

Abscisic Acid: Correlations with Abscission and with Development in the Cotton Fruit

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ABSTRACT

Abscisic acid was measured in developing cotton fruit (*Gossypium hirsutum*) by means of gas-liquid chromatography. High levels of abscisic acid occurred in correlation with abortion and abscission of young fruit, with low germination of immature seed, and with senescence and dehiscence of mature fruit. Declining or low levels of abscisic acid occurred in correlation with the period of most rapid fruit growth and with high germination of immature and mature seed. Young fruit of cultivar Acala 4-42 contained about twice as much abscisic acid as young fruit of cultivar Acala SJ-1, and this difference is correlated with a higher rate of young fruit abscission in Acala 4-42. Young fruit abscising late in the fruiting season contained about twice as much abscisic acid as young fruit abscising early in the fruiting season.

United States Cotton Research Station, Shafter, California, in the summer of 1967. The plants were grown in rows 101.6 cm (40 inches) apart and thinned to one plant every 30.4 cm (12 inches). The cultural practices of irrigation and cultivation were the standard ones of the Station. Flowers at anthesis were tagged on two dates: 6 July (early) and 8 August (late). Only one flower per plant was tagged. On the early date the tagged flowers were either the first or second on the plant; on the late date the plants had numerous flower buds, flowers, and fruits in various stages of development. Fruits were collected at anthesis (day 0) and at 2, 4, 6, 8, 10, 15, 20, 30, 40, and 50 days after anthesis.

Collected fruits were frozen on solid CO₂ and stored at -20 C until 5 min before extraction. Fifteen fruits 0 and 2 days old, 10 fruits 4, 6, and 8 days old, and five fruits each in the samples of 10 days and older were extracted. Before extraction bracts and pedicels were removed from the fruits, and fruits 15 days and older were separated into (a) fruit walls and (b) lint and seed, and the two portions extracted separately. Samples were ground in 80% (v/v) acetone, acid-base fractionated, and the acid fraction further purified by column chromatography. The trimethylsilyl derivative was prepared by reaction of the purified fraction with bis(trimethylsilyl) acetamide and assayed by gas-liquid chromatography. The amounts of ABA present were estimated by comparison of peak heights with those from known amounts of racemic ABA (sample courtesy of Shell Development Co.). The methods of extraction, purification and chromatography have been previously described by Davis *et al.* (8).

Abscisic acid was isolated and identified from the young fruit of cotton (12, 13) and was demonstrated to be an abscission-accelerating hormone of young cotton fruit (3). It occurs in the fruits of a wide variety of plants (see 4, 10); however, knowledge of the occurrence of ABA in correlation with the development and/or abscission of fruits is still quite limited. Van Steveninck (22) found that the occurrence of an inhibitor, now known to be ABA (14), was correlated with the abscission of young lupin fruit. Rudnicki and colleagues found ABA to increase with ripening of strawberry fruits (16) and of pears (17) and during the development of apple fruits (18).

In bioassays for ABA the interference of other hormones has been a serious hazard. However, the recent development of chemical assays with a high degree of specificity (6, 8) enables measurement of ABA independently of the other hormones. The research reported in this paper was undertaken with two objectives: (a) to confirm and extend with quantitative data the occurrence of ABA in correlation with young fruit abscission in cotton, and (b) to obtain similar data over the entire life-span of the cotton fruit and correlate that information with the events that take place in the course of the development and maturation of seed, fiber, and walls of the cotton fruit.

MATERIALS AND METHODS

The cotton used in these experiments was *Gossypium hirsutum* L., cultivars Acala 4-42 and Acala SJ-1, grown at the

RESULTS

Determination of the amounts of ABA in cotton fruit from the time of anthesis until dehiscence was done with fruit from the early tagging of cv. Acala SJ-1. The results are presented graphically in Figure 1. The more important aspects of these results include: (a) the amount of ABA in the fruit at anthesis was low and declined during the next two days. (A similar decline was observed in all of the samplings, both early and late. See Figs. 3 and 4). (b) The amount of ABA in the fruit increased over 15-fold between the 2nd and 10th days of development. (c) The amount of ABA decreased to a very low level by 20 days and was undetectable at 30 days. (d) by 40 days the amount had risen again and was above the previous high of the 10th day; and by 50 days when the fruit were mature and dehiscing, the highest amounts (4.7 μg/fruit) were found.

The various cultivars of cotton differ in their propensity to abscise young fruit. Of the two cultivars we investigated, Acala 4-42 abscises appreciably more young fruit than does Acala SJ-1. The young fruit abscission observed in our material is shown in Figure 2 (*cf.* 4-42 early and SJ-1 early).

