, man is challenged to seek new approaches to managing his water supply and its utilization. River diversions, dams, aqueducts, and ever deeper wells have typified man's approach to supplying water. However, man must now face the reality that few suitable dam sites remain which can be developed without seriously jeopardizing resources valued for other, equally important reasons. Man must seek, for the first time in this nation, solutions to water problems which fully acknowledge that the resources customarily utilized are finite and that the direct and indirect effects of water projects on the total environment are important.

Plants compete with man for the world's finite water supply. Since 99% of the water absorbed by most plants is lost directly to the atmosphere, plants constitute the least efficient step in the hydrologic cycle of an irrigated region. This fact provides man with a challenge to seek new imaginative ways to deal with water problems. Research directed toward the special uses and application of antitranspirants represents a significant effort to meet this challenge.

The most obvious use of antitranspirants is to conserve soil water and increase plant water potential. Maintenance of a high water potential at specific periods during the growth cycle of a plant may be of special benefit to some crops. Experiments indicate that antitranspirants applied just prior to harvest may aid in the final sizing of fruit, such as peaches and olives. They may aid in the survival of valuable plants in drought situations and the survival of transplanted seedlings. Antitranspirants also have the potential to help control growth, and improve the appearance and increase the yield and/or quality of some agricultural crops. In addition, film-forming materials may provide an effective barrier against insects, diseases, and salt and smog injury.

The growing competition between urban expansion and agriculture for favorable climate, land and water, forces agriculture to relocate into areas of less suitable climate and limited water supplies. Controlling the water balance in plants may allow some crops to be grown successfully in areas unfavorable to plants unless protected from excessive water loss. There are areas where there is sufficient water to sustain plant growth for only a part of the growing season. Here an antitranspirant could possibly keep plants in a favorable water balance until the water-stress period passed. There are areas where perennial plants can successfully survive once they become established. Often it is not feasible in such areas to provide the irrigation needed by the transplants until their roots can develop. This problem occurs on vast areas in reforestation projects and in ornamental plantings such as along highways.

It is possible to visualize myriad specific problems where the selective application of antitranspirants of the right form and duration may have substantial benefit. An antitranspirant unsatisfactory in one situation might be the answer to another. Therefore, the long range outlook is to foresee the development of a wide variety of antitranspirant materials, each formulated for use in a particular situation with a specific plant or crop.

Theory

There are three broad groups of antitranspirant sprays: 1) film-forming materials; 2) stomata-closing chemicals and 3) reflecting materials.

Film-forming antitranspirants: These include materials such as waxes, wax-oil emulsions, high alcohols, silicones, plastics, and latexes. They are sprayed as emulsions which dry on the foliage to form thin films. Ideally, these materials should be cheap, nonphytotoxic, resistant to breakdown and transparent to essential wavelengths of light, and should not affect growth. No known film materials, however, have a CO_2:H_2O ratio of more than one. This would suggest that, although transpiration and photosynthesis are both reduced, photosynthesis is likely to be reduced more. Several experiments, however, indicate that this is not the case.

Stomata-closing antitranspirants: Several chemicals which are capable of reducing stomatal apertures have been described by Dr. I. Zelitch of the Connecticut Agricultural Experiment Station. The most promising seem to be certain alkenylsuccinic acids and phenylmercuric acetate (PMA). The exact mechanisms by which these chemicals affect stomatal guard cells are not known. It is thought, however, that they may alter the permeability of the guard cell membranes, thereby making them more leaky to solutes. The solutes are required to drive an osmotic pump to get water into the guard cells and thereby open stomata. It is also possible that an energy system (such as photophosphorylation), which is required to enable the active intake of solutes, particularly potassium salts, is affected. A third possibility is that PMA retards the photosynthetic process, thereby causing a build-up of carbon dioxide in the intercellular spaces, with consequent stomatal closure.

Reflecting materials: These are emmulsions of white materials, such as lime or kaolinite which, after being sprayed on foliage, dry to form a coating with high reflectivity. The increased leaf albedo reduces leaf temperature and the vapor pressure gradient between the leaf and atmosphere, thereby lowering transpiration rates. Ideally, these materials should be applied to the upper surfaces of hypostomatous leaves, so as not to plug stomata and decrease photosynthesis.

The mode of action of a reflecting antitranspirant is quite different from that of a stomata-closing or film-forming type. The former acts by reducing the radiant energy necessary for transpiration and photosynthesis, while the latter two types affect the resistances in the water vapor and carbon dioxide pathways between the atmosphere and the leaf. Therefore, the following generalizations refer only to antitranspirants of the stomata-closing and film-forming type.

Equations, based on Fick's Law of Diffusion, for fluxes of water vapor (T) and carbon dioxide (P) between the leaf and the atmosphere, and their modification by the use of antitranspirants, are given below:

Antitranspirant	Transpiration	Photosynthesis
None	$T = \frac{\Delta H_2O}{r_a + r_e}$	$P = \frac{\Delta CO_2}{r_a' + r_e' + r_m'}$
Stomata-closing	$T = \frac{\Delta H_2O}{r_a + r_e + \Delta r_s}$	$P = \frac{\Delta CO_2}{r_a' + r_e' + \Delta r_s' + r_m'}$
Film-forming	$T = \frac{\Delta H_2O}{r_a + r_e + r_f - \Delta r_s}$	$P = \frac{\Delta CO_2}{r_a' + r_e' + r_f' - \Delta r_s + r_m}$

The flux of water vapor from a leaf (transpiration) is directly proportional to the water-vapor concentration gradient between the leaf and the atmosphere (ΔH_2O). It is inversely porportional to the resistances in the water-vapor pathway, namely, the resistance of the boundary layer (r_a) and the epidermis (r_e), which includes a variable stomatal resistance (r_s). Similarly, the rate of photosynthesis is directly proportional to the carbon dioxide gradient between the atmosphere and leaf (ΔCO_2) and inversely proportional to a boundary layer resistance (r_a'), and an epidermal resistance (r_e'), which includes the variable stomatal resistance (r_s'). The CO_2 pathway also contains a mesophyll resistance (r_m') that represents the impedance to carbon dioxide flux between the substomatal cavity and the chloroplasts. An increase in stomatal resistance (Δr_s and $\Delta r_s'$) caused by a nonphytotoxic stomata-closing compound will curtail both transpiration and photosynthesis. However, photosynthesis rates will be reduced less than transpiration rates, because of the large mesophyll resistance in the carbon dioxide pathway, provided r_m' is not also increased by the antitranspirant. Therefore, it should be possible to increase water use efficiencies with stomata-closing antitranspirants.

The respective resistances of a film antitranspirant to water vapor and to carbon dioxide are represented by r_f and r_f' in the equations. A decrease in rate of water loss caused by a film will, in all likelihood, increase the turgidity of the leaf and hence that of the guard cells. Consequently, the stomata may open further and thereby decrease stomatal resistance ($-\Delta\, r_s$ and $-\Delta\, r_s'$). However, it is unlikely that an external film will change the mesophyll resistance in any way. Thus, whether or not a film-forming antitranspirant increases water use efficiency will depend on the impermeability of the film to carbon dioxide and water vapor.

Therefore, under conditions conducive to stomatal opening, antitranspirants are expected to reduce photosynthesis. However, by conserving water in the soil and plant, antitranspirants maintain turgidity of the foliage and ensure high plant water potentials. Since high turgor is necessary for cell expansion, antitranspirants may be expected to increase growth under environmental conditions which would normally cause decreases in plant water potential.

While antitranspirants of the reflecting type cause a reduction in leaf temperature, the film-forming and stomata-closing types tend to increase leaf temperature (by curtailing transpiration rates and thereby reducing evaporative cooling). The heat budget of a leaf (Q) can be expressed by the following equation:

$$Q = R \pm C \pm LE \pm M$$

(Positive signs indicate heat loss by the leaf, and negative signs heat gain. Under most day-time conditions, heat is being lost by the leaf.)

Re-radiation (R) is by far the most effective means of heat dissipation, since heat loss by re-radiation is proportional to the fourth power of the temperature of the leaf. Heat dissipation by conduction and convection (C) depends on the difference between leaf and air temperature for conduction of heat, and on wind speed for forced convection. Some heat is lost by evaporative cooling (LE), and a small amount is used in the metabolic process of photosynthesis (M). In windy conditions if an antitranspirant reduces LE, R and C become more important, and in nonwindy conditions R becomes even more important as a means of heat dissipation. Therefore, an antitranspirant can raise leaf temperatures to lethal levels only if there is a drastic reduction in transpiration, an intense radiation load, and an absence of convection. Since such a combination of conditions is rare, and since R and C gain in importance as dissipators of heat when LE is reduced, antitranspirants increase leaf temperature but little. The fact that evaporative cooling is not vital to maintenance of nonlethal leaf temperatures is illustrated by many xerophytic plants, which transpire at extremely low rates under high solar radiation but still survive.

There is still some conflict as to the relative importance of active and passive mechanisms of ion uptake and transport, but it is generally agreed that transpiration does expedite ion transport in the plant. Over a long period, however, slower transpiration may have little effect on the ultimate concentration of ions in the leaves. In any event, when transpiration is reduced, the transpiration stream may possibly have a higher concentration of ions to offset the speed of their arrival at the leaves. It is unlikely that the reduction in transpiration by antitranspirants will be large enough to be deleterious to mineral nutrition, especially since antitranspirant effects decrease with time. When transpiration rate is reduced naturally (e.g., by cloudy, humid weather), a plant's mineral nutrition is not upset. The effect of an antitranspirant on mineral uptake is probably less important than its effect on reducing photosynthesis.

GENERAL COMMENTS ON EXPERIMENTAL PROCEDURES

Since the investigations with antitranspirants have been numerous and varied, specific procedures will be described as each experiment is reported. However, general experimental procedures are outlined below:

Antitranspirant application: Antitranspirant solutions or emulsions were usually sprayed on the plants. However, in some cases the plants were dipped in the solutions, and in other cases the material was applied with a small paint brush.

Foliar coverage: Distribution of the spray on plant surfaces was found by incorporating a fluorescent dye in the antitranspirant and then looking at the distribution of fluorescence under ultra-violet light. More recently, micrographs from a scanning electron microscope have been used to detect antitranspirant film on plant surfaces.

Transpiration: This was usually assessed gravimetrically by taking weight differences of potted plants and expressing transpiration rates as water loss per unit of leaf surface per unit of time. Transpiration rates of attached leaves were measured in a leaf chember by monitoring water vapor content in the air stream using a differential psychrometer.

Photosynthesis: Rates of photosynthesis were normally measured simultaneously with the differential psychrometer measurements for transpiration. Depletion of CO_2 in the air stream after passing through the leaf chamber was monitored by an infra-red gas analyzer.

Resistance: Changes in leaf resistance to diffusion of water vapor and carbon dioxide could be calculated in the leaf chamber apparatus for measuring transpiration and photosynthesis. Another instrument called a diffusion porometer or rate hygrometer (incorporating a humidity sensor and a thermistor) could be attached to leaves to determine diffusive resistance to water vapor leaving the leaf.

Leaf temperature: This could be measured using the thermistor bead in the rate hygrometer described above. Measurements were also made in the leaf chamber using 44 gauge copper constantan thermocouples and recording the output on a millivolt recorder.

Stomatal aperture: Direct measurements were made microscopically on epidermal peels from leaves. Indirect methods involved use of an infiltration technique and silicone rubber impressions of epidermal surfaces.

Water potential: An index of plant water potential was obtained by the relative water content technique, i.e., the ratio of the leaf water content at the time of sampling to the water content when maximum turgidity is achieved by floating on distilled water. Pressure potentials were measured by the pressure bomb technique. Dendrometer measurements of day-time shrinkage of tree trunks gave an index of the water balance of trees.

Growth: This was determined by measurements of yield, shoot elongation, leaf area, fruit size (using vernier callipers). Radial expansion and contraction of tree trunks were measured by Verner dendrometers (manually operated) and by Fritz dendrographs (automatic recording).

Soil Moisture: Changes in soil moisture were measured by gravimetric soil sampling, gypsum blocks, tensiometers, or a neutron moisture meter.

Location: Laboratory experiments were carried out 1) under a bank of quartz iodide and Gro-lux fluorescent lights, or 2) in a growth chamber (70" long x 30" wide by 40" high, inside dimensions) with light, temperature and humidity controls, or 3) in a walk-in growth room with light and temperature control. Some experiments were also carried out in polythene walled chambers located in a greenhouse. These chambers had temperature and humidity control and their floors revolved, thereby reducing positional variability. Most of the greenhouse experiments involved pots on benches. A heater and evaporative controller gave some temperature control, and whitewash on the glass roof reduced solar radiation in the summer. Field experiments were carried out in the University experimental fields or orchards at Davis, in University of California Field Research Stations, and in various commercial orchards.

RESULTS AND DISCUSSION

I. BASIC INVESTIGATIONS ON ANTITRANSPIRANTS

by

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BASIC INVESTIGATIONS ON ANTITRANSPIRANTS

Antitranspirant Materials

A major objective of this project is the search for new antitranspirant materials. Since we are neither staffed nor equipped for the chemical development of these materials, various chemical companies have been contacted to encourage research and production of antitranspirants. Our function, therefore, is to: 1) specify the desirable properties of antitranspirants; 2) evaluate the antitranspirants supplied by chemical companies; and 3) determine, through discussions with personnel in various agricultural and horticultural departments and through experimental results, the potential uses for antitranspirants. Desirable properties of an antitranspirant include: 1) ease of application, e.g., as a spray emulsion; 2) low surface tension of the spray to enable good wetting of vegetative surfaces; 3) durability of the film under outdoor conditions, e.g., resistance to physical abrasion , breakdown by sunlight and high temperatures, attack by microorganisms; 4) longevity of effect; (this could vary depending on the purpose for which the antitranspirant is being used); 5) high resistance to the passage of water vapor, but relatively low resistance to the passage of carbon dioxide and oxygen which are necessary for growth; (manipulation of the antitranspirant formulation to change the relative permeability of these gases would make the antitranspirant more versatile); 6) elasticity to enable the film to stretch to some extent as the leaf surface expands; 7) good shelf life; 8) inexpensive; 9) nontoxic to plants; 10) should leave no unsightly residue or residue which may be toxic to animal life if applied to edible plant parts.

A major undertaking was the initiation of correspondence to numerous chemical companies all over the United States to determine their interest in the development of antitranspirant materials. Not only was a great deal of interest shown, but we were surprised to learn of the numerous products which were already being marketed as antitranspirants, chiefly to the nursery industry. This enabled us to compile a list of commercially available antitranspirants, indicating their main ingredients, mode of action and source of availability. Copies of this list are available on request.

Samples of various experimental as well as commercially available antitranspirants were requested, and evaluation tests on these materials will be described later. Since facilities and time did not permit all of the basic and applied investigations with antitranspirants to be carried out with each and every antitranspirant material, we confined most of our investigation to one or two materials. Therefore, much of the experimental data with film-forming antitranspirants are from: 1) an experimental product (CS-6432) from the Chevron Chemical Company, Ortho Division, with whom we have been in close contact for several years, and 2) a commercially available product (Mobileaf) from the Mobil Oil Corporation, which is being used for tobacco transplanting in the east.

Some investigations have also been carried out with stomata closing antitranspirants, namely phenylmercuric acetate (PMA) and certain alkenylsuccinic acids. However, relatively little experimental work was done with reflecting materials. The reader is therefore referred to earlier investigators in this department by Aboukhaled.

Application to Plant Foliage

The effectiveness of an antitranspirant depends not only on the material itself, but also on the method of application. Evaluation of application methods was aided: 1) by the use of a rate hygrometer to determine resistance to water vapor diffusion from the leaves and 2) by incorporating a fluorescent dye to determine the effective distribution on the leaf surface. (The latter technique will be discussed in more detail under coverage.)

In an experiment with beans (Phaseolus vulgaris) a comparison was made between the following methods of application of the film forming antitranspirant CS-6432 (3%): 1) control (nothing applied); 2) fine spray (applied by aerosol applicator); 3) coarse spray (applied by hand operated piston-sprayer); 4) dip (leaves briefly submerged in antitranspirant solution). In one experiment the plants were placed in the sun, and in another they were kept in a greenhouse. In both cases, measurements were made with the rate hygrometer after the spray had dried. The results indicated that the largest increase in resistance occurred with the dip treatment, followed by the fine spray, and then the coarse spray (Table 1). However, in all cases the effectiveness (as indicated by increased resistance) was increased by the antitranspirant.

Table 1

Effect of method of application of the AT CS-6432 (3%) on diffusive resistance from Phaseolus vulgaris leaves. Each value is the average of readings made near the tip, middle and base of the lower surface of each leaf.

		Resistance (min cm^{-1})	
	Treatment	In Sun	In Greenhouse
	Control	0.07	0.14
CS-6432 (3%)	Dip	1.27	1.68
	Fine spray	1.02	0.80
	Coarse	0.34	0.26

The effectiveness of an antitranspirant is usually increased if it is applied in two doses, rather than a single application. The film antitranspirant, CS-6432 (3%), was applied on sugar beet (Beta vulgaris) leaves with a paint brush, as a single or a double application. The second coating of the double application was given as soon as the first one had dried. Inherent variability, which often occurs from leaf to leaf, was minimized by using half of a leaf for the treatment and the other half of the same leaf for control, the midrib being the dividing line between the two. Measurements of resistance to water vapor diffusion from the lower surfaces of the leaves showed that the CS-6432 was more effective in increasing resistance when given in two applications (6-fold increase in resistance) than in one application (3-fold increase in resistance). In table 2 the increased effectiveness was due to greater coverage and a thicker film.

Table 2

Effect of single and double* applications of the film-forming antitranspirant, (CS-6432, 3%), on resistance to diffusion of water vapor from the lower surfaces of sugar beet leaves. The midrib divided the control from the treated half of each leaf. (* A second coating was given after the first had dried.)

Single Application			Double Application		
Resistance (min cm^{-1})			Resistance (min cm^{-1})		
Control	CS	CS/Control	Control	CS	CS/Control
.18	.65	3.61	.10	.87	8.70
.18	.59	3.28	.13	1.07	8.23
.17	.54	3.18	.14	.55	3.93
.11	.45	4.09	.13	.69	5.31
.15	.50	3.33	.14	.79	5.64
.13	.58	4.46	.10	.86	8.60
.14	.37	2.64	.14	.86	6.00
.18	.41	2.28	.15	.60	4.00
.13	.37	2.85	.17	.66	3.88
.18	.41	2.28	.18	.86	4.78
Ave. .155	.487	3.200	.138	.779	5.907

In field experiments with almonds (to be described later) it was also found that a double spray of antitranspirant was more effective than a single spray in reducing day-time shrinkage of the tree trunks.

Even though a leaf may be completely wetted by a film-forming antitranspirant spray, a complete and uniform film is seldom formed because the hydrophobic leaf surface causes the liquid to runoff or accumulate in patches. The environmental conditions at the time of spraying probably influence the completeness of the film formed. Tests on bean leaves (Phaseolus vulgaris) indicated that a more effective film (determined by measurements of diffusive resistance to water vapor) could be formed by enhancing the rate of drying of the spray. In this experiment, the leaves were dipped in CS-6432 (2%) emulsion. One group of plants was left to drain and dry on the greenhouse bench under relatively cool conditions, while another group was placed under the greenhouse warm air blower. The antitranspirant spray in the first group of plants dried in approximately five minutes, whereas those under the blower dried in about one minute. The plants were then transferred to uniformly high lighting conditions in a growth chamber where readings were made on the lower surfaces of the leaves with a rate hygrometer. The resistance readings showed than an enhanced rate of drying of the spray doubled the realtive effectiveness of the antitranspirant film (Table 3).

Table 3

Effect of rate of drying of the film-forming antitranspirant, CS-6432 (3%), on diffusive resistance to water vapor of *Phaseolus vulgaris* leaves. Each value is the average of 8 measurements.

	Resistance ($min\ cm^{-1}$)		Resistance Ratio
Rate of Drying	Control	CS-6432	(CS-6432/Control)
Slow (approximately 5 min)	.019	.045	2.4
Rapid (approximately 1 min)	.024	.119	5.0

Since stomatal opening is dependent on light intensity, the lighting conditions at the time of antitranspirant application may have some bearing on its effectiveness. Because a film forming antitranspirant works by forming a barrier on the surface of the leaf, it is unlikely that the degree of stomatal opening, as affected by lighting conditions, would have any bearing on the subsequent effectiveness of the film. Experimental data (to be reported later) indicate that the stomata remain functional underneath the antitranspirant film, i.e., they are not glued in the open position if applied in the light, nor glued in the closed position if applied in the dark. However, stomata-closing antitranspirants act biochemically on the guard cells to reduce stomatal aperture, so that the degree of stomatal opening at the time of application may conceivably have an affect on the subsequent effectiveness of the material. The stomata closing antitranspirant, PMA, was therefore applied to one group of sugar beet leaves in the light and to another group in the dark, after allowing sufficient time for stomata to close in the latter case. Subsequent measurements in the light with the rate hygrometer showed that the PMA was effective in increasing resistance whether the treatment was given in the dark or in the light. In fact, the treatment in the dark appeared to be somewhat more effective than that in the light, though no explanation for this is offered (Table 4).

Table 4

Effect of PMA (150 ppm) on resistance to diffusion on water vapor from sugar beet leaves when treated in the light (open stomata) or dark (closed stomata). Each value is based on three replicates.

	Resistance ($min\ cm^{-1}$)			
Treated in:	Light		Dark	
Date	Control	PMA	Control	PMA
1/17/69	.08	.13	.07	.18
1/18/69	.09	.13	.09	.19
1/23/69	.11	.18	.09	.20

Further evidence that PMA treatment in the dark is effective in retarding stomatal opening in the light was obtained by microscopic examination of epidermal peels from discs of Vicia faba leaves. The leaf discs were floated for two hours in the dark on a solution of 10^{-4}M KCl. They were then transferred, still in the dark, to a solution of 100 ppm PMA. Control discs were not floated on PMA. After two hours the PMA discs were transferred to KCl solutions and were placed in the light, along with control discs. After one hour in the light (3000 fc) the apertures of control stomata were 12.0μ, and those treated with PMA were 6.9μ, indicating that PMA treatment in the dark did reduce stomatal opening in the light.

Previous experiments, as well as information in the literature (Gale, Waggoner, Davenport), indicate that antitranspirants will be most useful in conserving water under conditions which are conducive to large water losses, i.e., when soil and plant water potential are high. A good recommendation, therefore, is to apply an antitranspirant soon after an irrigation. However, in some circumstances this may not be possible. An experiment was therefore carried out to determine whether or not an antitranspirant is effective if applied to plants which are already in a stressed condition, but which would later be irrigated.

Eight pots of Phaseolus vulgaris were kept well watered and the soil in another eight was allowed to dry, so that the resistance to water vapor for leaves of the stressed plants (wet) was about 10 times that of the nonstressed plants (dry), and the transpiration rates were about 1/5 those of the nonstressed plants. Half the number of plants in the wet and the dry groups was sprayed with a film-forming antitranspirant (CS-6432, 3%) and transpiration (based on pot weighings) and resistance measurements were made. All of the pots in the dry treatment were then watered, and the measurements were repeated to assess the effect of the antitranspirant on never-stressed and prestressed plants.

Antitranspirant treatment increased resistance and decreased transpiration regardless of whether the plants were stressed or nonstressed (Table 5). During the stress period, application of an antitranspirant reduced water loss by 44%, the corresponding reduction for nonstressed plants being 31%. However, the percentage values alone can be misleading. In reality, only two units of water were saved in the dry pots and 10 units in the wet pots, because of the naturally high resistance (.54 min cm^{-1}) due to stomatal closure in the dry pots. It is of interest to note that the resistance of the film as a barrier to water vapor diffusion is small (.08 min cm^{-1}), compared to that offered by the consequences (chiefly stomatal closure), of natural desiccation (.54 min cm^{-1}). During the 24-hour period after irrigating the stressed plants (to the point of soil saturation), it was obvious that they had not completely recovered from the stress, since the controls transpired less and had greater resistance than the controls in the nonstressed pots. As a result, the antitranspirant appeared less effective on the prestressed plants than on the never-stressed plants.

Table 5

Effect of CS-6432 (3%) on transpiration and resistance to water vapor diffusion of Phaseolus vulgaris plants treated when well watered (wet) or stressed (dry). Each value is the average of four replicates. (Pre-antitranspirant spray data are also given to show inherent variability. Transpiration values cover a 24-hour period, but resistance values are instantaneous readings.)

	Transpiration (g/plant/day)			Resistance (min cm^{-1})		
Day	(1)	(2)	(3)	(1)	(2)	(3)
	Pre-spray	Post-spray	Post-spray Post-Irrig. of Dry Pots	Pre-spray	Post-spray	Post-spray Post-Irrig. of Dry pots
TREATMENT	(%)	(%)	(%)	(%)	(%)	(%)
Wet Control	26 (100)	32 (100)	33 (100)	.07 (100)	.05 (100)	.06 (100)
Wet CS-6432	31 (117)	22 (69)	23 (71)	.05 (71)	.08 (160)	.09 (150)
Dry Control	16 (100)	5 (100)	19 (100)	.28 (100)	.54 (100)	.09 (100)
Dry CS-6432	16 (101)	3 (56)	17 (89)	.37 (132)	.99 (183)	.12 (133)

A similar experiment was conducted on sugar beets (Beta vulgaris), except that in this case: 1) Mobileaf (1:5) was applied to the unstressed (wet) and stressed (dry) plants, and 2) only leaf resistance readings were made. At the time of treatment the resistance of the dry plants was double that of the wet plants (Table 6). The Mobileaf increased resistance 10-fold in the wet and 9-fold in the dry pots. After irrigating the dry pots, the antitranspirant remained effective, although the resistance of the treated leaves (.71-1.40 min cm^{-1}) was not as high as was observed when they were stressed (1.54 min cm^{-1}).

Table 6

Effect of Mobileaf (1:5) on resistance (min cm^{-1}) to water vapor diffusion from the lower surface of leaves of Beta vulgaris when well watered (wet) or stressed (dry). Each value is the average of five readings.

		Day	(1)	(1)	(1)	(3)
		Time	1030	1110	1430	1315
Wet	Control		.08		.07	.04
	Mobileaf		.80		.95	.59
Dry	Control		.17	Dry pots irrigated	.11	.08
	Mobileaf		1.54		1.40	.71

Thus, if an effective film antitranspirant is applied to already stressed (but not completely desiccated) plants, some curtailment of water loss can be expected. Furthermore, if the treated stressed plants are then re-irrigated the antitranspirant film continues to curtail water loss. This was also demonstrated in several other experiments involving CS-6432 and Mobileaf. It is not unreasonable to speculate that antitranspirant treatment of stressed plants would enhance recovery by a forthcoming irrigation because: 1) the severity of the stress would have been partially alleviated by reduced transpiration prior to irrigation, and 2) water uptake after irrigation would be able to 'catch up' to water loss more easily if transpiration is curtailed during the recovery period.

Foliar coverage

One of the reasons for variability in the effectiveness of antitranspirants appears to be the difficulty in obtaining good coverage of the foliage by the spray. The incorporation of a wetting agent ensures thorough wetting of the foliage, but as the spray dries it becomes difficult to detect, so that the distribution of the actual film on the leaf surface is not known. In order to assess where the antitranspirant spray lies on the leaf surface, a 0.1% solution (w/v) of the fluorescent dye, PTS, (sodium 3-hydroxy-5,8,10-pyrenetrisulfonate) was incorporated in the spray. The sprayed foliage was then observed in the dark under black light. It was assumed that the dye was part and parcel of the antitranspirant and that patches of fluorescence on the leaves corresponded to patches of antitranspirant film. It was surprising to note that although the foliage was thoroughly wetted, the coverage of the leaves by the spray after it had dried was never complete as indicated by the patchy pattern on the flourescence. The coverage was practically nil in the absence of a surfactant, but increasing the amount of surfactant (Vatsol) from 0.05 to 0.50 % did not increase fluorescence. Fluorescence was still visible on the foliage of the bean plants one week after spraying. The dye technique showed that the most consistently good coverage was obtained when a fine spray was given using an aerosol propellent.

