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Correlation between Loss of Turgor and Accumulation of Abscisic Acid in Detached Leaves

Margaret Pierce and Klaus Raschke*
MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824, USA

Abstract. Mature leaves of *Phaseolus vulgaris* L. (red kidney bean), *Xanthium strumarium* L. (cocklebur), and *Gossypium hirsutum* L. (cotton) were used to study accumulation of abscisic acid (ABA) during water stress. The water status of individual, detached leaves was monitored while the leaves slowly wilted, and samples were cut from the leaves as they lost water. The leaf sections were incubated at their respective water contents to allow ABA to build up or not. At least 8 h were required for a new steady-state level of ABA to be established. The samples from any one leaf covered a range of known water potentials (ψ), osmotic pressures (π), and turgor pressures (p). The π and p values were calculated from "pressure-volume curves", using a pressure bomb to measure the water potentials. Decreasing water potential had little effect on ABA levels in leaves at high turgor. Sensitivity of the production of ABA to changes in ψ progressively increased as turgor approached zero. At $p=1$ bar, ABA content averaged 4 times the level found in fully turgid samples. Below $p=1$ bar, ABA content increased sharply to as much as 40 times the level found in unstressed samples. ABA levels rose steeply at different water potentials for different leaves, according to the ψ at which turgor became zero. These differences were caused by the different osmotic pressures of the leaves that were used; ψ must equal $-\pi$ for turgor to be zero. Leaves vary in π, not only among species, but also between plants of one and the same species depending on the growing conditions. A difference of 6 bars (calculated at $\psi=0$) was found between the osmotic pressures of leaves from two groups of *G. hirsutum* plants; one group had previously experienced periodic water stress, and the other group had never been stressed. When individual leaves were subsequently wilted, the leaves from stress-conditioned plants required a lower water potential in order to accumulate ABA than did leaves from previously unstressed plants. On the basis of these results we suggest that turgor is the critical parameter of plant water relations which controls ABA production in water-stressed leaves.

Key words: Abscisic acid – *Gossypium* – Leaves (water relations) – Osmotic adjustment – *Phaseolus* – Turgor – Water stress – *Xanthium*.

Introduction

Abscisic acid (ABA) accumulates in leaves in response to water stress (Wright and Hiron, 1969; for reviews, see Hsiao, 1973; Milborrow, 1974). In order to understand how water stress causes ABA levels to increase it is necessary to characterize the response as a function of the parameters of leaf water status, notably water potential (ψ) and osmotic (π) and turgor (p) pressures[1]. Previous studies have, for the most part, dealt with the relationship between ABA content and leaf water potential. Zabadal (1974) studied accumulation of ABA in leaves of *Ambrosia* plants which had been placed in a desiccating environment. As the plants depleted the soil of water, ABA content increased only after leaf water potential declined to -10 to -12 bars. Zabadal introduced, therefore, the concept of a threshold water potential for the accumulation of ABA in water-stressed leaves. Similar results were obtained by Hemphill and Tukey (1975) for leaves of *Euonymus alatus* and by Blake and Ferrell (1977) for needles of Douglas-fir seedlings. ABA content increased steadily after ψ reached -8 to

* *Present address:* Pflanzenphysiologisches Institut, Universität Göttingen, Untere Karspüle 2, D-3400 Göttingen, Federal Republic of Germany

Abbreviations: ABA = abscisic acid; me-ABA = abscisic-acid methyl ester; ψ = leaf water potential; π = osmotic pressure; p = volume-averaged turgor; ε = volumetric modulus of elasticity

[1] π is a positive quantity in our notation, and, hence, $\psi = p - \pi$.

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-10 bars in leaves of maize and sorghum (Beardsell and Cohen, 1975). Wright (1977) used detached leaves in order to allow the same amount of time for production of ABA at each different ψ. He found that the ABA content of wheat leaves progressively increased with decreasing ψ.

The response curve which Wright found for wheat leaves became very steep below -9.3 bars, the water potential of plants showing early wilting symptoms. Beardsell and Cohen (1975) suggested that loss of turgor might correspond to the critical ψ at which ABA levels begin to increase. Davies and Lakso (1978) found in apple seedlings that declining turgor was better correlated with increasing ABA content than was declining ψ. How leaf turgor was related to the pattern of accumulation of ABA during water stress became an intriguing question.

Other work made us think that zero turgor might be important for ABA production. In 1974 Turner provided evidence that threshold ψ's which had been observed for stomatal closure in a variety of species were all associated with turgor pressures close to zero. ABA has been strongly implicated in causing stomatal closure during water stress (Little and Eidt, 1968; Mittelheuser and Van Steveninck, 1969; Wright and Hiron, 1969; Hiron and Wright, 1973), so one would expect stomatal closure to indicate that ABA was accumulating.

