

Concentrations of Abscisic Acid and Indoleacetic Acid in Cotton Fruits and Their Abscission Zones in Relation to Fruit Retention

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ABSTRACT

An experiment was conducted with field-grown cotton (*Gossypium hirsutum* L.) to determine the effects of drought and an increase in available photosynthate on the abscisic acid (ABA) and indoleacetic acid (IAA) contents of 3-day-old bolls and their abscission zones. Photosynthate availability was manipulated by removing about two-thirds of the plants to permit increased irradiance, and thus photosynthesis, in the plant canopy. The demand for photosynthate was decreased by removing all bolls from the remaining plants. The thinning and defruiting operations were performed about 3 weeks after first flower. Control plants were neither thinned nor defruited. Effects of water deficit were observed by making three harvests at different times during a 2-week irrigation cycle. Increasing the availability of photosynthate increased boll retention, but had relatively little effect on the concentrations of ABA and IAA in bolls. However, it did increase the concentration of IAA in abscission zones. Water deficit increased the ABA content of bolls and abscission zones and decreased the IAA content of bolls and abscission zones. Across all treatments, the IAA content of abscission zones was positively correlated, and the ABA content of bolls was negatively correlated, with boll retention. The results indicate that stresses change the hormonal balance in ways that are consistent with observed increases in fruit abscission.

of IAA in young bolls. Rodgers (26) used a bioassay to estimate the IAA content of bolls and reported that abscising bolls contained much less IAA than retained bolls. Differences were not evident, however, until the bolls were more than 5 d old. Bolls frequently abscise within 5 d of anthesis (13). Changes in hormone concentrations must precede abscission if they are to cause abscission.

The concentration of IAA in the abscission zone may be more important than the IAA content of bolls in regulating boll abscission. Auxin transport inhibitors stimulated leaf abscission (3, 21, 22) presumably because they decreased the concentration of IAA in the abscission zone. It is possible that water deficit affects the IAA content of abscission zones by decreasing auxin transport (5). Water deficit and auxin transport inhibitors had similar effects in promoting leaf abscission induced by ethephon (23). No direct data are available, however, on the effects of water deficit, or a shortage of photosynthate, on the concentration of IAA in abscission zones of either leaves or fruits.

The purpose of the work reported here was to determine the effects of a nutritional stress (shortage of photosynthate) on the ABA and IAA contents of young cotton bolls and their abscission zones in relation to boll retention rates. Because harvests were made at different times during an irrigation cycle, information was also obtained on the effects of water deficit on ABA, IAA, and boll retention. The results support the hypothesis that stresses decrease boll retention because they affect the hormonal balance in bolls and their abscission zones.

MATERIALS AND METHODS

Plant Culture. A field experiment was conducted at the Western Cotton Research Laboratory in Phoenix in 1985 with cotton (*Gossypium hirsutum* L. cv Deltapine 61). Seeds were planted April 8. After germination, seedlings were thinned to approximately 99,000 plants per ha. Treatments were imposed to increase photosynthesis and to decrease the demand for photosynthate about 3 weeks after first flower. The stand was thinned to about 33,000 plants ha⁻¹ on July 1 to permit better light penetration into the plant canopy and thus increase photosynthesis. All bolls were then removed from the remaining plants in those plots to decrease the demand for photosynthate and thus increase the amount available to newly developing fruits. Plants in control plots were neither thinned nor defruited in July.

Each plot was 4 rows (4 m) wide by 8 m long, and the treatments were replicated four times in a randomized complete block. The two center rows were used for boll and abscission zone harvests (one row) and for determination of boll retention rates (the other row).

Flowers were tagged on the day of anthesis with dated tags (both center rows). Bolls and their abscission zones were harvested 3 d later from one of the two center rows. Three harvests were made during the July 3 to July 18 irrigation cycle. (Plants

Abscission of cotton (*Gossypium hirsutum* L.) fruits (bolls) is strongly affected by the balance between production of and demand for photosynthate (10, 15, 20). The early work of Mason (20) led to the nutritional theory of boll abscission which stated that "the cotton plant retains only as many bolls as it can supply with carbohydrates, nitrogen, or other nutrients" (6). Research by Eaton and co-workers (7–9) indicated some inadequacies of the nutritional theory, and much of the subsequent research concentrated on hormonal regulation of abscission. The demonstration that nutritional stress (11) and water deficit (12) increase ethylene evolution in young cotton bolls provided an explanation for the increased boll shedding that occurs when the demand for photosynthate exceeds the supply or when plants are subjected to water deficit (16).

