Abscisic Acid and Cutout in Cotton

Gene Guinn*
United States Department of Agriculture, Agricultural Research Service, Western Cotton Research Lab,
Phoenix, Arizona 85040

ABSTRACT

A decline in growth, flowering, and boll (fruit) retention is referred to as cutout in cotton (Gossypium hirsutum L.). Fruit load affects cutout, possibly through hormonal effects. Experiments were conducted to test the hypothesis that fruits are a source of abscisic acid (ABA) that moves into fruiting branches and growing points where it inhibits growth, flowering, and boll retention. Removal of the flower or young boll at the first node of fruiting branches did not decrease the ABA content of fruiting branches or the abscission zone at the second node. Effects on ABA content of the boll at the second node varied. In one field test, ABA content of bolls at the second node decreased with successive harvests as bolls were removed from first node positions of several fruiting branches. Thus, the effect was cumulative and was not limited to individual branches. Removal of the flower or boll at the first node increased boll retention at the second node. Removal of all flowers during the first 3 weeks of flowering delayed the decreases in growth, flowering, and boll retention that occurred as fruit load increased. But, the ABA content of fruiting branches and mainstem apices was not decreased by early deflouring and did not increase with increasing fruit load. The results do not support the hypothesis that fruits are a source of ABA that moves into fruiting branches and growing points where it then inhibits growth, flowering, and boll retention.

Cotton (Gossypium hirsutum L.) is an indeterminate plant. Nevertheless, most modern cultivars exhibit cycles in growth, flowering, and fruit retention. Growth and flowering slow or stop and fruit retention decreases as fruit load increases. This hiatus in growth, flowering, and fruit (boll) retention is commonly referred to as cutout (19). If cutout occurs too early in the season, it may decrease yield. Conversely, complete and permanent cutout late in the season could be beneficial by depriving insects of a food source before they enter diapause (4). Therefore, elucidation of factors involved in the regulation of cutout is important.

Effects of cutout have been suggested. Fruit load appears to be a major factor; as plants become loaded with bolls, the flowering rate slows and boll retention decreases (11, 19). This behavior could result from competition for nutrients, a change in hormonal status, or both.

Balls (3) referred to cutout as senescence, and suggested that it was caused by “self-poisoning.” Eaton and Ergle (10) suggested that “boll shedding is controlled by the balance between auxin produced in the leaves and an anti-auxin or inhibitory material from developing bolls that is moved out into the fruiting branches.” The subsequent isolation and identification of ABA from cotton bolls provided support for their suggestion (1).

Several lines of evidence suggest that ABA may be a cause of cutout. It was detected in cotton bolls on the basis of its anti-auxin properties (6) and its ability to stimulate abscission (1, 7). Other reports indicated that ABA inhibits nucleic acid and protein synthesis, strongly inhibits growth, and promotes dormancy of buds (2, 17, 24).

Circumstantial evidence also suggests a causal role of ABA in cutout. Water deficits increase the ABA content of leaves (16) and bolls (12), and decrease growth (5), flowering (13), and boll retention (14). Likewise, nitrogen deficits increase the ABA content of cotton (20); decrease growth, flowering, and number of bolls produced (23); and hasten the occurrence and prolong the duration of cutout (Guinn, unpublished data).

Older fruits may produce ABA which then moves to young fruits of soybean (18) and Phaseolus (22) and to axillary buds of Phaseolus (21). Removal of older fruits from Phaseolus plants caused a rapid resumption of axillary bud growth and decreased the ABA content and abortion rate of young fruits (21, 22). Creelman and Sabbe (8) suggested that ABA, produced in cotton bolls, could decrease the rate of elongation of the fruiting branch and decrease the production of fruiting sites.

We conducted experiments with cotton to determine whether the presence of a boll at the first node of a fruiting branch affects growth of that branch; ABA content of the branch, abscission zone, and boll at the second node; and abscission of the boll at the second node. We also conducted a field experiment to determine the effects of fruit load (as it changed during the season and as modified by partial deflouring) on growth, flowering, and boll retention, and on the ABA content of fruiting branches and mainstem apices. These experiments were conducted to test the hypothesis that fruit load causes cutout because fruits increase the ABA content of the cotton plant.

MATERIALS AND METHODS

1981 Field Test. Cotton (cv ‘DPL 70’) was grown at the University of Arizona Cotton Research Center in Phoenix. The seed were planted on April 2 and the seedlings thinned to about 97,000 plants per ha in rows 1 m apart. Irrigations were applied about every 2 weeks from May 21 through August 24. Nitrogen was applied before planting and on June 2 to give a total of 148 kg ha⁻¹. Insect pests were suppressed by insecticide applications as needed.