The performance of an antitranspirant is related to the amount of wetting of the vegetative surface which depends on the surfactant added to the spray material. As pointed out earlier, the fluorescent pattern on the leaves depends on whether or not a surfactant is added. The data in Table 7 on the effects of the adjuvant, Bio-film (0.5%): 1) by itself, and 2) when added to a film-forming antitranspirant (CS-6432), on resistance to water vapor diffusion illustrates the importance of a surfactant. In this experiment *Phaseolus vulgaris* plants were grown in pots until primary leaves had fully expanded. In each pot one of the leaves was used as a control and the other was treated. Measurements of resistance of the lower leaf surfaces were made on the day of treatment using the rate hygrometer. The Bio-film by itself had no effect on increasing resistance; the antitranspirant, which normally wets the leaf fairly well, increased resistance by a factor of 3.7; the addition of Bio-film to the antitranspirant doubled its effectiveness by increasing the resistance by a factor of 6.7. The results of this test are not necessarily conclusive since interactions may occur between method of application, type of antitranspirant material and nature of the plant surface. Therefore, more studies are required with various types of antitranspirants and wetting agents on different plant species.

Table 7

Effect of a surfactant (Bio-film 0.5%), and an antitranspirant (CS-6432, 3%) on resistance to water vapor diffusion from *Phaseolus vulgaris* leaves.

	Resistance (min cm^{-1})	Ratio $\left[\frac{\text{Treated}}{\text{Control}}\right]$
Control	.035	0.97
Bio-film + water	.036	
Control	.030	3.66
CS-6432	.110	
Control	.030	6.67
CS-6432 + Bio-film	.200	

Observations on coverage using the fluorescent dye technique were made on bean leaves sprayed with PMA to which varying amounts of surfactant (Vatsol) has been added. Control leaves, because of their chlorophyll content, appear red under u.v. light. Similarly, leaves which had been sprayed with antitranspirant without any dye also appeared red so that no conclusions on coverage could be made. PMA without any surfactant, but with added dye, dried slowly on the leaves and subsequent distribution of fluorescence after drying was fairly poor. The addition of 0.05% surfactant greatly improved coverage, but increasing the amount from 0.05 to 0.5% made very little difference in the degree of flourescence. In general, coverage appeared to be better on the top than on the bottom side of the leaf, probably because of the more hairy nature of the lower side. Similar observations were noted using a film-forming antitranspirant instead of PMA. It was interesting to note that observation of the sprayed leaves under u.v. light while still wet showed that the coverage was complete and fluorescence was very bright. However after drying, the distribution of the fluorescence in nearly every case was patchy, indicating that there is much scope for improvement of antitranspirant coverage on vegetative surfaces. However, complete coverage of the leaf with a film of antitranspirant may not always be advantageous if CO_2 and O_2 passage between the leaves and the air are severely restricted by the film.

A correlation between film coverage (as determined by the fluorescent dye technique) and film effectiveness (as determined by resistance readings with the rate hygrometer) was found with the following experiment on sugar beet leaves. Each leaf was taped to the laboratory bench, though still attached to the plant, and its left side covered with plastic so that it could be used as a control, i.e., no spray. The right side of the lower surface of the leaf was then sprayed with the film-forming antitranspirant CS-6432 (2%) to which PTS fluorescent dye had been added. Different leaves were given various amounts of spray, varying from light to medium to heavy. By taping the leaves flat, complications arising from spray runoff and redistribution over the leaf surface were minimized. Differences between the light, medium and heavy sprays were clearly visible while the spray was still wet, but after it had dried the subsequent film and its distribution on the leaves was no longer visible, making its location dependent upon observation under ultraviolet light. After the sprays had dried completely, the degree of fluorescence on each leaf was observed and measurements made with the rate hygrometer. Since fluorescence was not evenly distributed over the whole of the treated leaf surface, the rate hygrometer was attached to that portion of the leaf which fluoresced in proportion to the treatment description, i.e., light fluorescence, medium fluorescence, or heavy fluorescence. The light and the medium sprays showed relatively small increases in resistance, whereas the heavy spray (which fluoresced the most) more than doubled the resistance (Fig. 1).

A Cambridge "Stereoscan" Scanning Electron Microscope Mark 2A has been utilized to more accurately evaluate coverage. The instrument has the combined capabilities of: 1) examining surface areas of fresh samples without fixation; 2) magnifying, over a wide range; and 3) detecting cathodoluminescence. These capabilities make it possible to study the relationship of antitranspirants to the stomata. Through the addition of a dye, CS-6432 yields a cathodoluminescent image. Mobileaf does not require the dye. With cathodoluminescence detection it is possible to accurately locate the distribution of these films and to distinguish them from natural waxes. Samples taken from areas thought to have excellent coverage show that a high percentage of the stomata are not covered (Plate I). In many instances, only a very thick film, such as at the edge of a droplet, actually submerges the stomata. Samples from treated leaves which have shown untimely yellowing show a very complete film over the stomata, indicating suffocation as a probable cause for the toxicity.

When spraying on a large scale under field conditions it is virtually impossible to get complete wetting of all the leaf surfaces on a plant, and varying degrees of impartial coverage result. It was postulated that partial coverage by an antitranspirant on a stomatal bearing leaf surface may slightly increase the water potential of the leaf and thereby increase the stomatal apertures by making the guard cells more turgid. This would be of relatively small consequence for those stomata which were covered by the film, but it would result in increased water loss from those stomata which were not covered by the film on the same leaf. An experiment was therefore conducted with cowpeas (Vigna sinensis) which have stomata on both the lower and the upper leaf surfaces, the stomatal frequency being less on the upper surfaces. Measurements of diffusive resistance were made with the rate hygrometer on both surfaces of the leaves at a light intensity of about 2000 foot candles. Half of a leaf was used for the treatment, and the other half of the same leaf for control, the midrib being the dividing line between the two. The treatment consisted of: 1) applying the film-forming antitranspirant CS-6432 (2%) on the lower surface, right-hand half of the leaf, and noting its effect on the upper surface, right-hand half of the leaf; the lower and upper left-hand halfs of the same leaf served as equivalent controls; 2) treating the upper surface of the right-hand side of the leaf with antitranspirant and noting its effect on the lower right surface. Three replicate pots were used and the whole experiment was replicated twice. The following results were noted (Fig. 2): 1) the antitranspirant greatly increased the resistance of the leaf surface to which it was applied; 2) when applied to the lower leaf surface, the resistance of the upper surface immediately above the treated half was less than the resistance of the upper surface above the untreated half; 3) when antitranspirant was applied to the upper leaf surface the resistance of the lower leaf surface immediately below it was less than that of the lower leaf surface on the untreated half of the

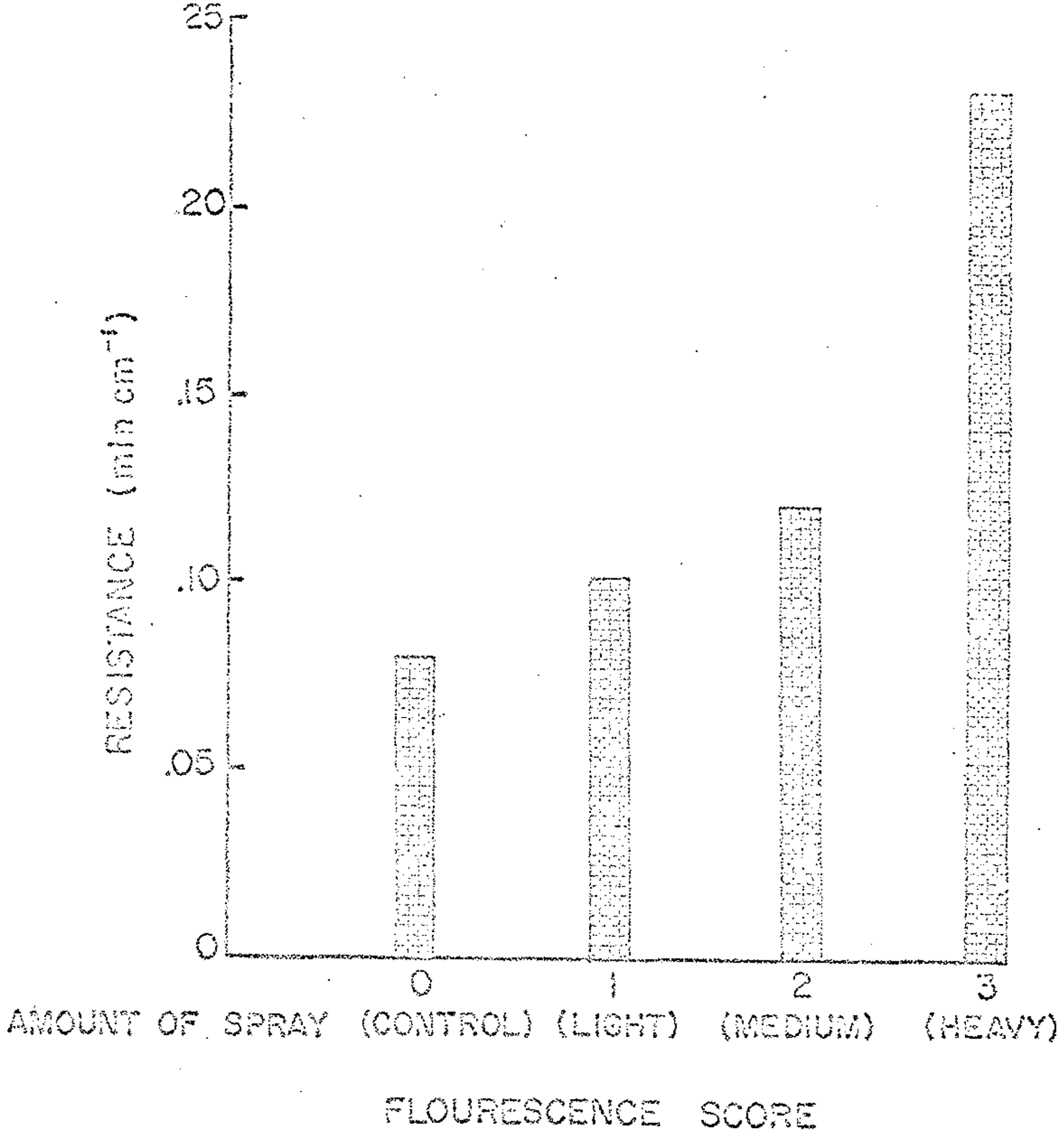


Figure 1: Effect of antitranspirant (CS-6432, 2%) spray coverage on resistance to water vapor diffusion from sugar beet leaves. (More fluorescence indicates greater coverage.)

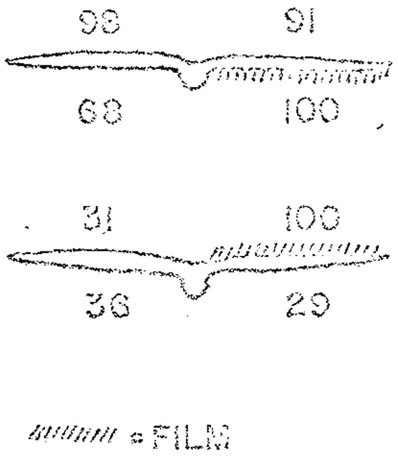


Figure 2: Influence of an antitranspirant film, applied to the lower (or upper) surface of a sugar beet leaf, on resistance to water vapor diffusion from the untreated surface above (or below). Each diagram is a leaf in cross section, and the numbers indicate resistance relative to that of the treated surface (100).

PLATE I

[Note: This plate is from a paper submitted for publication and the numbering pertains to that text.]

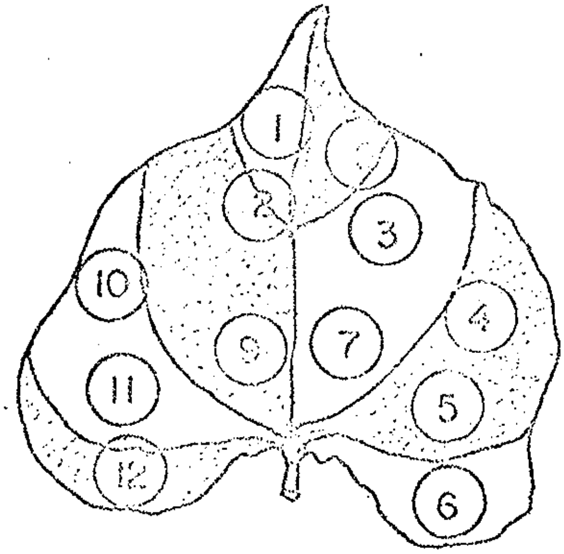
Hedera canariensis (Algerian Ivy) under-surface of a leaf as seen with the scanning electron microscope. Figure 4 (a & b): a droplet of Mobileaf 1:5 [1 cm = 90X]. Figure 5 (a &b): a film of Mobileaf 1:5 [1 cm = 190X]. Figure 6 (a & b): a film of 2% CS-6432 + the dye Brilliant Yellow 6G Base [1 cm = 190X]. Part *a* of each figure is the secondary electron image and part *b* is the cathodoluminescent image.


4a
5a

leaf. It is possible that the influence of the antitranspirant film on stomatal apertures of untreated surfaces of the same leaf is not restricted to the surface immediately above or below the film, but may also affect apertures of untreated surfaces on the same side of the leaf.

In an experiment with potted bean plants (Phaseolus vulgaris) the effect of a patchy application of the film-forming antitranspirant CS-6432 (3%) to both surfaces of one of the primary leaves was observed. The antitranspirant, with a fluorescent dye incorporated, was painted in patches between veins, leaving intervening patches untreated. The patchy coverage of the film was confirmed by observing under ultraviolet light, and the treated and untreated patches on the leaf were given location numbers (Fig. 3). The second primary leaf in each pot served as a control. Observations of resistance to water vapor diffusion were made with the rate hygrometer at the various locations on the lower surface of the treated leaf, and on exactly corresponding locations on the control leaf. In Figure 3, resistance readings on the treated and unstreated patches are listed in separate columns, and the corresponding values for the control leaf are given in parenthesis. On the treated leaf resistance values measured on the antitranspirant patches were about six times greater than those on the untreated patches. There was very little variability between resistance readings of the various locations on the control leaf (.04 to .05 min cm^{-1}), but there was much greater variability among positions of the untreated patches on the treated leaf (.02 to .05 min cm^{-1}). Thus the average resistance for untreated patches on the treated leaf (.033 min cm^{-1}) was less than that of the control leaf in equivalent locations (.045 min cm^{-1}). It therefore appears that partial coverage by an antitranspirant on a leaf can decrease diffusive resistance (by increasing stomatal apertures) of those portions of the leaf which were not covered by the antitranspirant film.

Further data on the effects of partial antitranspirant coverage on leaf diffusive resistance were obtained in another experiment with Phaseolus vulgaris leaves. Six pots of bean plants, with their primary leaves fully expanded, were used for this experiment. In each case, one of the primary leaves was an untreated control and the second leaf on the same plant was treated on its upper surface with a film-forming antitranspirant CS-6432 (2%); the lower surface was not treated. The pots were put in a growth chamber at 2000 f.c., 80°F, and 30-45% relative himidity. Resistance to water vapor diffusion of the treated upper surface was approximately 2.4 x that of the upper surface of the control leaf. However, the resistance of the lower surface of the treated leaf was about 26% less than that of the lower surface of the control leaf (Table 8).



Untreated Areas

Location	Resistance (min cm^{-1})	
6	.03	(.04)
11	.05	(.04)
7	.02	(.05)
10	.05	(.05)
3	.02	(.05)
1	.03	(.04)
Average	.033	(.045)

Treated Areas

Location	Resistance (min cm^{-1})	
12	.32	(.05)
5	.10	(.05)
9	.16	(.05)
4	.24	(.04)
2	.04	(.04)
8	.07	(.05)
Average	.205	(.047)

Figure 3: Effect of patchy film coverage of a bean leaf on resistance to water vapor diffusion. Shaded areas on the leaf indicate antitranspirant patches, and numbered circles indicate resistance measurement locations. The table shows resistance values at each location. Values in parenthesis are for corresponding positions on a control leaf.

Table 8

Effect of the film antitranspirant, CS-6432 (2%), applied only to the upper surface (C) of a Phaseolus vulgaris leaf, on resistance to water vapor diffusion of the lower surface (D). The resistance value of each surface of the treated leaf is relative to that of the corresponding control leaf surface. Each value is the average of six replicates. The letters indicate the measurement locations on the leaves

$$\text{Control leaf}\quad \frac{A}{B}\ )_T(\ \frac{\overset{C}{\text{CS-6432}}}{D}\quad \text{Treated leaf}$$

	Relative Resistances	
Leaf surface	Control leaf	Treated leaf
Upper	1.00 (A)	2.40 (C)
Lower	1.00 (B)	0.74 (D)

Thus, an effective antitranspirant film applied only to the upper surface of the leaf does decrease the resistance (and therefore increase transpiration) of the lower leaf surface of that same leaf. However, it should be pointed out that although this effect was consistent in all of the replicates of this experiment, it was not observed on all other occasions. This would suggest that there is probably some interaction with environmental conditions or, more specifically, the water status of the plant. Thus, although the average reduction in resistance for the lower surface in Table 8 was 26%, the reductions ranged from 36% for partially stressed plants to only 7% in nonstressed plants. In the sugar beet leaf experiment described earlier, where only half of the upper or lower surface of the leaf was treated, the effect of partial coverage may have extended both vertically and laterally on the same leaf. However, in the Phaseolus experiment just described the effect would be predominately vertically, i.e., between the upper and lower surface. The influence of the film on the upper surface of the treated leaf probably had no significant effect on resistances of the control leaf on the same plant. It is interesting to note that in our field experiments with olive trees, where only partial coverage of the leaves by the antitranspirants spray could be achieved (because of the large number of leaves and their geometric positioning), it was found that a leaf, which was visibly well sprayed on its stomata bearing lower surface, had a relatively large diffusive resistance and high water potential. However, a neighboring leaf on the same twig, which obviously received no to little spray (as indicated by low resistance values) had a relatively low water potential, indicating that the effect of increasing diffusive resistance by the antitranspirant was fairly localized and did not extend to any great degree to the neighboring leaf. This will be discussed in more detail in the description of the orchard experiments.

Some experimental evidence, found by direct microscopic examination, indicates that the apertures of stomata directly under an antitranspirant film can be greater than those on corresponding control leaves (not covered by antitranspirant film). In such cases, decrease in transpiration is entirely dependent on the resistance offered by the film to water vapor diffusion.

Rates of Transpiration and Photosynthesis

Numerous data have been gathered on the effects of antitranspirants on transpiration reduction as measured by gravimetric techniques, e.g., changes in weight of potted sugar beet, bean, dichondra, and other plants. These are described elsewhere in this report. This section will describe, in some detail, the apparatus and some of the results obtained in the simultaneous measurements of rates of transpiration and net photosynthesis of individual attached leaves in a leaf chamber.

The rates of transpiration (T) and photosynthesis (P) can be represented by the following equations:

$$T = \frac{\Delta H_2O}{R} \qquad\qquad P = \frac{\Delta CO_2}{R'}$$

where $\Delta\ H_2O$ = water vapor concentration between leaf and air; R = total resistance to water vapor diffusion from the leaf; $\Delta\ CO_2$ = gradient of carbon dioxide between air and leaf; R' = resistance to carbon dioxide flux between the air and the leaf.

The differential psychrometer method (developed by R. G. Wylie and used by Slatyer and Bierhuizen) enables simultaneous measurement of both T and $\Delta\ H_2O$. It consists of a pair of matched wet bulb thermometer elements. Air of identical temperature is passed over each element, but in one case the air comes from the leaf chamber and is therefore enriched with water vapor. This element, therefore, has a higher temperature (because of reduced evaporative cooling of the wet bulb) than the element that is flushed by air which does not pass through the leaf chamber. The temperature and humidity of the air (which was usually passed through the system at the rate of 150 liters per hour) could be adjusted by passing it through temperature controlled water baths.

The water circulation system of the apparatus: 1) helps to keep the chamber temperature close to that of the air stream entering it; 2) cools the quartz-iodide lights via a water jacket system; and 3) provides a heat sink in the water bath between the lights and the leaf chamber. The leaf chamber should be designed so that the leaf position is reproducible and the air stream through the chamber is evenly distributed. A clear plexiglass chamber, measuring 13 x 9.5 x 1.5 cm, surrounded by a water jacket, was used for single leaf measurement. (A larger chamber, in which a small pot could be placed, was also used for certain experiments.) A leaf, still attached to the plant, was lightly bound on a frame with nylon thread and inserted into the single leaf chamber for measurements. The end section of the frame was fastened to the chamber with wing nuts and the hole around the petiole was sealed with caulking compound. A combination of three 1500 watt quartz iodide incandescent lamps and several 20 watt fluorescent lamps, two to three feet above the leaf chamber, provided adequate light intensity and quality (wave lengths) for transpiration and photosynthesis studies.

Determination of transpiration rates depended on accurate measurements of temperature at various points in the system. This was done by copper constantan thermocouples, the voltage outputs of which were printed on a multipoint millivolt recorder. A convenient reference temperature for the thermocouples was 0°C, achieved by placing the reference junctions in a thermos containing melting ice. The thermocouples used in the differential psychrometer consisted of 34 gauge copper-constantan thermo-junctions which were threaded through a central hole in a plexiglass mounting. The two psychrometers were used as wet bulbs by enclosing them in wicks which dipped into water reservoirs. A differential psychrometer is formed by connecting the constantan wires of each thermocouple together and using a common constantan reference junction. Two air streams, one by-passing the leaf chamber and the other passing through the leaf chamber, had their temperatures equalized by passing them through a constant temperature water bath before passing over the wet bulb psychrometers. A thermocouple in the constant temperature bath provided the "dry bulb" temperature. Thermocouples (44 gauge, copper constantan) in the leaf chember provided a measure of leaf temperature.

After determining Δ H_2O and T experimentally, R was calculated. However, R consists of the sum of the boundary layer resistance (r_a) and the epidermal resistance of the leaf (r_e). By substituting a piece of green wet blotting paper, having albedo and geometry similar to that of the transpiring leaf, into the leaf chamber, we can eliminate the factor of r_e. Thus, $r_a = \Delta H_2O/E$, where E = rate of evaporation from the paper. The epidermal resistance of the leaf can then be found by difference, i.e., $r_e = R - r_a$.

Rates of photosynthesis were found by passing air samples: 1) from the air stream by-passing the leaf chamber, and 2) from the air stream after passing through the leaf chamber into a Beckman IR215 Infrared Gas Analyzer. The analyzer determines the differential in CO_2 content between the two air streams, thus indicating how much carbon dioxide has been depleted by absorption for photosynthesis by the leaf. By finding P and Δ CO_2 (assuming CO_2 content in the leaf to be 0), the various resistances in the carbon dioxide path could be calculated.

In order to eliminate possible variations which occur from leaf to leaf, the experimental leaf was run through the apparatus as a control, i.e., with no antitranspirant treatment. The leaf was then treated with antitranspirant, and after the material had dried it was rerun through the apparatus to determine the effect of the antitranspirant on transpiration, photosynthesis and resistance. Thus, all measurements were run in pairs, i.e., a pre-treatment and a post-treatment measurement on the same leaf, and three replicate leaves were used for each treatment, although replicates had to be made with time since only one leaf chamber could be used at any one time. The leaves were never detached from the plant. Before each run the plants were placed under the lights so that their stomata could adjust to the new lighting conditions, and the experimental leaf was kept in the chamber for about half an hour before final transpiration and photosynthesis measurements were made, to enable it to adjust to the lighting, temperature and air flow conditions inside the leaf chamber. The following measurements were necessary for the calculation of transpiration, photosynthesis and resistances: 1) thermocouple outputs from the "dry bulb" water bath, the "wet bulb" psychrometers, and the leaf chamber; 2) ppm CO_2 from the infrared gas analyzer; 3) rate of air flow from a flow meter; and 4) leaf area. Rates of transpiration and photosynthesis were expressed as mg dm^{-2} leaf area h^{-1}, and resistances as sec cm^{-1}.

The effects of a stomata closing antitranspirant (phenylmercuric acetate, 150 ppm) and a film-forming antitranspirant (CS-6432, 3%) on transpiration, photosynthesis, and resistances of oleander (*Nerium oleander*) leaves are shown in Table 9. The rates of transpiration and photosynthesis, measured a few

TABLE 9. Effect of the stomata closing antitranspirant, PMA, and the film-forming antitranspirant CS-6432 on the rates of transpiration and photosynthesis of oleander leaves measured 2 h after treatment. The calculated total and component resistances in the water vapor and carbon dioxide pathways are also given.

	TRANSPIRATION		PHOTOSYNTHESIS		WUE	RESISTANCES							
						TRANSPIRATION			PHOTOSYNTHESIS				
	mgH_2O dm^{-2}h^{-1}	%	mgCO_2 dm^{-2}h^{-1}	%	% × 10^2	$r_a + r_e$ (sec cm^{-1})	r_e (sec cm^{-1})	$r_e/(r_a+r_e)$ x 100 = %	$r_a'+r_e'+r_m'$ (sec cm^{-1})	r_e' (sec cm^{-1})	$r_e'/(r_a'+r_e'+r_m')$ x 100 = %	r_m' (sec cm^{-1})	$r_m'/(r_a'+r_e'+r_m')$ x 100 = %
Control	1420	100	10.45	100	50	3.63	2.17	60	18.5	3.71	20	12.3	66
PMA (150 ppm)	1190	84	6.76	65	39	5.18	3.72	72	28.4	6.37	23	19.5	69
Control	1410	100	7.42	100	36	3.85	2.39	62	26.4	4.08	15	19.9	75
CS-6432 (3%)	810	57	3.93	53	34	8.51	7.05	83	53.4	12.05	25	38.8	70

WUE = Water use efficiency = carbohydrate produced per unit of water transpired = (g CH_2O)/(g H_2O)

r_a = boundary layer resistance to water vapor = 1.46 sec cm^{-1}

r_a' = boundary layer resistance to carbon dioxide = 2.50 sec cm^{-1}

r_e = epidermal resistance to water vapor

r_e' = epidermal resistance to carbon dioxide

r_m' = mesophyll resistance to carbon dioxide hours after treatment, were decreased by both PMA and CS-6432. At the concentrations used in this trial, the percentage reduction in photosynthesis by the antitranspirants was greater than the reductions in transpiration. As a result, the water use efficiencies were also decreased, though to a smaller degree by CS-6432. Measurements made two days later (not shown in Table 9) indicated that the 150 ppm concentration of PMA was phytotoxic and resulted in even more drastic reductions in photosynthesis and water use efficiency. On the other hand, the CS-6432 reduced transpiration to a greater extent than photosynthesis two days after spraying, and therefore increased water use efficiency. This suggests that the resistance of the CS-6432 film to carbon dioxide was decreasing with time.

The various resistances in the transpiration and photosynthesis pathway were increased by both of the antitranspirants. It should be noted that any increase in the epidermal resistance (r_e) caused by PMA, would be due to stomatal aperture, whereas an increase in r_e caused by CS-6432 would be due to the film lying over the stomatal surface. It can be seen that for the low ventilation conditions in the leaf chamber, the boundary layer resistance comprised about 40% of the total resistance in the water vapor pathway, and the epidermal resistance about 60%, for control leaves. However for PMA treated leaves the epidermal resistance comprised about 72%, and for CS-6432 about 83%, of the total resistance. Because of the presence of r_m' the corresponding proportion of the total resistance in the carbon dioxide pathway contributed by the epidermal resitance (r_e') was much smaller, i.e., about 15 to 20% for control leaves and about 23 to 25% for treated leaves. The total resistance in the carbon dioxide pathway ($r_a' + r_e' + r_m'$) was increased by the antitranspirants, but it was approximately doubled by the CS-6432. It is of interest to note that 2 days later the CS-6432 had lost some of its influence on increasing the total resistance to CO_2, i.e., the resistance was increased by a factor of only 1.4 instead of 2.0.

