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Abscisic Acid and Abscission of Young Cotton Bolls in Relation to Water Availability and Boll Load[1]

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ABSTRACT

A field experiment was conducted to determine whether two factors, water deficit and fruit load, that are known to affect fruit abscission might also affect the concentrations of ABA in young cotton (*Gossypium hirsutum* L. 'Deltapine 70') bolls. The effects of water deficit were estimated by comparing ABA concentrations and abscission rates of bolls in control and stressed plots, and by following changes during irrigation cycles. The effects of fruit load were determined by removing about ½ of the flowers in partially defruited plots, and by comparing changes as boll load increased during the season.

Both ABA concentration and abscission rate of bolls increased with water deficit and decreased with relief of stress by irrigation. Boll abscission rates increased as boll load increased, and were lower in the partially defruited than in the fruited plots. Bolls in the partially defruited plots contained less ABA on some, but not all, harvest dates. Water deficit apparently had a greater effect than boll load on the ABA content of young bolls.

The results indicate that water deficit increases the ABA content of cotton bolls, and they provide additional circumstantial evidence that ABA is one regulator of young boll abscission in cotton. However, differences in ABA content did not account for increased boll abscission rate with increasing boll load during the season nor for differences in abscission between fruited and partially defruited plants.

Additional index words: ABA, Boll-shedding, Competition, Ethylene, Fruit-loss, Gossypium hirsutum L., Hormones, Irrigation, Stress.

Abscission is hormonally regulated. Ethylene and abscisic acid (ABA) are thought to stimulate abscission whereas auxin usually inhibits abscission (1). A difference of opinion has existed for a number of years as to the relative importance of ethylene and ABA in causing abscission of cotton bolls (fruits). Davis and Addicott (6) compared abscission rates and ABA contents of Acala 4-42 and Acala SJ-1 bolls. Acala 4-42 contained more ABA and shed more bolls than SJ-1. Furthermore, abscising bolls contained more ABA than retained bolls late in the season (but not early). Bhardwaj and Dua (2) found more "abscisins" in the pericarps of 'H 14', a high-shedding cultivar, than in those of a low-shedding cultivar. Dale and Milford (5) and Cognée (4) questioned the effectiveness of ABA in causing boll shedding because application of boll extract (Dale and Milford) or ABA (Cognée) did not stimulate abscission in their tests. Conversely, Varma (23) reported that application of ABA promoted abscission of flower buds and bolls. Work by Morgan and co-workers (e.g., 16) established ethylene as a potent abscission-promoting hormone.

Boll abscission is stimulated by water deficit (18), as is ethylene evolution (12). Water deficit can cause a rapid increase in ABA content of leaves, including cotton leaves (17, 19), but no reports have been published showing the effects of water deficit on ABA content of cotton bolls.

Boll abscission rate also increases during the season (24), but is decreased by partial defruiting (8, 20). As with water deficit, the effects of fruit load on ABA content of cotton have not been reported, but Tamas et al. (22) investigated the effects of older fruits of bean (*Phaseolus vulgaris* L.) on the ABA content and abortion rate of younger fruits. Fruit removal decreased the ABA content and increased the retention of younger fruits. Cotton bolls contain a higher concentration of ABA than leaves (Guinn, unpublished data). If older bolls are a source of ABA for younger bolls, and if ABA causes boll abscission, an increase in ABA could be a cause of the increased rate of boll abscission that has been observed with increasing boll load.

This paper reports experiments conducted to determine the effects of water deficit and partial defruiting on ABA content and abscission rate of cotton bolls under field conditions. ABA contents and abscission rates were also measured through the fruiting season to determine possible changes with increasing boll load.

MATERIALS AND METHODS

Experiments were conducted at the Univ. of Arizona Cotton Research Center in Phoenix in a field that was dead level (established with a laser plane). The soil is an Avondale clay loam (a member of the fine-loamy, mixed, hyperthermic family of Typic Torrifluvents). A preplant irrigation was applied to wet the soil to field capacity to a depth of more than 160 cm after a rainy winter. Cotton (*Gossypium hirsutum* L. 'Deltapine 70') seeds were planted on 14 Apr. 1980, and the seedlings hand thinned to a stand of about 75,000 plants per ha. Berms were constructed to give 36 basins 12 rows wide by 30 m long. Six irrigation treatments were replicated six times in a Latin square, but only four of the treatments were used for the tests reported here. The irrigation treatments differed until mid-July, after which all plots were irrigated on 15 July, 29 July, and 12 August. Earlier irrigations were as follows: A was irrigated 28 May, 11 and 24 June, and 8 July. B, E, and F were irrigated as shown in Table 1. Approximately 12 cm of water were applied at each irrigation, except for 15 cm on 28 May and 4 June. Boardwalks between Rows 4 and 5 permitted access during and after irrigation with minimal soil compaction.