To determine the effects of a boll at the first node of fruiting branches, we applied dated tags to the petiole of the leaf at that node on the day the flower opened. The flower was either removed (DF) or left to develop into a boll (Control). The treatments were replicated four times in a randomized block. When possible, 20 branches were tagged in each plot each day. The fruiting branches were removed 15 d later for measurements. The presence or absence of bolls at the second node was used to calculate percentage retention at the second node. Bolls at the first node were removed and discarded. Bolls at the second node were removed, freeze dried, and analyzed for ABA. Branch weights (beyond the first node) were determined with leaves and floral buds (squares) present. Leaves and squares were then
removed and branch lengths determined. The branches were cut into short pieces and freeze dried for later ABA analysis.

1982 Greenhouse Test. Seeds of ‘DPL 70’ were planted January 12 in a mixture of equal parts by volume of peat, sand, and vermiculite in 80 14-L plastic containers. The seedlings were thinned to two per container and given a complete nutrient solution three times per week.

This test differed from 1981 field test. Rather than removing the flower at the first node on the day of anthesis, we removed the boll at the first node (BR-1) when a flower appeared at the second node. Because the interval between successive flowers on a fruiting branch is about 7 d, young bolls remained about 1 week before they were removed at the first node. The flower at the second node was tagged with a dated tag. Flowers that were tagged on Mondays, Thursdays, and Fridays were harvested 4 d later for ABA analysis. Flowers that were tagged the other 4 d of each week were left for subsequent boll retention measurements, determined by comparing the numbers of applied and retained tags.

1982 Field Test. Cotton (‘DPL 70’) was grown in the same field as in 1981 and at the same population. Nitrogen was applied before planting and on June 16 to give a total of 155 kg ha⁻¹. Seeds were planted April 7 and irrigations were applied every 2 weeks from May 27 through August 25. Insecticides were applied as needed. The same treatments were imposed as in the previous experiment. Only branches with a boll at the first node and a white (1st d) flower at the second node were selected. The boll was removed at the first node (BR-1) or was not removed (Control). Treatment areas were one row by 8 m with untreated rows on both sides. The treatments were replicated six times in a randomized block. Flowers were tagged from July 8 through 29. Those tagged on July 9, 15, 19, 22, and 29 were harvested 4 d later for ABA analysis, whereas those tagged on all other days were left for boll retention measurements.

1982–1983 Greenhouse Test. To determine the influence of a boll at the first node on the ABA content of the abscission zone of the second node, another greenhouse test was conducted. The ‘DPL 70’ seeds were planted September 17 and cultured in 80 14-L containers of peat, sand, and vermiculite as before. The boll was removed at the first node (BR-1) or was not removed (Control) on the day a flower opened at the second node. The branch was removed 4 d later and abscission zones were harvested at the second node by cutting 2 mm on each side of the junction of the peduncle and the fruiting branch. The 4-d-old bolls were also saved for ABA analysis.

1983 Field Test. The effects of fruit load on growth, flowering, and boll retention—and on the ABA contents of fruiting branches and mainstem apices—were determined in 1983. Seeds of ‘Deltapine 61’ were planted on the Arizona State University Experimental Farm in Tempe on April 25. Seedlings were thinned to about 99,000 plants per ha. Irrigations were applied on May 27, June 20 and about every 2 weeks thereafter. Measurements were made as the fruit load increased during the season, and comparisons were done between control and partially defruited (DF) plants. All flowers were removed as they appeared during the first 3 weeks of flowering (through July 27) in the DF plots while control plants were permitted to fruit normally. The control and DF plots were replicated four times in a randomized block. Each plot was one row by 10 m.

We harvested fruiting branches and mainstem apices weekly from July 6 through August 10. A white flower (in anthesis) at the first node of a fruiting branch was the criterion used to determine the tissues harvested. The mainstem above that fruiting branch was harvested, and the fruiting branch beyond the white flower was harvested. The harvested mainstem apices and fruiting branches were rinsed in cold distilled H₂O and transported, over ice, to the laboratory in insulated chests. Leaves and petals were removed and discarded. Growth was estimated by measuring lengths of fruiting branches and mainstem apices. They were then cut into short pieces, quickly frozen, and lyophilized.