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Acala 4-42 abscised more than twice as many young fruit on the 6th day as did SJ-1, and although subsequent abscission was about equal in the two cultivars, total young fruit abscission by Acala 4-42 was 45% more than that by SJ-1. Measurement of ABA in fruits of the two cultivars disclosed that their young fruit abscission behavior was correlated with the amounts of ABA in the fruits. The results (Fig. 3) show that the young fruit of 4-42 contained appreciably more ABA than did SJ-1, two or more times as much at most sampling dates. The larger amount of ABA in the fruits of 4-42 at 4 days is correlated with their greater abscission at 6 days.

Late in the growing season there is much more abscission of young cotton fruit than there is early in the season. Data collected from our material illustrate this behavior pattern (Fig. 2, *cf.* SJ-1 early and SJ-1 late). Between the 4th and the 6th days, the rate of abscission of the young late fruit was about three times that of the (young) early fruit. Total abscission of the late fruit was more than twice that of the early fruit. Measurement of ABA in the early and late fruit showed, as expected, that the abscising late fruit did indeed contain more ABA than did the abscising early fruit (Fig. 4, *cf.* late abscised and early abscised). However, an inverse correlation was found for ABA in the early and late nonabscising fruit (Fig. 4, *cf.* early retained and late retained). The probability that other hormones are interacting strongly in the retained fruits, especially early in the season, is considered in the "Discussion."

Investigation of ABA in early and late fruit was coordinated with a parallel and equally important investigation of ABA in abscising and nonabscising young fruit. Abscising young fruit can be removed from the plant by gentle pressure, breaking away at the developing abscission layer (in the abscission zone) at the base of the pedicel. Such fruit are designated "abscised" in Figure 4. Nonabscising fruit can be removed from the plant only by the exertion of considerable pressure and with these the pedicel breaks near the base of the fruit some distance from the abscission zone. These fruit are designated "retained" in Figure 4. For the late samplings, the measurements show that the abscised young fruit contained much more ABA than the retained young fruit. For the early samplings, the measurements show a similar, higher ABA in the abscised fruit at the 6th day, but there was essentially no difference at the 8th day. An explanation of this seeming inconsistency is presented in the "Discussion."

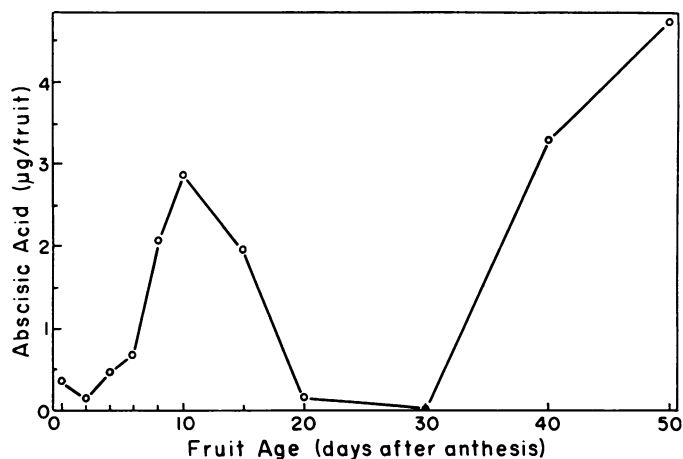


FIG. 1. Abscisic acid in cotton fruit during the course of development. Fruit were from cv. Acala SJ-1 that had flowered early in the season. Each point is the average of four determinations. Note the rapidly rising levels of ABA during the period of young fruit abscission (5th to 10th days), and during the approach of fruit wall senescence and dehiscence (30th to 50th days).

In an earlier, preliminary investigation relatively high amounts of ABA had been detected in the walls of young cotton fruit, while little or none had been found in the seeds, or in the placentae and interlocular walls (3). Since that investigation used inhibition of coleoptile curvature as a measure of ABA, it appeared desirable to repeat it with the gas chromatographic method. At the same time the scope was extended to include fruit up to the time of dehiscence. Each fruit was divided into (a) the "fruit wall" which included the central placental tissue and the interlocular walls, and (b) the "lint and seed" which comprised the contents of the locules. The results (Fig. 5) showed that the walls of the younger fruit contained at least twice as much ABA as the lint and seed, confirming the earlier investigation. In fact, no ABA was detected in the lint and seed at 15, 20, and 30 days; and in keeping with the results of the whole fruit measurements (Fig. 1), no ABA was found in the fruit wall at 30 days. The rapid rise of ABA in the fruit walls after 40 days is correlated with the senescence and dehiscence of the walls. The equally rapid rise of ABA in the lint and seed between 30 and 40 days and