It is emphasized that the resistance values in Table 9 are not direct measurements, but are calculations based on the measured rates of transpiration and photosynthesis and on the diffusion coefficients for water vapor and carbon dioxide through air. One of the shortcomings of the use of this system for determining resistances with film antitranspirants is that the diffusion coefficients for carbon dioxide and water vapor through the film medium are not known. The mesophyll resistance, which occurs in the carbon dioxide but not in the water vapor pathway, is really nothing but 'fudge factor' calculated by subtraction. It therefore, includes all resistances to CO_2 between the atmosphere and the chloroplasts, other than those accounted for by the boundary layer and stomatal resistances. These other resistances include the diffusion of carbon dioxide through the liquid phase of the cell walls to the site of absorption in the chloroplasts, and in the case of film antitranspirants, must also include factors related to the permeability of the film to carbon dioxide diffusion. Thus, r_m' was increased by CS-6432 because of the nature of the external film, and by PMA probably because of internal phytotoxicity which may have affected the photosynthetic system in a way other than simply increasing stomatal resistance.

Measurements of transpiration and photosynthesis were also made on pots of dichondra (Dichondra repens) which were inserted into a transparent plexiglass chamber that was larger than the one used for single leaf measurements. The dichondra was grown in 4-inch pots and carefully watered and fertilized with nutrient solution until a thick uniform matt of foliage was produced. About

100 such pots were prepared, and being of uniform size and appearance, they were strictly comparable, thereby reducing errors otherwise resulting from variable leaf areas and angles of inclination. The dichondra pots were useful since, upon completion of an experiment with antitranspirants, foliage could be cut allowing regrowth of fresh leaves, and thereby eliminating the need for replanting the experimental material. Internal measurements of the plexiglass chamber were 7 inches square by 5 inches high. The side walls were water-jacketed for cooling. Special baffles for air entry and exit were provided and air mixing was possible, when desired, with a 2.5 inch diameter four bladed fan inside the chamber, which was run by an external motor. The pot was inserted through a hole in the bottom plate of the chamber so that only the top surface of the pot was actually inside the chamber, and an airtight seal was achieved by inserting a circular rubber tube (inner tube of a bicycle tire, cut to size) around the pot rim, and inflating the tube until a complete seal was made. Pots of different size and shape could be inserted into the chamber, by changing the base plate.

In the experiment to be described, unlike the one with the sugar beets, the pot used as a control (no spray) was not later sprayed as an antitranspirant treatment. In other words, the control and various antitranspirant treatments were different pots. The measurements were not replicated. The treatments consisted of film forming and stomata-closing antitranspirants as well as combinations of the two, the stomata closing antitranspirant being sprayed first, followed by the film material on the same foliage. The various treatments and results are shown in Table 10.

Table 10

Effects of a stomata-closing (PMA) and two film-forming [CS-6432 (CS) and Allied (A)] antitranspirants on rates of transpiration and photosynthesis of Dichondra (Dichondra repens). The treatments include various concentrations as well as a 'combination' of stomata-closing and film-forming materials, i.e., PMA sprayed first, followed by CS or A.

	Transpiration		Photosynthesis	
Treatment	($mg\ dm^{-2}\ h^{-1}$)	% Reduction	($mg\ dm^{-2}\ h^{-1}$)	% Reduction
Control	1471	0	8.70	0
PMA (50 ppm)	1133	23	6.30	28
PMA (100 ppm)	1034	30	5.50	37
PPM (200 ppm)	875	40	2.19	75
PMA (300 ppm)	660	55	2.43	72
A (2%)	1321	10	7.56	13
CS (2%)	1061	28	6.52	25
PMA_{100} + A 2%	960	35	6.04	31
PMA_{100} + CS 2%	1184	19	7.10	18
CS 4%	976	34	7.24	17

All of the antitranspirants reduced the rates of transpiration and photosynthesis. In most cases both of these measurements were curtailed to the same extent percentagewise. However, one of the materials (CS-6432, 4%) reduced transpiration far more than photosynthesis, whereas the higher concentrations of PMA had the opposite effect, suggesting that there was some phytotoxicity involved. PMA (200 ppm), for instance, reduced transpirations about 40%, but reduced photosynthesis 75%. The effects of the PMA and film combination were not additive.

The boundary layer resistance (r_a) over a leaf depends largely on the ventilation rate. The fan in the dichondra pot plant chamber enabled studies of transpiration and photosynthesis under high and low ventilation rates, i.e., with small and large boundary layer resistances, respectively. In one experiment with dichondra, use of the fan inside the chamber increased transpiration rates by about 75% and photosynthesis by about 35%. Theoretically, if the boundary layer resistance is reduced, the other resistances (epidermal resistance for water vapor diffusion and epidermal and mesophyll resistances for carbon dioxide diffusion) become relatively more important in the total resistance pathway. Therefore, any increase in epidermal resistance, say by antitranspirant treatment, should have a greater effect in reducing transpiration and photosynthesis under ventilated than under non-ventilated conditions. In the experimental data with dichondra, the percentage reductions due to the antitranspirant were fairly similar whether the fan was on or off. However, in absolute units both transpiration and photosynthesis were reduced to a greater extent under ventilated than under non-ventilated conditions. These data and further discussion on the interactions of antitranspirant effects with wind speed will be presented later in this report.

In other experiments with dichondra it was found that various alkenylsuccinic acids, which are stomata-closing antitranspirants, reduced both rates of transpiration and photosynthesis. These data will be presented later in this report. Transpirantion and photosynthesis measurements were also used as a criterion for comparing the effect of various commercial and experimental antitranspirant products. Data on these products as well as any other materials which are developed in the future will be presented in a separate report at a later date.

Leaf Temperature

Stomatal apertures, and therefore leaf resistance and leaf temperature, vary with time (over minutes, hours, and diurnally). Data on the variations with time of leaf temperature as measured with a rate hygrometer are shown in Figure 4 in the section on duration of antitranspirant effects. It can be seen in this figure that during an 8 hour period, temperatures of antitranspirant treated leaves are consistently higher than those of control leaves throughout all phases of the variation. The relatively small magnitude of leaf temperature increase, compared to that of resistance increase, is also evident from this figure.

The relatively small influence of antitranspirants of the stomata closing and film-forming types on leaf temperature are illustrated by data from the following experiment on sugar beet (*Beta vulgaris*) leaves, using the rate hygrometer and its built-in thermistor for measurements of diffusive resistance and leaf temperature. Experimental error was minimized by using one-half of a

TABLE 11

Effects of antitranspirants (CS-6432, 3% and PMA, 110 ppm) on the temperature and resistance of the lower surface of sugar beet leaves in a greenhouse. (Average air temperature and relative humidity were 27°C and 60%, respectively.

	Temperature (°C)			Resistance (min cm^{-1})			Temperature (°C)			Resistance (min cm^{-1})		
	Control	CS-643	(CS-Con)	Control	CS-6432	(CS-Con)	Control	PMA	(PMA-Con)	Control	PMA	(PMA-Con)
	28.2	29.2	1.0	.10	.86	8.60	29.6	29.4	-.2	.12	.15	1.25
	29.5	30.1	.6	.14	.84	6.00	30.1	29.7	-.4	.13	.16	1.23
	29.4	29.9	.5	.15	.60	4.00	30.1	30.2	.1	.17	.26	1.53
	30.3	30.6	.3	.17	.66	3.88	30.2	30.3	.1	.17	.22	1.29
	30.2	30.6	.4	.18	.86	4.78	30.7	31.3	.6	.20	.28	1.40
Average	28.5	30.1	.6	.15	.76	5.45	30.1	30.2	.04	.16	.21	1.34

leaf as an untreated control and treating the other half with antitranspirant, the mid-rib being the boundary between the two halves. Although both the upper and lower surface of the treated half were treated, measurements were made only on the lower surface. The film-forming CS-6432 (3%) antitranspirant increased resistance by a factor of 5, but concurrent increases in leaf temperature were very small but consistent (Table 11). In this experiment PMA increased resistance relatively less than CS-6432. The differences in leaf temperature between the control and treated half of the leaf were also very small, and an increase in leaf temperature by PMA did not occur when the increase in resistance was relatively small. The elevation in leaf temperature by an antitranspirant may have been minimized in this experiment because of possible conduction of heat from the treated to the untreated half of the leaf. However, when the CS-6432 treated leaves were split down the mid-rib from the tip to near the base of the leaf, although the xylem serving the two halves of the leaf were still intact, differences in leaf temperature between the treated and control halves of the leaf were the same order of magnitude as with the unsplit leaves. Furthermore, similar results were obtained when measurements were made on separate control and treated leaves of bean plants, as described in the next experiment.

The rate hygrometer was used to assess the effects of film-forming antitranspirants on leaf temperature and resistance of bean (Phaseolus vulgaris) plants growing in pots in a controlled environment chamber at 21-22 °C, 50% relative humidity, 2500 f.c. light. The entire plant was either left unsprayed (control) or was sprayed with CS-6432 (2%) or Mobileaf (1:5). There were 5 replicate pots of each treatment, and numerous replicate measurements were made with time.

Table 12 gives average leaf temperature data for the lower surfaces of control and treated leaves under well watered conditions in five separate experiments. Elevations in leaf temperature by the antitranspirant did not always occur, and when they did, they were small.

Table 12

Effects of two film antitranspirants (AT) on temperatures of lower leaf surfaces of well watered bean (Phaseolus vulgaris) plants in a growth chamber.

		Temperature (°C)					
		1 day before treatment		1 day after treatment		2 days after treatment	
Expt. No.	Antitranspirant	Control	AT	Control	AT	Control	AT
1	CS-6432 (3%)	31.6	31.4	33.0	32.8	31.4	31.7
2	CS-6432 (3%)	32.0	32.5	31.8	32.8	30.9	31.6
3	CS-6432 (3%)	29.7	29.3	30.3	31.8	32.4	32.2
4	Mobileaf (1:5)	31.7	31.7	30.9	31.2	30.9	31.5
5	Mobileaf (1:5)	30.2	30.4	30.1	31.2	31.2	31.9

In experiments which used the leaf chamber for transpiration and photosynthesis measurements, copper constantan thermocouples (44 gauge) inserted in the chamber measured 1) leaf temperature after ensuring that the thermojunction was in contact with the lower surface of the leaf, and 2) air temperature by keeping the thermocouple just below the leaf. Leaf minus air temperature tended to be higher for antitranspirant than for control leaves. However in all measurements by thermistors or thermocouples, there is always some doubt as to the degree of contact between the measuring element and the leaf. Although the data reported above are fairly consistent, they should be compared with other techniques of measurement such as remote sensing. In future investigations it is planned to use a Barnes infrared thermometer for measurements of temperatures of individual leaves as well as plant canopies which have been treated with antitranspirants.

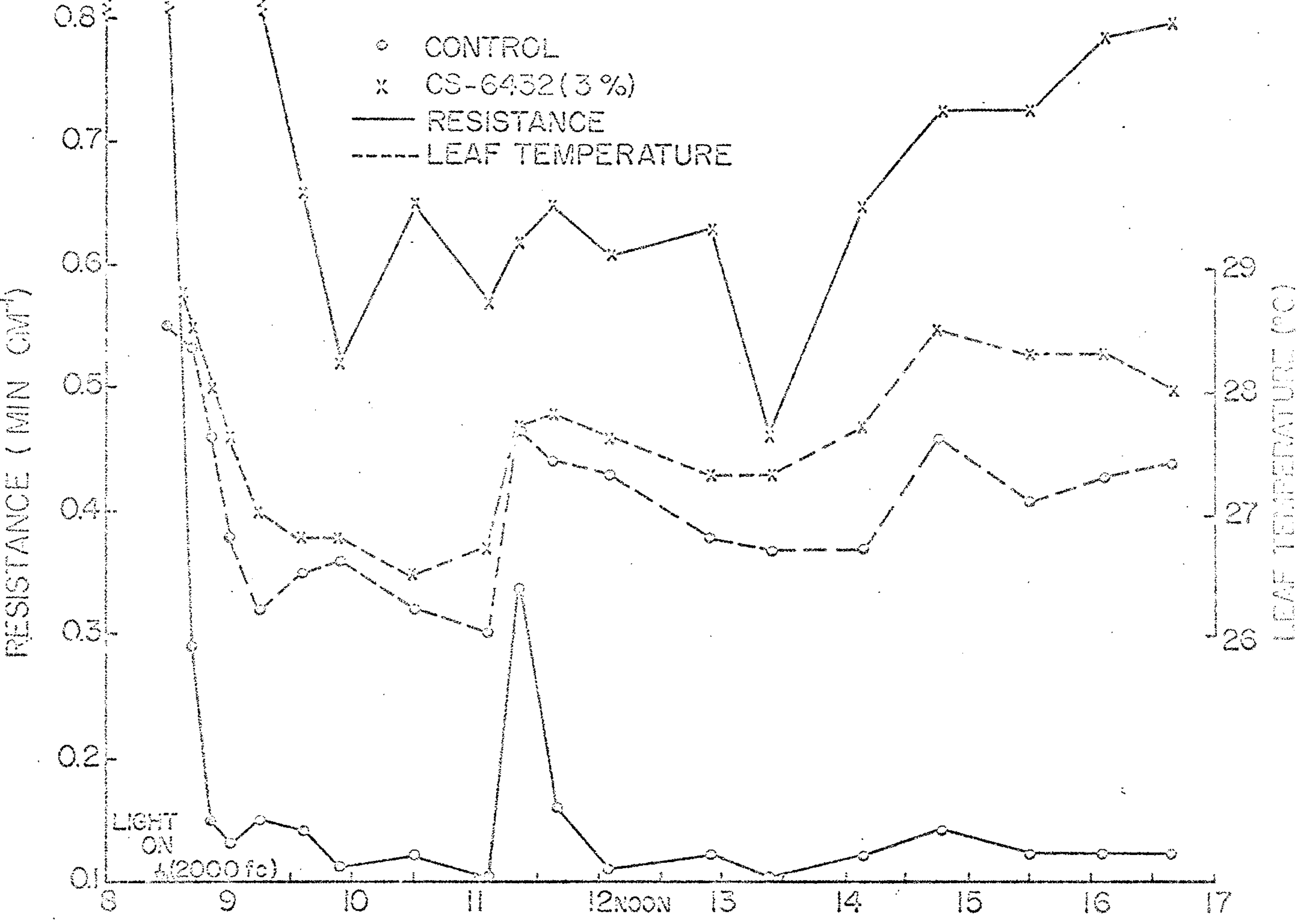
Although relatively little work was done with antitranspirants of the reflecting type during the 3-year period covered by this report, it is relevant to note the findings of Abou-Khaled using white coatings of kaolinite on citrus leaves. He reported that at radiation intensities of about 1 cal. cm^{-2} min^{-1} kaolinite reduced citrus leaf temperature by 4°C and transpiration by about 25%.

Duration of Effect

Before describing the long-term duration of an antitranspirant's effectiveness it is of interest to note the changes with time of resistance to water vapor diffusion over a period of several hours under constant environmental conditions. The film-antitranspirant CS-6432 (3%) was applied to the upper and lower surfaces of one-half of a sugar beet leaf, the other half being control. On the next day the sugar beet plant was placed in light of 2000 f.c. intensity, air temperature of about 25°C and relative humidity of 40%. Observations were made with the rate hygrometer on the lower surfaces of the control and treated halves of the leaves. Although there was a good deal of fluctuation in resistance with time, the CS-6432 surface had a consistently higher resistance (about a 4-fold increase) than the control half (Figure 4). Leaf temperatures also fluctuated with time over the 8-hour period, but the temperature of the treated half of the leaf was always slightly higher than that of the control half.

The longevity of antitranspirant effectiveness depends on the antitranspirant material, 2) environmental factors, such as soil moisture and atmospheric evaporative demand, which influence the water potential of the plant and therefore stomatal opening, 3) plant factors, such as the amount of new foliar growth after antitranspirant application, and 4) spray factors, such as the degree of coverage achieved. In order to eliminate complications caused by the interactions between antitranspirants and the various influencing factors, film-forming antitranspirant was painted on mature ivy leaves which had essentially ceased their expansion in leaf area. Resistance readings were then made on the lower surfaces of the leaves, at the same time each day, under fairly uniform conditions in a greenhouse. Variability was further reduced by the technique already described of using half of the leaf for control and the other half for antitranspirant. In the first ivy experiment, the increase in diffusive resistance caused by CS-6432 (2%) lasted for 6 days, and thereafter resistance readings on the treated side of the leaf were either the same or less than those on the control side of

Figure 4: Time fluctuatic of resistance and temperature of untreated and CS-6432-treated sugar beet leaves.



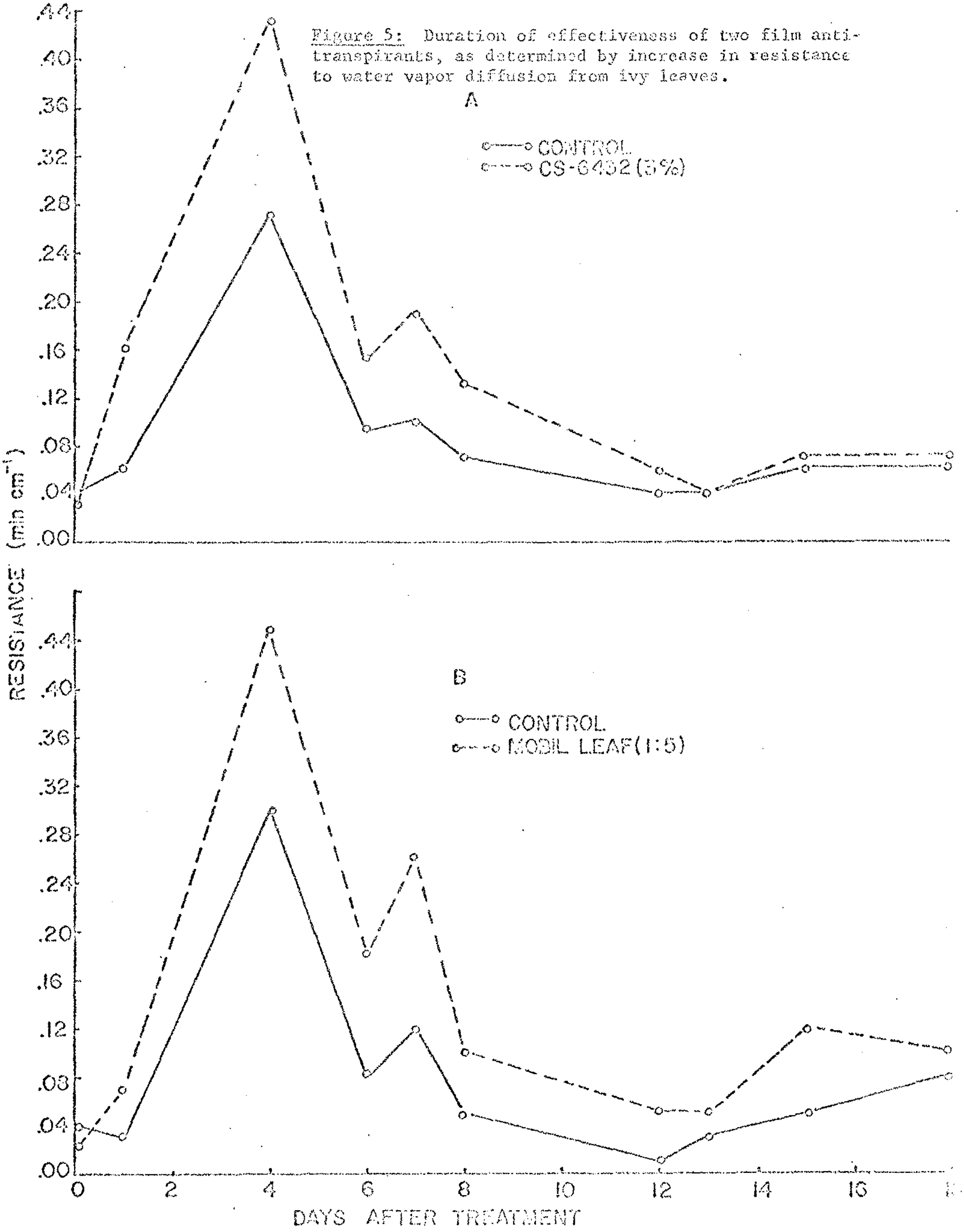


Figure 5: Duration of effectiveness of two film anti-transpirants, as determined by increase in resistance to water vapor diffusion from ivy leaves.

the leaf. However, the effect of CS-6432 (3%) lasted for 12 days (Figure 5A). In other experiments, CS-6432 (2 and 3%) appeared to effectively increase the resistance of ivy leaves for only 2 days. Thus, the duration of effectiveness of the CS-6432 film is somewhat inconsistent. However, it is possible to envision certain cases where an antitranspirant of relatively short duration would be more advantageous than one of longer and more persistent duration. In parallel experiments with Mobileaf (1:5), the duration of effect on ivy leaves continued for at least 18 days (Figure 5B). The relatively longer effectiveness of Mobileaf in increasing resistance was also observed in other experiments with the essentially nonexpanding ivy leaves.

The long-term effectiveness of CS-6432 (3%) on resistance of sugar beet leaves can be seen in Figure 6. In this experiment, unlike the data reported earlier, the CS-6432 was effective for approximately one month, although its effectiveness decreased from a 2.2 fold increase in resistance just after treatment to a 1.2 fold increase about one month later.

A similar experiment with sugar beets was conducted with phenylmercuric acetate to determine the duration of its effectiveness when applied in concentrations varying from 0 PMA (solvent of water and ethanol + X-77 surfactant) to 200 ppm PMA. Figure 7 shows that the higher concentrations of PMA (150 and 200 ppm) became effective immediately (within a few hours) after application, whereas the lower concentrations took a little more time to become effective but did increase resistance within 24 hours, although this is not shown in the figure. The experiment was terminated after 4 weeks, at which time all of the PMA concentrations still showed increased resistances to water vapor diffusion, the higher resistances occurring with the greater PMA concentrations. The resistance ratio for the PMA solvent alone remained at unity throughout the 4-week observation period, indicating that it behaved essentially the same as a control leaf. All of the observations were made under artificial lighting conditions at about 3000f.c., after allowing the plants to equilibrate with the lighting conditions.

Oleander plants (Nerium oleander), growing in 1 gal. containers in a greenhouse, were sprayed with CS-6432 (3%), PMA (110 ppm) or water + X-77 (control). The pots were irrigated frequently to avoid severe stress, and were bagged with polythene to prevent evaporation from the soil. Before spraying, transpiration rates amongst the pots were similar, but thereafter, the antitranspirants reduced water loss, initially by about 40% (Figure 8). A delayed irrigation on September 11 caused a decrease in effectiveness on September 10 - 11 due to soil moisture stress (see section on Interactions later in this report), but some effectiveness was required after re-irrigation. The effects continued for approximately 3 weeks, but were more-pronounced for CS-6432 than for PMA, the reduction in transpiration after the third week being 10% and 5%, respectively.

The overall effectiveness of an antitranspirant spray will be of relatively short duration when applied to plants that continue to produce new leaf surface, especially if the new growth occurs at the outer extremities of the plant where transpiration rates are highest. This is illustrated by comparing the transpiration rates of antitranspirant treated pots of dichondra (Dichondra repens) with untreated pots (Figure 9). Initial reductions in transpiration exceeding

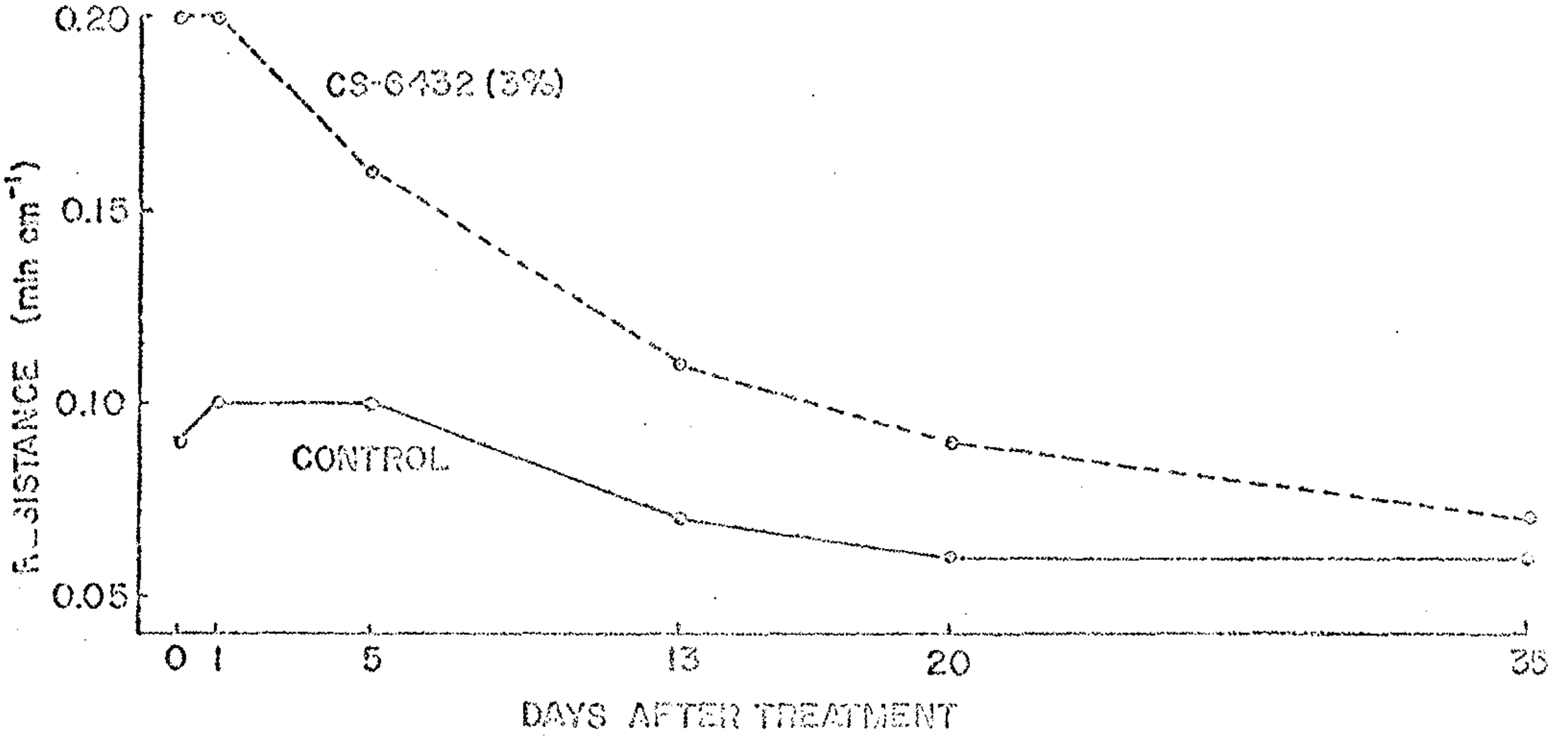


Figure 6: Duration of effect of CS-6432 (3%) on resistance to water vapor diffusion from sugar beet leaves.

