Abscisic Acid Levels in Dormant Peach Flower Buds[1]

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Abstract. A growth inhibitor isolated from dormant peach flower buds was identified as abscisic acid (ABA). Peach bud extracts were assayed for relative growth inhibition by the total acid fraction and for growth inhibition of a purified ABA fraction. Inhibition by both the total acid and ABA fractions increased in the fall until about the time of leaf abscission, and inhibition by both fractions decreased near or shortly after the end of the rest period. The inhibition by the total acid fraction decreased relatively more than the ABA fraction, possibly indicating interaction with growth promoting compounds in the total acid fraction. Inhibition increased as buds swelled, after termination of rest.

THE interest in growth inhibitors relative to the rest period of fruit plants has led to the isolation and identification of naringenin (9) and prunin (4, 8) from peach flower buds. Both flavanones inhibit elongation of the wheat coleoptile in the straight growth test (4, 8). Relative concentrations of naringenin have been correlated with the rest period (7, 10) in peach flower buds, but Dennis and Edgerton (6), Corgan (3), and Samish and Lavee (17) were not able to show a close correlation.

We have dissected large samples of peach flower buds (1,000 buds) (unpublished data) and could not detect any flavanone in the floral cup using a colorometric test similar to one described by Walker (7). This test is more sensitive than the assay used by Dennis and Edgerton (6) but the results substantiate their findings that naringenin is either absent, or in extremely low concentrations in the flower portions of the buds. When extracts of these samples were chromatographed on paper with water and assayed using the wheat coleoptile straight growth test, a zone of inhibition occurred at Rf 0.90, far different from Rf's of either naringenin (.13) or prunin (.40).

Work commenced in December, 1967 to assay peach flower buds for relative concentration changes of this growth inhibitor in the flower portions of the buds, and to determine its identity.

MATERIALS AND METHODS

Beginning in December, 1967, 3,000 bud samples of 'Redhaven' peach flower buds were collected at approximately weekly intervals until full bloom. The buds were homogenized 2 minutes in methanol and placed in a cold room at 0–5° C for 24 hours. The homogenate was filtered, the filtrate evaporated, and then partitioned between acetonitrile and hexane. The acetonitrile fraction was evaporated, taken up in warm water, and filtered. The filtrate was adjusted to pH 3.5 with sulfuric acid and extracted 3 times with equal volumes of diethyl ether. The ether fraction was extracted 3 times with 5% sodium bicarbonate solution. The sodium bicarbonate portions were combined, adjusted to pH 8.0, and extracted 3 times with ether to remove naringenin and other weak acids. The aqueous fraction was then adjusted to pH 3.5 and extracted with ether. The ether fraction

was evaporated and the residue streaked on a 72 cm × 90 cm Whatman #1 chromatography paper. The paper was developed in water and the portions Rf 0–0.6 were discarded. The portions Rf 0.6–1.0, free of prunin and naringenin, were eluted with methanol and the eluate made up to a volume of 10 cc in methanol. This eluate was designated the "total acid fraction."

Aliquots of the extract were evaporated in culture tubes and assayed for growth inhibition by the wheat coleoptile straight growth test (15). The wheat cultivar 'Tascosa' was used for all the assay work at Las Cruces, New Mexico. The quantity of extract required for 50% inhibition was determined from a plot of percent inhibition against volume of extract assayed for each sample date. The reciprocal of this value was plotted as relative growth inhibition (Figs. 3 and 4).

The bud collection and assay procedures in 1968–69 were the same as described above, but the extraction procedures were similar to those described by Milborrow (14) for abscisic acid (ABA) extraction. The buds were homogenized in 80% methanol and were extracted for 10 days with 3 changes of solvent. The filtrate was evaporated to the aqueous phase. Water insoluble material was removed by centrifugation. The supernatant was adjusted to pH 3.5 with sulfuric acid and extracted 3 times with one-tenth its volume of ether. The ether fraction was extracted 4 times with small amounts of sodium bicarbonate solution and water alternately to give a final volume approximately 15% of the ether. The combined aqueous fraction was adjusted to pH 7.5 and partitioned once with an equal volume of ether. The aqueous fraction was then adjusted to pH 3.0 and extracted 4 times with ether.

