Plant Physiol. (1984) 76, 1029–1035
0032-0889/84/76/1029/07/$01.00/0

Abscisic Acid Metabolism in Relation to Water Stress and Leaf Age in *Xanthium strumarium*[1]

Received for publication April 4, 1984 and in revised form August 18, 1984

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ABSTRACT

Intact plants of *Xanthium strumarium* L. were subjected to a water stress-recovery cycle. As the stress took effect, leaf growth ceased and stomatal resistance increased. The mature leaves then wilted, followed by the half expanded ones. Water, solute, and pressure potentials fell steadily in all leaves during the rest of the stress period. After 3 days, the young leaves lost turgor and the plants were rewatered. All the leaves rapidly regained turgor and the younger ones recommenced elongation. Stomatal resistance declined, but several days elapsed before pre-stress values were attained.

Abscisic acid (ABA) and phaseic acid (PA) levels rose in all the leaves after the mature ones wilted. ABA-glucose ester (ABA-GE) levels increased to a lesser extent, and the young leaves contained little of this conjugate. PA leveled off in the older leaves during the last 24 hours of stress, and ABA levels declined slightly. The young leaves accumulated ABA and PA throughout the stress period and during the 14-hour period immediately following rewatering. The ABA and PA contents, expressed per unit dry weight, were highest in the young leaves. Upon rewatering, large quantities of PA appeared in the mature leaves as ABA levels fell to the pre-stress level within 14 hours. In the half expanded and young leaves, it took several days to reach pre-stress ABA values. ABA-GE synthesis ceased in the mature leaves, once the stress was relieved, but continued in the half expanded and young leaves for 2 days.

Mature leaves, when detached and stressed, accumulated an amount of ABA similar to that in leaves on the intact plant. In contrast, detached and stressed young leaves produced little ABA. Detached mature leaves, and to a lesser extent the half expanded ones, rapidly catabolized ABA to PA and ABA-GE, but the young leaves did not. Studies with radioactive (±)-ABA indicated that in young leaves the conversion of ABA to PA took place at a much lower rate than in mature ones. Leaves of all ages rapidly conjugated PA to PA-glucose ester. Furthermore, when half expanded leaves were stressed on the intact plant, their rate of ABA catabolism was enhanced, an effect not observed in the young leaves.

In conclusion, young leaves on intact *Xanthium* plants produce little stress-induced ABA themselves, but due to import and a low rate of catabolism accumulate more ABA and PA than mature leaves.

It is well established that endogenous ABA levels increase considerably when plants are water stressed (3, 11, 19). The relationship between ABA accumulation, stomatal resistance, and water potential has been described for mature leaves of several species as water stress developed and was subsequently relieved (1, 4, 9). In these studies, the ABA content increased as the water potential and stomatal conductance decreased. Upon relief of stress, ABA decreased rapidly, but stomatal conductance returned to pre-stress values more slowly. When leaf age was considered, it was found that stress-induced ABA accumulated to the greatest extent in the youngest leaves (17, 24). This occurred despite the fact that mature leaves wilted earlier than younger ones. On the other hand, young *Xanthium* leaves, that were stressed following excision, produced little ABA (24). As ABA is rapidly transported throughout the plant (24), it is probable that the high levels in young leaves, on stressed plants, are mainly due to import from older leaves, and not to *in situ* production.

In this paper, we present data on the effects of drought and its subsequent relief on the levels of ABA and its catabolites, PA[2] and ABA-GE (22), in *Xanthium* leaves of different ages on intact plants. Various parameters of water relations were monitored throughout the stress-recovery cycle. The capacity of leaves of different ages to produce stress-induced ABA *in situ* was assessed by stressing detached leaves, while radioactive ABA was employed to determine the rate of ABA catabolism in the various leaves.

MATERIALS AND METHODS

Culture of Plant Material. *Xanthium strumarium* L., Chicago strain, was grown in a greenhouse under the same conditions as described (22). For the duration of an experiment, the plants were transferred to a controlled environment chamber (22).

In all experiments, leaves of three different ages were compared: mature leaves, 12 to 15 cm long (the youngest fully expanded lamina on each plant); half expanded leaves, 7 to 10 cm long; young leaves, 1.8 to 3.2 cm long. The percentage dry weight of turgid leaves, excluding the midrib and major veins, was as follows: mature leaves, 18.7%; half expanded leaves, 20.1%; young leaves, 20.1%.

Intact Plants. *X. strumarium* plants were transferred to the controlled environment chamber and well watered for a week before the stress treatment was started. Measurements of leaf length, stomatal resistance, and water potential were made throughout this period and the subsequent stress and recovery periods. The plants were stressed by withholding water and rewatered 3 d later. Eight plants were selected for similar water status during the early stages of the stress cycle and randomly assigned to two replicate groups (four plants per group). Leaf discs, 9 mm in diameter, were punched with a cork borer from these plants at intervals throughout the stress-recovery cycle. Discs were taken from the central region of mature and half expanded leaves; major veins were avoided. Individual leaves could not supply all the plant material harvested, so two of the

[1] Supported by the National Science Foundation Grant PCM 78-07653 and by the United States Department of Energy under Contract DE-AC02-76ERO-1338

[2] Abbreviations: PA, phaseic acid; ABA-GE, β-D-glucopyranosyl abscisate; PA-GE, β-D-glucopyranosyl phaseate; Ψ, leaf water potential; Ψ_s, solute potential; Ψ_p, pressure potential.