Other plant hormones also affect fruit abscission. IAA usually delays or prevents abscission, probably because it prevents synthesis of the specific cellulase in the abscission zone that weakens cell walls and causes abscission (1, 19, 24). ABA, in contrast, may cause abscission of young fruits (2, 4, 25), possibly by stimulating the production of ethylene (27).

The concentrations of ABA and IAA may be affected by stresses. The concentration of ABA in young bolls increased in response to water deficit (14) and to a decrease in photosynthesis (13). A deficiency of photosynthate could limit the production

Table 1. *Boll Weight, Percentage Boll Retention, and ABA and IAA Concentrations in 3-Day-Old Bolls and Their Abscission Zones as Influenced by Thinning-defruiting and Date of Harvest*

Bolls and their abscission zones were harvested 3 d after anthesis for ABA and IAA analyses. The plants were irrigated on July 3 and 18. Values are averages of four replications \pm SE.

| Treatment | Date Harvested | | |
|-------------------|-----------------|---|-----------------|
| | July 8 | July 12 | July 15 |
| | | <i>dry wt per boll, mg</i> | |
| Control | 243 \pm 6 | 254 \pm 4 | 215 \pm 4 |
| Thinned-defruited | 283 \pm 7 | 289 \pm 6 | 252 \pm 8 |
| | | <i>% retained</i> | |
| Control | 74.0 \pm 4.2 | 51.4 \pm 1.8 | 2.8 \pm 1.3 |
| Thinned-defruited | 89.0 \pm 1.7 | 79.2 \pm 6.3 | 14.8 \pm 1.6 |
| | | <i>$\mu\text{g ABA g}^{-1}$ dry wt of bolls</i> | |
| Control | 2.63 \pm 0.07 | 3.42 \pm 0.15 | 6.40 \pm 0.04 |
| Thinned-defruited | 2.09 \pm 0.09 | 2.75 \pm 0.23 | 5.03 \pm 0.19 |
| | | <i>$\mu\text{g ABA g}^{-1}$ dry wt of abscission zones</i> | |
| Control | 0.51 \pm 0.10 | 1.38 \pm 0.06 | 1.81 \pm 0.02 |
| Thinned-defruited | 0.52 \pm 0.10 | 1.23 \pm 0.06 | 1.79 \pm 0.08 |
| | | <i>ng IAA g⁻¹ dry wt of bolls</i> | |
| Control | 105 \pm 8 | 114 \pm 2 | 61 \pm 4 |
| Thinned-defruited | 92 \pm 5 | 130 \pm 14 | 71 \pm 3 |
| | | <i>ng IAA g⁻¹ dry wt of abscission zones</i> | |
| Control | 91 \pm 2 | 64 \pm 1 | 36 \pm 4 |
| Thinned-defruited | 120 \pm 3 | 84 \pm 7 | 69 \pm 4 |

were irrigated about every 14 d throughout the growing season.) Flowers were tagged on July 5, 9, and 12, and 3-d-old bolls were harvested on July 8, 12, and 15. Tags on retained bolls in the other center row of each plot were retrieved in October for calculation of percentage boll retention.

Plant Material. A portion of the fruiting branch was removed with the boll at harvest. The subtending leaf was removed and the branch was cut about 2.5 cm on each side of the abscission zones before the harvested material was rinsed in tap water and cold deionized H₂O. The samples were transferred, over ice in a small ice chest, to the laboratory. Excess peduncle and fruiting branch were quickly removed by cutting with a razor blade about 2 mm on each side of the junction of the peduncle and the fruiting branch. The resulting abscission zones were immediately placed on ice and then transferred to a -85°C freezer. Bolls were cracked or cut open to facilitate drying and also quickly frozen at -85°C . The tissues were lyophilized, weighed, ground to pass a 40-mesh screen, and stored in sealed vials at -85°C .