The residue (after centrifugation above) was triturated with saturated sodium bicarbonate solution and filtered. The filtrate was adjusted to pH 3.5 with sulfuric acid and partitioned 3 times with ether. This ether fraction was added to the ether fraction above and partitioned alternately 4 times with saturated sodium bicarbonate solution and water. The sodium bicarbonate phase was adjusted to pH 7.5 and partitioned once with an equal volume of ether. The aqueous phase was adjusted to pH 3.5 and partitioned 3 times with ether. The ether was evaporated and the residue chromatographed on paper with water as described above to remove naringenin and prunin. Extracts were assayed for growth inhibition as described above.

Portions of the 1967–68 extracts left over from the dilution assays were combined. Aliquots were chromatographed on paper with 5 solvents and the Rf's of the inhibiting zones compared to Rf's of ABA (Shell Dev. Corp.).

The 1968–69 extracts were taken to the University of California at Davis in August, 1969. In the laboratory of G. C. Martin, Department of Pomology, a composite sample was made up from the portions of each extract. This composite sample was chromatographed on silica gel GF254 thin layer plates with a known sample of ABA (Reynolds Tobacco Co.) in n-butanol; n-propanol; .88 N ammonia: water (2:6:1:2 V/V). A small portion of the zone with Rf corresponding to ABA was assayed for growth inhibition. The remainder of the zone was eluted with methanol and rechromatographed with benzene: acetic acid: water (8:3:5) (BAW). The active zone again was eluted with methanol and rechromatographed with benzene: ethyl acetate: formic acid (80:20:5). The active zone (Rf 0.40) was eluted with methanol and evaporated. The residue was dissolved in ether, filtered, and the ether evaporated. This residue was utilized for determination of UV spectrum in comparison with a known sample of ABA. Both the ABA standard and a blank for reference were prepared from thin layer plates developed in the last solvent and eluted the same as the inhibitor.

The samples by collection date were also assayed for relative ABA activity at Davis after thin layer chromatography in BAW. BAW was selected because MacMillan's data (13) on the Rf's of gibberellins indicated that it would probably separate ABA from gibberellins 1 through 9. Duplicate 10 μl samples (representing 3 buds) were spotted on silica gel G plates. Plates were developed 2 times in BAW for a distance of 10 cm above the origin. They were steamed 30 minutes to remove the acetic acid, then 1 cm² portions of the plates were scraped and assayed for inhibitor activity. The percent inhibition of wheat coleoptiles by the ABA fraction (Rf 0.05–0.15) was plotted. Wheat cultivar 'Genesee' was used for all assays at Davis.

In mid-January, 1968, peach twigs were treated with ABA as follows to determine whether ABA would delay bud development: plastic electrician's tape (¾″ wide) was placed around upright twigs so as to create a small reservoir which would retain liquid in contact with the stem for at least 24 hours. A solution of 10 ppm ABA (Shell Dev. Corp.) was placed in these cups and the bark was then wounded with a needle to facilitate uptake. Twenty such twigs were treated on 2 trees and bud development and flowering were noted in comparison to untreated check trees and also untreated twigs on the same trees.

The end of rest was determined as the date on which twigs brought into the greenhouse opened 50% of their flowers in 3 weeks.

RESULTS

Identification of Inhibitor. The Rf's of the inhibitor and ABA on paper in 5 solvents were similar. Also, with thin layer chromatography, in purification of the sample for UV analysis, the Rf's corresponding to ABA in each of the 3 solvents caused strong inhibition of wheat coleoptiles. These data, plus the similarity in UV absorption characteristics in both acidic and basic ethanol (Fig. 1), are good evidence that the inhibitor is indeed ABA.

