

Fruit Age and Changes in Abscisic Acid Content, Ethylene Production, and Abscission Rate of Cotton Fruits

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ABSTRACT

The relationships of fruit age, abscisic acid (ABA) concentration, ethylene evolution, and abscission rates were studied in an effort to determine why cotton (*Gossypium hirsutum* L., cv. Deltapine 16) fruits rarely abscise more than 15 days after anthesis. Because abscission of cotton fruits is increased by conditions that limit photosynthesis, greenhouse-grown plants with fruits of various ages were placed in dim light for 3 days to induce high rates of fruit abscission. Abscission rates, ABA concentrations, and ethylene evolution rates were determined for fruits of various ages. Almost all of the young fruits abscised, but abscission rate declined with age until almost no abscission was observed in fruits that were 15 or more days past anthesis.

Dim light increased the ABA concentrations of fruits that were 6 to 11 days old but did not increase ABA concentrations in fruits that were younger or older. The concentration of ABA declined with fruit age from peak values at 4 and 6 days after anthesis. Dim light also increased ethylene evolution from fruits up to 10 days old but had little effect on ethylene production or abscission of fruits more than 11 days old. Ethylene evolution declined with fruit age from peak values at 4 and 6 days after anthesis. Fruits of various ages (from plants not exposed to dim light) were sliced to induce high rates of wound ethylene production. The results indicated that the capacity for ethylene production declined with fruit age, parallel with a decline in abscission rate. Decreases in ABA concentration and ethylene evolution with fruit age indicate that change in the capacity to synthesize these hormones, especially in response to stress, is one cause of the decline in abscission rates as cotton fruits become older.

Cotton fruits (bolls) abscise at variable rates, depending upon cultivar, fruit load, water deficit, irradiance, night temperature, and wounding (e.g. by insects). However, they rarely abscise more than 15 d after anthesis (3, 4, 16). The reasons for this declining tendency to abscise have not been elucidated, but structural and hormonal changes are probably involved.

Ethylene and ABA have both been correlated with abscission of cotton fruits (4, 13, 14, 19). Davis and Addicott (4) reported that ABA content per fruit increased during the first 10 d after anthesis and then declined. An increase in fruit abscission up to 10 d after anthesis paralleled the increase in ABA content, but abscission rates for older fruits were not reported. Rodgers (19) found the highest concentration of ABA in cotton fruits at anthesis and 7 d later. Rodgers (18) also observed maximum rates of abscission at 5 and 10 d after anthesis; by 20 d, the rate had decreased to near zero. The ABA content of fruits was also quite low by 20 d after anthesis (4, 19).

Lipe and Morgan measured ethylene evolution from attached cotton fruits daily (13) and during the day and night (14). Maxi-

imum rates of ethylene evolution occurred on the day of anthesis and then declined to very low values 4 d later in fruits that did not abscise, but they increased in fruits that did abscise. Maximum rates of ethylene production preceded or coincided with abscission which, in tests by Lipe and Morgan (14), occurred 2 to 4 d after anthesis. They did not report ethylene evolution or abscission rates for fruits more than 6 d after anthesis.

Factors that decrease photosynthesis or increase respiration increase cotton fruit abscission (8). Three d of dim light (9) or darkness (21) increased ethylene evolution and abscission of young cotton fruits. Vaughan and Bate (21) also estimated ABA-like substances in fruits of control and darkened plants. Darkness increased the ABA content of their fruits. Unfortunately, fruit age and days of exposure to darkness were confounded, because they used only one population of fruits that were all 3 d old when the dark treatment started. Unlike Davis and Addicott (4) and Rodgers (19), Vaughan and Bate (21) found no change in ABA content between 4 and 10 d after anthesis in fruits of control plants.

Research reported in this paper was undertaken to (a) establish a relationship of fruit age to abscission rate in response to low irradiance, (b) determine effects of age and low irradiance on ABA concentration and ethylene evolution of cotton fruits, and (c) determine the capacity of fruits of different ages to produce ethylene.