four plants were sampled at each time. Every possible pair within each replicate of four plants (*e.g.* 1 and 2, 1 and 3, etc.) was sampled in successive harvests. The pair sequence was rotated until the experiment was completed. The small size of the young leaves made repeated sampling of the same leaves impossible and discs were therefore taken from other plants with half expanded leaf stomatal resistances most similar to the eight selected plants. The leaf discs were frozen in liquid N_2 immediately following each harvest and stored at −20°C in the dark until used. An additional disc of each leaf age was cut from each replicate at every harvest for leaf water potential measurements. Preliminary trials showed that repeated cutting of discs from the central region of half expanded and mature leaves had no discernable effect on the accumulation of ABA in either stressed or turgid leaves.

Detached Leaves. Mature, half expanded and young laminae were detached from turgid *X. strumarium* plants. Four leaves of each age were stressed in a stream of air at room temperature until they had lost 12% of their fresh weight and placed in plastic bags. All the leaves were incubated in the dark for 12 h. After this time, one leaf disc was cut from the central region of each leaf. The discs were pooled into pairs to provide two replicates of each treatment and leaf age. The leaf discs were frozen immediately in liquid N_2 and stored as above.

Measurement of Leaf Water Status. Stomatal resistance of mature and half expanded leaves was measured with a Li-Cor LI-65 autoporometer (Lambda). The autoporometer actually measures the total resistance, which includes both stomatal resistance and a minor cuticular component. Stomatal resistance of the upper epidermis only has been presented as this was more closely correlated with Ψ than was the stomatal resistance of the lower epidermis. The young leaves were too small to be measured with this method. Ψ was measured with a Wescor HR-33T dew point microvoltmeter equipped with C-52 sample chambers (Wescor) according to the methods of Nelsen *et al.* (12). Ψ_s was determined in the same discs after freezing in liquid N_2 and thawing. Ψ_p was calculated from the difference between Ψ and Ψ_s. A pressure bomb was used (18) to determine the Ψ of comparable leaves to provide an independent check on the hygrometer readings.

Experiments with Radioactive ABA and PA. In all experiments, radioactive ABA or PA was placed in vials and dissolved in 10 to 20 μl of ethanol and distilled H_2O containing 0.1% Tween 20 was added. The solution was applied via the cut petioles and the leaves were kept in a light box to enhance rapid transpiration. After the original solution and several rinses with water containing Tween 20 had been absorbed by the leaves, they were placed in the dark.

In one experiment, leaves of three ages were detached from unstressed plants, labeled with (±)-[^{14}C]ABA (36 μCi·mmol^{-1}), and placed with their petioles in an aqueous solution of 0.1% Tween 20. Twelve young leaves were each fed 0.1 μCi and four of these were pooled for analysis after 12, 24, and 48 h; four half expanded leaves were each fed 0.5 μCi and two of these were pooled for analysis after 12 and 24 h; two mature leaves were each fed 1 μCi and one leaf was analyzed after 12 h, the other after 24 h. Radioactive profiles of the leaf extracts were made as described below (Extraction and Purification Procedures).

Most of the [^{14}C]PA (0.20 μCi) produced by the leaves in the above experiment was divided and fed to seven leaves (four young, two half expanded, one mature) detached from nonstressed plants. The leaves were incubated as before for 24 h. Radioactive profiles of the leaf extracts were made.

An additional experiment was designed to determine if water stress provides a trigger for ABA catabolism in half expanded and young leaves. Leaves were treated as follows: (i) leaves were detached from nonstressed plants and labeled; (ii) as treatment i, but the leaves were stressed after radioactive (±)-ABA had been applied until they had lost 12% of their fresh weight; (iii) leaves were detached from wilted plants, labeled, then restressed to the same degree as before radioactive ABA was applied; (iv) leaves were detached from turgid plants that had undergone a stress-recovery cycle, and then labeled; (v) as treatment iv, but the leaves were stressed after labeling until they had lost 12% of their fresh weight. All the leaves in treatments i and iv were incubated for 24 h with their petioles in 0.1% Tween 20; those in the remaining treatments were placed in plastic bags before incubation. Ten half expanded leaves were each fed 0.1 μCi (±)-[^{14}C] ABA, and the two leaves in each treatment were pooled for analysis. One mature leaf was fed 0.2 μCi (treatment iv) to provide an additional control. A second set of half expanded leaves was fed 0.5 μCi (±)-[^{3}H]ABA (specific activity adjusted to 36 μCi·mmol^{-1}). A mature leaf was fed 1 μCi and placed in treatment i as a control. The final number of replicates per treatment of half expanded leaves (two leaves pooled per replicate, all incubated for 24 h) was: (i) 3, (ii) 2, (iii) 4, (iv) 5, (v) 4 replicates. In addition, twelve young leaves were each fed 0.05 μCi (±)-[^{14}C]ABA (two leaves in treatments i, ii, iv, and v; four leaves in treatment iii). The leaves in each treatment were pooled for analysis.