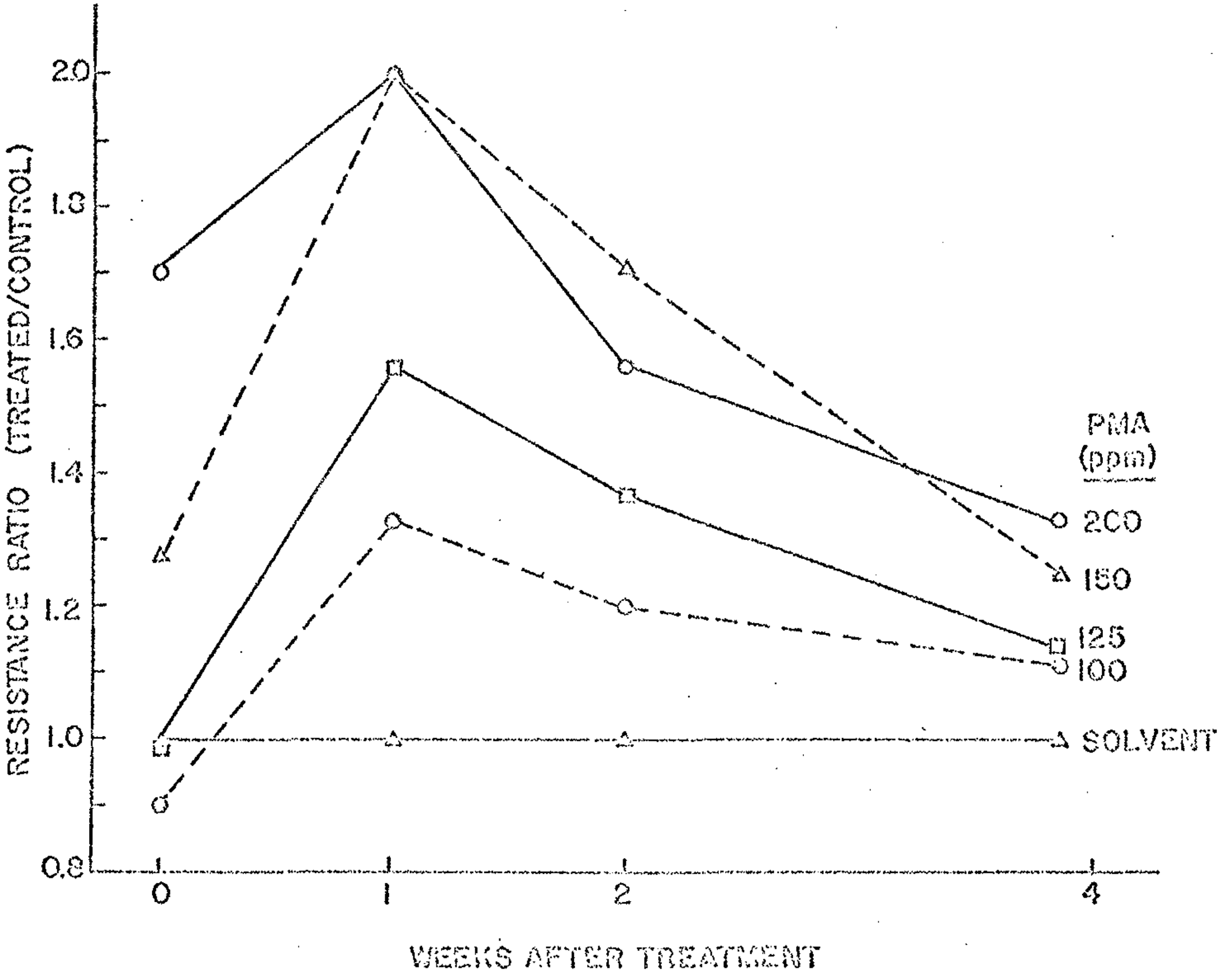


Figure 7: Duration of effects of various PMA concentrations on relative resistances to water vapor diffusion from sugar beet leaves.

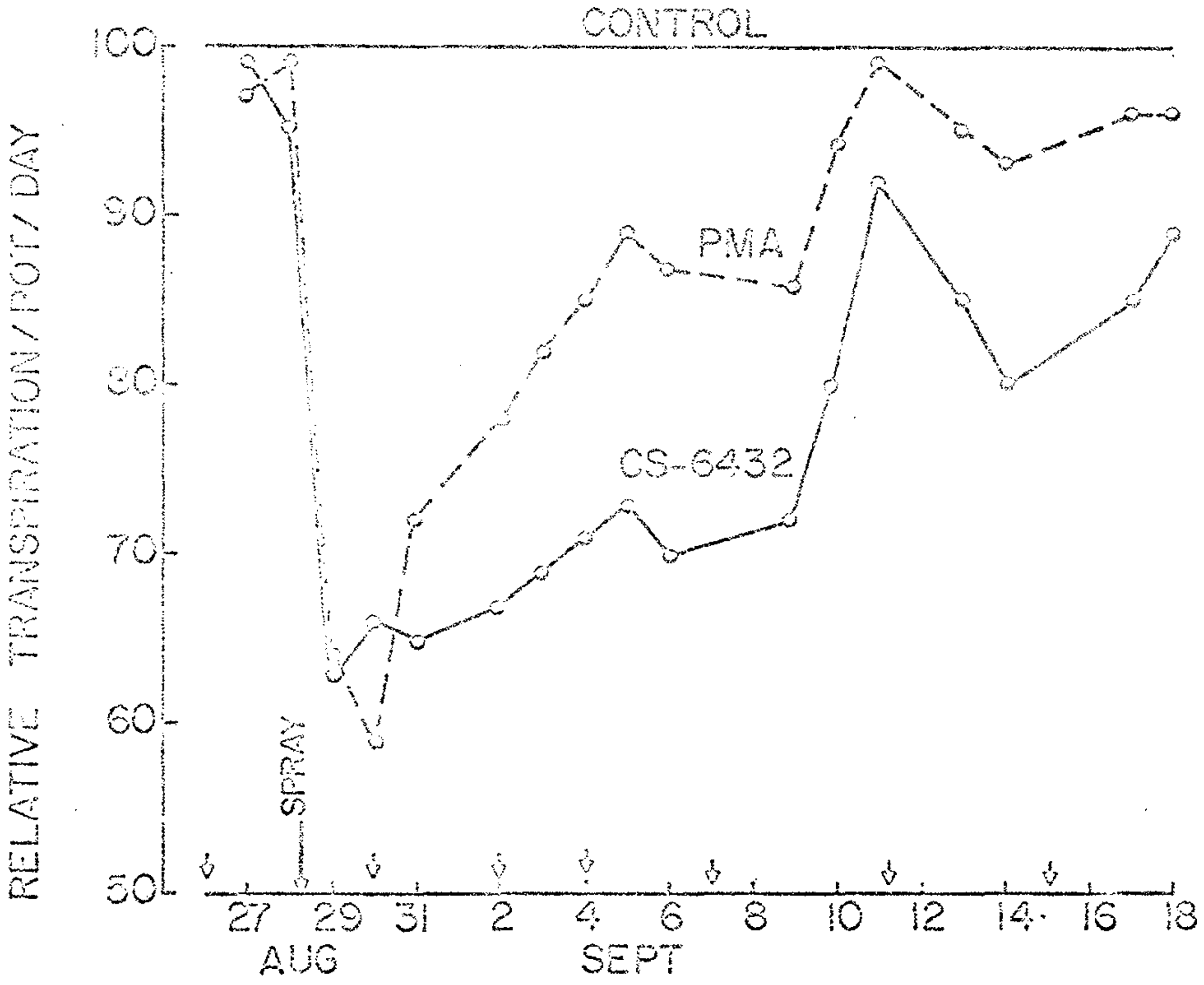
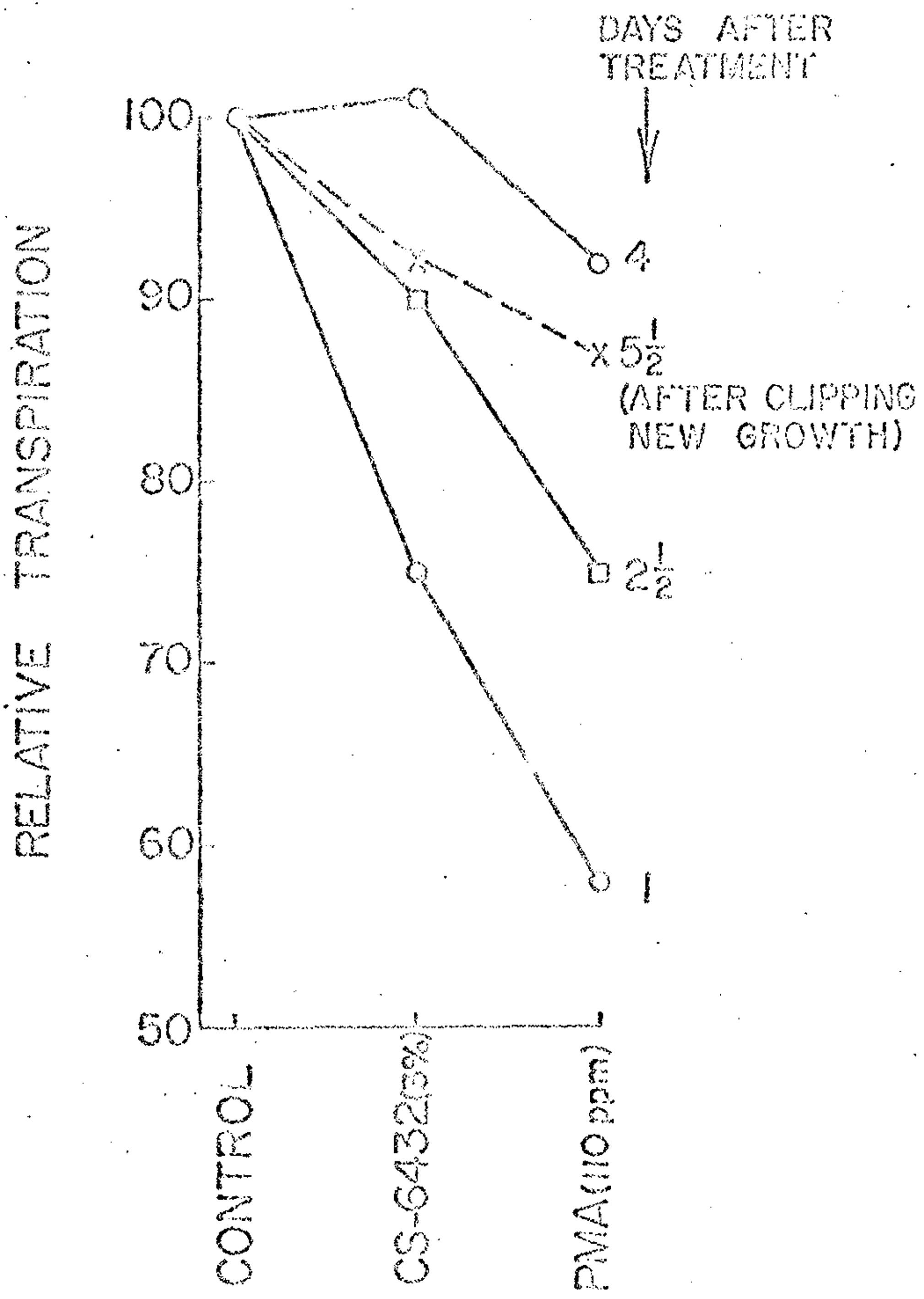


Figure 8: Duration of effects of CS-6432 (3%) and PMA (110 ppm) on transpiration from oleanders.

Figure 9:

EFFECTS OF ANTITRANSPIRANTS ON TRANSPIRATION OF DICHONDRA (NEW GROWTH WAS CLIPPED ON THE 5th DAY AFTER TREATMENT)



40% were obtained with PMA, and of 25% with CS-6432 (3%). However by the fourth day after treatment, a new flush of leaves had practically eliminated the effects of both antitranspirants. When these new leaves were clipped off, the reduced transpiration rates of the treated plants were manifested again although differences between sprayed and unsprayed plants were smaller than observed on the day after spraying.

The effects of new foliage appearing after spraying were also shown in experiments on 5-year old almond trees near Davis, using dendrometers to assess the effects of film-forming antitranspirants. The first trial was made in the early summer of 1967, when a considerable amount of new foliage was being produced on the periphery of the trees, and the second trial was made in the same orchard later that summer, when there was little, if any, new foliage growth. Because of these differences in new foliar growth, the applied antitranspirant limited water loss for only a week in the first experiment, but for over a month in the second trial, as indicated by measurements of shrinkage and growth of the tree trunks.

Information on the duration of an antitranspirant's effect was also noted in other field experiments. Measurements with dendrometers on the almond trees indicated that the antitranspirant continued to reduce shrinkage of the tree trunks for as long as one and a half months. Furthermore, the antitranspirant increased relative turgidities of the leaves, sampled in the late afternoon when shrinkage was maximum, for as long as two and a half months after spraying, thereby lending support to the long-term effects observed on shrinkage. Measurements of resistance of the leaves of prune trees showed that the antitranspirant CS-6432 (1%) was effective for at least 16 days after spraying, and possibly longer. Eighteen days after treating peach trees, the resistances of leaves sprayed with Mobileaf (1:9) was nearly 3 times those of control leaves, and leaf water potentials were approximately -5 atm. higher than for control leaves. On olives, it was noticed that water loss from harvested fruit (from trees which had been sprayed with CS-6432 (1½%) three weeks before harvest) was reduced by about 50% as compared with fruit from unsprayed trees.

In the numerous experiments with antitranspirants, the longevity of the effect has varied from a few days to several weeks. Some of the variability can be accounted for by plant and environmental factors, but in many cases it is probably due to some as yet unexplained property in the antitranspirant material itself. These problems may be related to age of the antitranspirant material, method of application, etc. Inconsistent and variable data have been reported to the companies producing the materials so that they can study the problem with a view to modifying formulations, emulsifiers, etc. in order to obtain more consistent effects.

Concentrations

Antitranspirant phytotoxicity may be caused by the material itself, or more particularly by the emulsifier present in the film forming antitranspirants. Although the degree of phytotoxicity depends on the sensitivity of the plant species, it can usually be regulated by adjusting antitranspirant concentration, without totally sacrificing the effectiveness of the material.

Thus, Mobileaf at the recommended concentration of 1:5 caused yellowing of the leaves of several fruit trees, necessitating determination of a lower, but yet effective, concentration. Various concentrations of Mobileaf were therefore applied to leaves of sugarbeets (which are a convenient experimental medium) to determine the rates of transpiration and photosynthesis in the leaf chamber apparatus. Phytotoxicity at the 1:5 concentration may well have been due to suffocation, since CO_2 intake (and possibly O_2 exchange) was practically eliminated by the film. (On a field scale, such overall drastic reductions would not be expected since coverage by the spray would be relatively incomplete compared to that achieved on the sugar beet plants on a laboratory scale.) Decreasing the concentration of Mobileaf gave correspondingly smaller reductions in transpiration and photosynthesis, though the reduction in photosynthesis was always greater than in transpiration (Table 13). The effects of decreasing Mobileaf concentrations on diffusive resistance (measured by the rate hygrometer in a separate experiment) are also shown in the table.

Table 13

Effects of various concentrations of Mobilleaf on transpiration and photosynthesis of sugar beet (Beta vulgaris) leaves. The effects on diffusive resistance relative to control, as measured by a rate hygrometer are also shown.

Concentration of Mobileaf	% Reduction Below Control		Resistance ratios (ML/Control)
	Transpiration	Photosynthesis	
1:5	85	100	12.6
1:7	70	80	5.8
1:9	60	70	5.3
1:11	25	50	2.0

Phytotoxicity trials were also conducted on sample twigs in the orchard on various fruit trees, using the same mobileaf concentrations listed in Table 13. These trials showed that a concentration of 1:9 was nonphytotoxic to leaves of apricots and peaches, and of 1:7 to leaves of olives. Since fairly substantial reductions in transpiration (and photosynthesis) could still be achieved at these concentrations, they were selected for spraying in experiments described later in this report.

The effects of various concentrations of phenylmercuric acetate on rates of transpiration and photosynthesis from dichondra (Dichondra repens) can be seen in Table 10. Increasing concentrations of PMA gave greater reductions in both transpiration and photosynthesis. In general, a 100 ppm (about $10^{-3.5}$ M) concentration of PMA was found optimum for most plant species in terms of reduced transpiration (and photosynthesis) and absence of phytotoxicity. The effects of various concentrations of other antitranspirants of the stomata closing type on transpiration rates of dichondra can be seen later in this report in the section of alkenylsuccinic acids.

Cotton plants in 5 inch pots were placed in a rotating chamber in the greenhouse. Transpiration rates were measured by weight differences over a period of several days after bagging the pots to eliminate evaporation from the soil. Pre-treatment data on transpiration rates was used to group the the plants into statistical blocks, and variation, due to position in the chamber, was further reduced by the continuous rotation of the pots on the turntable. The leaves of the cotton plants were then sprayed with distilled water plus X-77 surfactant (control), or PMA at 100, 150, or 200 ppm concentrations. The effects of the PMA over an 8½ hour period of daylight one day after spraying is shown in Figure 10. The minimum concentration of PMA required to effectively reduce transpiration from the cotton plants under the conditions of this experiment was probably less than 100 ppm.

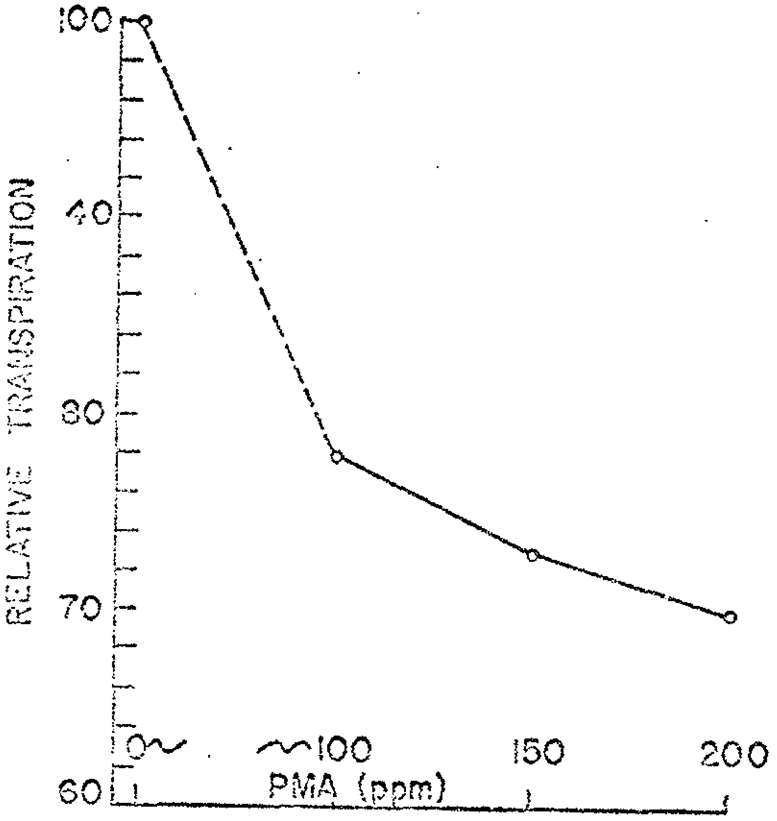


Figure 10: Effects of various PMA concentrations on transpiration (relative to that of control) of cotton plants.

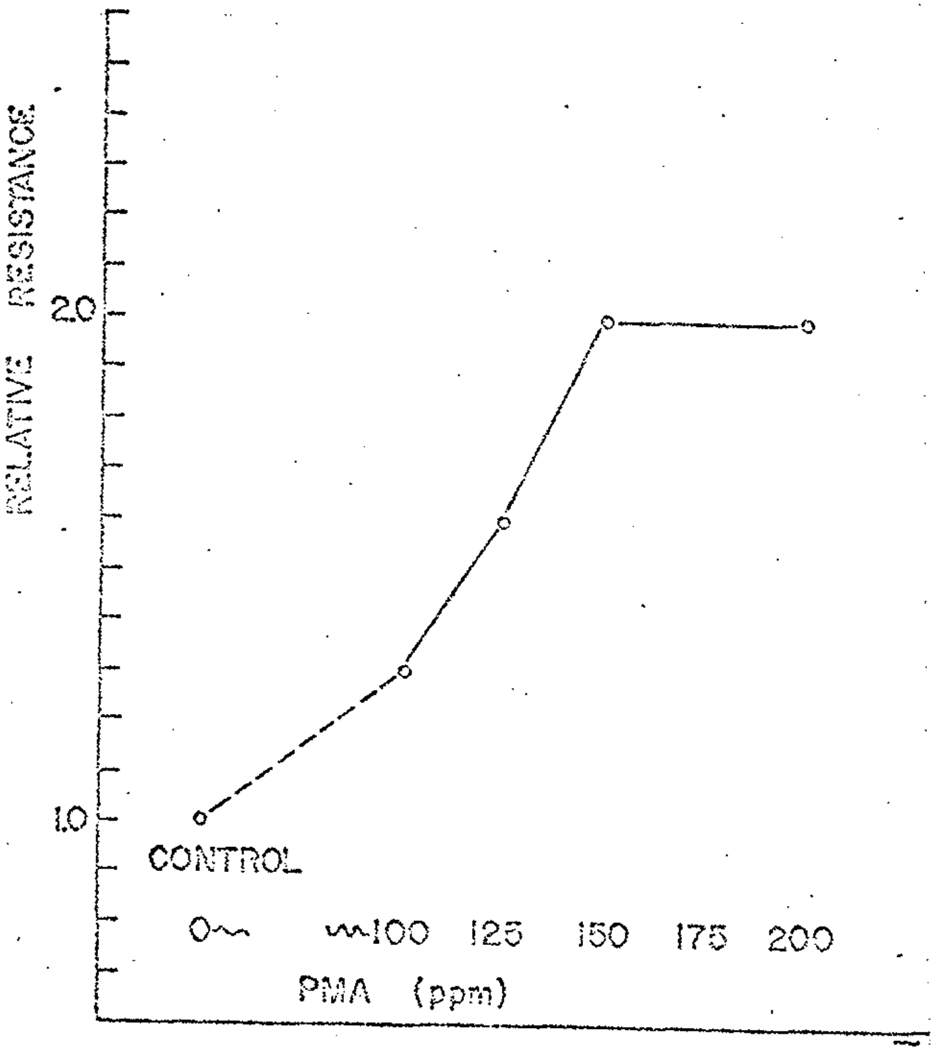


Figure 11: Effects of various PMA concentrations on relative resistance to water vapor diffusion from sugar beet leaves.

The effects of increasing concentrations of phenylmercuric acetate on resistance to water vapor diffusion from sugar beet leaves, measured one week after application at a light intensity of 3000 f.c., is shown in Figure 11. Measurements were made on the lower leaf surface and half of each leaf served as a control, the other half being treated. The data in Figure 11 are expressed as resistance relative to that of the control half of each leaf. It appears that no further gain in effectiveness can be obtained with concentrations higher than 150 ppm of PMA. Although no phytotoxicity was observed in this experiment, other investigations on sugar beet have shown that concentrations of 150 ppm and more do damage the leaves.

The effects of various concentrations of antitranspirants of the film-forming type appears to be very dependent on the nature of the leaf surface and its angle of inclination. Using the technique already described of treating half of a sugar beet leaf with antitranspirant and keeping the other half as a control, the following experiment was done with sugar beets and CS-6432 at various concentrations, using the rate hygrometer to assess the effects on diffusive resistance to water vapor. After treating half of each sugar beet leaf with CS-6432 at 2, 4, or 6% concentrations and allowing the material to dry, the pots were placed under artificial light at 2000 f.c. and resistance readings taken on the lower leaf surfaces. The CS-6432 increased diffusive resistance, as compared with readings on the control half of each leaf, but there was no trend of increased effectiveness with higher concentrations (Figure 12). A possible explanation for this is that the antitranspirant runs off the leaf as soon as it is applied in the liquid form, because of the natural angle of inclination of the leaf. This hypothesis was therefore tested in another experiment with sugar beet leaves in which all factors were kept the same as in the previous experiment except that during application and drying of the CS-6432 the leaves were taped to a flat surface (lower surface of the leaf facing up). In this case, diffusive resistance was greatly increased as the CS-6432 concentration increased from 2% to 4% to 6% (Figure 12). These experiments therefore suggested that under normal conditions and leaf angles, increasing concentrations of film antitranspirants may provide little if any added effectiveness, except in patches where the antitranspirant liquid may accumulate during the process of drying. It is not unreasonable to speculate that the lack of effectiveness of increasing concentrations would be less likely under conditions of more rapid drying of the spray (see section on antitranspirant application earlier in this report). However, it should be kept in mind that the choice of an antitranspirant concentration is often governed by factors of phytotoxicity rather than by degree of effectiveness alone.

Interactions

Antitranspirants are not equally effective under all environmental conditions. Since both transpiration and photosynthesis are affected by a number of different factors, several interactions are expected. An antitranspirant will be most effective in curtailing the magnitude of water loss from leaves when transpiration is not being restricted by natural stomatal closure in response to leaf deficits. Therefore, any environmental factor that induces stomatal closure (such as low light intensity or high evaporative demand, causing guard cells to lose turgidity because of a lag of water uptake behind transpiration) will tend to increase stomatal resistance, thereby reducing the usefulness of an antitranspirant as a barrier against transpiration losses.

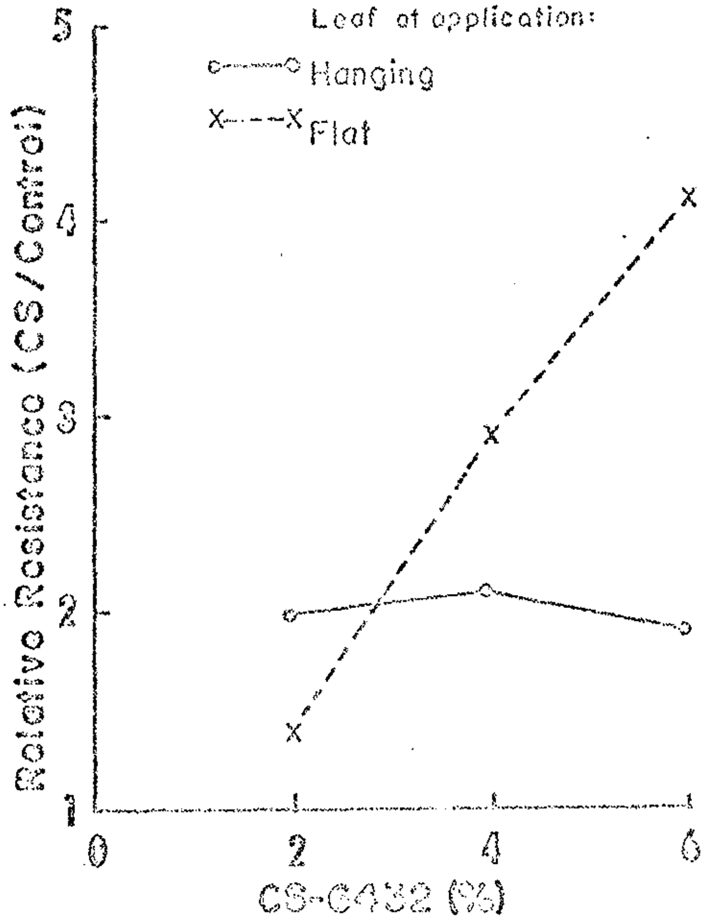


Figure 12: Effect of sugar beet leaf angle on the effectiveness of various concentrations of CS-6432.

This argument is particularly true of antitranspirants of the stomata closing type. However a film antitranspirant forms an additional resistance in series over the resistance already offered by a stomate. If however, environmental conditions are such that complete stomatal closure occurs, i.e., transpiration is nil, then an antitranspirant of the stomata closing or film forming type would obviously be of no use. Environmental factors which affect stomatal apertures (and therefore the effectiveness of antitranspirants) include light intensity, evaporative demand, and soil-water potential. Since the boundary layer resistance (r_a) is a part of the total resistance pathway for water vapor diffusion out of the leaves, its magnitude also determines the effectiveness of an antitranspirant. In still air r_a will be large and the epidermal resistance (r_e) will become relatively less important, thereby minimizing the effects of any additional resistance (stomatal closure or a film) created by an antitranspirant. The interactions between antitranspirant effects and various environmental factors are described in the following paragraphs.

Interaction with light: Half leaves of sugar beets were treated with a stomata closing antitranspirant PMA (150 ppm), the other half of each leaf being untreated controls. Measurements of resistance to water vapor diffusion from the lower surfaces of the leaves were made with the rate hygrometer at light intensities of 3000 f.c. and then at 1000 f.c. Control resistance was about 3 ½ times greater at the low than at the high light intensity (Table 14). At 3000 f.c. the PMA doubled resistance, whereas at 1000 f.c. it increased resistance only slightly.

Table 14

Effect of PMA on resistance to water vapor diffusion from sugar beet leaves at high and low light intensities.

	Resistance (min cm^{-1})	
	After 20 min. at 3000 f.c.	After 10 min. at 1000 f.c.
Control	.08	.27
PMA (150 ppm)	.16	.31

Using the same half leaf technique on sugar beet leaves, the interaction of the film forming antitranspirant, CS-6432 (3%) with light intensity was studied. The rate hygrometer measured diffusive resistances from the lower leaf surfaces. Table 15 shows the results of 3 separate replicated trials, each including measurements at a high and low light intensity. Comparisons should be made within each trial rather than between trials. It is evident that the largest difference in resistance between the treated and control halves of leaves always occurred at the lower light intensity in each trial.