MATERIALS AND METHODS

Fruit Age and Abscission Rates. Cotton (*Gossypium hirsutum* L., cv. Deltapine 16) plants were grown in a potting mix in 16-L containers in a greenhouse during the fall. Twelve containers were used with two plants in each. The containers were spaced to minimize shading. A complete nutrient solution was added twice weekly when the plants were small and 3 times weekly when they grew larger. Deionized H₂O was added at other times as needed. Flowers were tagged daily on the d of anthesis during the entire month of November. The plants were moved into a dimly lit (4 $\mu\text{E m}^{-2}\text{s}^{-1}$) room on November 24th and kept there until November 27th, when they were moved back into the greenhouse. Fruits up to 23 d old were present at the start of the dim-light treatment. Dated tags were gathered daily as fruits abscised, and the data were recorded by dates of anthesis and abscission. When abscission stopped, remaining tags were recorded by date of anthesis. These data were used to calculate the time required for abscission to occur and the percentage of fruit abscission in each age group.

ABA Content, Ethylene Evolution, and Abscission Rates as Influenced by Fruit Age and Irradiance. A second test was conducted 11 months later to determine the effects of irradiance on ABA content and ethylene evolution of cotton fruits of various ages. A different cultivar, Deltapine 70, was cultured in 80 containers in a greenhouse as described above, but with only one plant per container. Because of the large number of containers, they were not spaced as in the first test. Flowers were tagged on

the d of anthesis. The plants were divided in two groups of 40 each, and one group was moved into a dimly lit room at 0800 h on October 24th and back to the greenhouse at 0800 h on October 27th. The controls remained in the greenhouse. The two groups were further subdivided into plants used for hormone measurements and those used for abscission measurements (20 containers of each in each treatment). Fruits were removed from the darkened plants immediately after they were returned to the greenhouse on October 27th. Rates of ethylene evolution were estimated by GC (9) on fruits at an even number of d past anthesis. Four replicates of two or more fruits each were used for each age. Fruits that were an odd number of d past anthesis were immediately rinsed and frozen in a deep freeze at -90°C for later ABA analysis. Fruits used for ethylene measurement were also frozen, after ethylene measurements, for later ABA analysis. Fruits were harvested from control plants the next d (October 28th) for ABA and ethylene measurements. The subset of plants used for abscission measurements was kept until November 4th, at which time remaining tags were recovered so that total abscission rates could be calculated.

Fruits used for ABA analysis were freeze-dried and ground to pass through a 40-mesh screen. ABA was extracted from 200-mg portions with 30 ml of 80% methanol overnight on a magnetic stirrer at 4°C . Ten ml of 1% (w:v) NaHCO_3 were added to each sample, and the methanol was evaporated *in vacuo*. Lipids were removed by three extractions with ethyl acetate. The pH of the aqueous phase was adjusted to 3.0 with HCl, and the ABA was extracted into ethyl acetate (3×10 ml).

The samples were dried *in vacuo*, dissolved in 1 ml of methanol followed by 4 ml of 0.001 N HCl, and loaded onto a 2×4 -cm column of preconditioned Polyclar AT (11). The ABA was eluted with 0.001 N HCl; the first 20 ml were discarded and the next 55 ml retained. Others (5, 12) have used Polyclar AT at higher pH values for partial purification of ABA extracts. However, it is much more effective at low pH (7); 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 into diethyl ether, which was then evaporated *in vacuo*. The ABA was transferred to small glass tubes with diethyl ether and methylated with diazomethane (20), evaporated to dryness with a stream of N_2 , and dissolved in 0.1 ml of pyridine followed by 0.4 ml of hexane (2). A gas chromatograph with electron capture detector was used to quantify the ABA. A 0.32×183 -cm column of 3% OV-1 on Chromosorb W(HP) 80/100 mesh was maintained at 220°C . Nitrogen at a flow rate of $30 \text{ cm}^3 \text{ min}^{-1}$ was the carrier gas. Injector and detector temperatures were 300 and 310°C , respectively. An internal standard of $1 \mu\text{g}$ of a racemic mixture of ABA, added at the start of extraction, permitted correction for losses. Pure *trans*, *trans*-ABA was not available. The peak area for *trans*, *trans*-ABA was about 2.1 times the area for *cis*, *trans*-ABA in the external standard, and this ratio was used to calculate the net peak area due to native ABA. No appreciable change in the ratio occurred during the extraction and purification procedure. Identity was confirmed by cochromatography with authentic *cis*, *trans*-ABA and by observing a partial change of native ABA to the *trans*, *trans* isomer after irradiation with UV light (12). Recovery of ABA varied between 60 and 80%.

Fruit Age and Capacity for Ethylene Production. Ethylene evolution is stimulated by carbohydrate deficiency (9), water deficit (10), and wounding (22). Because of variation in the ratio of surface area to volume, rate of water loss differs in fruits of different sizes (15). Therefore, wounding was selected as a method for estimating maximum capacity for ethylene production by cotton fruits of different ages.