The following experiments were designed to test the prediction that species should differ in the leaf water potential required for accumulation of ABA, according to the water potential required for elimination of turgor. Excised leaves of *Phaseolus vulgaris*, *Xanthium strumarium*, and *Gossypium hirsutum* were used to study the relationship between ψ, π, p, and abscisic acid content. We also wished to find how gradual or abrupt was the onset of accumulation of ABA in the approach to zero turgor. Uncertainty because of variation between leaves could be eliminated by cutting a series of samples from individual leaves as they lost water. Monitoring the same leaves for decreasing ψ and fresh weight permitted calculation of π and p from "pressure-volume curves" (Scholander et al., 1965; Tyree and Hammel, 1972; Talbot et al., 1975). A preliminary report of this work was presented at the annual meeting of the American Society of Plant Physiologists (Pierce and Raschke, 1978).

Materials and Methods

Plants

Plants of *Xanthium strumarium* L. (cocklebur) (from a strain collected near Chicago, Ill., USA and propagated in California and later in Michigan) and plants of *Phaseolus vulgaris* L. (red kidney bean), cv. Mecosta (seeds from Foundation Seed Co., East Lansing) were grown in a potting mixture in a greenhouse. Air temperature maxima were between 23 and 29° C, and the relative humidity was generally 70–80%. The *X. strumarium* plants were kept pruned to the top five or six leaves and did not flower under the long-day conditions provided by extending the natural light period to 20 h/day by Sylvania (Danvers, Mass.) Gro-lux fluorescent lamps giving an energy fluence of about 0.3 W m^{-2} at plant level. Fully developed leaves from *X. strumarium* were taken for the experiments when the plants were 10–14 weeks old. Plants of *P. vulgaris* were also cultivated in a growth chamber, which had a 16-h photoperiod at 85 W m^{-2} of light from General Electric (Cleveland, OH, USA) coolwhite fluorescent lamps. Day temperature was 27° C; night temperature was 21°; relative humidity was 80%. The terminal leaflets of fully developed *P. vulgaris* leaves were used for the experiments when the plants were 4.5–6 weeks wold. Both *X. strumarium* and *P. vulgaris* plants were kept well-supplied with water.

Plants of *Gossypium hirsutum* L. (cotton), cv. Acala SJ-1 (seeds from C.A. Beasley, University of California, Riverside) were cultivated in two groups under different conditions. For 7 weeks both groups were exposed to a 13.5-h photoperiod, composed of 12 h of 60 W m^{-2} of light from General Electric cool-white fluorescent lamps plus 1.5 h of light from incandescent lamps only (4.5 W m^{-2}). Day temperature was 32° C; night temperature was 22°; relative humidity was 85%. In this growth chamber the plants were watered daily. After 7 weeks one group was transferred to another growth chamber where the plants were exposed to a 13.5-h photoperiod with a peak irradiance of 230 W m^{-2} of light from General Electric lamps H 400 DX 33-1 (mercury vapor) and LU 400 (high-temperature sodium vapor). Day temperature was 27° C; night temperature was 21°; relative humidity was 67%. Water was withheld from these plants until the mature leaves were visibly wilted. After 8 stress/recovery cycles of 2–3 days duration each the plants were watered regularly for 3 days before an experiment was performed. The 10th or 11th oldest leaves were used from plants 10 weeks old in both the unstressed and stress-conditioned groups of *G. hirsutum*. All light measurements were made with an Eppley (Newport, R.I., USA) pyranometer behind a Corning (Corning, N.Y., USA) No. 4600 infrared-absorbing glass filter.

Extraction and Purification of Abscisic Acid

Samples were cut from leaves with a razor blade to have an area between 4 and 8 cm^2. The samples were frozen and lyophilized. They ranged in dry weight from 10 to 50 mg. For these samples the extraction procedure of Zeevaart (1974) was simplified. Each freeze-dried sample was homogenized at room temperature in 15 ml methanol. The methanol extract was separated from the debris by vacuum filtration, and the debris was re-extracted by shaking overnight in another 15 ml of methanol. The second methanol fraction was combined with the first. Tritiated ABA (15,000 dpm) was added to the methanol extract for monitoring recovery. In some experiments chlorophyll concentration was determined in the methanol solution according to Holden (1965). Five ml H_2O were added to the methanol extract, and the methanol was evaporated under reduced pressure. Material insoluble in H_2O was removed by filtration through a Millipore (Bedford, Mass., USA) AP prefilter. The aqueous phase was acidified to pH 2.5 with HCl and extracted 3 times with equal volumes of ethyl acetate. ABA in the ethyl acetate extract was further purified by thin-layer chromatography and methylated as described by Zeevaart (1977). Recovery of ABA from the original methanol extract averaged 70–75%.

The amounts of me-ABA in the samples were determined with a Varian (Palo Alto, Cal., USA) 3700 gas chromatograph equipped with a ^{63}Ni electron capture detector. A 1.8-m glass column, 2 mm internal diameter, packed with 3% SE-30 on 80/100 Supelcoport

(Supelco, Bellefonte, Pa., USA) was used. Carrier gas was N_2, flowing at a rate of 20 ml min^{-1}. The column oven temperature was 200° C; the detector temperature was 300° C. Measurement of ABA by gas-liquid chromatography was not difficult even in the leaf sample which contained the least amount of ABA (2.7 ng). In that case, when one fiftieth of the sample was injected, the detector response at the retention time for me-ABA produced a peak 30 mm high and well-resolved from any other peaks. Calibration curves were prepared by injecting known amounts of standards. The concentration of (±)-me-ABA in a stock solution was measured by spectrophotometry in methanol using an extinction coefficient of 20,900 $M^{-1}cm^{-1}$ at $\lambda_{max}=265$ nm (Milborrow and Robinson, 1973).