Table 15

Interaction between the effects of CS-6432 (3%) and two levels of light intensity in three separate replicated trials. The values in the table are resistance to water vapor diffusion (min cm^{-1}) from the lower surfaces of sugar beet (*Beta vulgaris*) leaves.

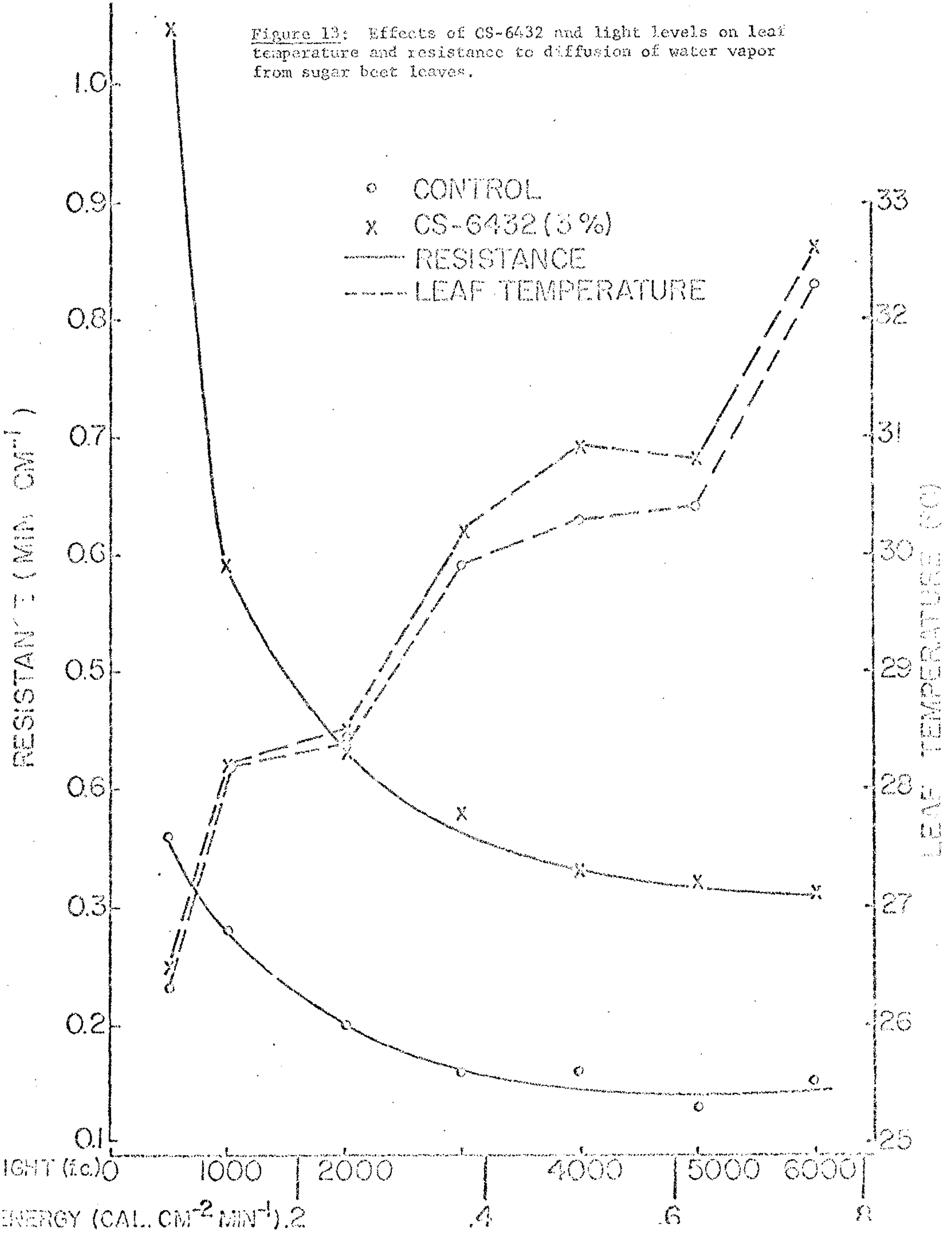
Trial	1		2		3	
Light (f.c.)	5500	300	3000	40	2000	120
Control	.06	.13	.06	.78	.07	.15
CS-6432	.09	.26	.15	.93	.09	.21
(CS - Con.)	.03	.13	.09	.15	.02	.06

A similar interaction was observed in another experiment with sugar beet leaves at light intensities ranging from 500 to 6000 f.c. (approximately .06 to .75 cal cm^{-2} min^{-1} radiant energy for this light source)--Figure 13. (The corresponding leaf temperatures are also shown in this figure; note that temperatures on the treated side of the leaf are only slightly higher than on the control side at all levels of radiation.) Another incidental observation in Figure 13 is the curvilinear response to changes in light intensity for both the control and treated halves of the leaves, indicating that the stomata under the antitranspirant film remain functional and responsive to environmental conditions, i.e., they are not "gummed up" by the film.

The seemingly greater effectiveness of the antitranspirant at low than at high light intensities is contradictory to statements made earlier in this section. However, it should be kept in mind that effectiveness is probably more realistic in terms of transpiration than diffusive resistance, because of the curvilinear relationship between transpiration and resistance. Thus, in Figure 14 (based on data of Waggoner and Zelitch, 1965, expressed originally as a linear relationship between resistance and the reciprocal of transpiration) it can be seen that in the lower range of resistances, a unit increase in resistance results in a large decrease in transpiration, but at higher levels of resistance (which would occur at low light intensities) it results in only a small decrease in transpiration. Therefore, in Figure 13, the amount of water saved by antitranspirant application would be far greater at 6000 f.c. than at 500 f.c.

This is illustrated in data on transpiration from potted oleanders growing in a greenhouse over periods of daylight (about 10,000 f.c.) and at night (0 f.c.). The results of two such trials are shown in Table 16, where it can be seen that both the film forming CS-6432 and the stomata closing PMA reduced transpiration during the day by as much as 70%. At night, the CS-6432 saved only a few grams of water because the magnitude of transpiration was small (and resistance high). PMA on the other hand, slightly increased the night-time

Figure 13: Effects of CS-6432 and light levels on leaf temperature and resistance to diffusion of water vapor from sugar beet leaves.



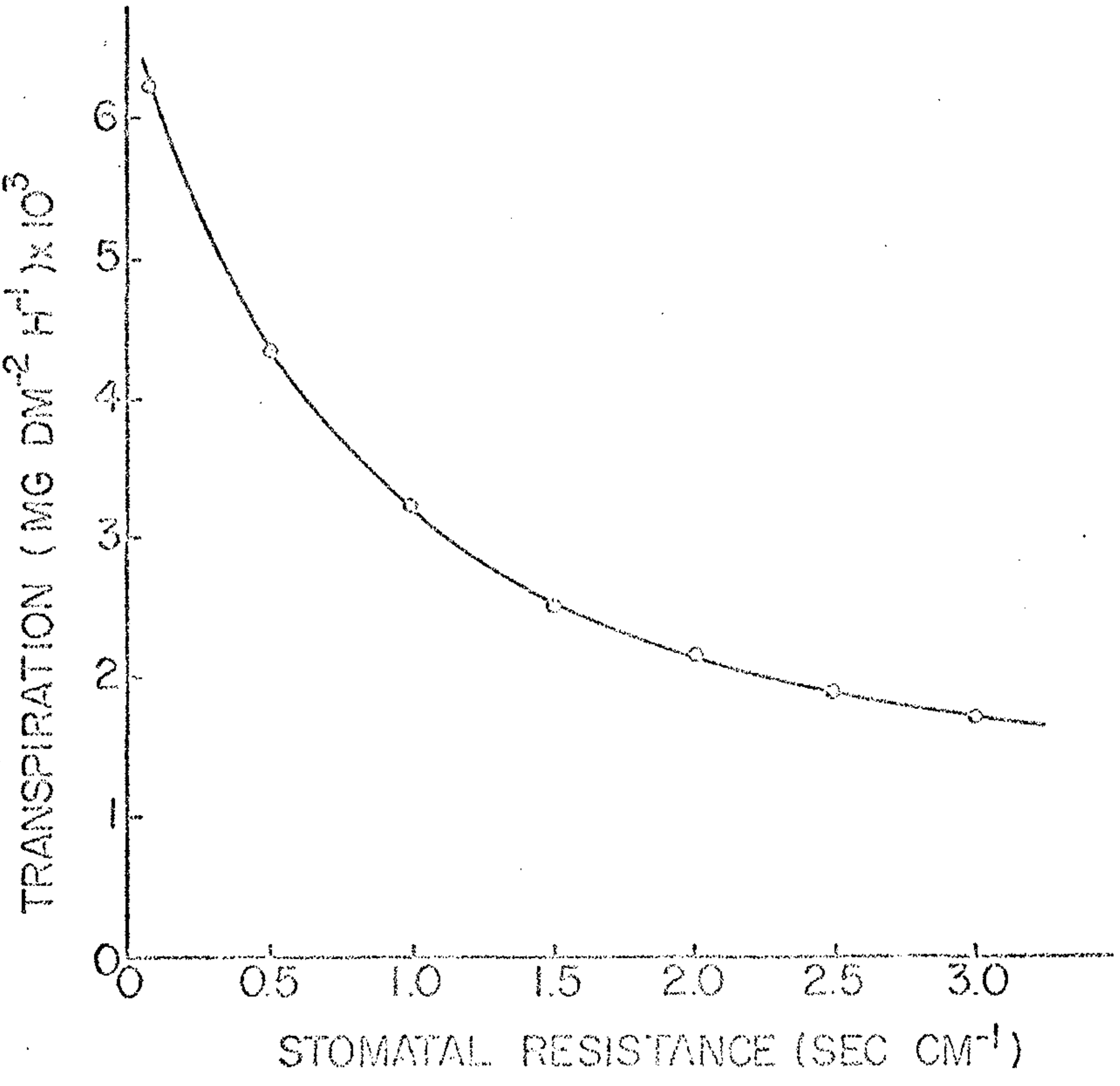


Figure 14: Relation between transpiration and stomatal resistance of tobacco leaves. (Based on data of Waggoner and Zelitch, 1965).

Table 16

Effects of antitranspirants on daytime and night-time transpiration of oleanders. (Means of 3 replicates)

	Water Transpired (g)			
	Trial 1		Trial 2	
	Day	Night	Day	Night
Control	96.3	6.0	184.7	9.0
CS-6432 (3%)	64.7	5.7	117.3	7.7
PMA*	31.0	7.3	127.3	12.0
S.E. of Means (±)	7.7	0.7	5.6	0.9

*150 ppm in Trial 1, and 110 ppm in Trial 2 water losses, indicating that in total darkness PMA retards stomatal closure. This effect of PMA will be discussed in more detail in the section on stomata closing antitranspirants.

Interaction with evaporative demand: Very little experimental work was done on this particular interaction. The effectiveness of the antitranspirant will depend on the joint influence of evaporative demand and soil-water potential on the water potential of the plant. If the evaporative demand and soil-water supply are such that large quantities of water are being transpired without causing drastic wilting and stomatal closure, an antitranspirant will be very effective in decreasing the magnitude of water lost. If, however, the evaporative demand greatly exceeds the rate of water uptake from the soil so that the water potential of the plant is reduced and natural stomatal closure occurs, the antitranspirant will have a relatively smaller effect in decreasing transpiration.

Interaction with soil moisture: Any increase in resistance to the flow of water from the soil into the root will cause a greater water deficit in the leaves and consequent stomatal closure. As a result, the effectiveness of the antitranspirant will be decreased. Thus, an antitranspirant will generally not be useful if the stomatal opening is already appreciably restricted by limited availability of soil water. Obviously, then, antitranspirants are not a cure for wilting, although they can be beneficial by retarding water loss prior to wilting, thereby at least delaying wilt and the undesirable effects on plants associated with a loss of turgidity.

Some of the interactions between soil moisture and antitranspirant effects can be seen in Table 5. In another experiment, *Phaseolus vulgaris* plants were kept either well watered (wet) or had irrigation withheld (dry) from the pots in which they were growing. During the five-day observation period the control 'dry' plants lost about half as much water as the control 'wet' plants. The effects of CS-6432 (2%) on transpiration rates in both water regimes can be seen in Table 17. In the 'wet' regime the antitranspirant reduced transpiration by about 25%, but in the dry regime there was no decrease in transpiration, indicating that partial stomatal closure, resulting from low soil-water potential, was as effective a barrier against water loss as was the antitranspirant film.

Table 17

Interaction between transpiration reduction by (CS-6432) and soil moisture. The degree of moisture stress is indicated by the lower transpiration rates in the dry pots of *Phaseolus vulgaris*.

	Transpiration (mg dm^{-2} da^{-1})	
	'wet'	'dry'
Control	870	480
CS-6432 (2%)	664	498

The effect of CS-6432 under irrigated and non-irrigated conditions was noted in an experiment with cowpeas (Vigna sinensis) growing in 5 inch pots in a growth chamber at about 22 to 25°C and 3000 f.c. light. When the plants were about 6 inches high, the pots were bagged with polythene to prevent soil evaporation, and transpiration was measured by periodic weighings of the pots. Half of the pots were kept irrigated and the other half were given only 2 initial irrigations; in each group half of the pots served as controls, the other half being treated with CS-6432. Since all of the pots had been filled uniformily with the same quantity of soil, it was possible to find a relationship between pot-weight and soil moisture percentage at the end of the experiment. The effects of the various treatments on changes in soil moisture content during the ten day experiment can be seen in Figure 15. During the first 3 days of the experiment, all of the pots were irrigated and reirrigated to 'pot capacity' (37% soil moisture w/w), and thereafter the unirrigated pots were allowed to dry up to the tenth day. Before spraying, the rate of soil moisture depletion amongst the various pots was fairly uniform, but after spraying with 2% CS-6432 on day one, and again with 6% CS-6432 on day three, the rates of soil moisture depletion were retarded by the antitranspirant treatment. The average soil-moisture content in the irrigated pots never dropped below 30% (w/w), whereas the control pots in the dry regime dropped as low as 17% at the end of the experiment, compared with about 24% for the antitranspirant sprayed plants in the dry regime. The interaction between soil moisture and antitranspirant effects from the data in Figure 15 can be seen more easily in Table 18, which shows the change in soil-moisture content between days 3 to 5, when mild soil-moisture stress occurred in the non-irrigated pots (soil-moisture content equals 29.6% for controls compared with 37% in the wet pots), and days 7 to 9 when more severe soil-moisture stress occurred in the non-irrigated pots (soil-moisture content in the non-irrigated control equals 20.8%, compared with 37% in the irrigated control). The amount of water depletion was always curtailed by the antitranspirant, and it is interesting to note that between days 3 to 5 the non-irrigated control lost slightly more soil moisture (3.9%) than the irrigated CS-6432 (3.6%). In both A and B of Table 18, more moisture was saved in the irrigated than in the non-irrigated pots, but whereas 1.6 times more water was saved by the antitranspirant as a result of irrigation under the mild stress conditions, 2.6 times more water was saved under the more severe stress conditions. In other words, the wetter the soil, the more efficient the antitranspirant in curtailing soil moisture depletion.

The rates of transpiration in the cowpea experiment described above are shown in Figure 16. The ups and downs in the curves are attributed to: 1) not maintaining constant soil moisture in the irrigated pots; 2) environmental variation from day to day caused by occasional changes in the growth chamber temperature, humidity and light duration settings. However, the environmental conditions were the same for all treatments in any one observation period. Before treatment, the transpiration rates for the controls and the plants to be treated with antitranspirant, were similar; the lower transpiration rates from the pots which were to be stressed, than from the irrigated pots, is coincidental. The effectiveness of the CS-6432 can be seen by a decrease in transpiration rates after the first day, under conditions which caused increases in transpiration rates of the untreated pots. Thereafter, transpiration rates of the un-irrigated control plants and the irrigated antitranspirant-treated plants were similar after the 6th day, indicating that

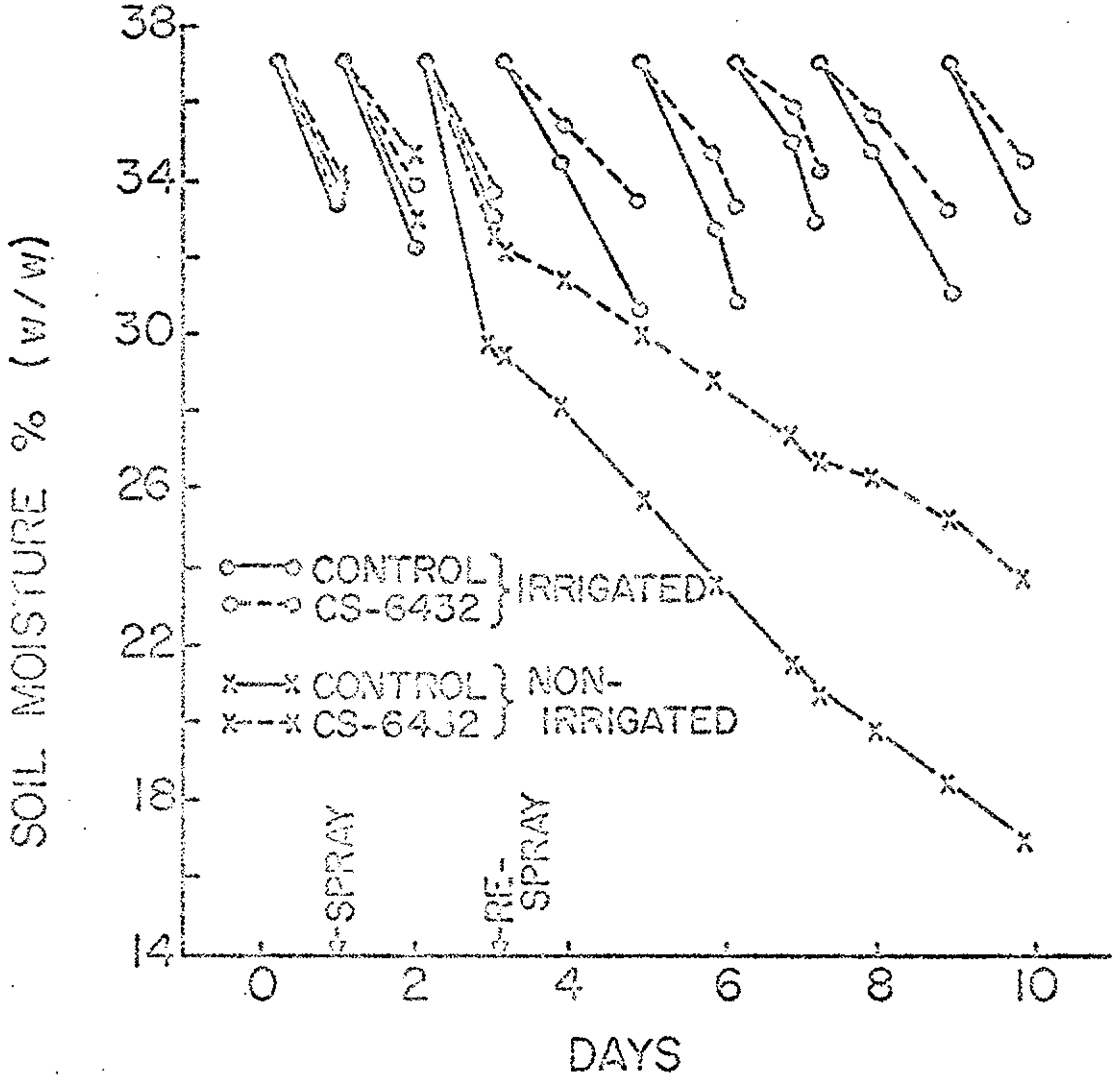


Figure 15: Effects of CS-6432 and irrigation on soil water depletion by cowpeas.

Figure 15: Effects of CS-6432 and irrigation on rates of transpiration of cowpea plants.

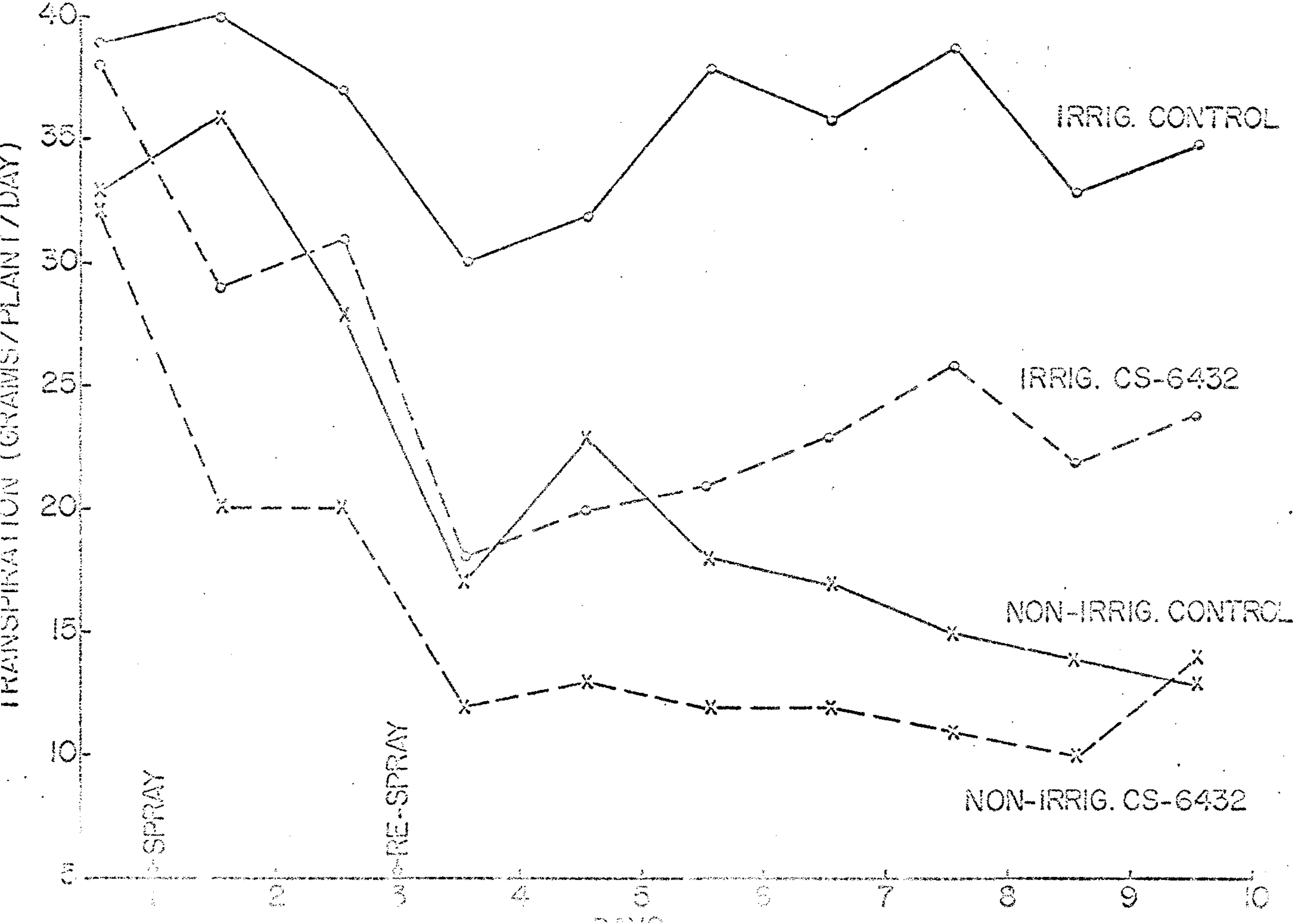


Table 18

Effects of CS-6432 (6%) and irrigation on soil moisture depletion by cowpea (*Vigna sinensis*) plants, (A) during mild soil moisture stress in the non-irrigated pots, and (B) during severe soil moisture stress in the non-irrigated pots.

	Soil Moisture % (w/w)				
A.	Day 3	Day 5	Depletion	Moisture Saved (Con-CS)	Ratio of Moisture Saved (Irrig/Non-irrig)
Irrig. Control	37.0	30.6	6.4	2.8	1.6
Irrig. CS-6432	37.0	33.4	3.6		
Non-irrig. Control	29.6	25.7	3.9	1.8	
Non-irrig. CS-6432	32.1	30.0	2.1		
B.	Day 7	Day 9			
Irrig. Control	37.0	31.2	5.8	2.1	2.6
Irrig. CS-6432	37.0	33.3	3.7		
Non-irrig. Control	20.8	18.4	2.4	0.8	
Non-irrig. CS-6432	26.8	25.2	1.6		

under relatively mild soil moisture stress (soil moisture content of unirrigated control pots on day 4 was 28%, compared with a 'pot capacity' of 37%), an antitranspirant can substitute for an irrigation. However, after the 5th day, a lack of soil moisture in the unirrigated controls curtailed transpiration to a greater extent than was achieved by CS-6432 in the irrigated pots. In the unirrigated pots the antitranspirant reduced transpiration until the 9th day when the environmental demand caused increases in transpiration in all pots which still had sufficient soil moisture; this included the CS-6432 unirrigated pots (24% soil moisture), but not the control unirrigated pots (17% soil moisture). (At this time the resistance to water vapor diffusion from the lower surfaces of the leaves in the unirrigated controls was 7.7 x that of the irrigated controls.) The fact that the antitranspirant-treated plants did respond to the increased evaporative demand, suggests that the CS-6432 effectiveness was wearing off. It is of interest to note that between days 8 and 9 the CS-6432 reduced transpiration rates by 11 grams in the irrigated pots, but only 4 grams in the unirrigated pots. This can be attributed to less soil moisture being available in the latter case, rather than to a difference in the film itself on the unirrigated plants, relative to that on the irrigated plants. If the experiment had been continued, the treated unirrigated plants would no doubt have depleted their soil moisture to the point of permanent wilt in about a week, but the untreated non-irrigated plants would already have wilted about 5 days earlier.

The effects of CS-6432 and irrigation on resistance to water vapor diffusion from the cow pea leaves on day 4 can be seen in Table 19. Resistances were higher 1) on the upper than the lower leaf surfaces, 2) for the antitranspirant treated leaves than for the control leaves, and 3) for the unirrigated than the irrigated plants. Once again, it should be remembered that an increase in resistance (as by an antitranspirant) at lower levels of resistance (irrigated pots) results in a greater reduction in transpiration than an equal increase in resistance occurring at higher levels of resistance (unirrigated pots).

Table 19

Effects of CS-6432 (6%) and irrigation on resistance to water vapor diffusion from the upper and lower surfaces of cow pea (*Vigna sinensis*) leaves. (Soil moisture content (w/w) of the various treatments at the time of measurements are also shown; 'pot capacity' = 37%.)

	Resistance (min cm^{-1})		Soil
	Lower	Upper	Moisture (%)
Irrig. Control	.23	.39	34.5
Irrig. CS-6432	.72	1.81	35.5
Non-irrig. Control	.42	.96	28.1
Non-irrig. CS-6432	1.26	1.84	31.5

The relationship between the effectiveness of PMA (150 ppm) in retarding transpiration and of soil moisture is illustrated by an experiment with oleanders growing in a light soil in one-gallon containers in a greenhouse. In Figure 17 the transpiration rates from PMA treated plants, relative to those from control plants is plotted against decreasing pot weight. Using a linear relationship between pot weight and soil moisture content found at the end of the experiment, it was possible to determine the soil moisture content (percent w/w) at the times of observation. The moisture contents plotted along the axis showing pot weights serve as an index for the soil moisture status of the experiment. (Because of the initially low transpiration rates in the PMA pots, their moisture contents which were not measured in this experiment, were probably higher than those of the control pots.) The duration of the experiment was approximately one week, and the time interval between the first and second irrigations was 5 days. All of the pots were initially irrigated, and after spraying, transpiration measurements were made by taking differences in the weights of the pots after covering the soil surface to prevent evaporation. PMA effectively reduced transpiration while the soil was moist but by the time soil moisture had dropped to 7% in the control pots (thereby causing stress and closing their stomata), transpiration rates were higher than those of control plants until these also exhausted their moisture supply. All of the pots were then irrigated, the moisture content being about 19.6% after all drainage had ceased. Once again the PMA decreased transpiration, though not quite as much as was observed initially, under the wet soil conditions.

