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Nutritional Stress and Ethylene Evolution by Young Cotton Bolls[1]

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ABSTRACT

Previous results have shown that a nutritional stress increases the rate of young boll abscission from cotton (*Gossypium hirsutum* L.) plants. Tests were conducted to determine whether a nutritional stress affects the rate of ethylene evolution by 4-day-old bolls. Fruiting cotton plants were subjected to conditions that should decrease photosynthesis (dim light, short days), increase respiration (long warm nights), or change the demand for products of photosynthesis (partial boll removal, changing boll load during a growing season).

As little as one day of dim light caused a measurable increase in ethylene. Partial boll removal slightly decreased the effects of subsequent exposure to dim light. Bolls from plants exposed to three consecutive long warm nights produced more ethylene than bolls from control plants. Rate of ethylene evolution by bolls of field-grown plants increased almost eightfold from June 25, when the boll load was light, to July 30, when the boll load was heavy.

Sugar analyses indicated significant negative correlations between sugar content of bolls and their rate of ethylene evolution.

The results indicate that a nutritional stress increased rates of ethylene evolution by young cotton bolls. This additional ethylene is probably a causal factor in the increased rates of boll abscission that occur when cotton plants are subjected to conditions that promote a nutritional stress.

Additional index words: Gossypium hirsutum L., Boll abscission, Light intensity, Night temperature, Photoperiod, Photosynthesis, Respiration, Sugars.

STARTING, perhaps, with the work of Mason (14) in the early 1920's, the nutritional theory of shedding was used to explain much of the abscission of young cotton (*Gossypium hirsutum* L.) bolls. According to this theory, the cotton plant retains only as many bolls as it can supply with organic nutrients. However, some of the results of Eaton and Rigler (7) and Eaton and Ergle (5, 6) indicated shortcomings of the nutritional theory of boll shedding. Later work on the roles of auxin in inhibiting abscission and of abscisic acid and ethylene in promoting abscission showed the importance of hormones in controlling the process. As a result, the nutritional theory of shedding has been largely ignored.

The nutritional and hormonal theories for the control of abscission are not necessarily contradictory or exclusive. A nutritional stress may alter the hormonal status of plant tissue, as suggested by Addicott and Lynch (1) and Brown (2). Ethylene, the most potent abscission-promoting substance known (17), was shown by Lipe and Morgan (13) to be a regulator of young fruit abscission in cotton. Ethylene production is stimulated by many kinds of stress (15, 18). This paper reports the effects of a nutritional stress on the rate of ethylene production by young cotton bolls.

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MATERIALS AND METHODS

To test the effects of nutritional stress on ethylene evolution by young bolls, separate treatments were selected that should (i) decrease photosynthesis (dim light or short days), (ii) increase respiration (long, warm nights), and (iii) change the demand for products of photosynthesis (partial boll removal or the change in boll load during a growing season).

'Deltapine 16' was used in all experiments. The effect of an increasing load during a growing season was tested with field-grown plants. All other treatments were tested with plants cultured in a complete nutrient solution in a climate-controlled greenhouse as reported earlier (9).

Dim Light. After plants started blooming and setting bolls, they were moved into a dimly lit room (650 lux) at about 8:30 AM and kept there 24 or 48 hours at a constant temperature of about 25 C. They were then moved back into the greenhouse for the rest of the experiment. Greenhouse temperatures were programmed by cam from a minimum of 20 C at 6 AM to a maximum of 35 C at 2 PM. Four different batches of 40 plants each were subjected to dim light on January 21 and 22, January 28, September 10 and 11, and September 16. Control plants remained in the greenhouse during the treatment periods. Daytime light intensity in the greenhouse was seldom $<$ 108,000 lux.

Long Warm Nights. To decrease photosynthesis and increase respiration, plants were moved into a dimly lit room at 4 PM and back into the greenhouse at 8 AM on June 24, 25, and 26. Temperature in the dimly lit room was kept at about 29 C during this treatment. Control plants were also moved into the room at 4 PM to duplicate any effect of moving the plants, but were immediately moved back into the greenhouse. Except for this brief interval of not more than 5 min each day, control plants remained in the greenhouse under prevailing long days (about 14.4 hours from sunrise to sunset) and cool night temperatures (down to an overnight low of 20 C). After three consecutive long warm nights the treated plants were returned to the greenhouse for the rest of the test.