Extraction and Purification Procedures. Leaf discs were analyzed for ABA, PA, and ABA-GE content as follows. The discs were lyophilized, weighed, and then pulverized with a glass rod. Small amounts of (±)-[^{3}H]ABA (16.4 Ci·mmol^{-1}), (±)-[^{3}H]ABA-GE (149 mCi·mmol^{-1}), and [^{3}H]PA (160 mCi·mmol^{-1}) were added to each sample to determine losses during the purification procedure. All results reported for leaf discs are corrected for recovery losses. The leaf tissue was extracted with 80% and 100% acetone (acetone, 1% acetic acid, 0.01% 2,6-di-*tert*-butyl-*p*-cresol) and shaken in the dark at room temperature. The supernatant was drawn off with a Pasteur pipette and more solvent added; this was repeated until the supernatant remained colorless. The supernatants were dried and purified by semipreparative reverse-phase HPLC on a μBondapak C_{18} (10 μm particle size), 30- × 0.78-cm column (Waters Associates). To separate PA and ABA-GE (2 min apart), the samples were eluted by means of a linear gradient from 10% to 35% ethanol containing 1% acetic acid in 40 min, with a 2-min pause before the gradient began. The flow rate was 2.5 ml·min^{-1}. After elution, the fractions containing ABA and PA were dried and methylated with ethereal diazomethane. The fractions containing ABA-GE were dried and hydrolyzed with 2 M NH_4OH at 60°C for 2 h which yielded free ABA. These samples were then dried and methylated. Quantification of the methyl esters of ABA and PA was performed with a Hewlett-Packard 5840A gas chromatograph equipped with a ^{63}Ni-electron capture detector. Samples were dissolved in ethyl acetate with an internal standard of the ethyl ester of ABA, and analysis was done on a Durabond DB-1 (J & W Scientific, Inc., Rancho Cordova, CA) gas capillary column (30 m × 0.32 mm × 0.25 μm). GLC conditions were: oven temperature, 165°C for ABA, 160°C for PA; H_2 carrier flow, 10 ml·min^{-1}; split ratio, 5:1; argon-methane (95:5) was used as make-up gas and had a flow at the detector of 80 ml·min^{-1}. Absolute amounts of methylated ABA and PA were determined by comparisons with standard material (20). Overall recovery of [^{3}H]ABA added to the samples was between 75% and 100%; recoveries of [^{3}H]PA and [^{3}H]ABA-GE (measured as [^{3}H]ABA) were between 70% and 90%.

Leaves from the radiolabeling experiments were frozen in liquid N_2 and homogenized in 80% acetone with a Polytron homogenizer (Brinkmann Instruments). The samples were extracted overnight at 4°C. The supernatant was decanted and replaced with 100% acetone for another day. The leaf extracts were dried until an aqueous solution remained which was frozen

and lyophilized. The leaf extracts were fractionated by preparative reverse-phase HPLC on the Bondapak C_{18}/Porasil B (Waters Associates) column previously described (21). The fractions were eluted by means of a linear gradient from 20% to 50% ethanol in 1% aqueous acetic acid in 30 min, at a flow rate of 9.9 ml·min^{-1}. Fractions were collected at 1-min intervals from 8 to 32 min. Fractions from the first labeling experiment containing [^{14}C]PA were pooled and purified using the μBondapak C_{18} column and conditions described above for the leaf disc analyses. The [^{14}C]PA was further purified by TLC (25).

RESULTS

Intact Plants

Leaf Growth. Mature leaves had essentially completed their growth prior to the onset of stress (Fig. 1). The leaves slightly wilted about 20 h after the final watering, followed by a recovery during the night. They wilted again during the 2nd d without water and, as the stress became more severe, the leaves substantially shrank (Fig. 1). Upon rewatering, the leaves regained their original size and turgor rapidly (see "Water Relations" below). Half expanded leaves entered the stress period during their linear growth phase, but growth ceased after 14 h. A slight shrinkage of these leaves was also observed in the latter stages of stress. On rewatering, the leaves regained turgor and their pre-stress size and rapidly recommenced elongation (Fig. 1). The young leaves behaved as the half expanded ones, except that no shrinkage was observed. Rapid growth rates occurred after rewatering as these leaves entered the linear growth phase (Fig. 1).

Stomatal Resistance. The effect of water stress on *X. strumarium* plants is clearly illustrated by changes in the stomatal resistance of the upper epidermis of the mature and half expanded leaves (Fig. 2). The resistance of the leaves of both ages increased 14 h after the final watering, which coincides with the cessation of leaf elongation. Stomatal resistance continued to rise throughout the stress period. On rewatering, the resistance declined in both types of leaves, but several days passed before pre-stress levels were attained.

Water Relations. The internal effects of the stress can be clearly seen by following changes in the Ψ throughout the stress-recovery cycle (Fig. 3). In leaves of all three ages, the Ψ had decreased after 12 h. During the 12-h dark period, the decline in Ψ was halted and returns to higher Ψ were then observed. Ψ fell rapidly in all leaves during the 2nd d of stress and only slight relief occurred during the next dark period. The mature leaves had the lowest Ψ of the three leaf types after the midpoint of the stress period. Ψ continued to fall rapidly after the 3rd dark period. At the end of the 3rd d of stress, the severely wilted plants were rewatered. Recovery was rapid and pre-stress Ψ were reached in all the leaves after 14 h.

The Ψ_s remained quite stable in all three types of leaves during the first half of the stress period, but declined steadily throughout the remainder of the stress period (Fig. 4). The older the leaves, the lower were the Ψ_s values attained. A substantial recovery occurred during the first 14 h after rewatering, but the return to pre-stress levels took considerably longer than for Ψ.