The [^{3}H]ABA, specific activity 22.5 Ci/mmol, was purchased from Amersham Corporation, Arlington Heights, Ill., USA, prepared according to Walton et al. (1977). The starting material for the synthesis of [^{3}H]ABA, 1-hydroxy-4-keto-α-ionone, was a gift from R.J. Reynolds Tobacco Co., Winston-Salem, N.C., USA.

Methods of Measuring Leaf Water Status

(I) Pressure-Bomb Method. The pressure-bomb technique developed by Dixon (1914) and by Scholander et al. (1965) was used to determine leaf water potential. The chamber (PMS Instrument Co., Corvallis, Ore., USA; models 600L and 1000) was lined with moist filter paper to reduce water loss from the leaf during measurements of ψ. Chamber pressure was increased by adding compressed nitrogen at a rate approximately 0.05 bar s^{-1} until xylem sap appeared at the cut surface of the petiole. At this point the chamber pressure is called the "balancing pressure" (p_{bomb}); $-p_{bomb}=\psi+\pi_{xylem}$. The osmotic pressure of the xylem sap was determined to be negligible for the leaves used in our experiments: all tests of turgid and wilted leaves gave π_{xylem} readings of less than 0.5 bars in a dew point hygrometer (Wescor, Logan, U., USA; model HR-33(T) Dew Point Microvoltmeter with a C-52 sample chamber) used as a psychrometer. Therefore, balancing pressures were taken as measurements of leaf water potential.

Leaf osmotic and turgor pressures were determined from pressure-volume curves (Scholander et al., 1965). In our case, these were prepared by plotting inverse balancing pressures ($=-1/\psi$) versus decreasing leaf fresh weight (Talbot et al., 1975) as in Fig. 1. The theory relating balancing pressures as a function of tissue volume has been developed by Tyree and Hammel (1972). The reciprocal bulk leaf osmotic pressure can be read from the linear portion of the curve or its extrapolation to the ordinate. Leaf turgor can be derived from the curve as the difference between osmotic and balancing pressures.

(II) Dew-Point Method. The theoretical basis of the Wescor dew-point microvoltmeter for measurements of ψ has been described by Campbell et al. (1973). Samples consisted of 1-cm leaf discs. Repeating the readings after freezing and thawing the leaf discs 3 times provided measurements of osmotic pressure.

Preparation of Samples of Known ψ, π, and p

Plants were watered and placed in a dark cabinet in the evening before an experiment was performed. A mature leaf was cut under water and allowed to take up water for about 1 h still in darkness. Highly turgid *G. hirsutum* leaves were obtained by applying 0.5–1 bar positive pressure for 10 min to the water in which the cut petiole was placed. To enable pressure-bomb work with *P. vulgaris* the groove in the petiole was filled with silicone rubber (not acetate cured) (Wacker-Chemie, Munich, Germany) several hours before the leaf was cut.

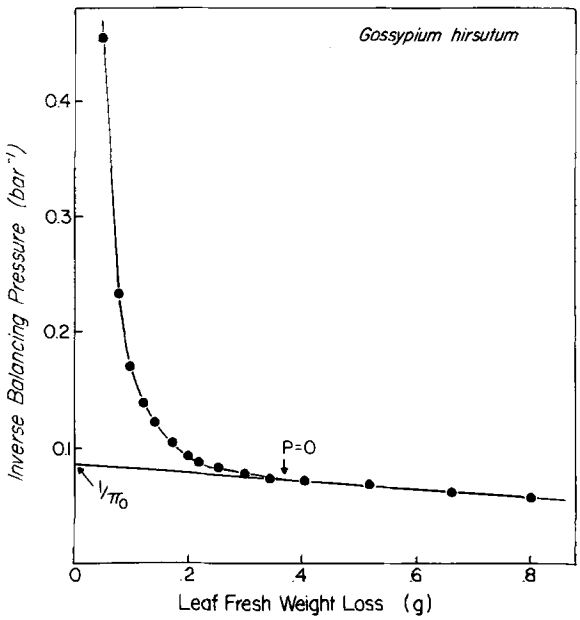

Fig. 1. A "pressure-volume curve" determined for a *Gossypium hirsutum* leaf. The same leaf provided the samples for ABA analysis, the results of which are shown in Fig. 4, *G. hirsutum*, watered daily. ($1/\pi_0$ = inverse original bulk osmotic pressure)

The dehydration procedure consisted of the following sequence. The leaf was weighed. A balancing pressure was determined. The bomb pressure was released at less than 0.1 bar s^{-1}. Two more minutes were allowed for ψ equilibration within the leaf. Then the leaf was taken from the chamber, and a sample was quickly excised and wrapped in pre-weighed foil. Sample plus foil were weighed; the rest of the leaf was left on the laboratory bench to lose water, and its weight was monitored until the leaf was judged to be at an appropriate water content for a new measurement of ψ with the pressure bomb. The cycle was repeated until enough samples had been taken to span the range from full turgor to several bars beyond the wilting point. At the beginning of an experiment samples had to be taken one after another as quickly as possible, but the rate of water loss dropped and the water loss requirement (per bar decrease in ψ) increased as the leaves wilted. To speed up the sampling rate, when necessary, toward the end of the experiment, the pressure bomb was used to force water from the cut end of the petiole (as in the original description by Scholander et al., 1965, for preparing pressure-volume curves).