ABA and IAA Analyses. ABA and IAA were extracted, purified, and estimated by HPLC as described earlier (17). Briefly, the method was as follows: Dry 200 mg samples were extracted with 80% methanol that contained BHT¹ and ascorbate as antioxidants. Methanol was removed *in vacuo* at about 35°C . Chl and lipids were extracted with hexane and the pH of the aqueous residue was adjusted to 8 with K₂HPO₄. Some impurities were extracted with ethyl acetate and the pH then adjusted to 2.8 with H₃PO₄. The acidic solution was passed through a C18 cartridge to trap and concentrate ABA and IAA. The cartridge was rinsed with 10 ml of 1 mM HCl. ABA and IAA were then eluted with 15 ml of 0.02 N NH₄OH and the pH of the effluent quickly adjusted to 2.8 with H₃PO₄. Water-washed diethyl ether, to which BHT had been added, was used to extract ABA and IAA from the acidic aqueous solutions. The ether was partitioned against 1 mM HCl and then evaporated *in vacuo*. The residue was

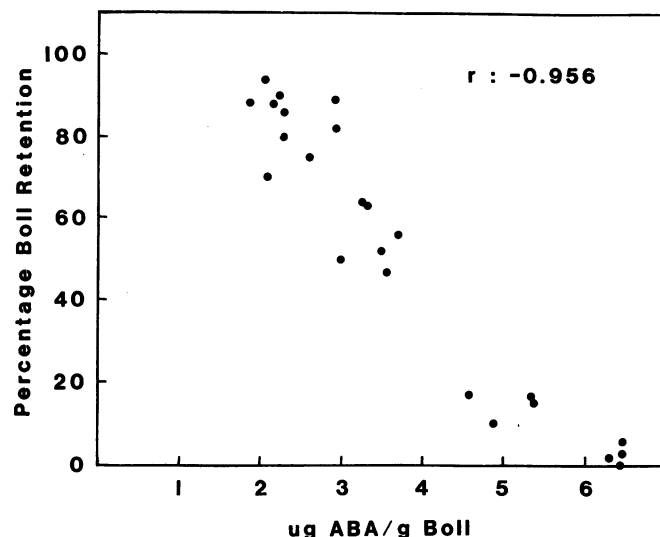


FIG. 1. Correlation of ABA concentration in 3-d-old bolls with percentage boll retention.

dissolved in acetonitrile and filtered with centrifugation through a 0.2 μm nylon filter (Schleicher and Schuell DF 102/1).² The solvent was evaporated under a stream of N₂ at about 35°C .

The samples were further purified by HPLC, first on a SAX column developed with 80% methanol-0.02 N acetic acid, and then on a C18 column developed with 50% methanol-0.02 N acetic acid. (Only the fractions that contained ABA and IAA were collected from the SAX column for further purification on the C18 column.) ABA was detected by *A* at 254 nm, and IAA was detected by natural fluorescence at 254 nm excitation and

¹ Abbreviations: BHT, butylated hydroxytoluene or 2,6-di-tert-butyl-*p*-cresol; SAX, strong anion exchange; T-DF, thinned-defruited.

² Names of products are included for the benefit of the reader and do not imply endorsement or preferential treatment by the United States Department of Agriculture.

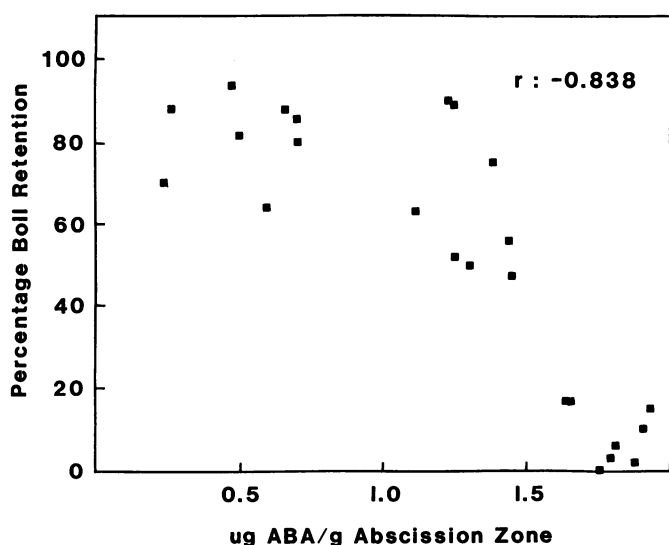


FIG. 2. Correlation of ABA concentration in abscission zones of 3-d-old bolls with percentage boll retention.