Deltapine 16 cotton was grown in a greenhouse as described above. Fruits of various ages were mounted in a microtome, and

2-mm-thick slices were obtained near the point of maximum diameter. Both carpel wall and ovulary tissue produced ethylene at high rates after this treatment. Two slices were enclosed in each 50-ml container with pieces of moistened filter paper, and ethylene evolution was determined by GC on a column of activated alumina (9). Six replicates were analyzed. Sampling was delayed about 1 h after slicing because of the lag period in wound ethylene production. The containers were ventilated after each sampling to prevent depletion of oxygen. Ethylene was collected for approximately 60 min during each of three consecutive periods. Rates are given as averages of the three periods.

RESULTS

Fruit Age and Abscission Rates. Virtually all of the young fruits abscised after plants were held in dim light for 3 d. Results were similar in the two tests, despite the fact that different cultivars were used (Fig. 1; Table I). Abscission rates declined with boll age in both tests and reached very low values for bolls that were 15 d old at the end of the dim-light treatment. Abscission rates of 2- and 3-d-old bolls were lower in the first test (Fig. 1) than they were in the second (Table I). Whether this was entirely due to a difference in cultivars or to some other factor(s) cannot be determined from the data. Because of the limited numbers of bolls involved in the abscission measurements (343 in 1979 and 232 in 1980 on the dim-light-treated plants), there was some scatter in the data. The number of bolls of specific ages ranged from 8 to 39.

Fruit ages shown were those at the end of the dim-light treatment. It is not possible, with a 3-d treatment, to specify an exact time during the treatment when the stimulus was adequate to initiate the abscission process. It probably varied with different fruits; obviously the full 3 d were inadequate to cause abscission of many of the older fruits. Additional time after the dim-light treatment was required for completion of the abscission process. The average delay was 2.7 d, but a few fruits required up to 6 d before they abscised (Fig. 2). The lag periods in each age group showed no consistent differences.

Statements concerning the age at which cotton fruits abscise seldom indicate whether it is the age at which the stimulus occurs or the age at which the fruit drops from the plant. The data in Figure 1 and Table I indicate that fruits were almost immune to abscission 15 d after anthesis. However, because of the lag period from stimulus to abscission (Fig. 2), a few fruits were as much as 24 d past anthesis when they finally abscised, even though they were no more than 18 d old at the end of the dim-light treatment.

ABA Content, Ethylene Evolution, and Abscission Rates as Influenced by Fruit Age and Irradiance. Three d of dim light increased the ABA concentration in fruits that were 6 to 11 d old at the end of the dim-light treatment, but did not increase the ABA contents of fruits that were younger or older (Table I). Although the abscission rate of younger fruits (less than 6 d old) was increased by dim light, the ABA content was not. ABA concentration tended to decline with fruit age after day 4 in controls and day 6 in the dim light treatment. ABA content and abscission rate of fruits that were 12 d old or older were little affected by the dim-light treatment. These results extend those reported by Vaughan and Bate (21). They reported an increase in ABA-like substances in fruits of dark-treated cotton plants, but they did not examine ABA in fruits that were more than 8 d old, nor did they examine fruits that were 2 or 3 d old at the end of the dark treatment. All fruits in their test were 6 d old at the end of the 3-d dark treatment.

With the possible exception of 4-d-old fruits, dim light increased the rate of ethylene evolution from young fruits, and the increases for all ages affected were greater (on a percentage basis) than were the increases in ABA (Table I). From peak values 4 and 6 d after anthesis in the control and dim-light treatments, respectively,

Table I. ABA Concentrations, Ethylene Evolution, and Abscission Rates of Cotton Fruits as Influenced by Fruit Age and Irradiance

Deltapine 70 cotton plants were grown in a greenhouse in 1980. Forty plants were subjected to 3 d of dim light, while 40 control plants remained in the greenhouse. Ethylene evolution and ABA concentration were determined at the end of the dim-light treatment, and the data are averages of four replications of two or more fruits per sample. SES of the means are shown. Abscission data are based on 236 and 232 fruits for control and dim-light treated plants, respectively.