Water Deficit. The effects of water deficit on ABA concentration and abscission rates of bolls were estimated by comparing irrigation Treatment B (control) with E and F (stressed), and by following changes in Treatment B during an irrigation cycle. White blooms were tagged in 8-m segments of Rows 4 and 5 of Treatments B, E, and F on the same day. Bolls were harvested 3 days later from Row 4 of each plot for ABA analysis, but were left on Row 5 for subsequent abscission measurements. Midday leaf potentials were determined with a pressure chamber for three leaves in each plot.

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Table 1. ABA concentration in 3-day-old bolls and boll abscission rates as influenced by water deficit.† Irrigation amounts and midday leaf water potentials are shown in parentheses.

Date	Irrigation treatment B		Irrigation treatment E		Irrigation treatment F	
	ABA content	Abscission	ABA content	Abscission	ABA content	Abscission
	μg/g	%	μg/g	%	μg/g	%
28 May					(Irrigated, 15 cm)	
4 June	(Irrigated, 15.2 cm)					
17 June	(Irrigated, 12.8 cm)					
24 June			(Irrigated, 12.2 cm)			
30 June	(Irrigated, 10.1 cm)					
2 July	(−19.1 bars)				(−27.8 bars)	
3 July	1.23 ± 0.10	19 ± 3			2.05 ± 0.28	68 ± 8
6 July	1.41 ± 0.15	34 ± 8			3.04 ± 0.22	80 ± 7
8 July			(−29.5 bars)		(Irrigated, 12.2 cm)	
10 July	1.87 ± 0.15	69 ± 9			2.46 ± 0.11	75 ± 8
10 July					(−22.5 bars)	
14 July	3.02 ± 0.29	89 ± 5	3.76 ± 0.22	92 ± 3		
14 July	(−28.6 bars)		(−29.7 bars)			
15 July	(Irrigated, 12.2 cm)		(Irrigated, 12.2 cm)			
17 July	(−22.8 bars)					
18 July	2.04 ± 0.21	59 ± 6	2.23 ± 0.32	51 ± 11		

† Data are averages of six replications and standard errors of the means are shown. In relation to bolls, dates refer to time of harvest of 3-day-old bolls.

Boll Load. The effects of boll load on ABA concentration and abscission rate of bolls were estimated by comparing normally fruited and partially defruited rows, and by following changes during the season as the boll load increased. To minimize the confounding effects of water deficit, this test was conducted in irrigation treatment A and white blooms were tagged 3 days after each irrigation. Row 5 was partially defruited by removing all visible pink blooms on Monday and all visible white blooms on Monday, Tuesday, and Wednesday of each week from 8-m of row in each of the six A plots. Blooms were not removed from Row 4. White blooms were tagged on Thursdays and Fridays in a 8-m segments of Rows 4 and 5 (normally fruited and partially defruited, respectively). Blooms tagged on Thursday were left for subsequent abscission measurements (both rows). Blooms tagged on Friday were harvested 4 days later for ABA analysis (both rows).

ABA Measurement. Freshly harvested bolls were frozen at −90 C, lyophilized, weighed, and ground to pass a 40-mesh screen. They were stored dry in a freezer until analyzed. Dry samples of 200 mg each were extracted with 30 ml of 80% methanol to which 1 μg of ABA (racemic mixture of ct and tt ABA) had been added as internal standard. Extraction was continued overnight on a magnetic stirrer at about 4 C in stoppered 125-ml flasks. Insoluble material was removed by centrifugation and 10 ml of 1% (w:v) $NaHCO_3$ was added to the supernatant fraction. Methanol was evaporated in vacuo and lipids were removed with three 10-ml portions of ethyl acetate. The pH of the aqueous fraction was adjusted to 3.0 with HCl and ABA was extracted with three 10-ml portions of ethyl acetate. The samples were dried in vacuo, dissolved in 1 ml of absolute methanol followed by 4 ml of 1 mM HCl, and loaded onto a 2 × 4-cm column of preconditioned Polyclar AT[3] (13). The ABA was eluted with 1 mM HCl; the first 20 ml were discarded and the next 50 ml retained. Others (9, 15) have used Polyclar AT at higher pH values for partial purification of ABA extracts. However, it is much more effective at low pH (10); most of the extracts were colorless after this treatment, even when highly concentrated.