Partial Defruiting. On January 10, all bolls were removed from half the plants that had been cultured from seeds planted on October 29. No differences in ethylene evolution between bolls from defruited and control plants were noted during the first six days after defruiting. Therefore, all plants were subjected to dim light from January 21 to 23 to induce a nutritional stress.

Seasonal Change. Cotton seeds were planted on March 19 in a field of Avondale clay loam on the Univ. of Ariz. Cotton Res. Cen., Phoenix. Rows were 102 cm apart, and the plants were thinned to a stand of about 42,200 plants/ha. Standard experiment station cultural practices were used to control insects and weeds, and to supply irrigation water (by the furrow method). White blooms were tagged each Friday from June 21 to August 9, and the 4-day-old bolls were harvested each following Tuesday. Care was taken to tag blooms on different plants each week, so that plants would not be used that had already had some bolls removed. Senescent bolls were avoided except on July 2 when almost all tagged bolls appeared to be senescent. Bolls were transported to the lab in a cooler over ice to prevent possible injury by heat.

Measurements. Blooms were tagged on the day of anthesis, and bolls were harvested four days later. Thus, all harvested bolls were 4-days-old. A razor blade was used to make a clean cut across the peduncle to minimize ethylene evolution due to wounding. Individual bolls were then placed in 50-ml polycarbonate centrifuge tubes. Tight-fitting caps, in which neoprene septa had been fitted, were placed on the tubes. Silicone vacuum grease was used to ensure gas-tight seals and was applied also to the lower side of the septa to minimize absorption of ethylene. About 1 and 5 hours after sealing the tubes, I determined their ethylene content by withdrawing 1-ml samples with a gas-tight syringe and injecting them into a gas chromatograph. A 0.32 $\times$ 183-cm stainless steel column of activated alumina was used to separate ethylene from other gases at a constant temperature of 120 C. Bolls were weighed, and

the changes in peak heights between the first and last ethylene measurements were used to calculate rate of ethylene evolution.

Bolls were rinsed in deionized water, cut open, freeze-dried, ground to pass a 40-mesh screen, and stored in a desiccator over silica gel until samples could be analyzed for sugars. Sugars were extracted and estimated by gas-liquid chromatography as outlined earlier (9).

RESULTS AND DISCUSSION

Subjecting plants to dim light (650 lux) during the day increased ethylene evolution by 4-day-old bolls (Table 1). The response was fairly rapid; rates of ethylene production had increased in bolls harvested 24 hours after plants were transferred to dim light. Since bolls were harvested only once per day, the data could not indicate precisely how soon ethylene evolution started to increase. Because ethylene is a potent abscission-promoting hormone, the increased rates of ethylene evolution that occurred when cotton plants were subjected to dim light could have caused the increased rates of boll abscission noted earlier by Dunlap (4) and by Guinn (9). Ethylene production started to decrease shortly after plants were returned to full sunlight (Table 1). However, the high rates of ethylene evolution that occurred soon after plants were placed in dim light probably initiated the abscission process in some bolls, so that they would have abscised even after plants were returned to full sunlight, as noted earlier (9).

Subjecting plants to long, warm nights for three consecutive days also increased rates of ethylene production by young bolls (Table 2). Boll abscission rate in this test increased from 35% for control plants to 47% for treated plants. Ethylene production subsequently dropped to control rates or below in bolls that developed after the long, warm-night treatment, even though they were present as squares (floral buds) during the treatment.

Removal of all bolls on January 10 did not immediately decrease ethylene evolution by bolls that subsequently developed on the defruited pants (Table 3). Daily boll removal for ethylene measurements decreased the boll load on all plants and probably prevented a nutritional stress from developing until the plants were placed in dim light. However, when plants were stressed in dim light on January 21 and 22, ethylene production initially increased more in bolls from control plants than in bolls from plants that were defruited on January 10. Dim light eventually caused very high ethylene production rates by bolls in both groups of plants. Unlike the results in Table 1, the rates of ethylene evolution did not decline when plants were returned to the greenhouse. Most of the bolls harvested on January 24 and 25 appeared senescent and abscised readily when touched.