A similar experiment was carried out with potted oleanders to note the effects of the film forming antitranspirant CS-6432 (3%) and the stomata closing antitranspirants PMA (150 ppm) under wet, and progressively drying, soil moisture conditions during a ten day period. All of the pots were irrigated to 'pot capacity' (approximately 22% soil moisture w/w) after spraying the antitranspirants. Figure 18 shows the changes in soil moisture, expressed as percent of 'pot capacity', over a period of ten days, beginning with a soil moisture content of about 22% and ending with about 7% (w/w). Figure 19 shows the transpiration data for the same experiment, expressed as a percent of the transpiration from control plants. In Figure 18 it will be noticed that the soil moisture depletion of control plants ceased to be linear by about the 4th day when about 55% of the moisture which was available at the start of the experiment had been depleted. At this time the corresponding soil moisture contents for the CS-6432 and PMA treated plants were, respectively, 65% and 75% of the moisture content at the start of the experiment. Thus by the 4th day, soil moisture was becoming a limiting factor in the control, but not in the two antitranspirant treatments. Similarly in Figure 19, by the 4th day transpiration rates from the control plants were the same as those from the treated plants because of 1) partial desiccation and stomatal closure in the control plants, and 2) absence of severe soil moisture stress in the treated pots. After the fourth day, transpiration rates of the antitranspirant treated plants exceeded those of control plants, so that by the 7th to the 9th day their soil moisture depletion (Figure 18) was at the same level as that of control plants. However, the treated plants continued to transpire at slightly higher rates than the control plants. Since expression of transpiration from treated plants as a percent of control (Figure 19) gives a deceptive impression of the effectiveness of the antitranspirants, the actual magnitudes of transpiration rates for each

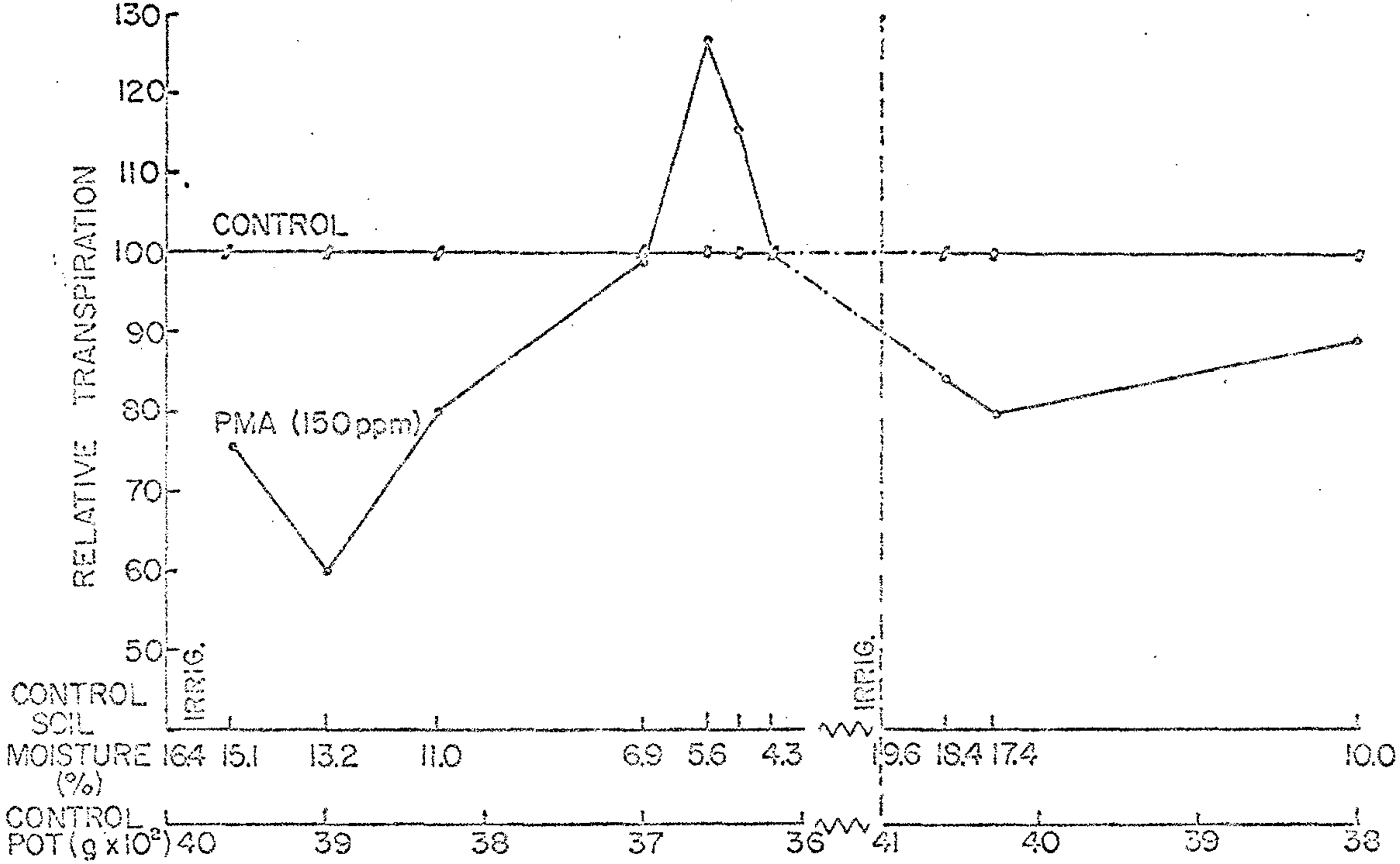
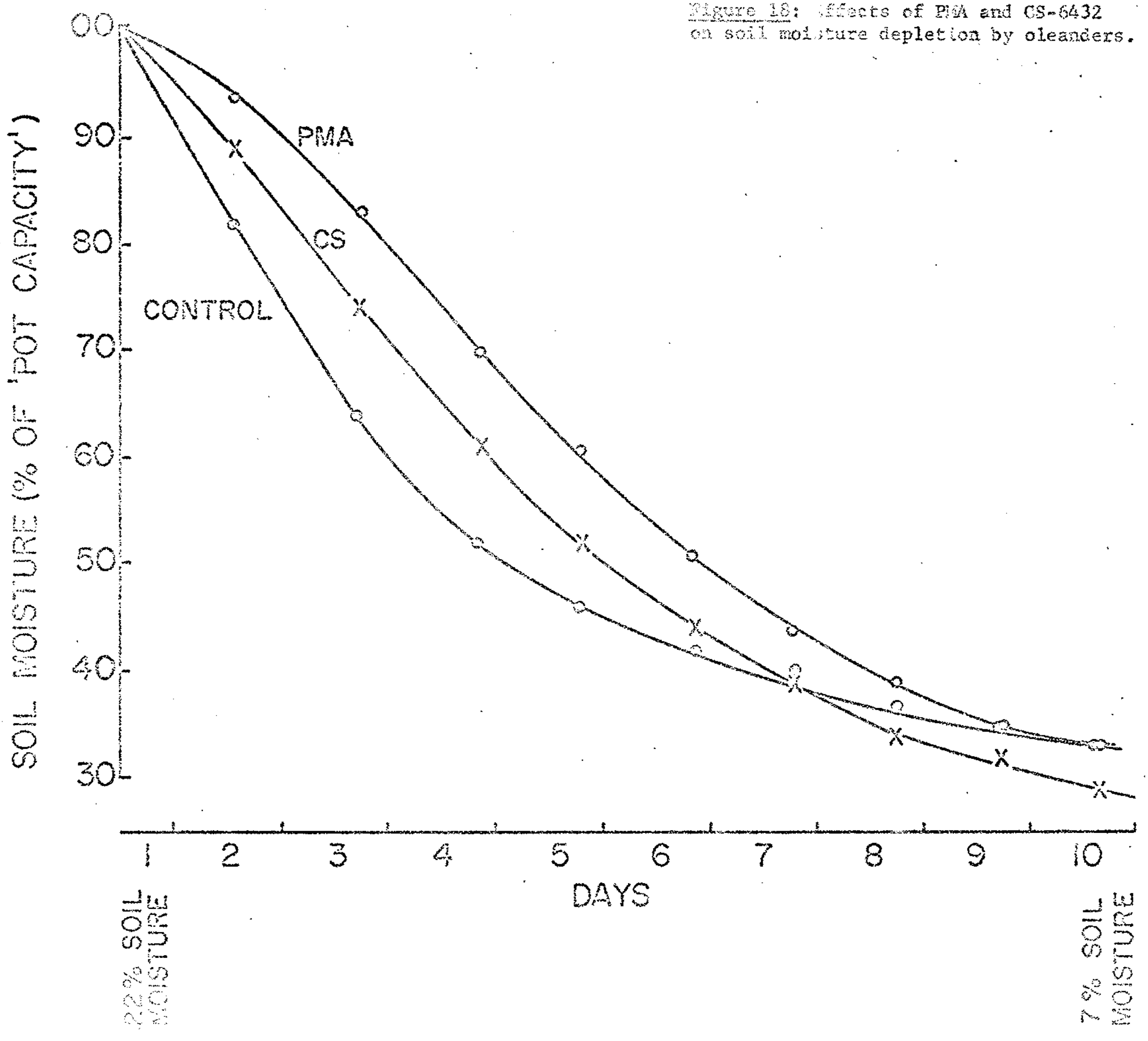


Figure 17: Effects of phenylmercuric acetate and soil moisture on transpiration of oleander plants. (Weights and corresponding soil moisture contents for control pots are given on the abscissa.)

Figure 18: Effects of PMA and CS-6432 on soil moisture depletion by oleanders.



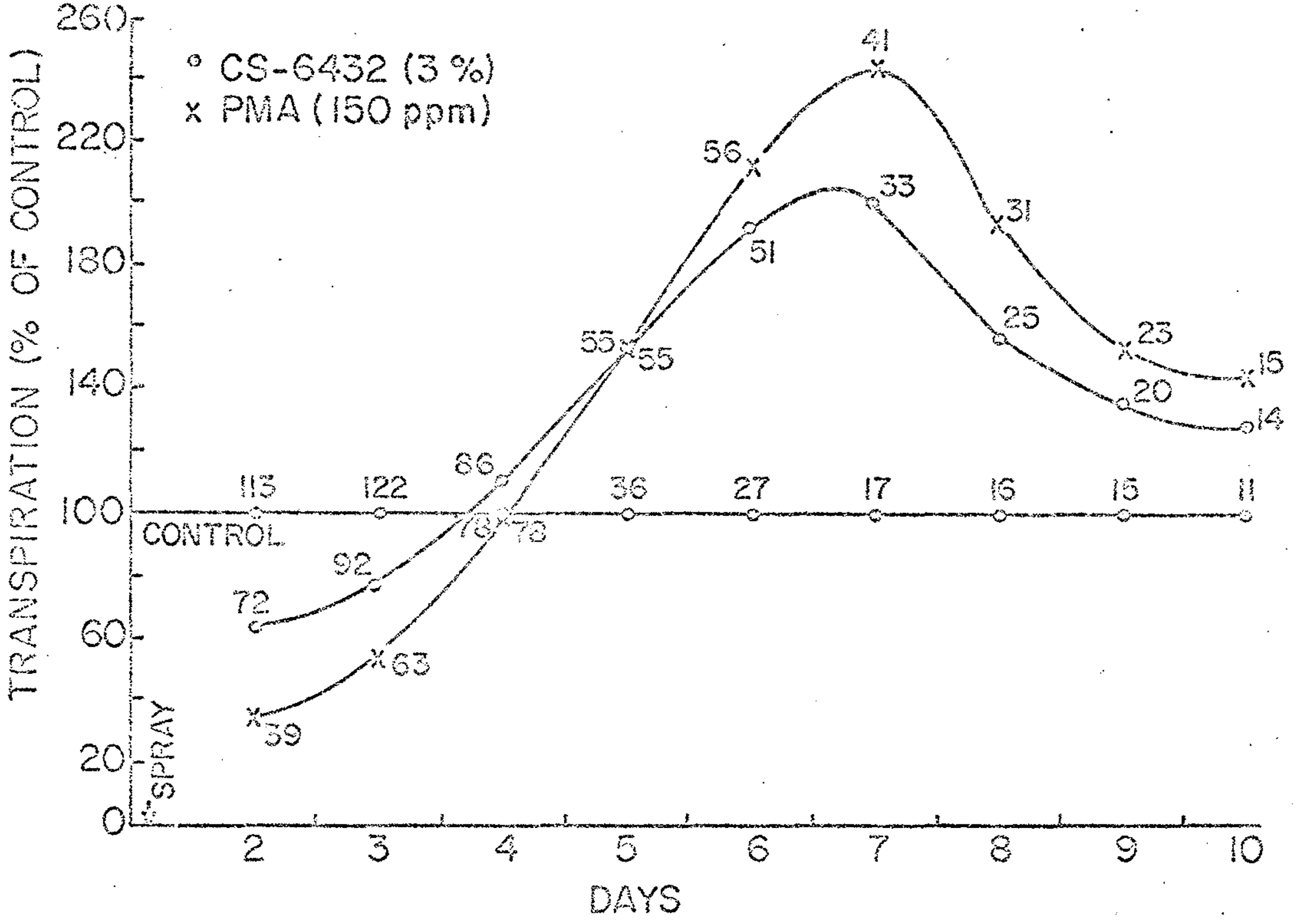


Figure 19: Effects of CS-6432 and PMA on relative transpiration (% of control) as soil moisture is depleted with time. (The numbers on the curves are grams water transpired each day.)

day have been given on the curves. Thus on day 2, PMA resulted in a saving of 74 grams of water per plant per day, whereas on day 7 PMA transpired only 24 grams of water per plant per day more than controls. Over the 10 day period, the total water transpired by the control, CS-6432, the PMA-treated plants were 434, 449, and 401 grams/plant, respectively. However, up to the 4th day, when control plants began to show signs of moisture stress, the cumulative water losses for control, CS-6432 and PMA-treated plants were 313, 250, and 179 grams/plant, respectively.

Interaction with wind: The environmental conditions of light, evaporative demand, and soil moisture just described affect the epidermal resistances (r_e) of leaves. Wind speed, on the other hand, affects another resistance in the water vapor and CO_2 pathways, namely the boundary layer resistance (r_a). Low ventilation rates, by increasing r_a, tend to decrease the importance of the effect of antitranspirants on r_a. However, this is only true if we assume that no other influence of wind (e.g., advection of heat, water vapor or dry air) becomes a dominating factor. No experiments were carried out to assess the interaction between antitranspirant effects and wind speed under outdoor conditions, but the following experiment with dichondra, using the small fan inside the plant chamber to create turbulence (and therefore decrease boundary layer resistance), is of interest.

The treatments on the dichondra consisted of no spray (control), CS-6432 (2%), CS-6432 (4%), PMA (100 ppm), and PMA (200 ppm). Simultaneous measurements were made of transpiration and photosynthesis rates using the method already described. Each plant was run with and without air turbulence in the plant chamber (fan off or on), but ventilation rates inside the chamber were not measured. The temperature, vapor pressure and flow rate of the incoming air stream were kept constant for all measurements. Use of the fan increased the rates of transpiration and photosynthesis by 75% and 35%, respectively for the control plants. The interaction between ventilation and antitranspirant effect can be seen in Figure 20. In all cases antitranspirant treatment decreased the rates of transpiration and photosynthesis. However, the amount of decrease was always greater when the fan in the chamber was switched on (turbulent air and small r_a), as indicated by the steeper slopes of the broken than of the solid lines. (A complete absence of interaction between antitranspirant effects and ventilation would have caused the solid and broken lines to be parallel in each case.)

The magnitude of interaction can be seen more easily in Table 20 which gives data from another experiment on dichondra involving measurements of the rate of transpiration and photosynthesis in the presence and absence of ventilation from the chamber fan. In each case, the effect of the antitranspirant, assessed by the difference between the control rate and antitranspirant rate, was greater in the ventilated than in the non-ventilated conditions. Thus, the effect of CS-6432 and the fan on transpiration (260 mg dm^{-2} h^{-1}) was nearly twice as big as its effect without the fan (140 mg dm^{-2} h^{-1}); the effect on photosynthesis was about 1½ times greater with, than without, the fan. In this experiment, PMA completely stopped photosynthesis, but the data still illustrate the greater effectiveness of PMA under ventilated, rather than non-ventilated, conditions.

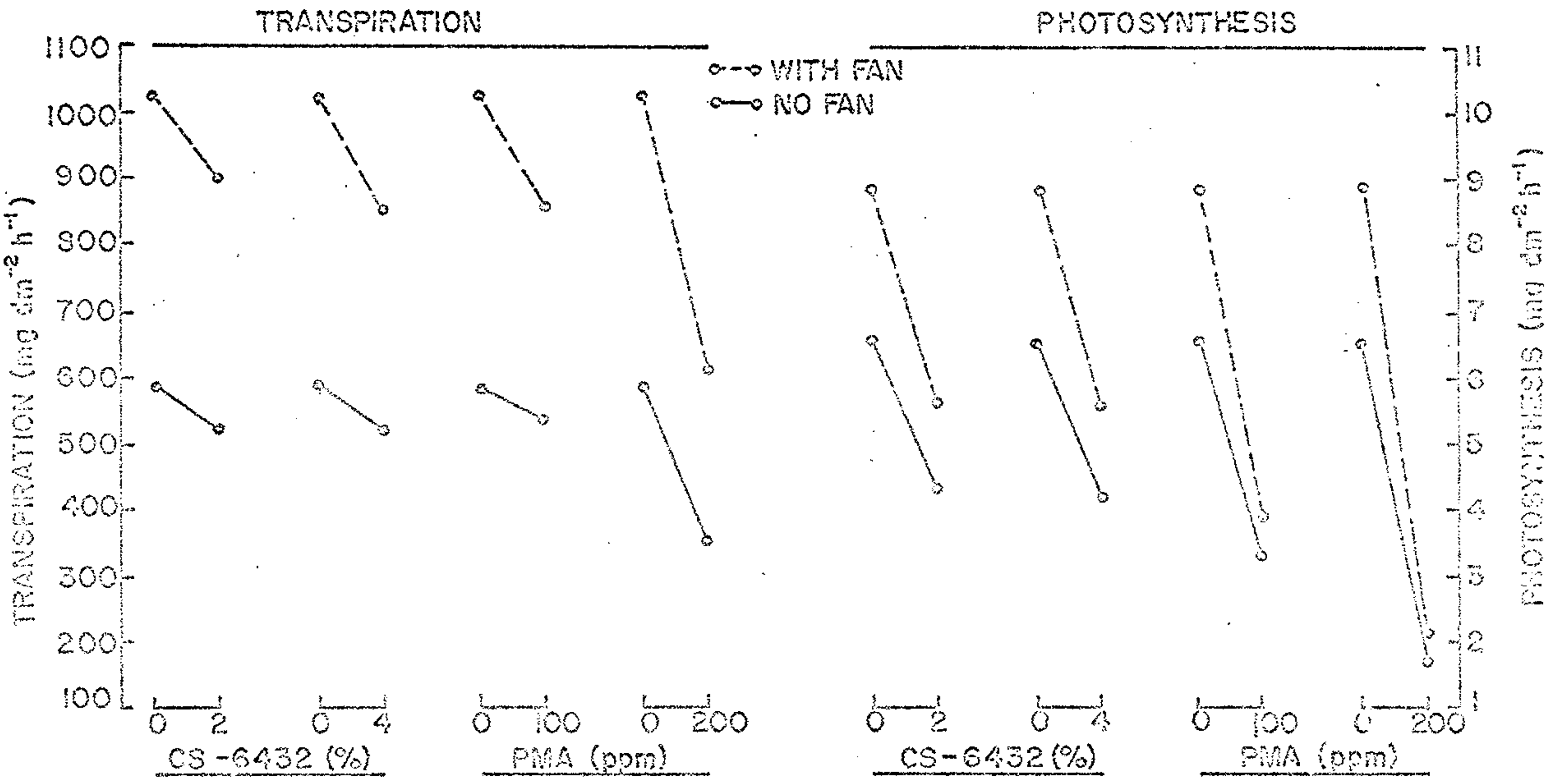


Figure 20: Effects of CS-6432, PMA, and air movement on rates of transpiration and photosynthesis of dichondra.

Table 20

Effects of antitranspirants and increased ventilation (fan) on transpiration and photosynthesis rates of dichondra (*Dichondra repens*).

	Transpiration ($mg\ dm^{-2}\ h^{-1}$)		Photosynthesis ($mg\ dm^{-2}\ h^{-1}$)	
	No fan	Fan	No fan	Fan
Control	870	1140	2.80	4.00
CS-6432 (3%)	730	880	1.27	1.70
Con-CS	140	260	1.53	2.30
Control	870	1140	2.80	4.00
PMA (110 ppm)	590	800	-1.23	-1.30
Con-PMA	280	340	4.03	5.30

Interaction with plant factors: Certain plant factors, particularly those concerned with water uptake (the roots) and water loss (stomates) influence the effectiveness of antitranspirants. Stomatal distribution on the leaves is important since the antitranspirant will be of little benefit if applied to non-stomatal-bearing surfaces of leaves. Thus, spraying only the upper surface of a hypostomatous leaf with a film-forming or stomata-closing antitranspirant will not affect transpiration or photosynthesis. An antitranspirant spray is, of course, effective in reducing transpiration only on those leaves to which it is applied. Antitranspirants will, therefore, have little value when applied to plants that continue to produce new leaf surface throughout the growing season, especially if the new growth occurs at the outer extremities of the plant, where transpiration rates are highest. The nature of the leaf epidermis influences an antitranspirant's effectiveness, since the leaves of some plant species are very difficult to wet with an antitranspirant spray. Depth of rooting often determines the duration of water supply from roots to leaves and, therefore, also governs the duration of effect of antitranspirants.

The influence of the above mentioned plant factors on the effectiveness of antitranspirants was always kept in mind when spraying in the laboratory, greenhouse and field. All of the orchard crops sprayed with antitranspirants in the field trials (to be described later in this report) bear their stomata exclusively on the lower sides of their leaves. These trees were, therefore, sprayed from the ground either with an orchard sprayer (at about 400 pounds/sq.inch pressure) or with a mist blower. The blast from these sprayers was powerful enough to turn the leaves and ensure wetting of their under surfaces. Several laboratory and greenhouse experiments showed no effect of either a film-forming (CS-6432) or a stomata-closing (PMA) antitranspirant when applied to the upper surfaces only of hypostomatous oleander leaves. The influence of rooting depth was noticed in a field experiment with oleanders which were not irrigated during the summer, but relied entirely on stored winter rainfall in a deep soil profile. After the roots in the upper layers had exhausted most of their moisture supply, the effects of an antitranspirant in retarding the rate of soil moisture depletion was noticed only at the lower soil depths (4 to 6 feet) where deeper roots were utilizing un-tapped soil moisture remaining from the winter rains.

Stomatal apertures

Stomatal apertures can be measured indirectly by methods such as: 1) qualitative observation of the infiltration of liquids of varying surface tension into the leaf; the more closed the stomata, the lower the surface tension of the liquid required to achieve penetration, which is judged by visual appearance of a "grease spot"; 2) mass flow porometry, which involves the measurement of rate of penetration of air under pressure through the leaf; the more open the stomata, the less the time required for a given pressure drop; 3) rate of water vapor diffusion using a rate hygrometer described earlier in this report. Direct measurements of stomatal aperture include: 1) impression of the leaf with silicone rubber which is coated with celulose acetate to obtain a clear "positive" imprint for viewing under the microscope; 2) microscopic examination of the epidermis after stripping it from the leaf.

Because of its qualitative nature, the infiltration technique was seldom used. Since film antitranspirants hindered the infiltrating liquids, this technique was restricted to leaves which were either untreated or treated with antitranspirants of the stomata-closing type. In field experiments with almonds, the infiltration technique (using moistures of iso-propanol and distilled water) did not show large differences in stomatal opening between leaves from unsprayed and PMA sprayed trees, but did show differences in degree of stomatal opening in the course of the day between sunrise and sunset. In laboratory experiments, mixtures of iso-butanol and ethylene glycol, were useful in deteriming the effects of PMA on stomatal closure of excised oleander leaves. The technique helped to confirm other data (to be described later in this report) which indicate that PMA not only retards stomatal opening, but also retards the rate of stomatal closure.

Before acquiring the rate hygrometer (also known as a diffusion porometer, since it measures the rate of water vapor diffusion from the leaves), a mass flow porometer, similar to the type designed by Alvim, was used to measure the effects of an antitranspirant on leaf resistance. The time required for a pressure drop of 20 mm mercury on the pressure gauge was taken as an index of the resistance to the passage of air being forced through the leaf. The half leaf technique was used to assess the effects of antitranspirants on cotton (Gossypium hirsutum) growing in pots in a greenhouse. One-half of each leaf was used as a control (painted with distilled water plus X77 surfactant), and the other half was treated with either PMA (110 ppm) or CS-6432 (2%). Porometer measurements were made on the lower surfaces of the leaves of 4 pots, using two leaves per pot, i.e., a total of 8 readings per treatment. Both the PMA and CS-6432 increased resistance to the flow of air through the leaves, as indicated by the longer time required for the given pressure drop (Table 21).

Table 21

Effects of PMA (110 ppm) and CS-6432 (2%) on the time required for a 20 mm Hg pressure drop, using a mass flow porometer on cotton (Gossypium hirsutum) leaves.

Treatment	Time (sec.)	%
Control	25	100
PMA	56	224
Control	32	100
CS-6432	61	191

Sufficient data have already been presented on the use of the rate hygrometer which measures resistance to diffusion of water vapor from leaves, rather than resistance determined by a mass flow of air through the leaves.