Cutting samples from the leaf meant that the running total of fresh-weight loss could not be used directly in plotting a pressure-volume curve. The weight of the samples and the fact that the amount of leaf left to lose water was continuously decreasing had to be taken into account. The weight losses between sampling were calculated according to the following formula:

$$\frac{(\text{Wt before sampling} - \text{sample wt}) - \text{wt after water loss}}{\text{fraction of leaf remaining}}$$

The fraction of leaf which a sample represented was estimated from dry-weight measurements.

Pressure-volume curves prepared as described were indistinguishable from those of control leaves from which no pieces were cut. Therefore, we concluded that no significant error was introduced, either by unavoidable cutting of vascular tissue during sampling or by calculating whole leaf equivalent weight loss. Cutting major veins during sampling was avoided.

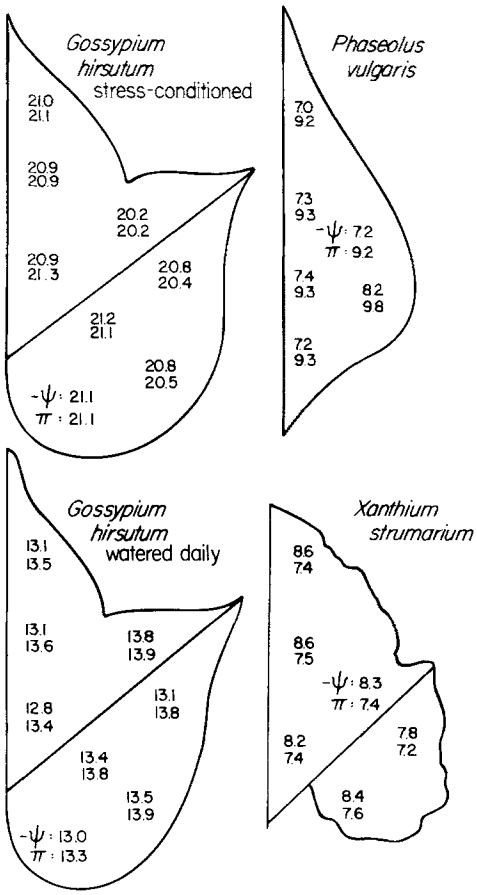

Fig. 2. Measurements of leaf water potential (ψ, upper number of each pair) and osmotic pressure (π, lower number) made with a dew point microvoltmeter on discs cut from leaves of *Xanthium strumarium*, *Phaseolus vulgaris*, and *Gossypium hirsutum*. Each value is the mean from measurements made on 3 leaves of similar ψ

Twelve experiments were performed which provided samples for analysis of ABA as a function of ψ, π, and p in individual leaves. Six examples are presented here.

Considerations for Sampling

In the procedure described above, samples for analysis of ABA were cut from random positions around the leaf. Variability would be introduced into our results if there were gradients of ψ or π along the leaf.

The positional distribution of ψ and π was checked on control leaves (no samples cut for analysis of ABA) which were close to wilting. The dew point method was used to measure ψ and π in discs cut from various positions on the leaf blades. Figure 2 shows the range of values obtained. In no case was there a discernable pattern in the variability in terms of the location on the leaf from which a sample was taken. We concluded that taking all samples for analysis of ABA from one leaf was justified and superior to using different leaves for each point.

Sample Incubation

When samples for analysis of ABA were excised from a leaf they were at known water potentials. After weighing, the foil-covered

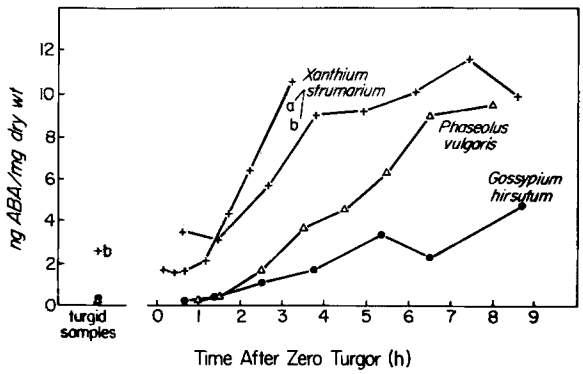

Fig. 3. Time course of accumulation of abscisic acid in detached, water-stressed leaves of *Xanthium strumarium* (a: $\psi = -10.1$ bars, b: $\psi = -11.1$ bars), *Phaseolus vulgaris* – greenhouse culture ($\psi = -8.4$ bars), and *Gossypium hirsutum* – culture watered daily ($\psi = -13.4$ bars)

samples were placed in Petri dishes lined with wet filter paper for incubation to allow ABA to accumulate or not. After the incubation period the samples were unwrapped, reweighed, then frozen with liquid nitrogen. Samples were lyophilized for dry weight determination. An unavoidable but small loss of weight occurred during sample incubation. In general the change in weight was around 1%, and samples generally weighed between 100 and 200 mg. Respiration during 10 h of incubation could account for roughly $^1/_3$ of the weight loss, and perhaps another $^1/_3$ of the loss happened during the post-incubation weighing. For fully turgid samples a loss in weight no matter how small represents a significant change in water potential, and the highly turgid samples may have declined in ψ during incubation by as much as 1 bar. For most of the samples, however, we concluded that the water status was essentially constant during the incubation period.