Ψ_p began to fall in all the leaves after 12 h without water (Fig. 5). The half expanded and mature leaves approached zero turgor at the same time that wilting symptoms became apparent. During the dark period, the leaves regained turgor, but on the 2nd d of stress Ψ_p fell sharply in all leaves. Zero turgor actually occurred at apparent Ψ_p values of -1.5 to -2 bar. These values were determined by a comparison between the hygrometer and pres-

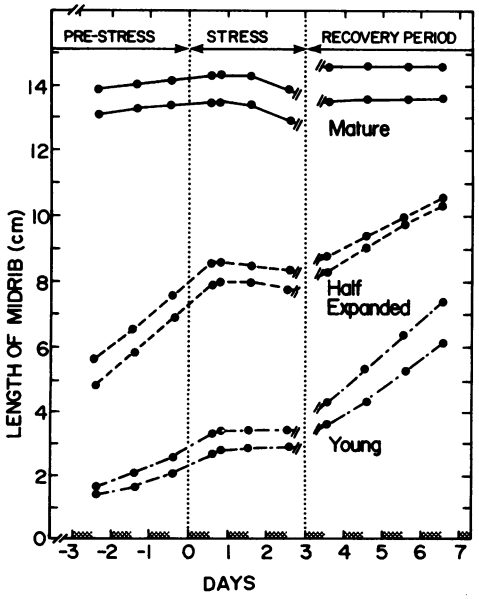

FIG. 1. Leaf elongation of six representative leaves throughout the stress-recovery cycle. (■), Dark periods.

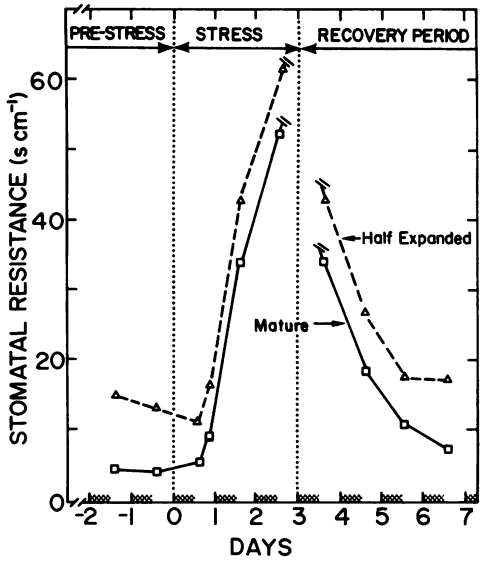

FIG. 2. Stomatal resistance of the upper epidermis of half expanded and mature leaves throughout the stress-recovery cycle. (■), Dark periods.

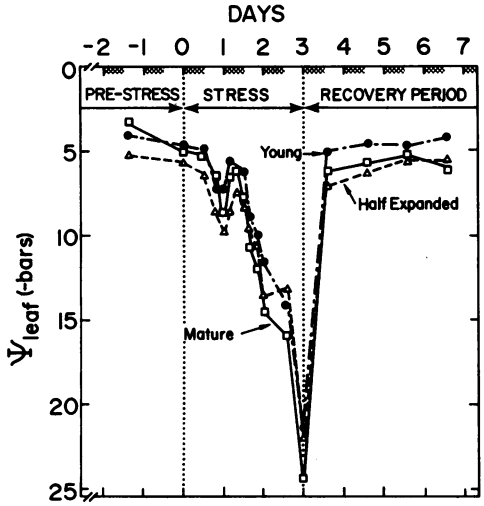

FIG. 3. Water potential of leaves of three different ages throughout the stress-recovery cycle. (■), Dark periods.

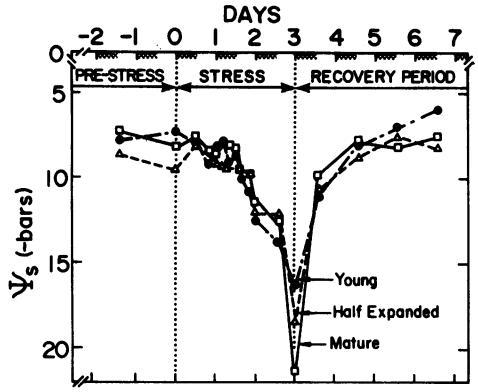

FIG. 4. Solute potential of leaves of three different ages throughout the stress-recovery cycle. (■), Dark periods.

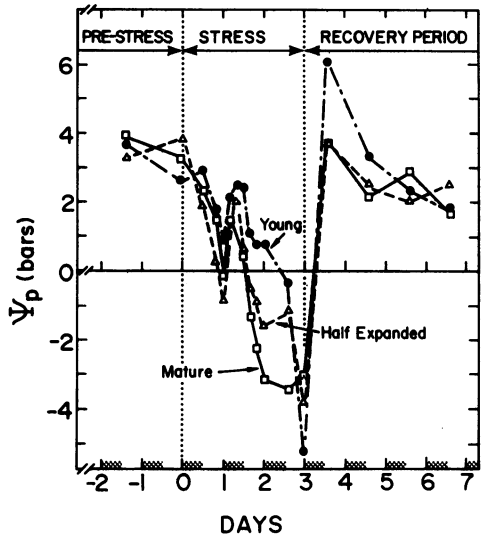

FIG. 5. Pressure potential of leaves of three different ages throughout the stress-recovery cycle. (■), Dark periods.

sure bomb methods used to measure the Ψ of *X. strumarium* leaves. Values of Ψ_p below −2 bar arose as no allowance was made for either matric potential, or for cell wall water that diluted the cytoplasmic solution in severely wilted tissue. A further complication arises as the immature leaves were expanding during the pre-stress and recovery periods. The discs cut from these leaves were not at equilibrium and, therefore, the values for Ψ_p (and Ψ) are probably slightly underestimated.