Fruit Age days	ABA Concentration $\mu\text{g/g}$		Ethylene Evolution $\text{nl/g}\cdot\text{hr}$		Abscission %	
	Control	Dim light	Control	Dim light	Control	Dim light
2	2.25 \pm 0.12	2.10 \pm 0.30	2.62 \pm 0.21	7.18 \pm 2.50	21	100
3	3.23 \pm 0.47	1.12 \pm 0.08			37	100
4	3.40 \pm 0.18	3.24 \pm 0.09	4.36 \pm 0.97	5.56 \pm 0.27	42	100
5	3.08 \pm 0.65	3.21 \pm 0.89			44	100
6	2.74 \pm 0.38	5.84 \pm 1.22	2.57 \pm 1.41	15.38 \pm 2.09	27	100
7	2.98 \pm 0.45	4.65 \pm 0.38			33	100
8	2.92 \pm 0.23	3.76 \pm 0.27	0.86 \pm 0.19	3.85 \pm 0.69	31	94
9	2.70 \pm 0.13	4.50 \pm 0.25			50	89
10	1.73 \pm 0.10	4.41 \pm 0.81	0.72 \pm 0.15	3.75 \pm 0.66	20	91
11	2.28 \pm 0.12	3.38 \pm 0.15			21	87
12	1.54 \pm 0.04	1.48 \pm 0.04	0.69 \pm 0.15	0.87 \pm 0.13	20	31
13	2.04 \pm 0.16	1.58 \pm 0.23			7	29
14	1.11 \pm 0.13	0.84 \pm 0.07	0.23 \pm 0.10	0.20 \pm 0.05	0	8
15	1.00 \pm 0.06				0	4

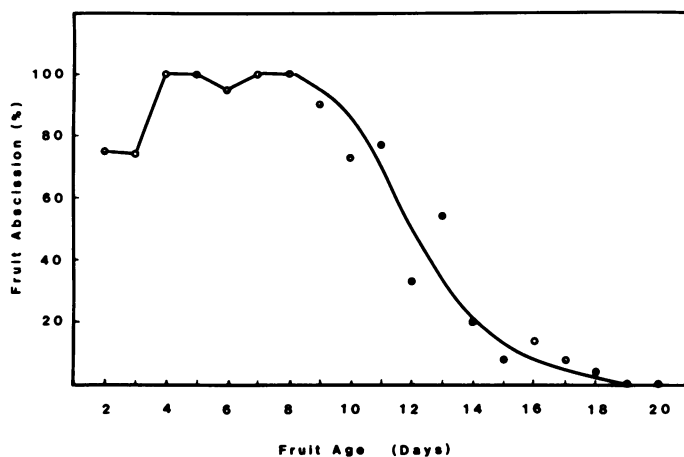


FIG. 1. Fruit abscission induced in Deltapine 16 by 3 d in dim light as influenced by fruit age at the end of the dim-light treatment in November, 1979. Values are the percentage of all fruits in each age group which eventually abscised.

ethylene evolution decreased with age almost 95% in controls and 99% in fruits of plants exposed to dim light. The influence of dim light on ethylene evolution decreased sharply with age; fruits that were 12 or 14 d old were unaffected. Likewise, their abscission rates were largely unaffected by the dim-light treatment.

Fruit Age and Capacity for Ethylene Production. Slicing caused very high rates of ethylene evolution (Fig. 3). The 4-d-old fruits gave the highest rate; younger fruits might have produced even more but could not be mounted in the microtome. Rate of wound ethylene production declined rapidly with increasing fruit age. Although the rates were much greater, this decline paralleled the decline in *in situ* ethylene evolution and preceded the decline in abscission (cf. Table I and Figs. 1 and 3).

DISCUSSION

The maximum ABA concentrations and ethylene evolution rates of young cotton fruits coincided with the highest incidence

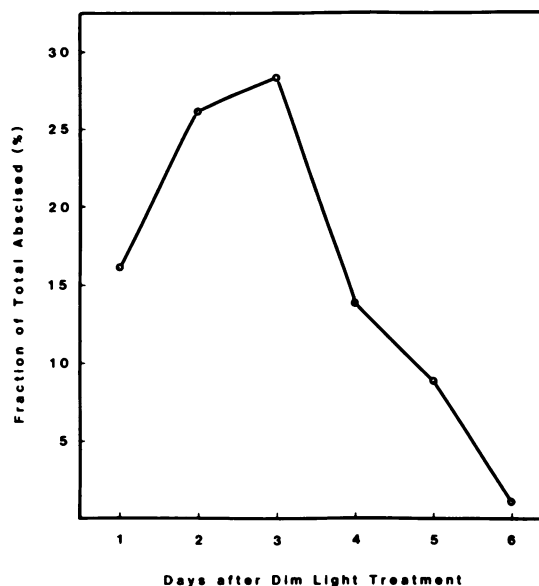


FIG. 2. Lag period for fruit abscission after the end of the 1979 dim-light treatment. Values show the fraction of fruit abscised each day after the dim-light treatment as a percentage of all fruits that abscised.