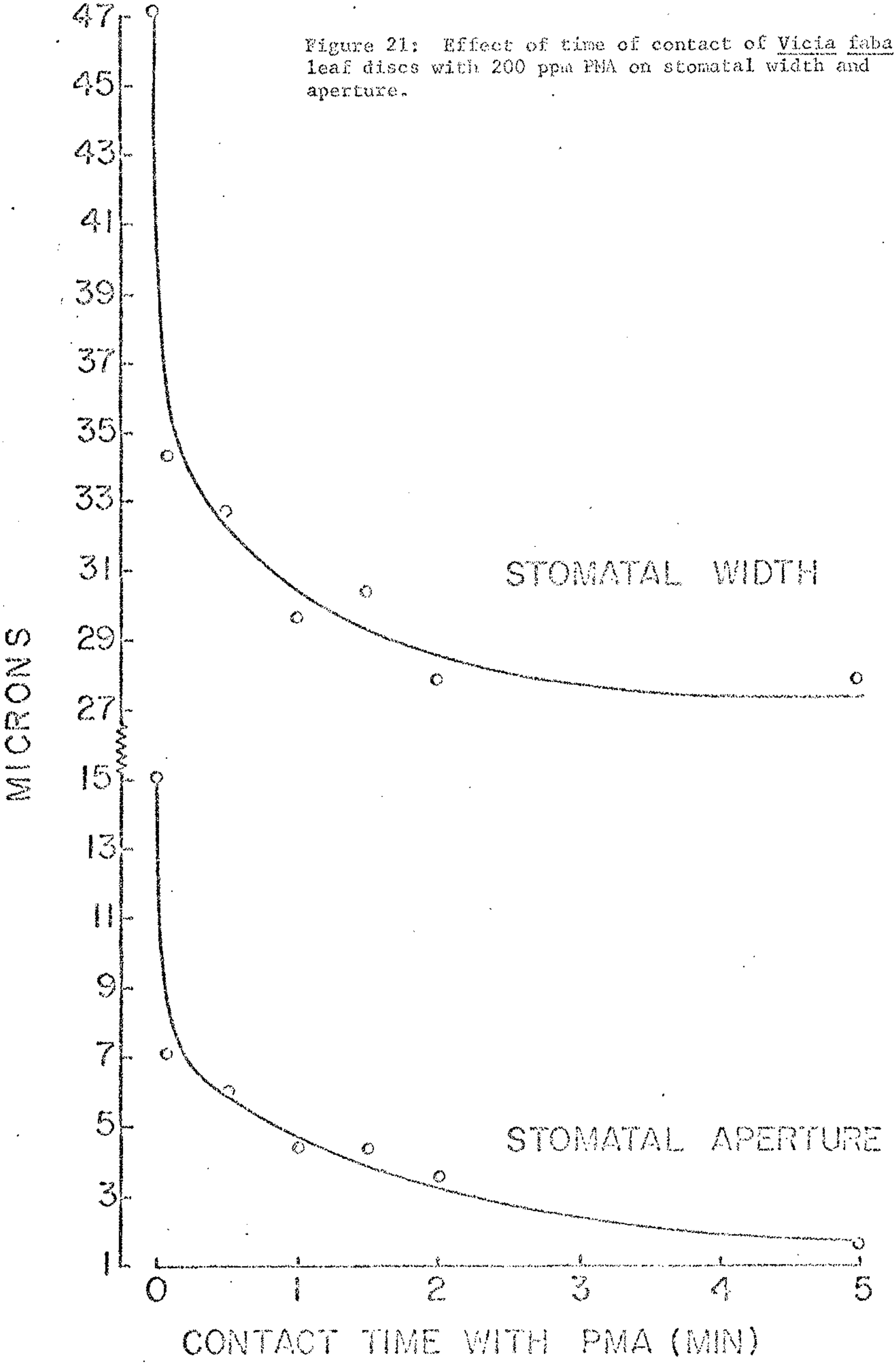
The silicone rubber method was more useful for determining the stomatal distribution than for accurate measurements of stomatal aperture. Direct microscopic measurements of stomatal apertures, as affected by antitranspirants, were therefore made on epidermal strips. Stomatal apertures were measured to determine the influence on them of humidity, light intensity, transpiration, water stress (with and without AT treatment) and the degree of film coverage. Sugar beets, oleander, snap beans, zebrina, sedum, and fava bean were screened as possible test plants. The first three did not yield epidermal peels, whereas the latter three did. The stomata of the zebrina and sedum were unresponsive in the test conditions, so the fava bean (Vicia faba) was chosen. The aperture readings were made from epidermal strips of either intact leaves or leaf discs. The discs were taken with a 6 mm diameter cork borer and floated in small petri dishes of liquid for treatment. Solutions of Tris-Maleate-$CaCl_2$, 10^{-4} M $CaCl_2$, 10^{-4} M KCl, and double distilled H_2O (DDW) were tested. The greatest apertures were achieved on KCl, so it was frequently utilized. Adequate opening was achieved on DDW and was often used for control discs in comparisons with PMA. Normally, the lower epidermis of the leaf was used as the test surface. When treating floating discs, it was necessary to have this surface in contact with the PMA solution. If the treatment had been applied to the intact plant, the discs were generally floated with the treated surface away from the liquid, although it was not essential. The stomata on the floating discs were found to be much more responsive to light intensities than those on the intact leaves. The illumination was much more uniform on the discs as they all floated at the same level. The stomata frequently, but not invariably, opened more rapidly and to a greater ultimate aperture, if the petri dish had a transparent cover. Convenience in the experiment determined whether covered or uncovered dishes were used. Measurements of the apertures were made as nearly as possible in the center of the disc to avoid cells damaged by sampling and treating techniques.

In the section on antitranspirant application earlier in this report it was shown that the stomata on epidermal peels of Vicia faba were affected by PMA treatment, whether the treatment was made in the light or in the dark. Another experiment with Vicia faba epidermal strips was done to determine the minimum contact time with PMA for stomatal closure to occur. Seven groups of leaf discs were floated in 200 ppm PMA solution for 7 different times, ranging from 0 time (control) to 5 minutes, before floating then on .1M KCl in a growth chamber at 3000 fc. They were then put into the dark for 12 hours, and then again in the light at 3000 fc for 2½ hours, at which time the epidermal strips were taken for microscopic measurements of stomatal apertures. A contact time of as little as 5 seconds was all that was necessary for the 200 ppm PMA to effectively reduce stomatal aperture (Figure 21). Other data suggest that the minimum contact time for effectiveness of PMA increases as PMA concentration decreases. It was also noticed that the stomatal apertures of leaf discs floating on PMA solution are affected only on the epidermis which is in contact with the solution, i.e., the PMA is not translocated through the leaf to the epidermis not in contact with the solution.

In the analysis of some of the theoretical aspects of antitranspirants, it was postulated that an antitranspirant film on a leaf may actually decrease the resistance of stomata lying immediately under it, i.e., increase stomatal apertures, as a result of the increased water potential of the leaf resulting from the antitranspirant treatment. This was tested on Vicia faba plants growing in well watered pots in a growth chamber at about 50% relative humidity and 2800 foot candles light. Mobileaf (1:5) was applied to Vicia faba leaves and, after the film had dried, stomatal measurements were made on epidermal peels under the microscope. The peels included the Mobileaf film which was sufficiently transparent to allow unhindered measurement of stomatal apertures. Stomatal observations were also made on epidermal peels from untreated leaves (located at the same node on the stem as the treated leaf). Thirty-six stomatal measurements were made for each treatment, comprising strips from 6 leaves with 6 measurements on each strip. Table 22 shows that for well watered plants the average stomatal aperture underneath the Mobileaf film was nearly 3 times greater than that of the untreated leaves. Total stomatal widths (across both guard cells) were also increased. For stressed plants, however, the apertures under the films were increased five fold. A similar effect was also noticed with the film antitranspirant CS-6432 (2%) when applied to Vicia faba leaves. In this case, half of each leaf was treated with CS-6432, the other half being untreated control. Leaf discs were then taken from the treated and untreated halves of the leaf and floated in 0.1 M KCl in covered petri dishes at 2300 foot candles light. Another set of measurements was made on discs which were kept at only 150 foot candles light. Stomatal apertures were increased under the CS-6432 film in both of the lighting conditions (Table 22).

Thus by increasing stomatal apertures, an antitranspirant film tends to defeat its own purpose, making it even more important to have a high resistance to water vapor to maintain its effectiveness. Wider stomatal apertures under the film should also offer less resistance to the entry of carbon dioxide. However, with presently available materials, the relatively low CO_2 permeabilities of the films, rather than the stomatal apertures, are the real limiting factors.

Figure 21: Effect of time of contact of Vicia faba leaf discs with 200 ppm PMA on stomatal width and aperture.



It should be rememberd that the stomata are not 'glued' open by the overlying film, and that they do remain functional in their responses to stimuli such as light, as pointed out in the data of Figure 13.

Table 22

Effects of film antitranspirants on apertures and total widths of the stomata covered by the films. Measurements were made on epidermal peels from *Vicia faba* leaves growing under various environmental conditions.

Anti-transpirant	Condition	Light (f.c)	Aperture (microns)		Width (microns)	
			Control	Treated	Control	Treated
Mobileaf (1:5)	Watered	2300	4.6	11.9	31.4	39.9
Mobileaf (1:5)	Stressed	2300	1.9	9.6	27.8	38.9
CS-6432 (2%)	Watered	2300	3.2	9.4	33.4	36.9
CS-6432 (2%)	Watered	150	1.4	2.3	27.6	30.0

In the experiment on the measurement of stomatal apertures of *Vicia faba* leaf discs just described, measurements were also made of resistance to water vapor diffusion from those areas of the leaf which were to have their epidermises stripped for stomatal observation. These data were then used to plot the inverse relation between stomatal aperture and diffusive resistance (Figure 22). The curves for the untreated and film treated leaves are similar in shape, but the latter is shifted to the right, indicating that at a given resistance the apertures are always wider under the film, than in the absence of a film. The shape of the curves also suggests that when stomatal apertures are wide, a unit decrease in aperture results in only a very small increase in resistance, but when stomatal paertures are narrow, a unit decrease in aperture results in a relatively large increase in resistance. These data are based on relatively few measurements, and further investigation is required to substantiate the relationship between stomatal paerture, resistance and transpiration, and the effects of antitranspirants of the stomata closing and film forming types on these relationships.

Stomata Closing Antitranspirants

Effects of phenylmercuric acetate (PMA): Other workers (Zelitch and Waggoner) have pointed out that stomata closing antitranspirants, such as PMA, prevent the guard cells from attaining complete turgidity, and thereby reduce

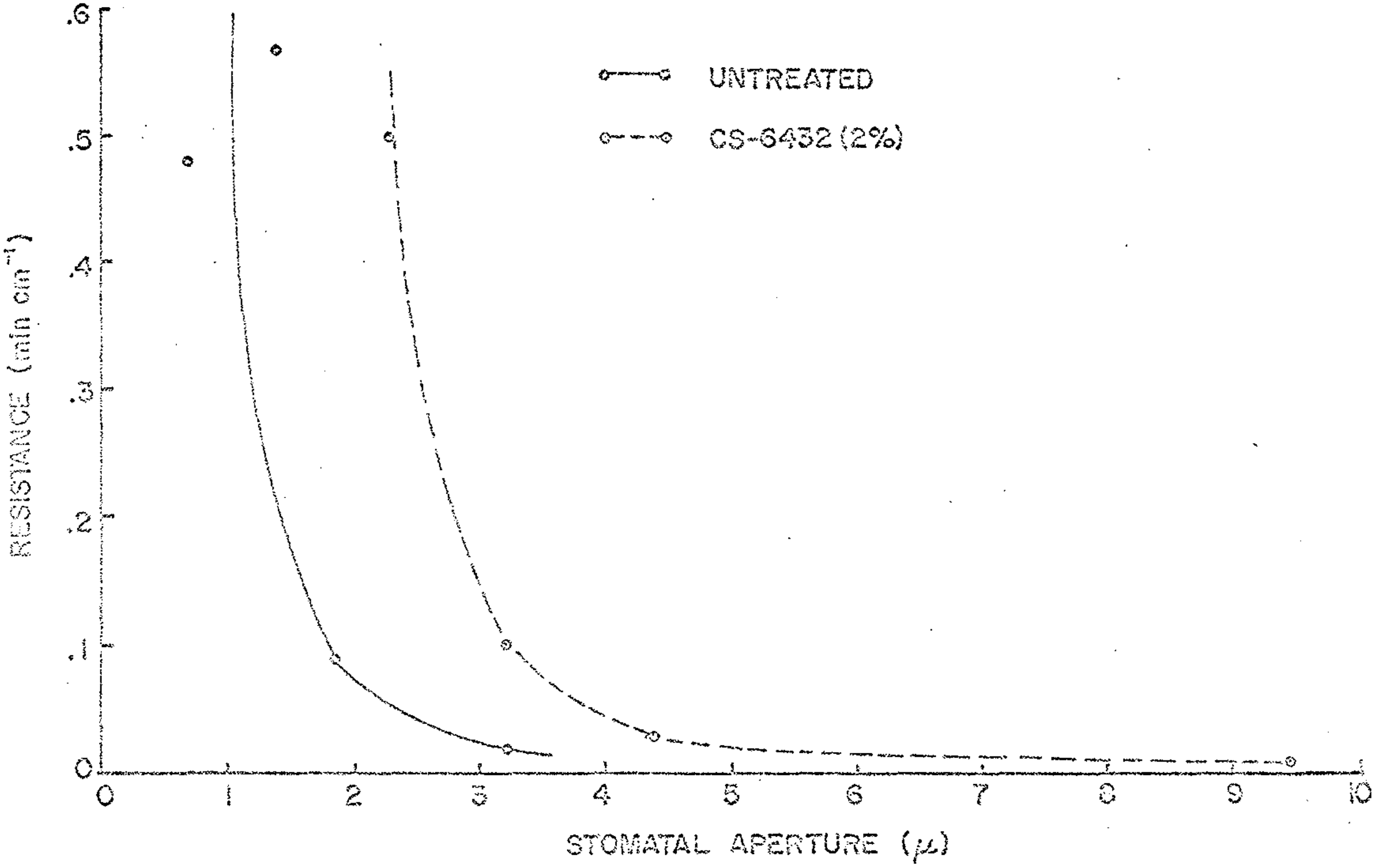


Figure 22: Effect of a film antitranspirant on stomatal aperture and on the relation between stomatal aperture and resistance of *Vicia faba* leaves.

stomatal apertures, transpiration and photosynthesis. However, Mansfield believes that PMA may also affect stomatal apertures indirectly by adversely affecting photosynthesis, resulting in a CO_2 build-up and consequent stomatal closure. It is not within the scope of this report to investigate the biochemical reactions involved when a stomata-closing antitranspirant affects the guard cells. However, various effects of antitranspirants observed by others have been confirmed, and some new effects, not reported in the literature, have been found and will be presented here.

In the last section, it was pointed out that only a short contact time between epidermis and PMA solution is required for effectiveness, and that the effect is localized. The effects of PMA of different concentrations on transpiration, photosynthesis, resistance, and leaf temperature under various environmental conditions have been illustrated earlier in this report.

In the course of the experiments with sugar beets on the interaction of antitranspirants with light (see section on interactions earlier in this report), it was noticed that PMA not only retards the rate of stomatal opening as light increases, but also retards the rate of closure, as light decreases. Thus in Figure 23A, PMA (150 ppm) retarded stomatal opening (indicated by the higher resistance of treated than control leaves) 30 minutes after transferring the plants from dark to 1000 foot candles, and then progressively to 2000 and 4000 f.c. light. However, when, the light was again reduced to 1000 f.c., the stomata of the control leaf surface responded more rapidly (steep increase in resistance) than those of the PMA treated leaf. Thus, for the final half hour of the observation period (at 1000 f.c.) the resistance to water vapor diffusion was lower for the PMA treated surface than for control. In Figure 23B, similar results were obtained using PMA (200 ppm). In this case, the light level was reduced progressively from 4,000 to 1,000 to 150 f.c. As expected, there was a rapid increase in resistance at the lowest light level for control leaves, but the PMA-treated leaves showed a relatively small increase in resistance. In other words, PMA retarded stomatal closure.

The first indication that PMA retarded stomatal closure was noted in an experiment with oleander leaves in 1968. PMA (120 ppm) was sprayed on the upper and lower surfaces of leaves still on the plant in the greenhouse. A similar group of leaves was sprayed with a film-forming antitranspirant (Wilt Pruf, 1:3), and a third group was unsprayed controls. About 1½ hours later, each leaf was cut from the plant, weighed immediately (to the nearest 0.1 mg), and then placed at a 45° angle, with the under surface facing up, in a growth room at 24°C, 40% relative humidity, and 1500 foot-candles of light. The leaves were periodically weighed to determine water loss, and the resulting transpiration rates were expressed as mg H_2O dm^{-2} h^{-1} (Figure 24). The PMA and Wilt Pruf slowed transpiration by about 30% initially, and then transpiration of controls and Wilt Pruf-treated leaves dropped sharply, presumably from stomatal closure since the leaves were detached from their water supply. Water loss from the PMA-treated leaves however, declined only slightly, suggesting that stomatal closure was inhibited. Thus, between excision and the end of the experiment, Wilt Pruf-treated leaves lost 10% less and PMA-treated leaves, 60% more water per unit of leaf area than

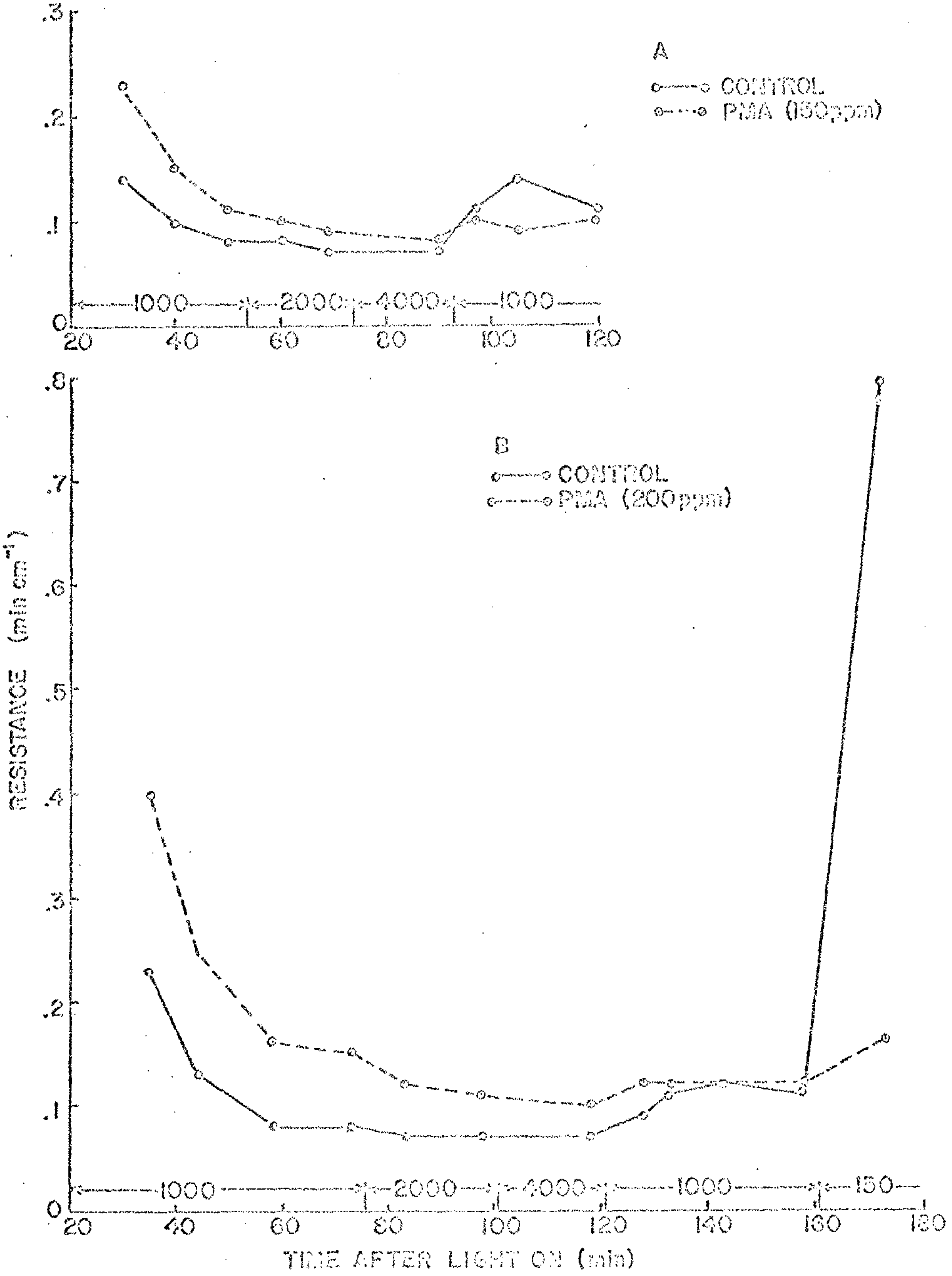
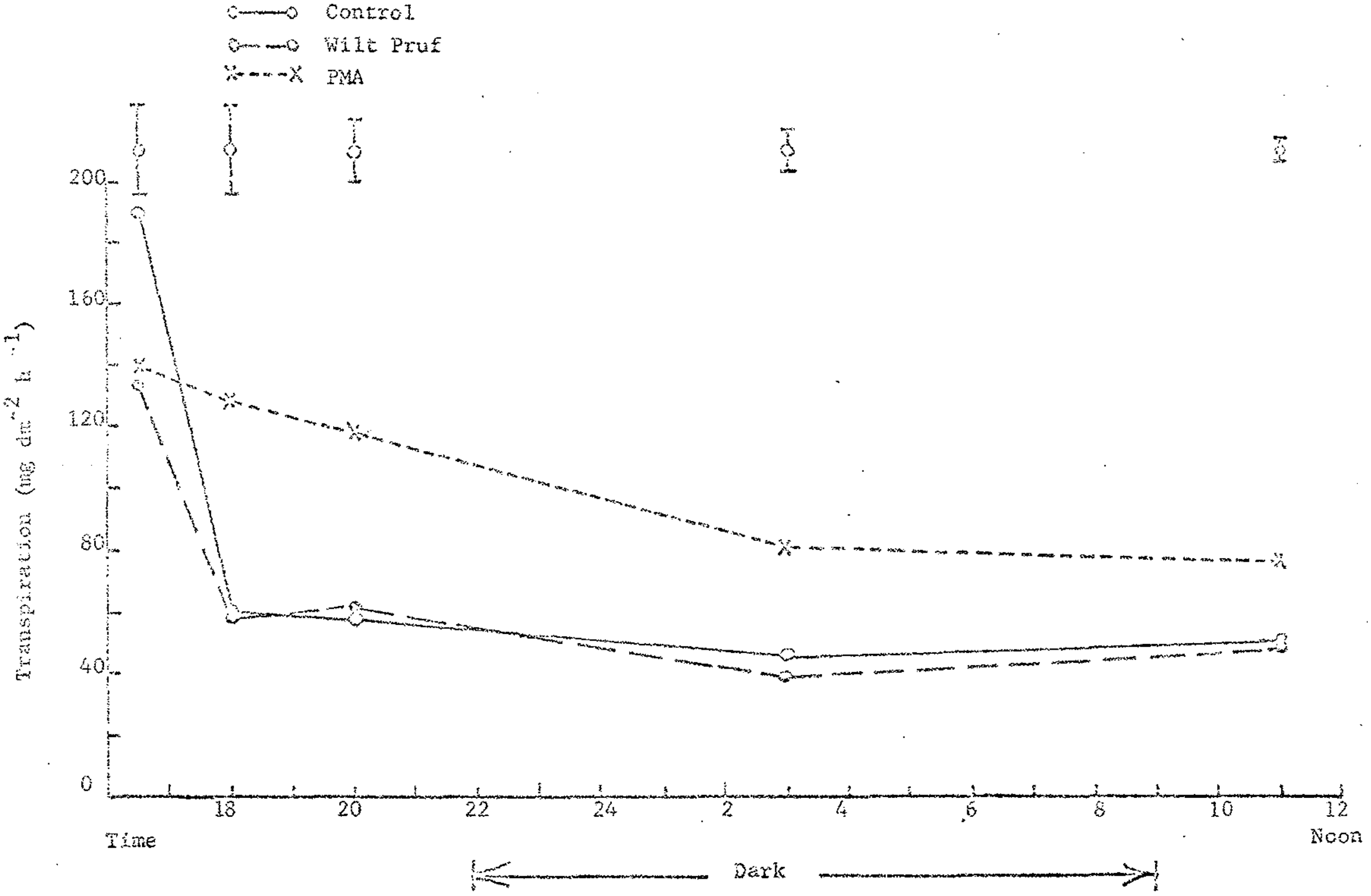


Figure 23: Effects of (A) 150 ppm and (B) 200 ppm PMA on resistance to water vapor diffusion from sugar beet leaves, as influenced by light. (Light intensities, in foot candles, are shown on the abscissa.

Figure 24: Effects of PMA (120 ppm) and Wilt Pruf (1:3) on transpiration rates of excised oleander leaves. (The vertical lines show ± standard errors of means at each observation point.)



did the control leaves. The higher rates of transpiration of the PMA treatment were significant at the 5% probability level. The stomata under the film of Wilt Pruf were apparently as functional as those in the control leaves, so that one hour after excision the film gave no additional conservation of water over that provided by stomatal closure. This also suggests that cuticular transpiration, which the film should curtail, was not large.

Oleander stomata occur in groups of 2 to 3, in sunken pockets, and are exclusively on the lower surfaces of the leaves. To confirm that the faster transpiration from excised leaves observed in the last experiment was due to the effect of PMA on stomata rather than on the cuticle, PMA (120 ppm) was painted on intact leaves in a greenhouse as follows: both lower and upper leaf surfaces treated; only lower surface treated; only upper surface treated; and control, i.e., no treatment. The leaves were excised 1½ hours later, and transpiration rates were determined according to the procedure described in the previous experiment. The experiment confirmed that with excised leaves PMA-treatment results in significantly higher transpiration rates than in controls, but this occurs only if the stomata bearing surface is treated. Thus, leaves which had only their upper surface treated responded essentially the same as control leaves, i.e., there was normal stomatal closure and reduced transpiration, indicating that the effect of PMA was on the stomata rather than on the cuticle. Another interesting observation was that about 10 hours after excision, leaves which had their lower surfaces treated with PMA were visibily drier and began curling because the prevention of stomatal closure resulted in their rapid desiccation. Thus, at the end of the experiment these leaves had lost about 90% more water per unit leaf area than controls. A third experiment with excised oleander leaves confirmed the effect of PMA in retarding stomatal closure, and showed the effect to occur with 200, 150, and 100 ppm PMA, but not with the PMA solvent (distilled water plus 4% ethanol plus 0.1% X-77 surfactant) by itself.

Since PMA increased water loss from excised leaves by preventing complete stomatal closure, the next step was to see if the same effect could be observed with intact oleander plants during the period of normal stomatal closure in the dark. The oleanders were grown in a greenhouse in well-watered one-gallon cans enclosed in plastic bags to prevent evaporation from the soil. Transpiration rates during the day and night were determined from weighings at sunrise and sunset. Before treatment, transpiration rates of all the pots were measured in a uniformity trial so that inherent differences between plants could be minimized by statistical design. The plants were then sprayed with: 1) distilled water + 0.1% X-77 (control); 2) CS-6432 (3%), an experimental film-forming antitranspirant; or 3) PMA (150 ppm). Each treatment was replicated three times. The results of two such trials are summarized in Table 16. In both of the trials the antitranspirants greatly reduced transpiration (by 31-68%) during the day, but at night only the film-forming antitranspirant reduced water loss, and by only a small amount. On the other hand, PMA increased transpiration at night by 22-33%, although that extra water loss at night was very small compared with the water saved during the day. These data provide further evidence that PMA prevents stomata from closing as much as untreated stomata.

The premise that PMA ratards stomatal closure has, so far, been based on transpiration rather than stomatal studies. Since the sunken stomata of oleander leaves are difficult to examine, the infiltration technique using iso-butanol diluted with various amounts of ethylene glycol, was used in an experiment with excised leaves. Periodic measurements made 1-6 hours after cutting the oleander leaves indicated that the stomata of PMA-treated leaves were slightly, though consistently, more open than those of control leaves.

A more direct test of the effects of PMA on stomatal behavior was made using epidermal peels from the lower surfaces of *Vicia faba* leaf discs. Microscopic examination of the peels from the discs after they had been floating with their lower surfaces in contact with solutions of 10^{-4}M KCl or 100 ppm PMA for 3 hours in the light, showed that PMA had reduced stomatal apertures by about 50% (Table 23). All of the discs (including those treated with PMA) were then floated on fresh 10^{-4}M KCl solutions and placed in the dark for 4 hours. At the end of that time, microscopic examination of epidermal peels showed that control stomata had closed appreciably in the dark whereas PMA-treated stomata were nearly twice as open as controls in the dark. Stomatal apertures are continuously changing over limited ranges both in light and dark. Therefore it is probably only fortuitous that PMA-treated leaves showed the same aperture in light and dark (Table 23). In other words, PMA does not 'fix' stomatal apertures.

Table 23

Effect of PMA on stomatal apertures of *Vicia faba* leaf discs after 3 h in light followed by 4 h in dark. (Based on 5 replicates.)

	Stomatal Aperture (microns)	
	After 3 hours in light	After 4 hours in dark
Control	15.2	3.9
PMA (100 ppm)	7.1	7.1
S.E of Means (±)	1.3	1.4

Snap beans (*Phaseolus vulgaris*) were grown in 5" pots, fertilized with Hoagland solution, and put in a growth chamber at 62°F, 68% relative humidity, and 2500 f.c. of light. Only the two primary leaves were used, with all secondary leaf buds nipped out. Plastic bags were placed over the soil surface to eliminate evaporation, and 8 such pots were weighed periodically to determine transpiration ($g\ H_2O/dm^2$ leaf area) in light and dark conditions. After pre-treatment data were obtained on variability in water use per pot, four of the plants were treated with PMA (140 ppm). Table 24 shows the data, expressed as transpiration from PMA relative to that from control.

The uniformity of the plant material prior to treatment is indicated by ratios of transpiration (PMA/Control) near unity, both in light and in the dark. After treatment, PMA caused a 16% reduction in transpiration in the light on day 2, and an 8% reduction in the dark. It was thought that the retarded transpiration by PMA in the dark might be due to high night humidity (80%) in the growth chamber, which might cause the stomata of control leaves to close to a lesser degree. Therefore, on day 3 the night humidity was lowered to 33%. Thereafter, dark transpiration was greater from PMA-treated leaves than from control leaves ($P < 0.01$), but PMA continued to reduce transpiration during the day ($P < 0.01$). Occasional measurements of diffusive resistance to water vapor from the lower surface of the Phaseolus leaves, made with the rate hygrometer, showed that: 1) stomata of both the control and the treated leaves did not close completely in the dark; and 2) at low night humidities, PMA reduced diffusive resistance as much as 60% below that of controls in the dark.