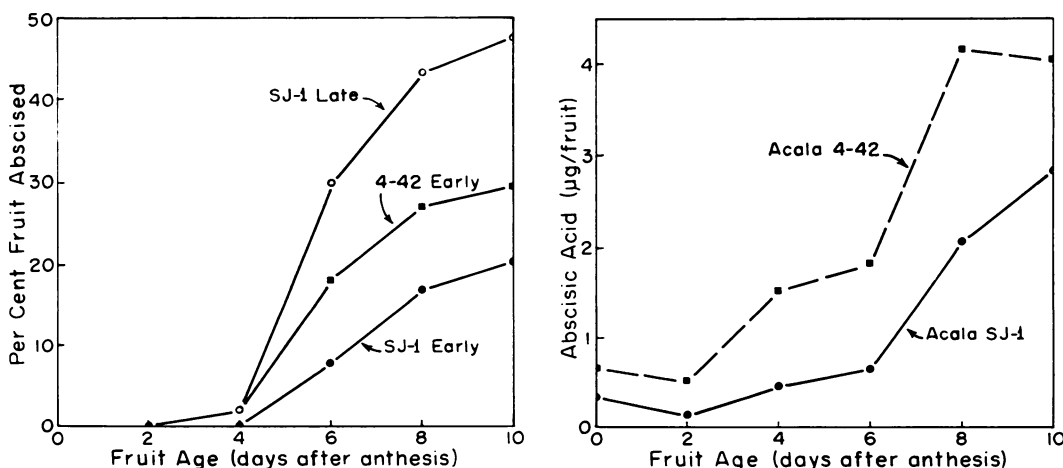


FIG. 2. Time course of young fruit abscission of cotton early in the fruiting season and late in the fruiting season. Tagged were: 1395 flowers of Acala SJ-1 early; 675 flowers of Acala 4-42 early; and 1791 flowers of Acala SJ-1 late.

FIG. 3. Abscisic acid content of young fruit of two cultivars of cotton collected early in the season. Each point is the average of four determinations. Note that the higher amounts of ABA in Acala 4-42 are correlated with greater abscission of young fruit (*cf.* Fig. 2).

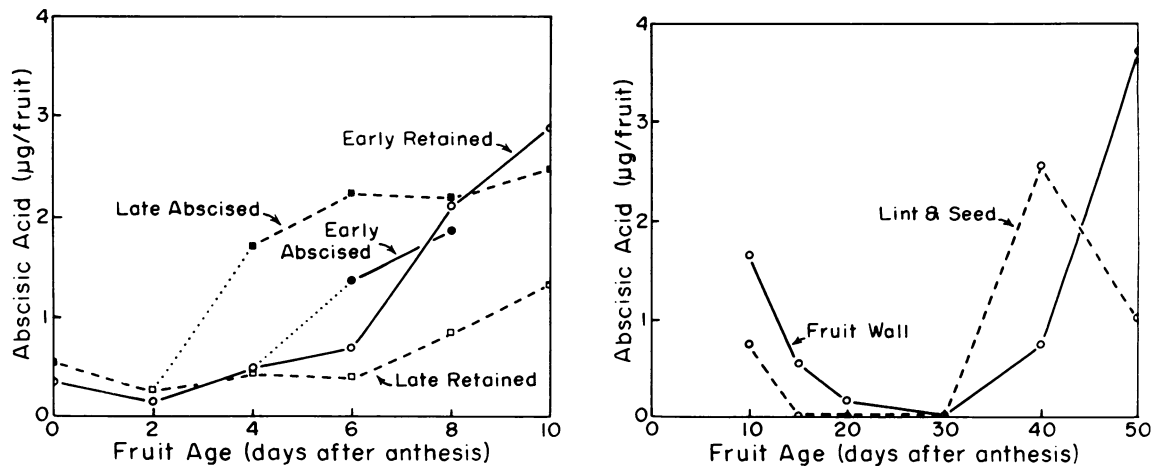


FIG. 4. Abscisic acid in "abscised" and "retained" (nonabscising) young cotton fruit of cv. Acala SJ-1, early and later in the fruiting season. Each point is the average of three or more determinations. The finely dotted lines indicate that abscission of the "early abscised" fruit did not begin until sometime between the 4th and 6th days, and abscission of the "late abscised" fruit did not begin until sometime between the 2nd and 4th days. Note the much higher levels of ABA in the late abscised fruit than in the late retained fruit. Curves for the early fruits are discussed in the text.