Relative Concentration of Inhibitor. Assays of thin layer chromatograms of the acid fraction by date in 1968–69 are presented in Fig. 2. The origin was not included in the assay because of the possibility of gibberellins in this fraction. The plate was divided into 10 equal sections from 0.5 cm above the origin to the solvent front. This procedure resulted in the concentration of the ABA in
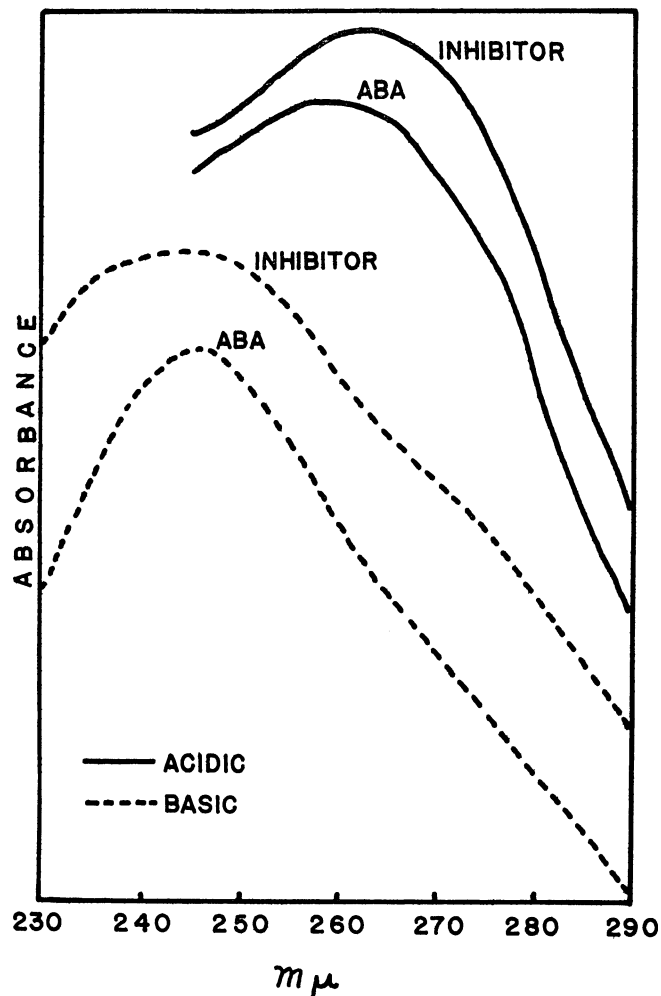
Fig. 1. Ultra violet absorption spectra of peach flower bud inhibitor and known ABA in .005 N ethanolic H_2SO_4 (solid line) and .05 N ethanolic ammonia (broken line).

the first fraction (Rf 0.1) and that is the only fraction which consistently caused coleoptile inhibition (Fig. 2). Assays of the ABA fractions (Rf 0.05–0.15 BAW on silica gel G) were repeated in quadruplicate and the averages by date presented in Fig. 4. The values for percent coleoptile growth inhibition in Fig. 4 represent the inhibition by extracts of 3 peach flower buds in 1 cc of growth medium.

Relative growth inhibition, as determined by dilution assay of the total acid fraction, increased in the fall (Fig. 4) until about the time of leaf fall. The January, 1968 sample (Fig. 3) caused complete inhibition at all dilutions tested. Near the end of rest, inhibition decreased sharply in both 1968 and 1969. In 1968, extracts of February 5, 12, and 19 caused slight growth promotion of wheat coleoptiles. As the buds expanded after rest, inhibition increased in 1968, but remained about constant in 1969 (Fig. 4). The November 20, 1968 sample (Fig. 4) has an extremely high level of inhibition, but it was later determined that this sample contained a significant quantity of prunin which had not been eliminated in the extraction procedures. Therefore, data for November 20, 1960 are not presented. Relative concentrations between the 2 lines in Fig. 4 are not directly comparable because different cultivars of wheat were used in the 2 groups of assays. At the date of greatest relative growth inhibition (November 6) an aliquot rep-

resenting 30 buds in 1 cc of growth medium was required for 50% growth inhibition.