Choice of the sample incubation period was based on time courses of accumulation of ABA for the three species (Fig. 3). The time courses were prepared as follows. After cutting a turgid control sample, a leaf was dehydrated until a pressure-volume curve indicated that the leaf had reached zero turgor. The leaf was removed from the bomb and divided into about 10 pieces, which were wrapped in foil and incubated. At various times a piece was frozen and later extracted for ABA. The control sample was incubated until the last wilted sample was frozen (Fig. 3, at the left), affirming that excision and long incubation by themselves do not result in accumulation of ABA. For Fig. 3, ABA content was plotted versus time from when zero turgor was reached. Considerable ABA had accumulated by 6 h, and there was a tendency for the rate of accumulation to decrease after that time; at least 8 h were required for a new steady-state level to be reached.

On the basis of the time courses all samples for a dehydration series were incubated until 8.5 h had elapsed from the time zero turgor was reached. It took about 1.5 h to reach zero turgor and a total of about 4 or 5 h to take a leaf through a pressure-volume curve. By freezing samples all at the same time the bias of having samples with lower water potential also having experienced water stress for a longer time (as occurs in soil-drying experiments) was eliminated. The samples should all have had close to a new steady-state level of ABA for their respective ψ's.

Results

These experiments all followed the same procedure. Individual leaves were allowed to wilt slowly. Samples

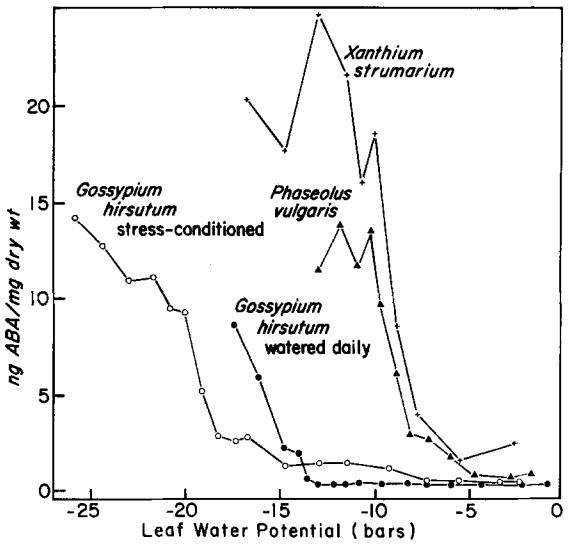

Fig. 4. The effect of leaf water potential on abscisic acid content in single, excised leaves of *Xanthium strumarium*, *Phaseolus vulgaris*, and *Gossypium hirsutum*

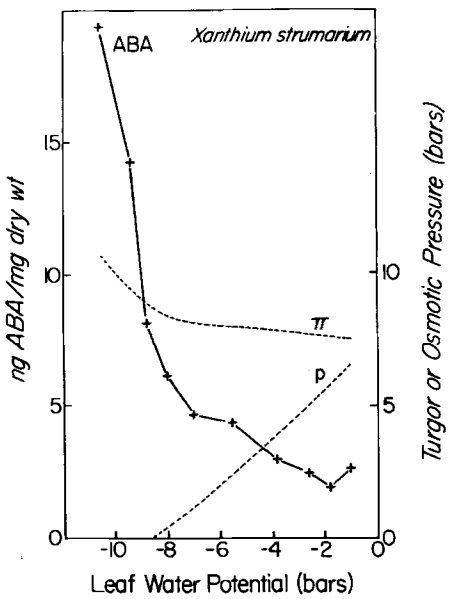

Fig. 5. The relationship between osmotic pressure (π), turgor (p), and leaf water potential, and the effect of water potential on abscisic acid content in samples from a single, excised leaf of *Xanthium strumarium*

for ABA analysis were cut from a leaf as it lost known amounts of water and incubated at constant water content to allow accumulation of ABA to a new steady-state level. Just before a sample was cut, the leaf water potential was determined with a pressure bomb. Pressure-volume curves were prepared from the ψ and weight-loss measurements (e.g. Fig. 1). These were used to determine π and p.