Ψ_p declined more rapidly the older the leaf under stress. The young leaves maintained turgor considerably longer than the older leaves (Fig. 5). The plants were rewatered once the young leaves showed wilting symptoms. Leaves of all three ages rapidly recovered, and the young leaves had initially a higher Ψ_p than their prestress values.

ABA, PA, and ABA-GE Levels. The ABA content of all three leaf types started to increase 20 h after the last watering (Fig. 6). This was followed by a slight decline in ABA content which corresponded to the overnight recovery of Ψ (Fig. 3). As the stress became more severe, the half expanded and young leaves rapidly accumulated ABA at a very similar rate, while in the mature leaves the ABA level increased more slowly. The ABA content of the half expanded and mature leaves declined during the final stages of the stress period, even though the stress continued to become more severe. However, the young leaves accumulated ABA throughout the entire stress period as well as during the 14-h period immediately following rewatering. In fact,

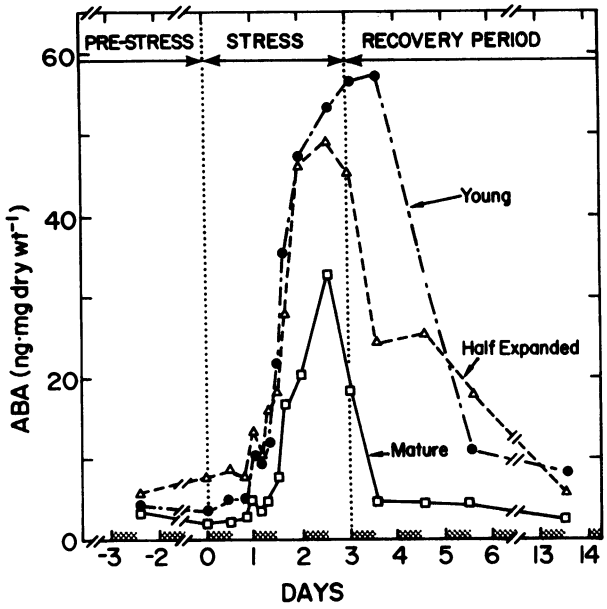

FIG. 6. Accumulation and degradation of ABA in attached leaves of three different ages throughout the stress-recovery cycle. (■), Dark periods.

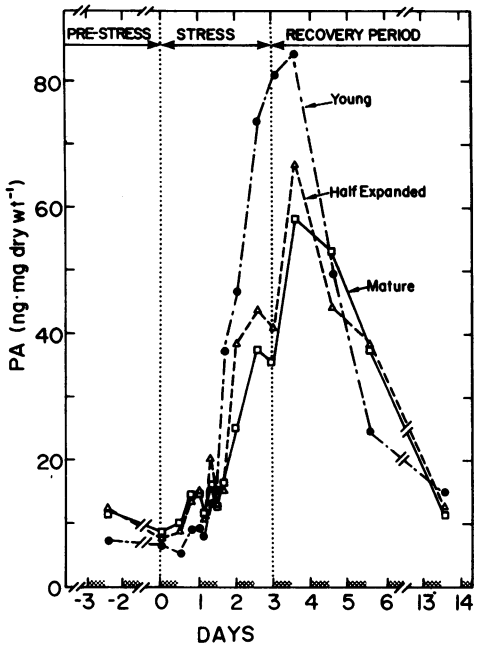

FIG. 7. Accumulation and degradation of PA in attached leaves of three different ages throughout the stress-recovery cycle. (■), Dark periods.

the young leaves accumulated higher levels of ABA during the stress than did the older leaves. This is in agreement with earlier work on *X. strumarium* (24) and has also been observed in *Euphorbia lathyrus* (17). Upon relief of stress, the level of ABA in the mature leaves declined to its pre-stress level within 14 h. ABA levels fell more slowly in the half expanded and young leaves, and took several days to reach pre-stress values (Fig. 6).

PA began to accumulate in the leaves 12 h into the stress period (Fig. 7), before the initial rise in ABA content. However, rapid accumulation of PA did not start until the 2nd d of stress. Unlike ABA, PA was first substantially accumulated by the young leaves, followed about 4 h later by the half expanded leaves. The young leaves accumulated PA throughout the stress

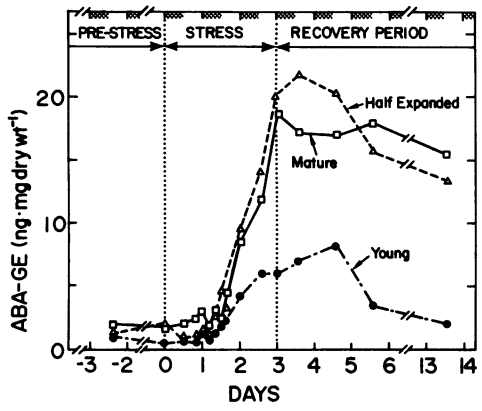

FIG. 8. Accumulation of ABA-GE in attached leaves of three different ages throughout the stress-recovery cycle. (■), Dark periods.