The ABA activity (Rf .05–.15 BAW) also increased during the fall of 1968 (Fig. 4) and, with the exception of one sampling date (October 24), corresponded closely to inhibition of the total acid fraction. After November 6, percent coleoptile inhibition (Fig. 4) fluctuated, with no particular trend for several weeks. ABA did not disappear with the termination of rest. The trend was perhaps downward for a few weeks before and after rest
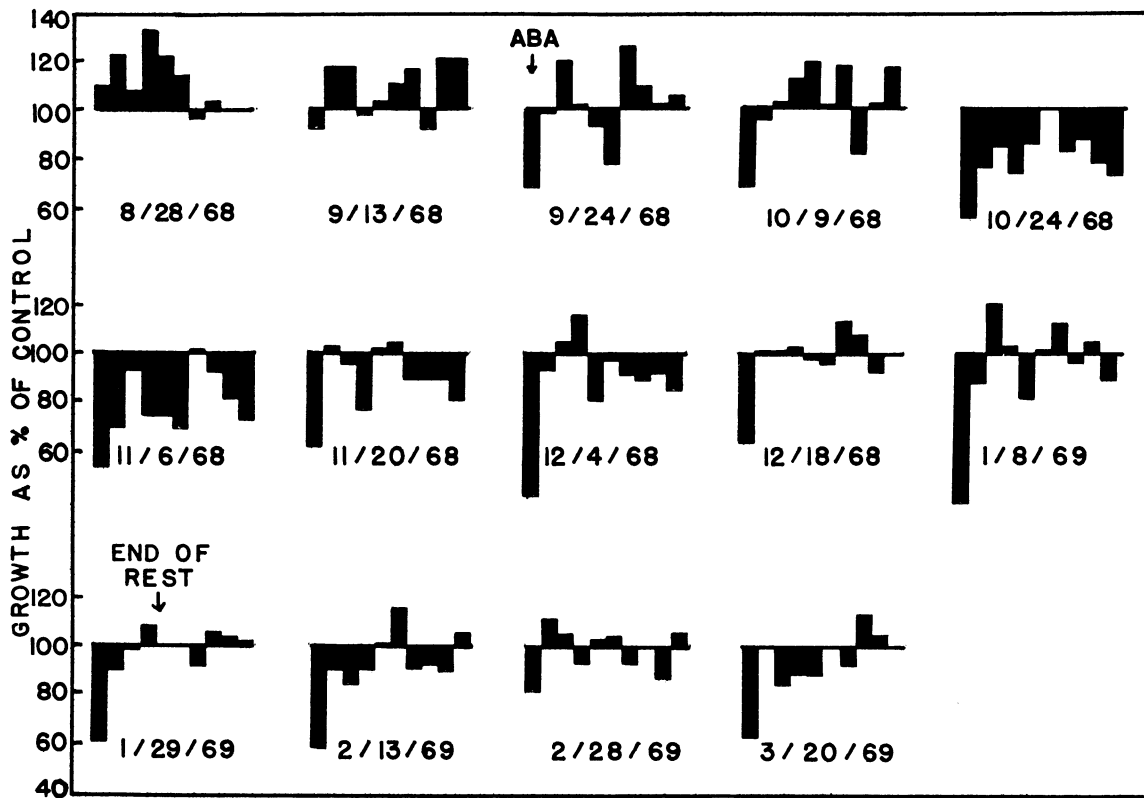