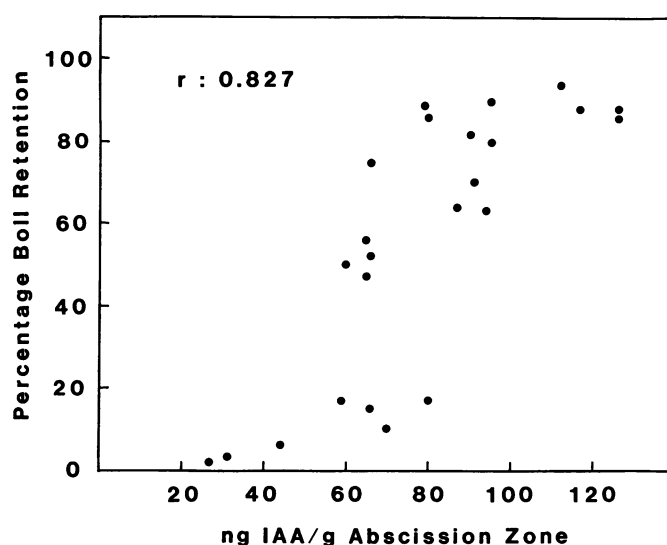


FIG. 4. Correlation of IAA concentration in abscission zones of 3-d-old bolls with percentage boll retention.

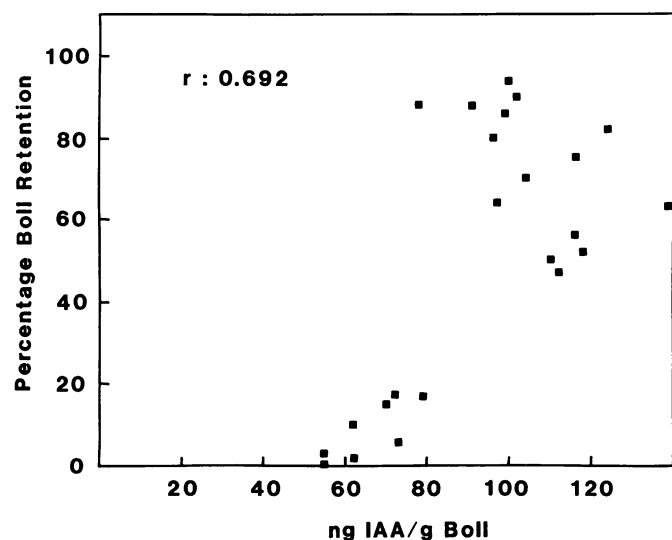


FIG. 3. Correlation of IAA concentration in 3-d-old bolls with percentage boll retention.

360 nm emission. [^{14}C]ABA and [^{14}C]IAA, added to samples at the start of extraction, were used as internal standards. All values were corrected for losses, which amounted to about 25% for ABA and up to 50% for IAA.

RESULTS AND DISCUSSION

Because sugars are metabolized to proteins, lipids, and cellulose in cotton bolls, dry weight was considered a more reliable indication of photosynthate availability than sugar content of the bolls. Weights of retained bolls were consistently higher on the thinned-defruited than on control plants (Table I). Furthermore, plants that were thinned and defruited retained a higher percentage of their young bolls than the controls (Table I), further indicating a difference in the amount of photosynthate allocated to bolls in the treated and control plants.

No rain occurred before the final (July 15) harvest. Plants were visibly wilted by 0900 h on July 15. Midday leaf water potential measurements with a pressure chamber on leaves from similarly wilted plants in the same field gave values of -2.90 ± 0.09 MPa compared with earlier, unstressed values of -1.69 ± 0.11 MPa 3

d after the previous irrigation.