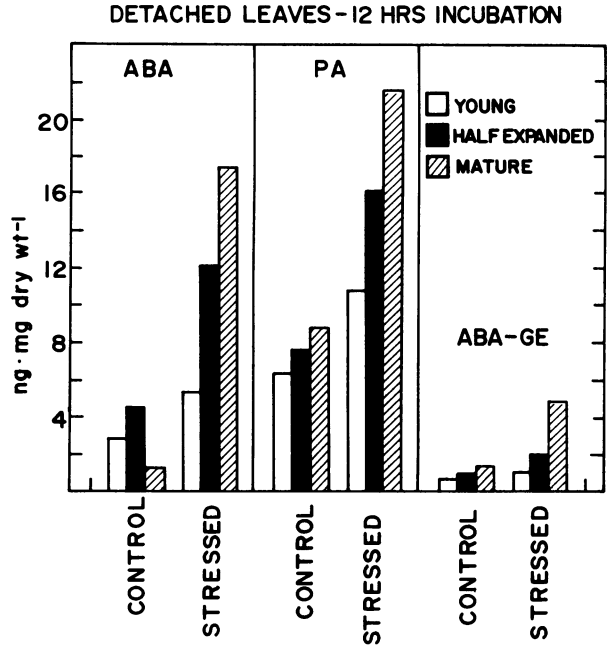

FIG. 9. Accumulation of ABA, PA, and ABA-GE in detached leaves of three different ages after 12 h incubation in the dark. Stressed leaves were detached and dehydrated to a 12% loss of fresh weight before incubation.

period and closely followed the overshoot of ABA accumulation in the 14-h period immediately following rewatering (Fig. 6). These leaves contained about 20 ng·mg^{-1} dry weight more PA than ABA at this time. The half expanded and mature leaves also rapidly accumulated PA as the stress took effect, but the PA leveled off after 2 d. Following rewatering, large quantities of PA accumulated in both these leaves, as ABA disappeared (Fig. 6). PA levels gradually declined in all three leaf types in the succeeding days (Fig. 7).

ABA-GE levels began to rise at the same time as ABA. The mature and half expanded leaves accumulated ABA-GE quite rapidly after the midpoint of the stress period (Fig. 8). However, these leaves contained much less ABA-GE than either ABA or PA (Figs. 6 and 7). In the young leaves, the level of ABA-GE increased only to a limited extent during stress. Once the stress was relieved, the mature leaves stopped accumulating ABA-GE but, in agreement with earlier work (22), this level of ABA-GE was maintained in the leaves for at least the next 11 d. The pattern of ABA-GE accumulation was somewhat different in the half expanded leaves. To interpret the results, dilution of ABA-GE, caused by the expansion of the leaf lamina upon rewatering, must be taken into account. For example, the half expanded leaves contained 21.7 ng·mg^{-1} dry weight 14 h after rewatering. The mean rate of expansion of the area of the leaf from which the discs were taken, between this and the next harvest, was estimated from Maksymowych (Ref. 10, Fig. 30) to be 37%. Furthermore, the dry weight per unit area of the leaves decreased as they expanded and necessitated an additional adjustment of 6% to standardize the ABA-GE levels in the leaves at the two harvests to the same area and dry weight. Thus, the expected ABA-GE content at the next harvest, if there was no further accumulation, would be 14.9 ng·mg^{-1} dry weight. The actual value of 20.5 ng·mg^{-1} dry weight was substantially higher. This implies that ABA-GE continued to accumulate in these leaves between these two harvests (14–38 h after rewatering). A similar calculation predicts that the leaves would contain 14.1 ng·mg^{-1} dry weight at the next harvest, which agrees quite well with the observed 15.7 ng·mg^{-1} dry weight. Hence, it is clear that, in contrast to the mature leaves, the half expanded leaves continued to accumulate ABA-GE for 2 d after the relief of water stress, although not thereafter. The young leaves also continued to accumulate ABA-GE slowly for at least 2 d after rewatering. The subsequent decrease in ABA-GE can also be attributed to dilution in these expanding leaves.

DETACHED LEAVES

Accumulation of ABA, PA, and ABA-GE in Response to Water Stress. The ability of detached leaves to both synthesize and catabolize ABA was investigated in leaves of three ages comparable with those used on intact plants (Fig. 9). When stressed, mature leaves produced an amount of ABA comparable with that accumulated by similar leaves on intact plants (Fig. 9). Detached, half expanded leaves also produced ABA when stressed, but considerably less than when attached to the plant. Detached young leaves produced little ABA under stress conditions. Stressed, detached leaves of all three sizes accumulated less PA and ABA-GE than when on the intact plant. For PA, this difference was most pronounced in the young leaves. Detached mature and half expanded leaves still catabolized their ABA to PA and ABA-GE to a considerable extent, in agreement with earlier work with mature leaves (22). The capacity to both synthesize and catabolize ABA under stress conditions increased with leaf age (Fig. 9). This was confirmed by analyses of variance that showed significant interactions of stress condition and leaf age for the three metabolites ($P < 0.01$). Similar results for detached leaves were also obtained with stress periods of 6 and 24 h.