Flowers were counted and tagged two other days each week. Boll retention rates and fruit loads through the season were calculated on the basis of numbers of tagged flowers that were retained.

ABA Analysis. Harvested plant material was freeze dried, ground to pass a 40-mesh screen, and stored dry at -20°C until it could be analyzed. Trans-ABA, purified by HPLC, was added as an internal standard to each sample when 80% methanol was added. ABA was extracted overnight as outlined earlier (12). Samples were filtered with suction, evaporated in vacuo to the aqueous phase, and lipids were extracted with three 10-ml portions of hexane. The pH of the aqueous residue was then adjusted to 2.5 to 3.0 with HCl. ABA was extracted into ethyl acetate (3×10 ml). The ethyl acetate was evaporated in vacuo and the ABA was dissolved in 1 ml of methanol. Four ml of 1 M HCl were added to the dissolved sample and it was then loaded onto

Table 1. ABA Content, Length, and Dry Weight of Fruiting Branches as Affected by a 15-Day-Old Boll at the First Node

<table>
<thead>
<tr>
<th>ABA</th>
<th>Length</th>
<th>Dry Wt</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.81 ± 0.04</td>
<td>93 ± 5</td>
<td>164 ± 12</td>
</tr>
<tr>
<td>DF</td>
<td>0.92 ± 0.05</td>
<td>94 ± 4</td>
<td>164 ± 10</td>
</tr>
</tbody>
</table>

Table 2. ABA Content, Dry Weight, and Percentage Retention of Bolls at the Second Node as Affected by a 15-Day-Old Boll at the First Node

<table>
<thead>
<tr>
<th>ABA</th>
<th>Boll Retention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.23 ± 0.13</td>
</tr>
<tr>
<td>DF</td>
<td>2.42 ± 0.14</td>
</tr>
</tbody>
</table>

Table 3. ABA Content and Percentage Retention of 4-Day-Old Bolls at the Second Node of Fruiting Branches as Influenced by the Presence of a Boll at the First Node

<table>
<thead>
<tr>
<th>Harvest Date</th>
<th>Control</th>
<th>BR-1</th>
<th>Control/BR-1</th>
<th>µg ABA g⁻¹ dry wt</th>
<th>Percentage Boll Retention</th>
<th>µg ABA g⁻¹ dry wt ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 13</td>
<td>4.71 ± 0.44</td>
<td>4.69 ± 0.63</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>July 19</td>
<td>4.71 ± 0.50</td>
<td>3.96 ± 0.26</td>
<td>1.19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>July 23</td>
<td>4.56 ± 0.50</td>
<td>3.49 ± 0.26</td>
<td>1.31</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>July 26</td>
<td>4.78 ± 0.89</td>
<td>3.33 ± 0.46</td>
<td>1.44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>August 3</td>
<td>5.93 ± 1.22</td>
<td>3.92 ± 0.56</td>
<td>1.51</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>4.65 ± 0.35</td>
<td>3.85 ± 0.21</td>
<td>1.21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined</td>
<td>22.0 ± 2.0</td>
<td>41.6 ± 2.9</td>
<td>0.53</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
a 16 cm² column of purified Polyclar AT¹ and eluted with 70 ml of 1 mM HCl. In earlier tests, the eluate was concentrated *in vacuo* at 35°C to about 10 ml and the ABA was extracted into ethyl acetate. In later tests, a Sep-Pak C18 cartridge was attached to the Polyclar drain line with a male luer fitting to collect the ABA as it eluted. (Because of its low polarity, ABA has a high affinity for C18 at low pH and is retained from the aqueous solvent.) The C18 cartridge was flushed with 20 ml of 1 mM HCl followed by air from a syringe. ABA was then eluted from the C18 with 20 ml of ethyl acetate at about 5 ml min⁻¹. Ethyl acetate was evaporated *in vacuo* and the ABA was dissolved in a small amount of methanol.

ABA in samples from the 1981 and 1982 experiments was quantified by GLC. The purified samples were methylated, fractionated on a glass column of OV-1, and the ABA was estimated with an electron-capture detector (12).