of abscission. Although dim light increased the ABA content of fruits that were 6 to 11 d old, it did not affect the ABA concentration in fruits younger than 6 d. Therefore, it is unlikely that ABA was involved in dim-light-induced abscission of very young fruits. It may be worth noting that 2- and 3-d-old fruits had lower abscission rates in the first test than did 4- to 8-d-old fruits (Fig. 1). Perhaps ethylene alone stimulated abscission in the younger fruits, whereas both ethylene and ABA affected abscission in the older fruits.

Other hormones may have suppressed abscission. Rodgers (17) found maximum gibberellin activity in extracts from 2- or 3-d-old cotton fruits, and he also found a relatively high concentration of IAA in 3-d-old fruits. Although he did not use a treatment to stimulate abscission, Rodgers (17, 18) reported a lower rate of

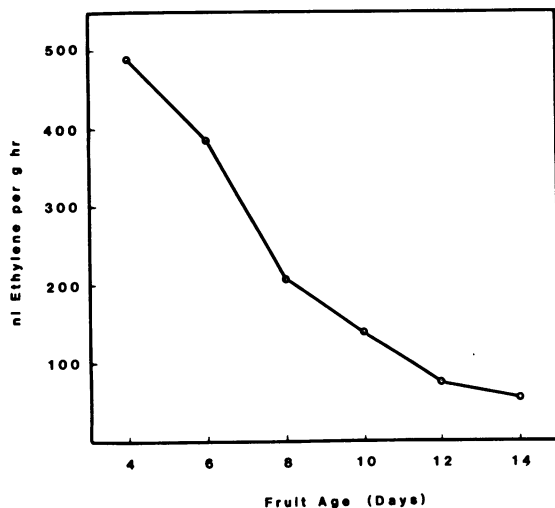


FIG. 3. Wound ethylene evolution from sliced Deltapine 16 fruits of different ages.

abscission for 3-d-old than for 5- to 10-d-old fruits (in agreement with the results of Fig. 1). He cited evidence that gibberellins decrease fruit abscission in cotton. His results, like mine, indicated a decrease in fruit abscission to near 0 at 20 d after anthesis. He reported maximum concentrations of IAA and cytokinins, and a second peak of gibberellin activity, in 15-d-old fruits. Therefore, the decrease in boll abscission with age may be due, not only to decreases in ethylene evolution and ABA content, but also to increase in hormones that suppress abscission.

The lag period between stimulus and abscission makes it difficult to specify just when a stimulus initiates the abscission process. The lag period would probably vary with temperature, fruit load, and intensity of the stimulus. Temperature during the dim-light treatment averaged about 25°C, and, in the greenhouse, it ranged between 22 and 35°C. Higher temperatures would probably shorten the time from stimulus to abscission.

The mechanism by which low irradiance increases ABA concentration and ethylene evolution has not been determined, but the ethylene response apparently is related to availability of photosynthate. Enriching the air with CO₂ decreased abscission, whereas warm nights, short days, or dim light increased fruit abscission (8) and ethylene evolution (9). Significant negative correlations between sugar contents and rate of ethylene evolution were reported for 4-d-old cotton fruits (9). Cloudy weather (6) and close spacing (1), which would increase mutual shading, greatly increased cotton fruit abscission.

It is fortunate that only young fruits abscise. This feature permits more rapid recovery from a temporary stress and minimizes loss of metabolites, because the fruits are small when they abscise. Maximum growth rates occur later when fruits are 10 to 20 d old (18).

Although IAA and gibberellins may have decreased abscission, especially about 2 and 15 d after anthesis (17), the results reported

here indicate that ethylene evolution and ABA content are positively correlated with abscission rates of different aged cotton fruits. When data for control plants only were analyzed, the correlation coefficients were 0.74 for ethylene *versus* abscission and 0.87 for ABA *versus* abscission. When the data for control and dim-light plants were combined, the correlation coefficients were 0.75 for ethylene *versus* abscission and 0.66 for ABA *versus* abscission. Furthermore, both the influence of an abscission-promoting stress (dim light) and the capacity for maximum rates of ethylene production (due to wounding) decreased with fruit age in parallel with a decrease in fruit abscission rate.

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