Thinning and defruiting increased boll retention above the control levels at all three harvest dates (Table I). Decreases in boll retention with time probably resulted from a combination of increasing demand for photosynthate (due to increasing boll load) and increasing water deficit (as the soil dried after the July 3 irrigation).

The concentration of ABA in bolls was higher in controls than in bolls from the T-DF treatment, and it increased with time as soil moisture was depleted (Table I). The concentration of ABA in abscission zones was only one-half to one-fifth that of bolls and was not significantly affected by the T-DF treatment. The ABA contents of both tissues increased with time, presumably because of increasing water deficit. The ABA content of bolls increased with water deficit in an earlier test (14), but the ABA content of abscission zones was not determined then.

The IAA content of bolls showed no consistent response to the T-DF treatment, but the IAA content of abscission zones was consistently higher in T-DF than in the controls (Table I). Furthermore, the IAA content of abscission zones decreased with time. The IAA content of bolls decreased only at the final harvest. Changes in the IAA content of bolls and their abscission zones could result from changes in synthesis, transport, conjugation, or destruction of IAA. The data in Table I do not provide evidence for definitive selection among these possibilities. Assuming that IAA is produced in bolls and then transported to abscission zones, similar to auxin transport in petioles (3, 21-23), a mild stress may decrease the movement of IAA from bolls to abscission zones, resulting in a slight accumulation of IAA in the bolls at the expense of IAA in their abscission zones. Severe water deficit, however, decreased the IAA content of bolls as well as that of abscission zones (Table I, July 15 harvest).

The ABA content of bolls showed a strong negative correlation with boll retention (Fig. 1). The ABA content of abscission zones also showed a negative correlation with boll retention (Fig. 2), although not as good as the correlation of ABA content of bolls with boll retention. The IAA contents of bolls (Fig. 3) and their abscission zones (Fig. 4) were positively correlated with boll retention. The IAA content of abscission zones showed a better correlation with boll retention than the IAA content of bolls (Table I; Figs. 3 and 4). The concentration of IAA in abscission zones was highest when boll retention was highest (July 8, T-DF plants), and was lowest when boll retention was lowest (July 15, control plants). The data were subjected to analysis of variance

with treatments as main plots and date of harvest as subplots. Differences in IAA content of abscission zones due to treatment were significant beyond the 0.001 level of probability. Differences with time were significant beyond the 0.01 level.

The IAA content of abscission zones is probably more important than the IAA content of fruits in regulating fruit abscission. Research with leaf abscission indicated that application of IAA to debladed petioles decreased and delayed the appearance of cellulase in the abscission zone that resulted from deblading or exposure to ethylene (1, 19, 24). Morgan and Durham (21) showed that auxin-transport inhibitors stimulated leaf abscission, both in the presence and in the absence of exogenous ethylene. Our results indicated a better correlation of IAA in abscission zones than of IAA in bolls with boll abscission (*cf.* Figs. 3 and 4).

The strong negative correlation of ABA content of bolls with boll retention (Fig. 1) indicates a role of ABA in boll abscission. Water deficit was probably the major cause of the increase in ABA, as was also indicated previously (14). Jordan *et al.* (18) found that water deficit enhanced ethylene-mediated leaf abscission in cotton and suggested that the effect of water deficit was due either to increased production of ethylene or to increased sensitivity to ethylene. ABA may stimulate ethylene production; for example, Sagee *et al.* (27) reported that ABA increased ethylene production in citrus. However, similar experiments have not been conducted with cotton. Water deficit increases the ABA content of young bolls (14) and stimulates their rate of ethylene production (12), but it has not been determined whether the effect on ethylene production is mediated through ABA. In addition, the decreases in IAA content of abscission zones (Table I) probably increased sensitivity to ethylene because these hormones have opposite effects on the production of cellulase in abscission zones (1, 19, 24).

Because all three growth regulators (ABA, IAA, and ethylene) changed in ways that should promote abscission, it is unlikely that any one regulator was solely responsible for decreased boll retention. The results reported here, combined with earlier results obtained for ethylene production (11–13), indicate that a deficiency of photosynthate and a water-deficit stress increase boll abscission rates because they modify the hormonal balance in young bolls and their abscission zones.

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