The results of leaf-dehydration series are shown in Fig. 4 for leaves of *Xanthium strumarium*, *Phaseolus vulgaris*, *Gossypium hirsutum*, and *G. hirsutum* which had been forced into osmotic adjustment by periodic withholding of water. Values for ABA content were plotted as ng ABA/mg dry weight, but lines of the same shape were obtained when ABA content was expressed on the basis of chlorophyll content. The curves of ABA content versus ψ all show a region of high ψ (right side of Fig. 4) over which little ABA accumulated, followed by gradual transition to a region of ψ over which ABA content rose steeply. The curves differed in the water potential at which the steepest slope occurred. The responses became very steep below -8 bars for these examples of *X. strumarium* and *P. vulgaris*, below -13.5 bars for a *G. hirsutum* leaf, and below -18.5 bars for a leaf from a stress-conditioned *G. hirsutum* plant. The variation appeared to arise from the leaves having different osmotic pressures and, hence, different ψ's at which loss of turgor occurred. At full hydration ($\psi = 0$) these leaves had bulk osmotic pressures of 8.3 bars for *P. vulgaris*, 8.6 bars for *X. strumarium*, 11.6 bars for *G. hirsutum* and 17.2 bars for stress-conditioned *G. hirsutum*. At zero turgor (when ψ reached $-\pi$) the leaves had π's of 9.6 bars for *P. vulgaris*, 9.7 bars for *X. strumarium*, 13.7 bars for *G. hirsutum*, and 21.5 bars for stress-conditioned *G. hirsutum*. Figure 4 shows that leaves with similar original bulk osmotic pressures had almost overlapping curves of accumulation of ABA in response to decreasing ψ. Leaves with different osmotic pressures, even of the same species, differed with respect to the water potential (and π) at which accumulation of ABA took place.

Data for π and p were added to a graph of ABA content versus ψ for another *X. strumarium* leaf in Fig. 5. Figure 5 illustrates the correspondence between loss of turgor and accumulation of ABA. Samples with low but still positive turgor developed levels of ABA which were up to 3 times the level of ABA in fully turgid samples. Zero turgor strongly affected the accumulation of ABA; the steepest increase in ABA content occurred between the first two samples having zero turgor.

Greenhouse and growth-chamber conditions produced *P. vulgaris* plants with slightly different osmotic pressures. Figure 6 shows that the small difference in osmotic pressures was reflected in an equivalent displacement between the curves of increasing ABA content. *P. vulgaris* samples with a turgor of 1 bar developed levels of ABA which were 4–8 times the level of ABA in fully turgid samples. Below $p = 1$ bar, ABA content increased sharply to as much as 40 times the level found in unstressed samples. As in Fig. 5,

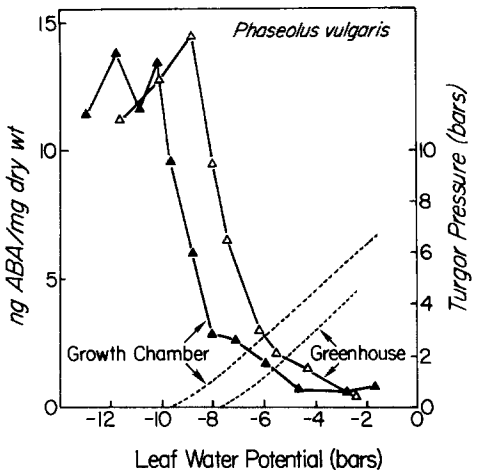

Fig. 6. The relationship between turgor (.....), and leaf water potential and the effect of water potential on abscisic acid content, (▲, △), in two leaves of *Phaseolus vulgaris*. The leaves were excised from plants cultivated in either a growth chamber or a greenhouse

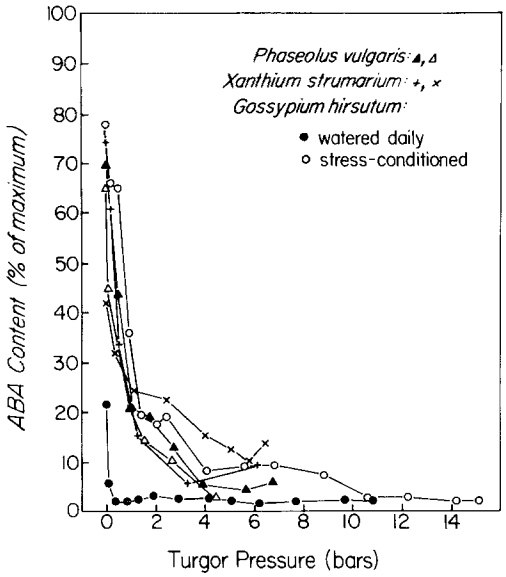

Fig. 7. Abscisic acid content as a function of turgor in single, excised leaves of *Xanthium strumarium*, *Phaseolus vulgaris*, and *Gossypium hirsutum*. ABA content is plotted as the percent of the maximum ABA content accumulated by the same leaves at any leaf water potential tested. The relationship between ABA content and ψ for these leaves is shown in Figs. 4, 5, and 6

both examples in Fig. 6 show that the steepest slope in the relation between ABA content and ψ occurred at zero turgor. Some of the curves of ABA content versus ψ in Figs. 4, 5, and 6 indicate a tendency for the response to level off within several bars of the point of zero turgor. Saturation of the response is particularly obvious in Fig. 6.