Fig. 2. Wheat coleoptile assays of peach bud extracts by date, chromatographed on thin layer silica gel G in benzene:acetic acid:water (8:3:5). Rf values are shown from 0.1 to 1.0, left to right for each date.
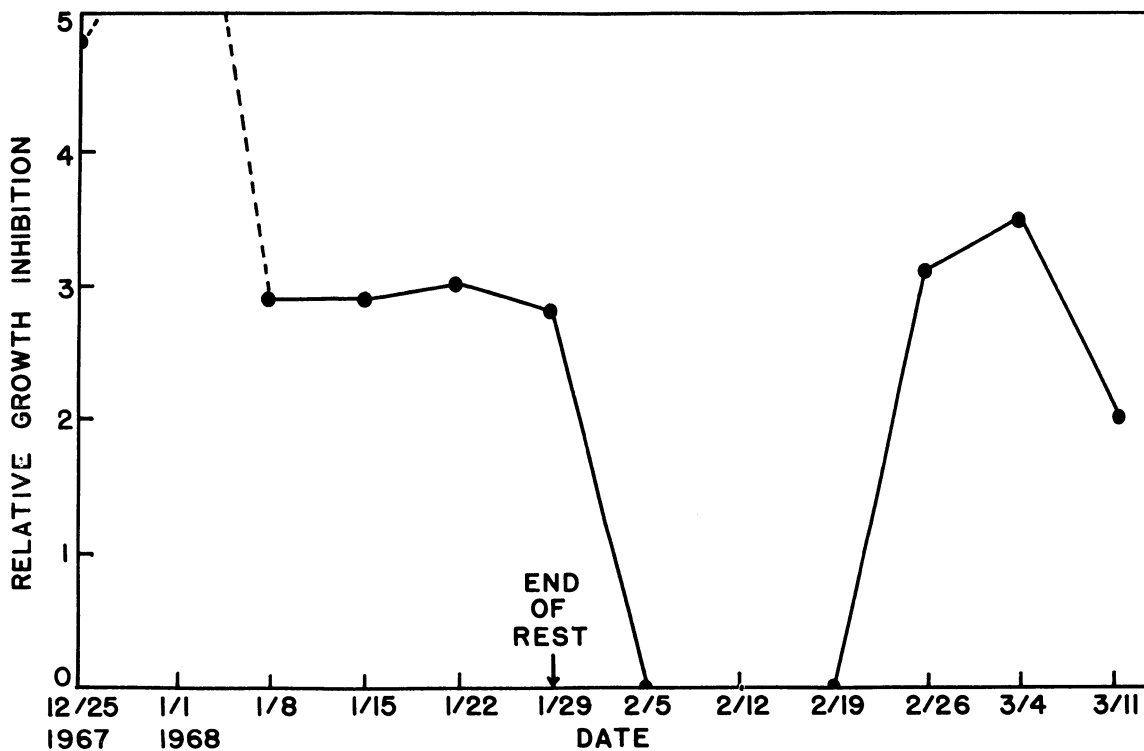