In another experiment with Phaseolus vulgaris, transpiration and diffusive resistance in light and dark conditions were measured more frequently. The techniques used were as in the previous experiment except that transpiration was based on only one pot each for control and PMA (120 ppm). Diffusive resistance was measured on the lower surfaces of the two leaves of each pot. Temperature in the growth chamber was maintained at 63°F, and humidities were 52% during the light period and 35% during the dark period. PMA decreased transpiration markedly in the light and tended to increase it slightly in the dark (Figure 25). On the other hand, PMA tended to increase resistance values in the light and decreased them in the dark. In the light, a large difference between the transpiration curves is associated with a relatively small difference in the resistance curves. Similarly, in the dark, a relatively large difference in the resistance curves is associated with a relatively small difference in the transpiration curves. This suggests a curvilinear relation between transpiration and resistance of the type shown in Figure 14.

Figure 26 gives a more detailed picture of the effect of PMA on leaf resistance to water-vapor diffusion. In these experiments, half of the bean leaf was left untreated, and the upper and lower surfaces of the other half were treated with PMA (120 ppm), the midrib being the boundary between control and treated. Four replicates of such leaves were used. Stomata closed more in control halves than in the PMA-treated halves within the first one-half hour after the onset of darkness. Measurements at 1-2 hour intervals throughout the night showed that PMA continued to prevent complete stomatal closure in the dark ($P< 0.01$), and also prevented complete opening when the lights were switched on.

Table 24

Effects of PMA on transpiration of *Phaseolus vulgaris* in light and dark conditions. (After the second day, the night humidity was lowered from 80% to 33%.)

	Pre-treatment		Post-treatment							
Day	1		2		3		4		5	
Relative humidity(%)	68	80	68	80	68	33	65	33	52	33
Light or Dark	L	D	L	D	L	D	L	D	L	D
Relative transpiration (PMA/Control)	1.01	1.04	.84	.92	.67	1.26	.83	1.30	.83	1.61

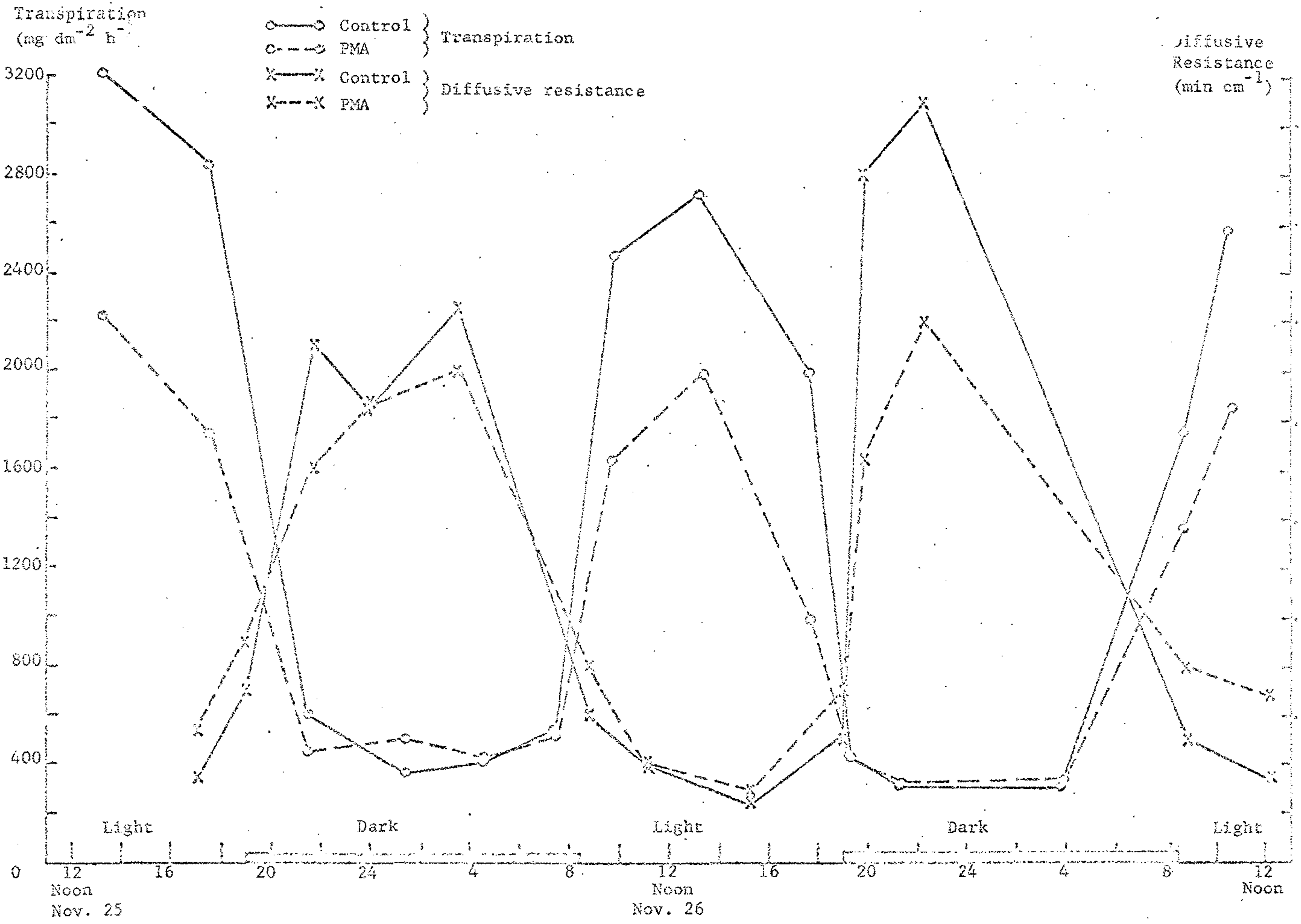


Figure 25: Effects of PMA (120 ppm) on transpiration rates and resistance to water vapor diffusion from leaves of *Phaseolus vulgaris* in light and dark.

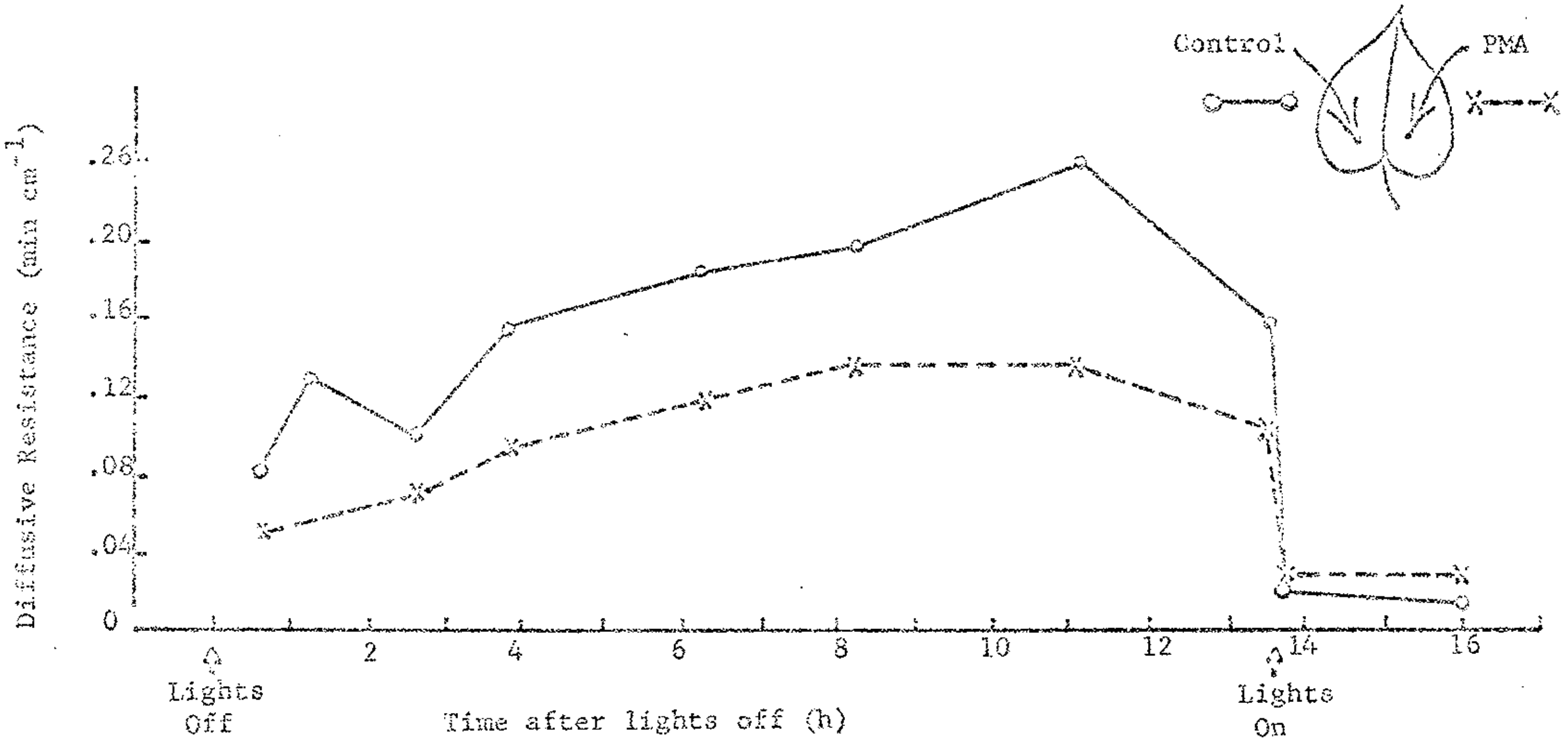


Figure 26: Effects of PMA (120 ppm), applied to one half of *Phaseolus vulgaris* leaves, on resistance to water vapor diffusion in the dark. (The standard error of differences between means for the entire dark period = ± 0.01 min cm^{-1}.)

It is emphasized that prevention of complete stomatal closure in the dark by PMA does not cancel the efficiency of this chemical as an antitranspirant, since under normal conditions day-time savings in transpiration greatly outweigh the increased night-time losses of water, which are usually relatively small. Perhaps the greatest disadvantage of PMA is its heavy metal content and the possibility that repeated sprays would accumulate mercury in plants, the soil, and the ground water system and may eventually result in animal toxicity. If partial stomatal closure occurs naturally during the day in untreated leaves because of reduced light or low plant water potentials (induced by drying soil and/or dry air), PMA may prevent stomata of treated leaves from closing to the same extent. This should result in increased rates of transpiration and photosynthesis. Thus, the smaller reductions in transpiration and photosynthesis by PMA from plants in dry than in wet soil, which have been observed by various workers, may have been caused partly by an improved plant water balance and partly by direct prevention of stomatal closure by PMA.

Effects of alkenylsuccinic acids: Zelitch of the Connecticut Agricultural Experiment Station listed a number of chemical inhibitors of stomatal opening amongst which were certain alkenylsuccinic acids. These materials probably prevent stomatal opening by causing the plasma membranes of the stomatal guard cells to increase in permeability so that they lose turgor. Alkenylsuccinic acids and their derivatives have the basic structure $CH_3 - (CH_2)_n - CH = CH{-}CH_2 - CH(COOH) - CH_2COOH$. Zelitch pointed out that since even partial neutralization of the carboxyl groups to form sodium, calcium, or ammonium salts results in a loss of stomatal closing activity, it is essential to use distilled water for preparing the solutions. He found that the free dodecenylsuccinic acid did not always bring about stomatal closure when used as a spray, whereas the monomomethyl ester of decenylsuccinic acid was reliable in closing stomata and reducing transpiration.

The following alkenylsuccinic acids were sprayed on pots of dichondra (Dichondra repens), and their effects on rates of transpiration and photosynthesis were determined by the methods already described: 1) nonenylsuccinic acid, 2) n-decenylsuccinic acid, 3) dodecenylsuccinic acid, and 4) n-dodecenylsuccinic acid. Each chemical was sprayed, along with the surfactant X-77, at rates of 0 (control), 100, 200, and 400 ppm. In Figure 27 the rates of transpiration and photosynthesis (determined as mg H_2O or CO_2 dm^{-2} h^{-1}) are expressed in relative units, taking control as 100. Nonenylsuccinic acid reduced transpiration and photosynthesis almost proportionately, the greatest reduction being about 30% with 200 ppm. With increasing concentration of n-decenylsuccinic acid, greater reductions in transpiration occurred with a maximum reduction of nearly 50% at 400 ppm. However, paradoxically, there was no decrease in photosynthesis at this concentration. No explanation for this can be offered, particularly since these experiments were unreplicated. However, because of this surprising observation, measurements on this plant were repeated and similar results were obtained. Dodecenylsuccinic acid at 100 ppm gave a 20% reduction in transpiration with no reduction in photosynthesis, whereas the 200 and 400 ppm concentrations reduced both transpiration and photosynthesis. N-dodecenylsuccinic acid appeared to be effective as a transpiration repressant only at 100 ppm, and not at higher concentrations, but photosynthesis was reduced by 10 to 15% at all concentrations.

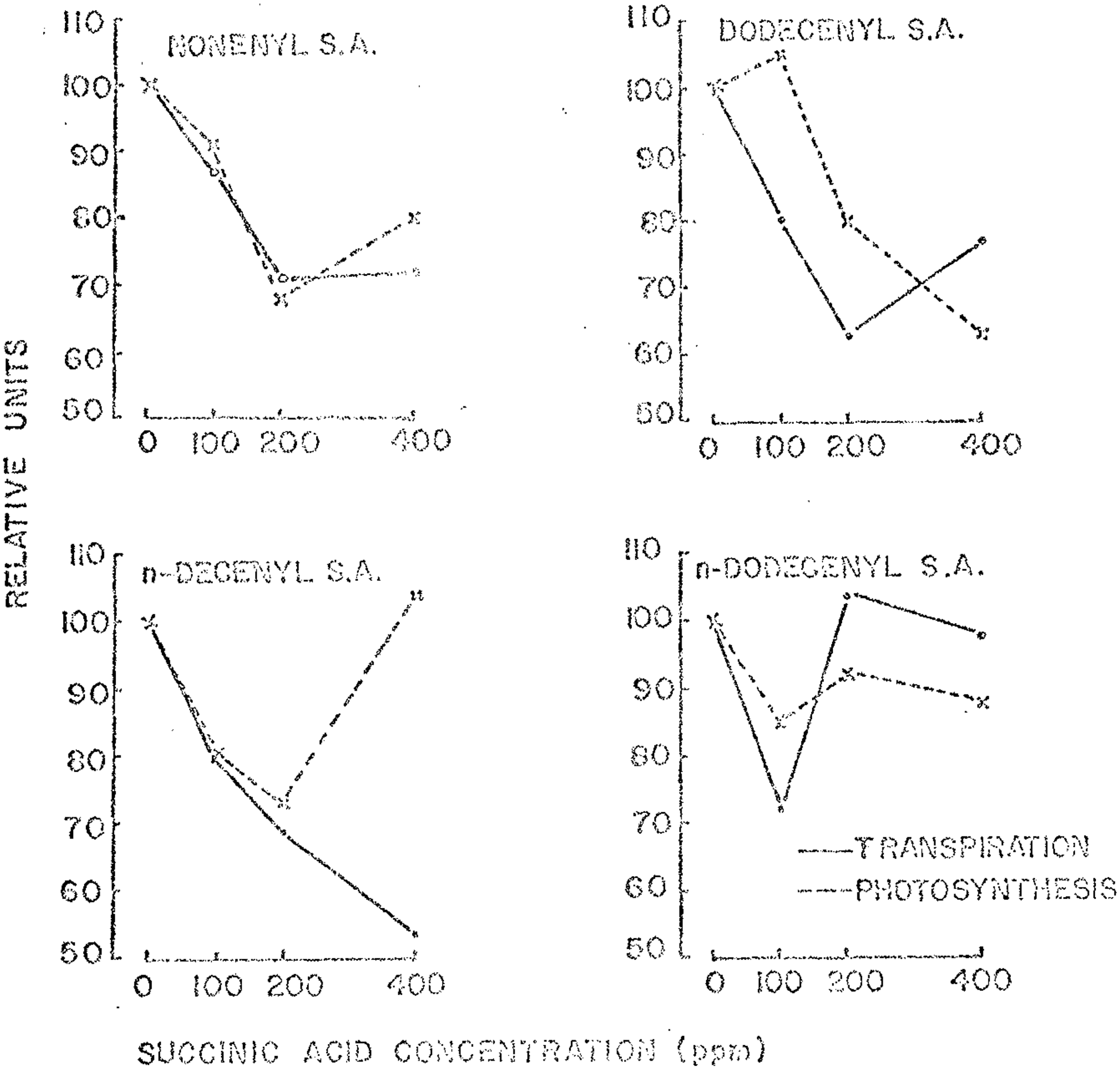


Figure 27: Effects of various concentrations and types of alkenylsuccinic acids on rates of transpiration and photosynthesis of dichondra plants.

The somewhat erratic effects of various concentrations of these alkenylsuccinic acids confirms the observation of Zelitch that not all of these materials are completely reliable, and suggests the need for further investigation with replicated experiments. The indication of greater reductions in transpiration than in photosynthesis for at least some of the data in Figure 27 shows some promise for the use of these materials as stomata closing antitranspirants. From a theoretical point of view, discussed earlier in this report, a greater reduction in transpiration and photosynthesis is to be expected, provided no phytotoxicity (or increase in mesophyll resistance) occurs. No phytotoxicity was, in fact, observed in these trials on dichondra. The requirement of alcohol and distilled water as solvents for alkenylsuccinic acids increases the price and inconvenience in the preparation of large quantities of spray. However, the absence of any heavy metals (unlike PMA) increases the range of usage of these materials, although mammalian toxicity trials have not yet been carried out with alkenylsuccinic acids.

Since stomata closing sprays are effective in extremely dilute concentrations, they may be expected to be less expensive than other antitranspirants if their unit costs do not appreciably exceed those of other types of materials. For example, PMA at 10 cents per gram used at the rate of 15 grams/acre (diluted in 100 gallons of water) would cost only $1.50 per acre. However, as already pointed out, the mercury content in PMA prohibits widespread, and particularly repeated, use of this chemical. PMA therefore serves a more useful purpose as a laboratory research tool in helping to elucidate questions concerning stomata.

Plant Water Potential

Several methods exist for the measurement of plant water potential or some index of it. Ideally, the most accurate method is the best one, but the criterion for selection is usually convenience, particularly for field measurements. In this study an index of plant water potential was obtained by 1) the relative water content method, and 2) the pressure bomb method, both of which were amenable to field experiments. Since most of these data will be presented in the description of the various field experiments in the second part of this report, only a few samples of data will be presented here:

Since the water balance of a plant depends on the relative rates of water uptake and loss, and since an antitranspirant decreases the latter, an improvement in the water balance (and therefore the water potential) of the plant is expected. Thus, after application of an antitranspirant to the foliage of fruit trees, it was found by dendrometer measurements that the day-time shrinkage of the tree trunks (a normal phenomenon resulting from transpiration exceeding water uptake) was reduced by about 50%. Thus, the water potential of the tree as a whole was increased by antitranspirant treatment, although the dendrometers provided only an indirect measure of this.

Relative water content: This measure is also called 'relative turgidity' and is the ratio of the water content of a leaf at the time of measurement, to the maximum water it can hold, expressed as a percentage.

$$RWC = \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100$$

Leaf discs were weighed after sampling, floated in distilled water at about 40 f.c. light for at least 6 h, re-weighed to obtain turgid weight, and finally dry weighted.

The relative water contents of almond leaves from antitranspirant treated trees, sampled in the late afternoon were always 4 to 7% (actual RWC values) greater than those of untreated trees, the effect being noticeable even 2½ months after spraying. Similarly, treatment of oleander (Nerium oleander) plants along the freeway with PMA (110 ppm) or CS-6432 (3%) resulted in increases of 2-3% of RWC values. It should be kept in mind that the effective range of relative water content for most plants is fairly small (60 - 100%) and that a small RWC increase can be equivalent to a relatively large plant water potential increase, depending on plant species.

When assessing the effect of a film antitranspirant on RWC, an error may arise from the technique itself, resulting in an under-estimation of RWC of film-coated leaves. This was found in a recent experiment using Mobileaf (1:5) on oleander leaves when no increase in RWC was observed as a result of the treatment. It was postulated that in the process of floating the discs for determination of turgid weights, water was not only being absorbed by the leaf to fill its deficit, but was also being taken up by the Mobileaf film on treated discs, resulting in higher turgid weights. Examination of the RWC equation above shows that an increased turgid weight (denominator) would result in a decreased value for RWC.

This hypothesis was therefore tested in an experiment with well watered oleander plants growing in pots in a greenhouse. Mobileaf (1:5) was painted on several leaves of a plant, leaving a corresponding leaf at the same node as a control, thereby eliminating variations in relative water content which may be caused by leaf age or position on the plant. Four replicates were used, each replicate weighing consisting of 12 discs. The relative water contents of antitranspirant treated leaves were about 6% (actual RWC values) lower than controls (Table 25). The reason for this can be seen in the second column where the water uptake (difference between turgid weight and fresh weight) was about 7.6 mg higher for the Mobileaf treated discs. It was also postulated that the effect of water uptake by the film would be relatively less important if most of the uptake were to occur by the leaf, i.e., by using a leaf with a large deficit. The same experiment was therefore repeated with stressed oleander plants. In this case relative water content was reduced by 3.3% (actual RWC values) by the antitranspirant, and water uptake was increased 7.6 mg, i.e., by about 17% compared with 77% for the unstressed plants.

Table 25

Influence of an antitranspirant film on relative water content (RWC) values and water uptake (U) by discs of oleander (Nerium oleander) leaves. (U = turgid weight minus fresh weight.)

	Unstressed		Stressed	
	RWC (%)	U(mg/12 discs)	RWC (%)	U(mg/12 discs)
Control	90.8	10.1	59.6	45.7
Mobileaf (1:5)	84.9	17.7	56.3	53.3
Difference	5.9	7.6	3.3	7.6
% increase in U		77%		17%

A decrease in relative water content might also occur if the antitranspirant decreased the fresh weight (numerator in the RWC equation). However, the average fresh weight per disc of Mobileaf treated discs was 0.2 mg greater than that of control discs, so that decreased RWC could not be attributed to any effect of the film on fresh weight. The only other possible explanation for the observed decreases in RWC by antitranspirant treatment, is that they are real, and that the increased water uptake by the treated discs was due to a greater deficit in the leaf, rather than because of water uptake by the film itself. However, since numerous other data indicate that antitranspirants actually increase plant water potentials, this is an unlikely explanation. Furthermore, when a thin layer of Mobileaf was coated on glass coverslips (an inert medium which did not take up water itself), there was a consistent increase in weight, after soaking in distilled water, for those slips which were covered by film, but no change in weight for uncoated slips. This, therefore, substantiates the hypothesis that the film itself takes up water and gives the artifact of decreasing relative water content as a result of antitranspirant application.

Pressure bomb: This method consists of increasing the pressure around the plant tissue, e.g., a leaf, until xylem sap appears at the end of the petiole, which extends outside the chamber and is exposed to atmospheric pressure. The pressure necessary to retain this condition is a function of the water potential of the leaf cells. Most of the data on water potential as measured by the pressure bomb have been collected in field studies, using leaves and fruit of various orchard crops, and these data will be presented in more detail in the second part of this report. The investigations usually involved measurement of resistance to water vapor diffusion from the leaves (using the rate hydrometer) while still attached to the tree, followed immediately by a pressure bomb reading on the same leaf after detaching it from the tree. A sample of such data is shown in Table 26. In each case the film antitranspirant increased the resistance to water vapor diffusion and thereby increased the water potential of the leaf, i.e., values were less negative. It should be kept in mind that the water potential values shown in this table are actually only estimates of the hydrostatic or matric forces in the intact xylem, and do not include the component of solute potential. Furthermore, the relation between pressure bomb values and water potential (measured psychrometrically), varies with plant species. The pressure bomb values presented are therfore only an index of water potential, but the method provides a very convenient means of assessing the effects of antitranspirants on the water status of leaves.

Table 26

Effects of film antitranspirants on resistance to diffusion of water vapor (measured by a rate hygrometer) and on leaf water potential (pressure bomb values) of three orchard crops.

Tree	Treatment	Resistance (min cm^{-1})	Water Potential (atm)
Prune	Control	.03	-9.5
	CS-6432 (1%)	.15	-6.7
Peach	Control	.06	-15.0
	CS-6432 (1%)	.11	-10.4
Apricot	Control	.07	-29.0
	Mobileaf (1:9)	.30	-19.0

SUMMARY OF BASIC INVESTIGATIONS ON ANTITRANSPIRANTS

Before embarking on any extensive field trials with antitranspirants, certain basic information concerning the materials, their application, effects, and interactions with environment are required. Materials are procured from various cooperating Companies after specifying the required properties and effects. Spray application of the antitranspirant is usually the most convenient method, but the amount of foliar coverage by the spray can be affected by environmental factors (e.g., drying conditions), the nature of the leaf surface, and the wetting agent present in the spray. The degree of coverage on a leaf surface was assessed by viewing under black light after incorporating a fluorescent dye. A more refined technique involved observation at the stomatal level with the help of a Scanning Electron Microscope. Complete coverage by a film is not always desirable, but partial coverage may slightly increase water loss from those portions of the leaf not covered by the spray.

In general, antitranspirants reduced both transpiration and photosynthesis rates by increasing the resistances in the water vapor and carbon dioxide pathways. Because of other methods of heat dissipation, the reduction of evaporative cooling by an antitranspirant raised leaf temperatures only slightly. The duration of effectiveness of an antitranspirant was variable depending on plant species, method of assessment, and environmental factors; the effective duration varied from two days to two months. Preliminary trials are always required to determine optimum concentration of antitranspirant for a particular species to obtain the maximum effect with the minimum phytotoxicity. The optimum concentration varies with plant species, environment and the specific purpose for which the antitranspirant is to be used.

Both theoretical and experimental evidence show the various interactions of antitranspirant effects. The materials are likely to be most effective in decreasing transpiration under conditions which normally lead to wide stomatal opening, e.g., high light intensity coupled with factors which raise plant water potentials. High wind speeds decrease boundary layer resistance and therefore increase the effectiveness of an antitranspirant. Plant factors such as stomatal distribution and rooting depth also influence the performance of an antitranspirant.

Antitranspirant effects on stomata were judged indirectly by porometry (mass flow or rate hydgrometer), and directly by microscopic measurement of stomatal apertures from epidermal peels. The relationship between diffusive resistance and stomatal aperture was determined. It was found that a film antitranspirant, by increasing plant water potential, actually increases the aperture of stomata lying immediately under it. Direct stomatal measurements enabled assessment of the effects of the stomatal-closing antitranspirant, phenylmercuric acetate (PMA). It was discovered that PMA not only retards stomatal opening, but also retards closure, thereby increasing transpiration under conditions of low light or low water supply. The effects of various alkenylsuccinic acids (stomatal-closing antitranspirants which do not contain heavy metals) on the rates of transpiration and photosynthesis were measured.

Since the usefulness of an antitranspirant arises not only out of its effect on water conservation but also because of its influence on the water status of the plant, the effects of antitranspirants on the relative water content of leaves and on water potential (as estimated by a pressure bomb) were measured. An antitranspirant by increasing the resistance to water vapor diffusion from leaves also increased the leaf water potential. The numerous possible applications of this latter effect will be reported in Part II on 'Applied Investigations with Antitranspirants'.

RELEVANT LITERATURE

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In addition to the above, we expect to prepare papers for publication on the following subjects dealing with antitranspirant research arising out of this project:

Antitranspirant application and foliar coverage.
Antitranspirant-environment interactions.
Effects of stomata-closing and film-forming antitranspirants on stomatal apertures.
Effects of antitranspirants on plant water potential and fruit growth.
Specialized uses for antitranspirants.

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