Ethylene evolution by young bolls of field-grown plants tended to increase as the season progressed and boll load increased (Table 4). Uncontrolled weather variations and moisture stress between irrigations undoubtedly affected the results. For example, afternoon temperatures in the field reached 44.5 C on June 27 and 44 C on June 28. Moisture stress was relieved by irrigation on July 1. The combination of hot weather and moisture stress probably caused high rates of ethylene production by bolls harvested

Table 1. Ethylene evolution by 4-day-old cotton bolls as influenced by one or two days' exposure of plants to dim light.†

Harvest date	Control	Dim light	% of Control
	μl/kg·hour		
	First test		
28 Jan (DL)	3.30 ± 0.84	2.94 ± 0.45	89
29 Jan (S)	1.31 ± 0.30	3.83 ± 0.44	292
30 Jan	2.62 ± 0.80	5.56 ± 0.94	212
31 Jan	2.00 ± 0.24	3.90 ± 1.35	195
1 Feb	2.41 ± 0.81	1.24 ± 0.46	51
	Second test		
10 Sept (DL)	1.16 ± 0.21	0.84 ± 0.20	72
11 Sept. (DL)	1.03 ± 0.33	2.07 ± 0.21	201
12 Sept. (S)	0.70 ± 0.11	3.69 ± 0.68	527
13 Sept.	0.67 ± 0.09	2.41 ± 0.42	360
14 Sept.	0.81 ± 0.11	0.86 ± 0.16	106
18 Sept	0.84 ± 0.07	1.44 ± 0.25	171
19 Sept	0.75 ± 0.10	0.96 ± 0.22	128

† Plants were exposed to dim light, 650 lux, on days indicated by (DL) and were returned to full sunlight in the greenhouse on days indicated by (S). Data are averages of 8 to 12 samples/treatment at each harvest date (except for five control bolls on 29 Jan and seven dim-light bolls on 30 Jan). Standard errors of the means are shown.

Table 2. Ethylene evolution by 4-day-old cotton bolls as influenced by exposure of cotton plants to long (16-hour), warm (29 C) nights on June 24, 25, and 26.†

Harvest date	Control	Long, warm nights	% of Control
	μl/kg·hour		
26 June	1.33 ± 0.31	2.51 ± 0.36	189
27 June	1.79 ± 0.63	2.82 ± 0.62	158
28 June	0.96 ± 0.22	3.79 ± 1.56	395
29 June	1.15 ± 0.50	1.80 ± 0.79	157
30 June	–	–	–
1 July	0.91 ± 0.13	0.73 ± 0.09	80

† Data are averages of 12 bolls/treatment at each harvest date. Standard errors of the means are shown.

Table 3. Ethylene evolution by 4-day-old cotton bolls as influenced by defruiting on January 10 followed by 2 days of dim light on dates indicated by DL.†

Harvest date	Control	Defruited	% of Control
	μl/kg·hour		
14 Jan	0.73 ± 0.07	0.77 ± 0.06	105
15 Jan	0.61 ± 0.04	0.56 ± 0.03	92
16 Jan	0.50 ± 0.04	0.66 ± 0.04	132
21 Jan (DL)	2.97 ± 0.53	1.93 ± 0.29	65
22 Jan (DL)	7.06 ± 1.77	3.18 ± 0.21	45
23 Jan (S)	7.31 ± 1.57	6.89 ± 1.19	94
24 Jan	9.20 ± 1.16	11.41 ± 1.03	124
25 Jan	22.12 ± 3.18	15.27 ± 2.96	69

† Plants were returned to the greenhouse on 23 Jan. Data are averages of 4 to 8 bolls/treatment at each harvest date. Standard errors of the means are shown.

on July 2. Practically all bolls harvested on July 2 showed signs of senescence and, therefore, were not saved for sugar analyses. The exceptionally high rates of ethylene evolution by bolls harvested on August 6 was at least partly due to damage caused by a hailstorm on the afternoon of August 5. The rather steady increase in ethylene production from July 9 to 30, however, was probably caused by a combination of increasing boll load and shortening photoperiod. Bolls that were harvested on August 13 showed a relatively low rate of ethylene production, possibly because older bolls had started to open and because these young bolls were near the tops of the plants, where light intensity was high.

Rate of boll abscission generally increases as the season progresses (5). Davis and Addicott (3) reported that young bolls abscising late in the season

Table 4. Ethylene evolution and correlation coefficients for sugars versus ethylene evolution by 4-day-old cotton bolls produced during a growing season on field-grown plants.