Catabolism of Radioactive ABA and PA. The ability of *X. strumarium* leaves of different ages to catabolize ABA was further investigated by feeding (±)-[^{14}C]ABA to turgid leaves. The amount of radioactivity in ABA and its catabolites, at various times after feeding, was expressed as a proportion of the total radioactivity recovered (Fig. 10). ABA is converted to PA by hydroxylation of one of the 6′ methyl groups. In *Xanthium*, PA is then conjugated to the glucose ester, PA-GE (23). ABA is also catabolized to ABA-GE by conjugation at the carboxyl group of ABA. Catabolite 'C' arises by hydroxylation of the 2′ methyl group of (−)-ABA (2). As shown in Figure 10, most of the labeled ABA in the mature leaves was catabolized within 12 h of feeding and little remained after 24 h. However, neither the young, nor the half expanded leaves, catabolized the applied ABA to a great extent (Fig. 10). In contrast, when [^{14}C]PA was fed to turgid leaves, all three leaf types were able to conjugate PA to PA-GE to approximately the same extent (Table I). This result indicates that the block in ABA catabolism in immature leaves is located between ABA and PA.

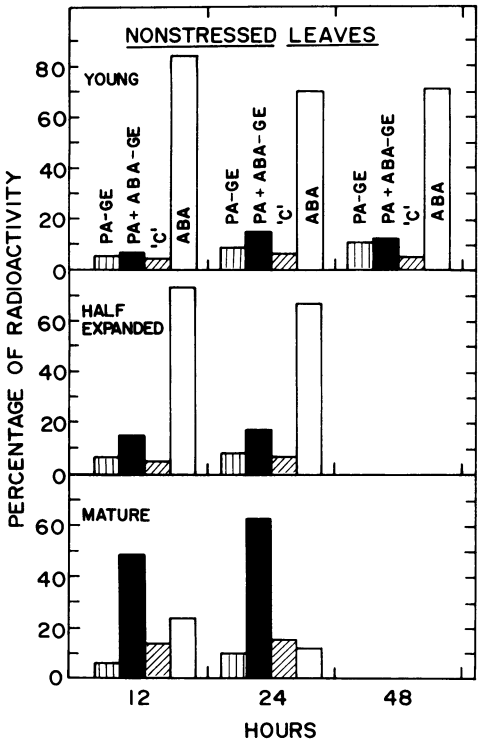

FIG. 10. Percentage of radioactivity in ABA and its catabolites after feeding (±)-[^{14}C]ABA to detached turgid leaves of three different ages. Total amounts of radioactivity recovered were as follows. Young leaves, after 12 h—0.26 μCi; 24 h—0.16 μCi; 48 h—0.23 μCi. Half expanded leaves, after 12 h—0.52 μCi; 24 h—0.63 μCi. Mature leaves, after 12 h—0.77 μCi; 24 h—0.81 μCi. (For the chemical nature of the catabolites, see "Results.")

Table I. *Total Radioactivity Recovered as PA and Its Catabolite PA-GE, and Their Ratio, 24 Hours after Feeding [^{14}C]PA to Detached, Turgid Leaves of Three Different Ages*

A total of 0.2 μCi [^{14}C]PA was divided and fed to the leaves: four young, two half expanded, one mature.

Leaf Type	PA	PA-GE	PA-GE / PA
	nCi		
Young	4.40	1.76	0.40
Half expanded	8.75	2.41	0.28
Mature	9.80	2.91	0.30

Turgid half expanded leaves did not catabolize ABA to any great extent in the labeling study (Fig. 10), but accumulated relatively high ABA-GE levels when stressed on the plant (Fig. 8) and in stressed detached leaves (Fig. 9). This suggests that water stress may trigger ABA catabolism in half expanded leaves. The results of the experiment designed to investigate this possibility (Table II) clearly show that stressing detached leaves did not affect the rate of ABA catabolism (treatments i and ii). However, when half expanded leaves were stressed on the intact plant, the rate of ABA catabolism, in subsequently detached leaves, was enhanced (treatments iii to v). Restressing the leaves after feeding the radioactive ABA had an additional effect (treatment iii).

Young leaves catabolized ABA in all five treatments at a rate similar to that observed in turgid leaves (Fig. 10). Thus, water stress did not stimulate ABA catabolism in the young leaves.

Table II. *Percentage of Radioactivity Remaining in ABA 24 Hours after Feeding Radioactive ABA to Detached Half Expanded Leaves*

Each leaf was fed 0.5 μCi (±)-[^{3}H]ABA or 0.1 μCi (±)-[^{14}C]ABA (36 μCi·mmol^{-1}). Mean recovery of radioactivity per leaf: ^{3}H, 0.14 μCi; ^{14}C, 0.044 μCi. One replicate of each treatment was fed (±)-[^{14}C]ABA, the remaining replicates were fed (±)-[^{3}H]ABA. Replication details in "Materials and Methods."

Treatment	Radioactivity in ABA
	%
(i) Non-stressed plant; leaf detached, labeled.	51
(ii) As (i), then stressed.	52
(iii) Stressed plant; leaf detached, labeled, restressed.	31
(iv) Stressed and recovered plant; leaf detached, labeled.	40
(v) As (iv), then restressed.	37

DISCUSSION

The results presented in this paper demonstrate the importance of the physiological age of leaves for ABA metabolism. The rates of accumulation and degradation, and the levels of the three compounds measured, ABA, PA, and ABA-GE, varied considerably with leaf age in both turgid and stressed plants.