The ABA-containing fraction from the column was concentrated to 10 to 15 ml in vacuo at no more than 25 C with a rotary evaporator attached to a freeze drier. The ABA was extracted with diethyl ether which was then evaported in vacuo. The ABA was transferred to small glass tubes with diethyl ether and methylated with diazomethane (21). The sample was then evaporated to dryness with a stream of N_2 and dissolved in 0.1 ml of pyridine followed by 0.4 ml of hexane (3). A gas chromatograph with electron capture detector was used to measure the ABA. A 0.32 × 183-cm column of 3% OV-1 on Chromosorb (W(HP)[3]80/100 mesh was maintained at 220 C. Nitrogen at a flow rate of 30 cm^3 min^{-1} was used as the carrier gas. Injector and detector temperatures were 300 and 310 C, respectively. Internal and external standards permitted correction for losses. (Pure tt ABA was not available. The peak area for tt ABA was about 2.1 times the area for ct ABA in the racenic mixture, and this ratio was used to calculate the net peak area due to native ABA.) Recovery averaged about 80%. Identity was confirmed by co-chromatography with authentic ct ABA, and by observing a partial change of native ABA to the tt isomer after irradiation with UV light (15).

Boll Abscission. Plants were harvested at the end of the season (18 September to 23 October). Tags remaining on non-abscised bolls were sorted by dates and used to calculate abscission rates (i.e., by comparing the numbers recovered with the numbers applied).

RESULTS AND DISCUSSION

Water Deficit

ABA content and boll abscission rate both increased with water deficit and decreased after stress was relieved by irrigation (Table 1). The correlation coefficient of ABA concentration in 3-day-old bolls with boll abscission rate was significant at the 0.01 level ($r = 0.70$, $n = 57$).

The results were influenced by the fact that conditions changed with time. Although irrigation Treatment B was intended as non-stressed control, the average midday leaf water potential decreased from −19.1 bars on 2 July to −28.6 bars on 14 July. Therefore, only the first two or three dates can be considered as control or non-stressed. The ABA content of bolls was probably influenced by water status during the 3 days from anthesis to harvest. Boll abscission was probably affected by water status from the day of anthesis until abscission occurred, which can be several days after anthesis (14). In relation to bolls, the dates in Table 1 refer to day of boll harvest rather than day of anthesis. Because bolls abscise at variable times after anthesis it is not possible to assign dates when boll abscis-

[3]Mention of a trade name or proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

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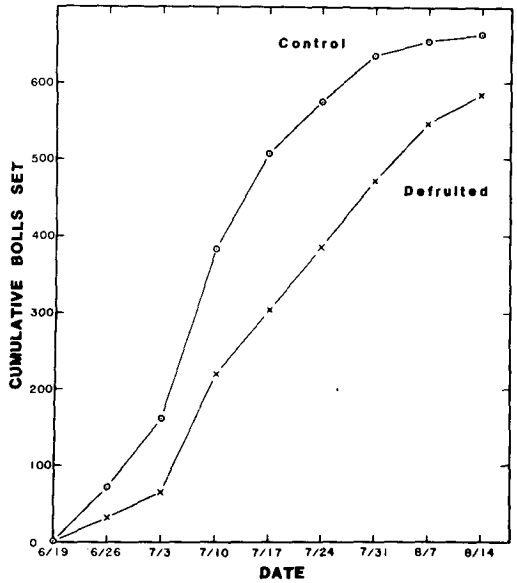

Fig. 1. Comparison of boll load in control and partially defruited plots during the season. Data points are averages of six replications and are cumulative numbers of bolls set from flowers that appeared by the dates indicated.

sion occurred. Recent results (Guinn, unpublished data) indicate that bolls abscise as soon as 2 days after anthesis and abscission rate increases to a maximum at 6 days after anthesis. Therefore, the dates do not indicate when bolls abscised but indicate that they were in the same chronological population as those harvested for ABA analysis. Bolls used for ABA measurement and abscission estimates were tagged at anthesis 3 days before the dates shown in Table 1.

Results obtained in 1981 (unpublished) indicate that leaf water potential increases rather slowly after irrigation and does not reach a maximum (least negative number) until 6 or 7 days later. Therefore, some stress may have persisted during the 3-day interval before the 18 July harvest in Treatments B and E, and before the 10 July harvest in Treatment F. In Treatment F, young bolls were exposed to 1 day of severe stress because irrigation did not occur until the day after anthesis. These factors may account for the relatively high ABA contents and boll abscission rates just after irrigation (Table 1).