FIG. 5. Abscisic acid in the fruit wall (including placenta and interocular walls) and in the lint and seed of cotton fruit during development (cv. Acala SJ-1, "early" collection). Each point is the average of two determinations. Note that after the 40th day ABA decreased in the lint and seed, and rapidly increased in the fruit wall. These changes are correlated with increased germinability of the seed and with senescence and dehiscence of the fruit wall.

its steep decline to 50 days is also noteworthy, and correlated with internal events. These correlations are further considered in the "Discussion."

DISCUSSION

The results of this investigation confirm and extend the observations of Carns *et al.* (5; see 3) that disclosed the presence of a substance in diffusates of young cotton fruit that could counteract auxin-induced curvature of *Avena* coleoptiles. They found the counteraction by the diffusates increased with fruit age to a peak in the vicinity of five to seven days, and then rapidly declined. There appears little question but that in their experiments they were measuring an interaction between the ABA and the auxin in the diffusates. If we may be permitted to interpret their curve for the net activity of ABA and auxin in the light of our curve for ABA, it can be suggested that levels of both ABA and auxin are rising in the young fruit. It appears that from the 5th or 6th day until the 10th day that auxin is rising even more rapidly than ABA, since Carns found a loss of net inhibiting activity during that period. After 10 days the influence of auxin continues to predominate as indicated by the decline of the growth inhibition in Carns' diffusates to almost zero. It is of interest to note that the period from the 6th to the 14th day after anthesis is the period of most rapid growth of the fruit (see Fig. 6). The above interpretation indicates that the first part of this growth period is characterized by a rapid rise in auxin, and the later part is characterized by a rapid decline in ABA. A recent investigation has disclosed a parallel increase in cytokinin activity in young fruit that peaks on the 5th or 6th day and then declines to very low levels by the 12th to 15th days (19).

In addition to ABA, two auxins have been identified in young cotton fruits, indoleacetic acid and ethyl indoleacetate, and the presence of a third auxin detected (3). Gibberellins are also present: GA_1 , GA_3 , and GA_4 have been tentatively identified and appear to be present in substantially higher amounts in young fruit than in old fruit (20). From the foregoing information and the known physiological properties of the hormones there can be little doubt that auxins, gibberellins,

cytokinins and ABA interact to influence both the development and abscission of young cotton fruit. Since information on the chemical nature and the metabolism of the hormones in the young fruit is still far from complete, it remains a matter of conjecture as to how they may be interacting under various conditions. In considering the results shown in Figure 4, ABA levels were well correlated with abscission behavior in the late fruit. The abscised fruit contained several times the amounts of ABA as did the retained fruit. Thus late in the season ABA may well be the predominant factor influencing young fruit abscission. In contrast, early in the season the differences in ABA levels were much smaller and only in fruit abscised on the 6th day was the ABA level substantially higher than in the retained fruit. Since it is probable that auxin and cytokinin levels are higher in the young, early plants than in the older, late plants it is suggested that these hormones may have a greater influence on the course of abscission in the early fruit than in the late fruit. For example, appropriate changes in auxin levels could either augment or counteract the abscission accelerating influence of moderate levels of ABA (2). It is possible also that in young fruit, synthesis of the growth promoting hormones, particularly the gibberellins, may take precedence over the synthesis of ABA: both gibberellins and ABA are products of the terpenoid pathway of biosynthesis. As the plants become less vigorous, synthesis of the gibberellins may decline permitting the synthesis of relatively greater amounts of ABA.

The striking fluctuations in amounts of ABA during the development of the cotton fruit (Fig. 1) are correlated with the morphological events that take place during the course of development. Some of the more important of these correlations are summarized in Figure 6. The decline in ABA during the first 2 days after anthesis was observed in all of our materials (Figs. 1, 3, 4) and is correlated with a loss of fresh weight. Petal abscission occurs during the 1st day after anthesis and fertilization occurs 20 to 30 hr after anthesis (9). And it should be noted that correlated with these low levels of ABA, young fruit abscission was not observed in any circumstances before the 4th day.