Harvest date	Ethylene evolution	Ethylene evolution vs.			
		Fructose	Glucose	Sucrose	Total sugars
	µl/kg·hour	r			
25 June	0.60 ± 0.08	−0.51*	−0.51*	−0.25	0.50*
2 July	4.02 ± 0.94	–	–	–	–
9 July	0.95 ± 0.17	−0.68**	−0.42	−0.23	−0.50*
16 July	1.06 ± 0.44	−0.73**	−0.61*	−0.59*	−0.69**
23 July	2.04 ± 0.55	+0.59*	+0.17	+0.59*	+0.31
30 July	4.75 ± 1.60	−0.71**	−0.78**	−0.59*	−0.73**
6 Aug	13.18 ± 5.59	−0.67**	−0.82**	−0.79**	−0.81**
13 Aug	1.25 ± 0.17	−0.26	−0.23	−0.07	−0.19
All dates		−0.34	−0.38**	−0.38**	−0.33**

*, ** Significant at $P > 0.95$ and $P > 0.99$, respectively. Data are averages of 15 to 21 samples at each harvest date. Standard errors of the means are shown.

Table 5. Correlation coefficients for sugars versus ethylene evolution by 4-day-old cotton bolls. Half the plants in each test were subjected to one or two days of dim light on dates indicated by DL.

Harvest date	Ethylene evolution vs.			
	Fructose	Glucose	Sucrose	Total
	r			
		First test		
15 Jan	−0.12	−0.08	−0.09	−0.11
16 Jan	−0.42	−0.27	0.08	−0.31
21 Jan (DL)	−0.43	−0.05	−0.13	−0.20
22 Jan (DL)	−0.75*	−0.82**	−0.79**	−0.82**
23 Jan (S)	−0.50	−0.59*	−0.60*	−0.59*
All dates	−0.75**	−0.76**	−0.77**	−0.76**
		Second test		
28 Jan (DL)	−0.30	−0.22	−0.24	−0.23
29 Jan (S)	−0.54	−0.46	−0.77**	−0.60*
30 Jan	−0.44	−0.28	−0.26	−0.38
31 Jan	−0.52*	−0.29	−0.15	−0.28
1 Feb	−0.63**	−0.44	−0.17	−0.55*
All dates	−0.44**	−0.35**	−0.25*	−0.41**
		Third test		
10 Sept (DL)	−0.31	−0.45	−0.22	−0.36
11 Sept (DL)	+0.03	−0.13	−0.45	−0.20
12 Sept (S)	−0.42*	−0.40	−0.49*	−0.45*
13 Sept	−0.52**	−0.57**	−0.56**	−0.57**
14 Sept	−0.38	−0.44*	−0.18	−0.47*
All dates	−0.37**	−0.40**	−0.20*	−0.42**

*, ** Significant at $P > 0.95$ and $P > 0.99$, respectively. Correlation coefficients were calculated for 4 to 12 pairs of values for each date.

contained about twice as much abscisic acid as those abscising early in the season. But, retained bolls contained less abscisic acid late than early in the season. My results indicate that increasing rates of ethylene evolution may also be a factor in causing increased rates of abscission as the season progresses. Rate of ethylene evolution increased almost eightfold from June 25 to July 30 (Table 4).

Although my results indicate that a nutritional stress increased rates of ethylene production by young bolls, correlation coefficients between sugar content and ethylene evolution by young bolls were not always statistically significant (Tables 4 and 5). Many conditions, besides a nutritional stress, can affect ethylent production. Furthermore, one should expect little correlation between sugar content and ethylene evolution when there is little or no nutritional stress. For example, correlations were poor before plants were subjected to dim light, but became highly significant when a stress was developed in dim light (Table 5).

It is difficult to establish an exact rate of ethylene production that will cause abscission, because the sensitivity of plant organs to ethylene varies with age, condition, and the levels of other hormones (10, 11, 13, 16). Lipe and Morgan (12) found that ethylene production rates for young cotton fruits were high at the time of anthesis but dropped to very low levels, in nonabscising fruits, four days after anthesis. In fruits destined to abscise, however, ethylene production rates reached a maximum two to four days after anthesis (13). The increased ethylene production by 4-day-old bolls that occurred when plants were subjected to dim light may explain why cloudy weather (8) and mutual shading of closely-spaced plants (2) often results in abnormally high rates of abscission.

The results of these tests indicate that the nutritional and the hormonal theories of shedding are not mutually exclusive, because a nutritional stress may increase ethylene evolution enough to promote abscission.

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