No attempt was made to correlate leaf water potentials with ABA and boll abscission data because they were not determined at or after all tagging dates. It is evident from the data in Table 1, however, that both ABA concentration and boll abscission rate were influenced by stress and irrigation; they both increased with stress and decreased with relief of stress.

Fruit Load

Partial defruiting decreased the number of bolls set by any given date (Fig. 1), but the decrease was not as great as might have been expected because (a) some flowers were missed during defruiting and (b) boll retention rate was higher in the partially defruited than in the fruited control plots (Table 2). Boll abscission rate increased during the season as boll load increased, but the rate for the

Table 2. ABA concentration in 4-day-old bolls and boll abscission as influenced by partial defruiting and increasing boll load during the season.†

Date of anthesis	ABA Content		Boll abscission	
	Fruited	Defruited	Fruited	Defruited
	μg/g		%	
Irrigated 24 June				
27 June	2.10 ± 0.17	1.91 ± 0.06	20 ± 10	13 ± 4
Irrigated 8 July				
11 July	1.99 ± 0.11	2.08 ± 0.17	38 ± 5	20 ± 6
Irrigated 15 July				
18 July	2.67 ± 0.17	1.79 ± 0.06	62 ± 10	36 ± 4
25 July	4.18 ± 0.27	3.15 ± 0.26	87 ± 6	43 ± 5
Irrigated 29 July				
1 August	2.50 ± 0.27	2.66 ± 0.31	70 ± 9	62 ± 8

† Data are averages of six replications and standard errors of the means are shown.

partially defruited plants was lower than that for the fruited controls.

Boll load apparently had little effect on ABA concentration in bolls. The ABA content of bolls increased very little as boll load increased during the season, and the ABA concentration was significantly higher in bolls of fruited than in bolls of partially defruited plants only for bolls that developed from 18 and 25 July blooms.

The data for 25 July were included to show the confounding effects of moisture stress. Bolls developed from these blooms during the period just before the next irrigation. The average leaf water potential decreased to −24.8 bars on 28 July. Stress may have been greater in fruited than in partially defruited plants. Eaton (7) reported that removal of all floral buds as they appeared approximately tripled the weight of roots. A more extensive root system might extract water from a greater soil depth and, thereby, decrease the severity of stress between irrigations. (Leaf water potentials were not determined separately for the fruited and partially defruited plants, however.)

The correlation of ABA concentration and boll abscission rate was low when only data for unstressed plants were included ($r = 0.35$, $n = 57$), but was considerably higher when the 25 July data were included ($r = 0.54$, $n = 69$). Moisture stress apparently had a greater effect than boll load on ABA in bolls.

The facts that abscission rate was higher in normally fruited than in partially defruited plants and that abscission rate increased as boll load increased during the season indicate that boll load influenced boll abscission rate. Lack of corresponding increases in ABA concentration indicates that factors other than, or in addition to, ABA content regulated boll abscission that was stimulated by increasing boll load. Ethylene was probably involved; Guinn (11) reported increasing rates of ethylene evolution with increasing boll load from 25 June to 6 August in an earlier field test. Significant negative correlations between sugar content of bolls and ethylene evolution were found on several dates; these and other data indicated that a nutritional stress stimulates ethylene production in young cotton bolls (11). As sink size (number of bolls) increases in relation to source size (net photosynthesis per plant) competition for available photosynthate could result in a nutritional stress that triggers additional ethylene produc-

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tion. Although abscission rates were not determined, they have been shown in other tests to increase during the seasons as boll load increased (20, 24, and Table 2 of this report).

Ethylene may also have been a factor in the increased boll abscission rates that resulted from a water deficit. An earlier report (12) indicated that water deficit does increase the rate of ethylene evolution from young cotton bolls.

A correlation is a necessary, but not a sufficient, condition for proving a causal relationship. Because ABA is only one of at least three hormones that may regulate abscission, it is difficult to prove a regulatory role for any one of them. A change in one may enhance or counteract a change in another. The poor correlation between ABA concentration and boll abscission rate in the defruiting test may indicate that ABA is not a major regulator of boll abscission that is caused by an increasing boll load (other hormones, such as ethylene, may play a more dominant role). The results do indicate that water deficit can increase both the ABA concentration in bolls and boll abscission rate. The significant correlation provides circumstantial evidence that ABA is involved in regulation of boll abscission that is caused by a water deficit.

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