The rapid rise in ABA between the 6th and 10th days is

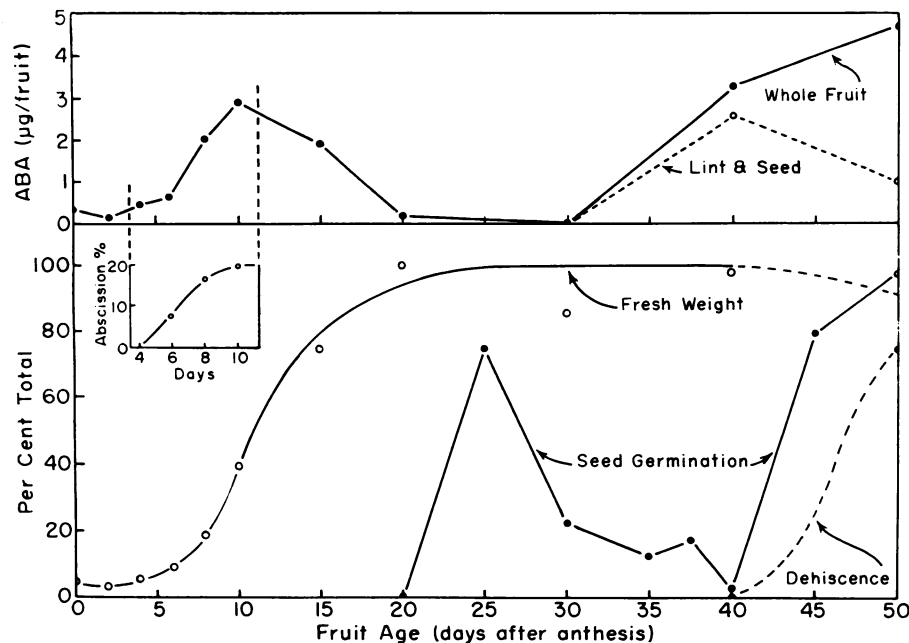


FIG. 6. Summary of abscisic acid and developmental changes in the cotton fruit from anthesis (day 0) to senescence (day 50). The upper curve shows the ABA contents (from Figs. 1 and 5). The inset shows the young fruit abscission of material used for the ABA determinations (cv. Acala SJ-1, early collection). The fresh weight curve also summarizes actual measurements from that collection. The seed germination curve is based on the data of Abdel-Al (1). The dehiscence was that shown by the Acala SJ-1 early collection. The correlations demonstrated here are considered in the text ("Discussion").

correlated with the active period of young fruit abscission (*cf.* Figs. 2, 3, and inset of Fig. 6). The level of ABA fell rapidly after the 10th day, and cotton fruit rarely abscise after that time. With the 10th day the fruit enters into its period of most active growth (note the fresh weight curve of Fig. 6) and during this period also the fiber hairs on the seed are elongating rapidly and complete their growth in length soon after the 20th day. The very low levels of ABA at 20 and 30 days are correlated with the active deposition of secondary wall thickenings in fiber cells and with the deposition of reserves in the seeds (9). These processes continue after 30 days but at declining rates.

As the seeds mature between the 30th and 40th days there is a rapid increase of ABA in the lint and seed fraction followed by a decline to less than half the maximum level by 50 days, the time of full maturity and fruit dehiscence. These fluctuations (Fig. 5) show a strong inverse correlation with the germination of immature seed observed by Abdel-Al (1) (Fig. 6: seed germination). He found that immature seeds develop considerable germinability between the 20th and 25th days when the ABA in the lint and seed is very low. As the ABA rises to a peak at 40 days, the germinability decreases, but it increases again to reach almost 100% at fruit maturity on the 50th day, by which time the ABA levels in the lint and seed have again fallen.

At maturity the fruit wall becomes senescent, chlorophyll gradually disappears and the tissues lose turgor. Dehiscence commences soon after the onset of these changes. The course of dehiscence observed in our material is indicated in the curve of Figure 6. It is correlated with a very rapid rise of ABA in the fruit wall (Fig. 5). Similar increases in levels of ABA have been found by Rudnicki and colleagues (16-18) in strawberries, pears, and apples as those fruits mature and senesce.

The fluctuations in levels of ABA during development are a reflection of changes in its metabolism. Mechanisms of biosynthesis and degradation of ABA are now under investi-

gation (*e.g.*, 11, 15, 21), and when the results can be coordinated with investigations such as are reported here, they will add a significant chapter to the knowledge of the physiology of abscisic acid.

In overview, it is quite apparent that hormonal control in fruit development involves a complex interplay of major plant hormones (see 2, 7). Thus it is quite understandable that the developmental behavior of the fruit is not always well correlated with the observed levels of ABA. However, the several strong correlations between ABA levels and developmental behavior are indeed noteworthy.

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