Fig. 3. Relative growth inhibition of wheat coleoptiles by the total acid fraction of bud extracts, 1967–68.
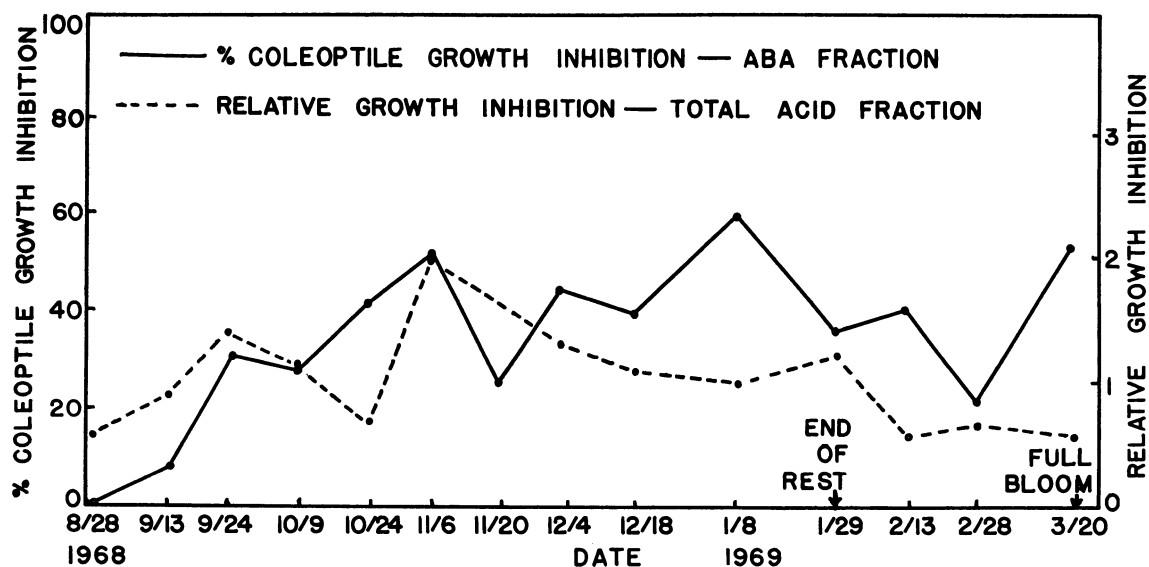
Fig. 4. Relative growth inhibition of wheat coleoptiles by the total acid fraction of peach flower bud extracts (broken line) and percent coleoptile inhibition by the ABA fraction (Rf .05–.15–benzene:acetic acid:water 8:3:5), 1968–69.

was ended, but the change was not dramatic, and ABA activity was again high after rest was over and the buds became most active. These data are based on relative ABA changes per bud. As buds expanded after rest was terminated, the quantities of tissue increased. Although the data indicate an increase in ABA after rest (Fig. 4) the percentage of ABA on a fresh or dry weight basis could be relatively low, compared to values earlier in the dormant season. The inhibition represented in Fig. 3 and by the broken line in Fig. 4 is an expression of the net activity of the acid fraction, and the chances of interaction with growth promoters are much greater than for values represented by the solid line in Fig. 4, which represents measurements after purification.

Applications of ABA to peach twigs had no effect on date of flower opening or growth of vegetative buds. A few buds enclosed in the plastic cups were soaked in 10 ppm ABA for 24 hrs., but still opened normally with the checks.

DISCUSSION

ABA has been isolated from many plants (1, 12, 14, 17, 20) including peach seeds (11), so its presence in peach flower buds is not surprising. Although the data presented are not rigid chemical proof that the inhibitor is ABA, the many reports to date indicate that ABA may occur in most, if not all, higher plants.

Evidence is accumulating that ABA has an important function in plant growth regulation. Wareing (20), Lipe and Crane (11), Martin (12), and others have found that levels of ABA correlate with the rest period of buds and seeds of woody plants. Lipe and Crane (11) were able to induce conditions indicative of rest in peach seedlings with externally applied ABA. Data presented here are good correlative evidence that ABA accumulation in fruit buds in the fall could function in the onset of rest. Accumulation in peach flower buds during the fall until about the time of leaf abscission is suggestive of inhibitor synthesis in the leaves during short days, as suggested by Phillips and Wareing (16), and translocation into the buds, where it accumulates. If ABA accumulation during the fall is dependent on leaf synthesis, then trees which retain their leaves would have potential for greater ABA accumulation and perhaps an extended rest period. This

could explain why peach trees which retain their leaves late in the fall as a result of late N applications, GA treatment (5), or other factors (2), tend toward late bloom and delayed foliation the following spring. At present, there is little evidence to substantiate this, other than the data presented here on fall ABA accumulation in the buds, and observations made correlating late leaf fall with late bloom (2).