Samples harvested in 1983 were quantified by HPLC. A Partisil SAX column was used for further purification, and a 25-cm by 4.6-mm column of 5-µm C18 was used for final fractionation and quantification. ABA was eluted isocratically from the SAX column with 20% methanol-0.05 N aqueous acetic acid (v:v) at 1.5 ml min⁻¹. The ABA fraction was collected, dried *in vacuo*, dissolved in 25 µl of methanol, and chromatographed on the C18 column with 50% methanol-0.1 N HCOOH at 0.8 ml min⁻¹ (isocratic). This procedure gave exceptionally clean chromatograms, and it was possible to use absorbance at 254 nm for quantification without resorting to GLC and its less stable electron-capture detector. *Trans-ABA*, added as internal standard, and native ABA were cleanly separated (baseline resolution). Recovery of the internal standard, added to the tissue before extraction, was usually 70 to 80%. Every sample contained an internal standard and all values were corrected for percentage recovery, whether determined by GLC or HPLC.

### RESULTS

#### 1981 Field Test
Neither the length nor the dry weight of fruiting branches beyond the first node was influenced by removing the flower at the first node (Table I). The presence of a boll at the first node did not increase the ABA content of the fruiting branch. On the contrary, the ABA content of the fruiting branch tended to be higher on most harvest dates in defruited branches (Table I). Likewise, leaves at the first node tended to contain more ABA when no boll was present than when the boll was present (data not shown).

As with leaves and fruiting branches, the presence of a boll at the first node did not increase the ABA content of the boll at the second node (Table II). Therefore, these results do not support the hypothesis that bolls are a source of ABA for other parts of the plant.

Despite the failure of a boll at the first node to increase the ABA content of the fruiting branch or the boll at the second node, its presence did influence boll retention at the second node. Fruiting branches retained a higher percentage of their bolls at the second node when the flower was removed at the first node than when it was permitted to develop into a boll (Table II). These results agree with those of Kerby and Buxton (15) who found that more bolls were retained at the second node when a boll failed to develop at the first node. They attributed the effect to competition between bolls at adjacent nodes for assimilates rather than to a hormonal effect.

The interval between flowers at adjacent nodes on a fruiting branch is about 6 or 7 d. Therefore, the boll at the second node was 8 or 9 d old at harvest 15 d after anthesis at the first node. It could be argued that bolls destined to abscise had already done so, and that abscised bolls might have had a higher ABA content before they abscised. Therefore, subsequent tests were conducted in such a way that bolls were harvested 4 d after anthesis at the second node (presumably before abscission occurred).

#### 1982 Greenhouse Test
Four-d-old bolls at the second node contained 2.83 ± 0.15 and 2.57 ± 0.08 µg of ABA g⁻¹ dry weight on control and BR-1 plants, respectively (averages of six harvest dates from April 2 through 20). Although the differences in ABA contents of bolls were small, boll retention at the second node was much higher on BR-1 than on control plants, 83.8% versus 50.0%. The experiment was terminated before boll retention rates declined to low levels.

#### 1982 Field Test
Boll retention at the second node was also increased in this test by removing the boll at the first node (Table III). Boll retention rates were lower than those obtained in the first two tests, probably because the test was continued until the plants were more heavily loaded and retention rates had declined to low levels. Four-d-old bolls from control and BR-1 plants...
Table VI. Growth of Mainstems and Fruiting Branches in Relation to ABA Content of Control and Partially Defruited Plants

<table>
<thead>
<tr>
<th>Date</th>
<th>C Length</th>
<th>DF Length</th>
<th>C ABA Content</th>
<th>DF ABA Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mainstem from fruiting branch to apex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>July 6</td>
<td>432 ± 9</td>
<td>142.0 ± 0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>July 13</td>
<td>359 ± 19</td>
<td>0.62 ± 0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>July 20</td>
<td>224 ± 15</td>
<td>1.40 ± 0.2</td>
<td>1.43 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>July 27</td>
<td>150 ± 14</td>
<td>1.46 ± 0.7</td>
<td>1.62 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>August 3</td>
<td>70 ± 18</td>
<td>0.96 ± 0.1</td>
<td>0.94 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>August 10</td>
<td>40 ± 8</td>
<td>1.41 ± 0.1</td>
<td>1.42 ± 0.27</td>
<td></td>
</tr>
</tbody>
</table>