The dependence of ABA production on loss of turgor is summarized in Fig. 7. ABA content progressively increased as turgor approached zero. Accumulation of ABA in the example from *G. hirsutum* plants which were well-supplied with water was exceptionally abrupt; all of the increase in ABA above unstressed levels took place below $p=0.5$ bar. For the other leaves, more than 80% of the increase in ABA above unstressed levels took place at less than 1 bar turgor.

Discussion

Leaf ψ and π Influence Production of ABA through Their Effect on Turgor

ABA content in water-stressed leaves increased at different water potentials for different leaves (Figs. 4–6), but in each case the capacity for accumulation of ABA rose sharply as turgor approached zero (Fig. 7). We interpret these results to mean that leaf water potential per se does not control ABA content. It is unlikely that bulk leaf π directly influences production of ABA either, for the following reasons: (1) Increases in ABA content were not uniquely related to π just as they were not uniquely related to ψ. (2) In most leaves the entire change in ABA content occurred over a range of π of about 2–4 bars, for instance from 7 to 9 bars in *P. vulgaris* or from 14 to 18 bars in *G. hirsutum* (cf. Fig. 5). Changes in π of this magnitude normally occur in leaves during the course of a day and may be attributed to solute accumulation rather than dehydration as Acevedo et al. (1979) reported for leaves of maize and sorghum. Even larger diurnal changes in π were observed in cotton leaves (Cutler et al., 1977) when solute accumulation was accompanied by significant dehydration during the day, but even so the stomata did not close, suggesting ABA levels did not rise in this case. The changes in bulk leaf π which we observed were almost certainly not responsible for the 10- to 40-fold accumulation of ABA that occurred. We also think it is unlikely that accumulation of ABA depends on the concentration of some individual component of π, despite a nearly linear relationship between π and ABA content that appears over a narrow range of π if ABA content is plotted versus π. The linearity results from the fact that with decreasing ψ only small changes in leaf π and ABA occur so long as turgor is positive,but that these changes increase after turgor has been lost (e.g. Fig. 5). We feel that the approxi-

mately linear relation between π and ABA content is coincidental because the relation holds only for a 2–4-bar range of π, which is just a 30% increase in solute concentration. Not all, but at least half, of the leaves showed saturation of the response within the range of water potentials tested (see Figs. 4, 6); increases in solute concentration above about 30% were less effective or not at all effective in promoting accumulation of ABA. We concluded that accumulation of ABA in water-stressed leaves is most probably a turgor-dependent process rather than a ψ- or π-dependent one.

How is Accumulation of ABA Related to Turgor?

Single mature leaves were used in these experiments in the hope of getting a clearer answer as to whether ABA production in response to water stress exhibits a threshold phenomenon or not. In one case – that of *G. hirsutum* well-supplied with water – the response curve versus ψ in Fig. 4 showed an abrupt change of slope coincident with the point of zero turgor. In the rest of the examples in Figs. 4–6 the curves were more sigmoid in shape, just as Wright (1977) found for detached wheat leaves, showing no clear threshold.

The results, when plotted against turgor (Fig. 7), showed a lack of response at high turgor and a progressive increase in accumulation of ABA by the leaf samples as they approached zero turgor. This pattern of response may reflect the nature of turgor pressure sensing by the individual cells of the leaf. The curves are of the same shape as that found for the turgor-dependence of K^+ influx into cells of *Valonia utricularis* (Zimmermann and Steudle, 1977). The electromechanical model of Zimmermann and colleagues (reviewed in Zimmermann, 1978) should be kept in mind as an aid in the investigation of ABA production in leaves.

Alternatively, the triggering of ABA synthesis in individual leaf cells may be more closely coupled to zero turgor than is shown by the response curves of leaf sections. Part of the accumulation of ABA in leaf samples having apparently positive turgor was certainly due to leaves not being homogeneous populations of cells. Inverse balancing pressures plotted versus tissue volume (or fresh weight) will not look like a straight line unless nearly all of the cells have lost turgor; only then will pressure-volume curves indicate zero turgor has been reached (Cheung et al., 1976). Thus, any variation in π between or within different cell types would spread elimination of turgor in some cells to higher water potentials than that corresponding to zero turgor according to the pressure-volume curve. Variation in the volumetric modulus of elasticity (ε), which affects the relative change in volume that occurs as turgor is lost, should do the same. According to this view, we could expect that the more uniform the cells of a leaf are, the more abrupt should be the onset of accumulation of ABA with respect to loss of turgor.

How Reliable are the Determinations of Turgor?

Irrespective of how gradual or abrupt the onset of accumulation of ABA was, in every leaf we analyzed for ABA content as a function of ψ, π and p, determined from pressure-volume curves, we found that accumulation of ABA was most sensitive to changes in ψ at turgor pressures less than 1 bar. We attempted to compare the results with coincident determinations of water status made by the dew point method. This method yielded ψ values which agreed fairly well with determinations of balancing pressures made with the pressure bomb. However, π (and hence p) values were always several bars lower than those indicated by the pressure-volume curve, displacing the point of zero turgor to higher ψ values and also resulting in some negative values for p (see also Fig. 2). While the measurement of π on frozen-thawed tissue or expressed sap seems adequate for relative measurements (as in Fig. 2), apparent under-estimation of π (Boyer and Potter, 1973; Tyree, 1976) produces ambiguous negative values for p, and makes this method questionable for determining the point of zero turgor. If psychrometric (or dew-point) measurements of leaf ψ are plotted as $1/\psi$ versus water content, p can be determined from the resulting graph just as it is from "pressure-volume curves" (Talbot et al., 1975). We found good agreement between determining the point of zero turgor by this method and by the pressure-bomb method.