PA began to accumulate in leaves of all three sizes 12 h after the final watering which implies that synthesis and degradation of stress-induced ABA had begun. Actual increases in ABA and ABA-GE levels were apparent after 20 h; the rate of ABA synthesis only then surpassed its rate of degradation. Ψ_P, in the mature leaves, had declined considerably by the time the first rise in PA concentration was apparent, although zero turgor had not yet been reached (Fig. 5). However, it is probable that a certain proportion of the mesophyll cells in mature leaves had lost turgor and provided the necessary stimulus for stress-induced ABA synthesis. The initial PA accumulation by the half expanded and young leaves probably arose from a rapid redistribution of the newly synthesized PA throughout the plant, as these leaves still maintained a high Ψ_p at this time (Fig. 5).

Stress-induced ABA metabolism in the plants was found to respond rapidly to quite small changes in water status; as the leaves regained turgor during the second dark period, ABA and PA levels concomitantly declined. Thus, it seems clear that zero turgor is the critical factor that triggers stress-induced ABA metabolism, which agrees with the conclusions of Pierce and Raschke (13, 14).

Comparisons of the metabolite levels in leaves stressed after excision, and when left on the intact plant, provide insight into the patterns of transport that take place when the entire plant is water stressed. It is significant that the young leaves accumulate high levels of ABA and PA, but very little ABA-GE, when the intact plant is stressed. In contrast, stressed detached young leaves accumulate much less ABA and PA. It has been shown that ABA and PA are both highly phloem-mobile (24), but there is no evidence that ABA-GE can be transported throughout the plant. The results suggest, therefore, that the high levels of ABA and PA observed in young leaves on the stressed intact plant arose predominantly through import from mature leaves which synthesized these metabolites. This conclusion is supported by the findings that the ABA and PA concentrations in phloem exudate of *Xanthium* increase after stress, and that radioactive ABA moves from mature leaves to the shoot tips (24). Movement of radioactive ABA from mature leaves to sink organs has previously been reported in a number of species (5, 6, 8, 15). Export of ABA and PA from individual mature leaves need not be great

to account for the levels accumulated by young leaves, as the ratio of mature to immature leaf tissue is high on *X. strumarium*.

Half expanded leaves, stressed on the intact plant, accumulate more ABA and PA than detached leaves, probably due to a combination of production and import of these metabolites. The decline of ABA in the latter stages of stress (Fig. 6) suggests that these leaves may change from a sink to a source mode and export ABA to the young leaves as the stress becomes more severe. This idea is supported by the results of another stress-recovery experiment of intact *X. strumarium* plants, where the water stress developed more slowly than in the present one (K. Cornish and J.A.D. Zeevaart, unpublished results). A steady decline of ABA in the half expanded leaves was seen during the final stages of the stress period, whereas the mature leaves maintained a constant ABA level.

As noted before (21), ABA levels quickly declined when mature *Xanthium* leaves were rehydrated (Fig. 6), whereas PA levels increased rapidly before declining rather slowly (Fig. 7). ABA fell to the pre-stress level within 14 h of rewatering, but the stomatal resistance was still high at this time (Fig. 2), which demonstrates a lack of correlation between 'bulk' leaf ABA and stomatal opening. It is possible that the high level of PA, which exists in the leaves at this time, is at least partially responsible for the after-effect of stress on stomatal aperture (16).

Turnover of ABA has been shown to be rapid (half-life of about 3 h) in both turgid and wilted bean leaves (7). However, it was suggested that the turnover rate in the turgid leaves may have been spuriously high (11), because it was determined after feeding excessive amounts of radiolabeled ABA. The fed ABA raised the concentration in the cytosol to levels comparable with those developed during wilting. This argument may also be applied to the turnover of ABA in turgid mature *X. strumarium* leaves. The rapid ABA catabolism seen in the labeled leaves may simply result from the high internal ABA concentration mimicking the stress condition. If a high level of internal ABA is all that is required to trigger its rapid breakdown, then detached turgid and stressed half expanded leaves also ought to increase their rate of catabolism. This did not occur (Table II), however, and it is probable that the rapid ABA catabolism in turgid mature leaves is not an artifact of feeding.

The block in ABA catabolism seen in the immature leaves (Fig. 10) may be related to the fact that the younger leaves on a plant are the last ones to reach zero turgor when drought occurs (Fig. 5). Half expanded leaves do have the ability to increase their rate of ABA catabolism when stressed on the intact plant. The increase in rate is possibly due to import of material from the mature leaves. The young leaves have not yet developed the same potential for a rate increase.

In *Xanthium*, the oxidation of ABA to PA is followed by the conjugation of PA to the glucose ester (PA-GE) (23). It is not clear why the enzyme responsible for this second step should be active in all the leaves, regardless of age (Table I), while the enzyme that presumably supplies its substrate, PA, is not.

In conclusion, mature leaves on intact *Xanthium* plants wilt before young ones as water stress takes effect. The young leaves synthesize little ABA themselves, but accumulate higher levels than the mature ones during stress due to import of ABA. The young leaves also catabolize ABA very slowly which aids in maintaining their high ABA level.

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