Fruiting branch from first node to tip

<table>
<thead>
<tr>
<th>Date</th>
<th>C Length</th>
<th>DF Length</th>
<th>C ABA Content</th>
<th>DF ABA Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 6</td>
<td>214 ± 11</td>
<td>1.52 ± 0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>July 13</td>
<td>187 ± 12</td>
<td>1.35 ± 0.1</td>
<td>1.40 ± 0.40</td>
<td></td>
</tr>
<tr>
<td>July 20</td>
<td>162 ± 11</td>
<td>1.67 ± 0.1</td>
<td>1.70 ± 0.14</td>
<td></td>
</tr>
<tr>
<td>July 27</td>
<td>113 ± 4</td>
<td>1.57 ± 0.1</td>
<td>1.92 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>August 3</td>
<td>45 ± 12</td>
<td>1.20 ± 0.2</td>
<td>1.06 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>August 10</td>
<td>39 ± 11</td>
<td>2.15 ± 0.3</td>
<td>2.50 ± 0.27</td>
<td></td>
</tr>
</tbody>
</table>

contained the same amount of ABA on July 13, but bolls from control plants contained somewhat more ABA thereafter. The difference increased with successive harvests, indicating a cumulative effect of removing bolls at the first node of fruiting branches (Table III).

1982-1983 Greenhouse Test. Removing the boll at the first node did not decrease ABA content of the abscission zone or boll at the second node. On the contrary, abscission zones and bolls at the second node contained slightly more ABA when the boll was absent at the first node (Table IV).

1983 Field Test. Flowering rate decreased as fruit load increased after August 1, and it decreased more in C than in DF plants (Table V). Fruit retention also decreased as fruit load increased, and was lower in C than in DF plants on all dates that flowers were tagged (Table V). These results indicate a strong effect of fruit load on flowering and fruit retention.

Growth of fruiting branches and mainstems also decreased as fruit load increased during the season. Length of mainstem apices decreased greatly from July 6 to August 10 (Table VI). Both fruiting branches and mainstem apices were initially shorter in DF than in C plants, probably because of a stunning effect of handling the plants. (Considerable manipulation was involved in searching for and removing flowers daily for 3 weeks in the DF plots.) However, growth rate of branches and mainstems eventually declined less in DF than in C plants (Table VI).

ABA content did not correlate with these changes. ABA content of fruiting branches and mainstem apices did not increase as growth rate, flowering, and fruit retention decreased. Furthermore, partial defruiting did not decrease the ABA content of fruiting branches or mainstem apices despite the fact that defruiting moderated the declines in growth, flowering, and fruit retention that occurred as fruit load increased.

DISCUSSION

The results do not support the hypothesis that ABA, produced in cotton bolls, moves to other parts of the plant where it inhibits growth, flowering, and retention of younger bolls. Removal of the flower or boll at the first node did not decrease the ABA content of that fruiting branch or the abscission zone at the next node, nor did such partial defruiting consistently decrease the ABA content of the boll at the second node of fruiting branches. Likewise, removal of all flowers during the first 3 weeks of flowering did not decrease the ABA content of fruiting branches or mainstem apices. Furthermore, the ABA content of these tissues did not increase as fruit load increased during the season even though their growth decreased greatly.

Boll retention rates were higher in partially defruited than in control plants, and decreased as fruit load increased during the season. However, defruiting had small and inconsistent effects on the ABA content of young bolls. It appears unlikely that ABA moved from older to younger bolls because the intervening fruiting branch tissue tended to contain less, rather than more, ABA when bolls were present at first nodes. Therefore, in those cases in which more ABA was found in bolls at second nodes, fruit load may have affected the production of ABA in young bolls (rather than transporting ABA from the older bolls to them).

Moisture status affects the ABA content of bolls (12) and may have been a confounding factor in these experiments. Bolls of field-grown plants contained more ABA than those of greenhouse plants. The greatest effect of partial defruiting on ABA content of bolls occurred in the 1982 field experiment. In that experiment, the difference increased with time, indicating a cumulative effect that extended beyond individual fruiting branches. Developing bolls compete strongly for organic nutrients and greatly decrease root growth (9). Therefore, removing the boll at the first node of several fruiting branches might gradually have improved root growth, increased soil moisture extraction capability, and improved the moisture status (relative to the control plants) of these field-grown plants.

Regardless of the cause, the differences in ABA content of bolls due to partial defruiting were relatively small. These results contrast with those of Tamás et al. (22) with Phaseolus. They found that the ABA content of young fruits on control plants was at least twice that of young fruits on partially defruited plants.

A role for ABA in cutout cannot be excluded even though the ABA contents of fruiting branches and mainstem apices did not correlate with fruit load, growth, flowering rates, or boll retention. Growth, flowering, and fruit retention are probably regulated by the balance of several growth regulators. A decrease in hormones such as cytokinins and IAA could make the tissues more sensitive to a given level of ABA. My results, however, do not support the hypothesis that cotton fruits cause cutout by increasing the ABA contents of fruiting branches, younger bolls, and mainstem growing points.

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