The emergence of peach buds from rest was closely correlated with a decrease in coleoptile inhibition by the total acid fraction in both 1968 and 1969. A portion of this decrease resulted from lowered levels of ABA, but this acid fraction also would contain any auxins or gibberellins present in the buds. The fact that growth promotion was obtained in the coleoptile assay on 3 dates after termination of rest in 1968 and that inhibition by the total acid fraction decreased relative to the ABA fraction in the late dormant season of 1969 indicate a possible interaction with growth promoting compounds. The modification of the ABA inhibition in 1969 could be a result of either auxin or gibberellin. Although gibberellin does not promote growth in the wheat coleoptile assay, it is known to reverse the growth inhibition caused by ABA (19). Ramsey[4] has shown an increase in gibberellin content of apricot buds just before the end of the rest period, but little if any decrease in the quantity of ABA. It appears that while a decrease in ABA may be one factor in terminating the rest period of peach flower buds, synthesis of growth promoting compounds which can overcome the inhibiting effects of ABA may be equally important.

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Phenylmercuric Acetate Effects on Water Loss of the Tomato[1]

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Abstract. One of paired tomato plants was sprayed with 100 ppm phenylmurcuric acetate (PMA). Transpiration rates were measured gravimetrically. During the initial daylight periods, PMA treatment reduced water losses in 2 tests. Conversely, night water losses were higher for the PMA treated plants in both tests. When moisture stress symptoms occurred, water losses by the treated plant were higher. The results indicate that PMA closes the stomates at some small aperature. This reduces transpiration when plants are not stressed for water. Relative increased water losses occur, however, when untreated plant losses would be minimal (dark, wilted).

RECENTLY, much research has centered on the chemical reduction of plant stomate aperature and resultant reduction in transpiration. The ability of phenylmercuric acetate (PMA) to reduce transpiration has been clearly demonstrated. Zelitch and Waggoner (8) report that PMA closed stomates of tobacco and corn. Slayter and Bierhuizen (3) reported that PMA in concentrations of 10^{-4} and 10^{-5}M caused proportionately greater reduction in transpiration than photosynthesis of cotton leaves. Hence, water-use efficiency, expressed as the transpiration ratio (g of water transpired/g of carbohydrates produced), was improved.

The results of Gale (1) and Slayter and Bierhuizen (3) show that transpiration was reduced by film-type plastic antitranspirants. However, Gale (1) noted that under conditions which lead to a high degree of plant moisture stress (hot, dry, sunny weather), the film-type anti-transpirants caused increased transpiration. This increase was thought to be due to delayed stomatal closure of the treated plants. Under field conditions, Gale showed an increase in water-use efficiency with film-type antitranspirants.

Use of PMA could result in improved use of existing water supplies in agriculture. Zelitch (6) described a delay in wilting of PMA-treated plants and stated that

"the longer time that the leaf hydration in the treated plants remained above the controls indicated the benefit that might be derived from the closure of stomates prior to a drought."

Zelitch (7) stated that if an applied substance works specifically to reduce the stomate aperature, transpiration rate will be inhibited to a greater degree than the photosynthetic rate. Also, that PMA is the most widely used material for closing stomates; that the probable mechanism of closure is via the formation of mercaptides with the sulfhydryl groups of proteins and membranes; and that PMA probably will not be translocated from a treated leaf or to newly formed ones.

There is a paucity of information on the effect of PMA on horticultural plants cultured in normal circumstances. Granger and Edgerton (2) observed the closure of stomata and injury to apple leaves at concentrations of PMA from 300 to 1000 ppm. Results obtained on PMA effects in a preliminary study on field grown tomatoes in New Mexico indicated that the PMA-treated plants were under a greater moisture stress; leaflets curled upward, exposing the developing fruit; and vegetative growth appeared to be substantially reduced. Much of the exposed fruit (both green and ripe) scalded in the desert sun.

From these results a further study was suggested to determine the effect of PMA on water losses. This paper summarizes results from a greenhouse study conducted

774

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