What are the Implications of the Observed Relation between Accumulation of ABA and Leaf Turgor?

Our experiments do not deal directly with stomatal behavior, but the requirement for low turgor in order for leaves to accumulate ABA has consequences concerning stomatal response to water stress. The guard cells themselves, if they possess the ability to make ABA, are probably the last cells in the leaf to be stimulated to do so. In general, guard cells have by far the highest osmotic pressures of any cell type in the leaf even when the stomata are closed (see review by Raschke, 1979); hence, the guard cells should be among the last cells in the leaf to reach

zero turgor during water stress. It is thus very unlikey that ABA production by the guard cells themselves initiates stomatal closure. Rather, if ABA does mediate stomatal closure during water stress, it most likely comes to the guard cells as a result of a rapid transfer from those leaf cells that are first to reach zero turgor. An insensitivity of stomata to changes in ψ in the region of high leaf turgor has been observed by many investigators (summarized in Turner, 1974). We think this insensitivity reflects lack of stimulation of the system producing ABA. However, as the results in Fig. 3 demonstrate, wilted leaves may require more than one hour before any increase in whole-leaf ABA can be seen, but stomata generally close within 10–20 min after a leaf loses turgor (e.g. Beardsell and Cohen, 1975). As Beardsell and Cohen (1975) have pointed out, in order for ABA to trigger stomatal closure during water stress, changes in ABA concentration in the vicinity of the guard cells must happen much faster than changes in whole-leaf ABA. Accumulation of ABA near the guard cells will be accelerated if a high rate of transpiration sweeps (pre-existing or newly-produced) ABA from the mesophyll to the epidermis.

The known ability of *G. hirsutum* plants to increase π in response to reduced water supply (Brown et al., 1976; Cutler and Rains, 1978) and thereby decrease the leaf ψ at zero turgor was used successfully to alter the relation between ABA content and ψ. Compared to the response of leaves from plants which were watered daily, increases in ABA content in leaves from stress-conditioned plants were displaced to lower water potentials (Fig. 4). Jones and Turner (1978) and Fereres et al. (1978) documented osmotic adjustment to dry environments in sorghum. One would expect that the critical range of water potential from -8 to -10 bars for sorghum below which ABA levels increased (Beardsell and Cohen, 1975) would be shifted to lower ψ's by prior stress conditioning. Further, our demonstration that stress conditioning of plants can displace accumulation of ABA to lower water potentials provides a likely explanation of why stress conditioning can also displace drought-induced stomatal closure to lower ψ's (e.g. Jordan and Ritchie, 1971; McCree, 1974).

Field-grown plants often resemble stress-conditioned plants in the respect that turgor-dependent processes such as growth by cell expansion and stomatal opening are extended to lower ψ's in these plants than in laboratory plants that do not normally experience water stress (e.g. Jordan and Ritchie, 1971; Cutler et al., 1977). Very small diurnal fluctuations in ABA content occurred in field-grown cotton regardless of whether the crop was irrigated or not (McMichael and Hanny, 1977). Our results lead us to suggest that the unirrigated ("stressed") field cotton failed to accumulate ABA during the day when leaf water potential fell to -30 bars because prior adaptation to drought allowed positive turgor to be maintained.

In addition to drought adaptation, conditions such as leaf or plant age or canopy position, by altering cell elasticity, volume, or solute content, affect what ψ and π are when $p=0$. Judging from the results of this investigation, one would expect leaves of different age or canopy position to accumulate ABA at different ψ's, according to the ψ at which turgor becomes zero. Obviously one cannot generalize for a given species or variety on what will be the most critical ψ for the accumulation of ABA. The point of zero turgor may even change during the course of a slow soil-drying experiment as a result of drought adaptation. Turgor cannot be predicted; it has to be determined.

Not only did stress conditioning of *G. hirsutum* displace accumulation of ABA to lower ψ's, but it also resulted in a more gradual increase in ABA content with declining p. This loss of sharpness in the onset of accumulation of ABA was probably the result of an increased variance in solute content and wall elasticity of the cells capable of producing ABA. Whether a gradual increase in ABA production offers an advantage to plants over an abrupt one in the toleration of drought remains to be investigated.

Our results are in agreement with reports of Most (1971) and Wright (1972) according to which some accumulation of ABA occurred under conditions of mild water stress where wilting symptoms were not seen. ABA content at $p=1$ bar averaged ca. 4 times the level found in fully turgid samples (see Fig. 7) when sufficient time was provided for development of a new steady-state level of ABA. Fig. 7 shows that a doubling of ABA content may occur below $p=$ 4 bars with as little as a 1 bar decrease in turgor. Therefore, any investigation into the chain of events between infliction of a stress and production of ABA should include a determination of whether a small change in turgor was involved.

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