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Water Deficit and Ethylene Evolution by Young Cotton Bolls

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ABSTRACT

Ethylene evolution and abscission of young cotton (*Gossypium hirsutum* L.) bolls were shown, in earlier papers, to increase when plants were subjected to conditions that decreased photosynthesis and sugar content of bolls (dim light, long warm nights). Moisture stress also increased ethylene evolution by young bolls, but it did not decrease their concentrations of fructose, glucose, or sucrose. When detached bolls were incubated for 16 or 24 hours at high or low humidity, their rate of ethylene evolution increased markedly at low humidity and slightly at high humidity. These results suggest that water deficit stimulates ethylene evolution by young bolls directly through partial desiccation, but do not exclude the possibility of a stimulus from moisture-stressed plants. Although attached and detached bolls both lost only a small percentage of their water content, detached bolls lost more for a given rate of ethylene evolution than bolls on moisture-stressed plants. The increased rate of ethylene evolution by young cotton bolls on plants subjected to a water deficit is probably adequate, in many cases, to cause their abscission.

Water deficit increases the rate of abscission of leaves and young fruits (bolls) of cotton (13, 15). Water stress was found to increase ethylene production by detached avocado fruits (1), orange leaves (2), *Vicia faba* plants (6), and intact cotton petioles (14). Lipe and Morgan (13) withheld water from cotton plants to promote leaf and fruit abscission and found that subjecting some of these plants to reduced atmospheric pressure (200 mm Hg) decreased the rate of abscission, presumably because the reduced pressure removed some of the ethylene.

Although drought may have increased ethylene evolution by cotton bolls, it seemed unlikely that this was a direct effect of desiccation because of the large volume to surface area ratio of bolls compared to that of leaves. Radin and Sell (20) found a low transpiration rate for bolls. Guinn (9) reported evidence that a nutritional stress, caused by dim light, long warm nights, or an increasing boll load, decreased sugar content and increased ethylene evolution by young bolls. Many reports have shown that water deficit decreases photosynthesis, primarily through stomatal closure (5, 18), but also by decreasing Hill reaction activity (5, 7), photophosphorylation (5, 21), and activity of enzymes involved in CO_2 fixation (19). Furthermore, water deficit was shown to decrease translocation of ^{14}C from leaves of corn (3) and sugarcane (10). It seemed probable that water deficit induced a nutritional stress in young cotton bolls which, in turn, increased their rate of ethylene evolution and abscission.

MATERIALS AND METHODS

Cotton (*Gossypium hirsutum* L.) plants were cultured in a greenhouse where the temperature was programmed from a minimum of 20 C at 6 AM to a maximum of 35 C at 2 PM with gradual changes between these extremes. Humidity was not controlled, but ranged from 20 to 50% because the greenhouse was cooled by refrigeration rather than by evaporative pads. Bolls were subjected to water deficit while on intact plants in the first two tests and after detachment from nonstressed plants in the remaining tests. Ethylene was collected only after bolls were removed from plants regardless of whether they were subjected to moisture stress before or after detachment.

Drought in Intact Plants. Cotton plants (cv. Empire Glandless) were cultured in redwood boxes 46 cm high × 75 cm wide × 152 cm long. The stand was thinned to give 40 plants in each box that contained 0.41 m^3 of a potting mixture of peat, sand, and vermiculite in a 1:1:1 (v/v/v) ratio. The plants were irrigated weekly with a complete nutrient solution (8) and, additionally, with deionized water as needed to keep the rooting medium moist until the drought treatment. Watering was discontinued in the drought treatment after adding nutrient solution on October 25 in the first test and on November 8 in the second. October 28, 29, and 30 were cloudy days and the plants showed no signs of wilting until October 31 (a clear day). The weather remained clear during the second test and the plants started wilting on November 12.

Partial Desiccation of Detached Bolls. Cotton plants (cv. Deltapine 16) were cultured in a complete, aerated, nutrient solution (8) in the greenhouse described above, with two plants in each 10-liter container. Blooms were tagged on the day of anthesis, and bolls were harvested from 1.5 to 4.5 days later. (Bolls harvested between 8:00 and 8:30 AM were considered to be a whole number of days old when harvested, and those harvested between 4:00 and 4:30 PM were considered to be *n* plus 0.5 days old.) In order to determine if water loss *per se* increased ethylene evolution by young cotton bolls, I removed bolls (with intact bracts) from the plants and kept them in a desiccating cabinet over silica gel or in a large jar with pieces of moistened filter paper for 2 to 24 hr before collecting and measuring ethylene. Because bracts have a much larger surface area to volume ratio than the rest of the boll, bracts were removed from bolls after the desiccating treatment, in some tests, and ethylene evolution and moisture contents of bracts and bolls were determined separately.

Measurements. Ethylene was collected by placing individual bolls in 50-ml polycarbonate centrifuge tubes. The caps were fitted with neoprene septa and were sealed with silicone stopcock grease. One-ml samples were withdrawn approximately 1 and 5 hr later with a gas-tight syringe, and ethylene was determined by gas-solid chromatography (9).

Bolls were weighed, rinsed in deionized water, sliced open, lyophilized, and weighed again. The difference between fresh and dry weights was used to calculate the moisture content of each boll. When used for sugar analysis, the dried bolls were ground to pass a 40-mesh screen.

Sugars were extracted with hot 70% ethanol, purified, dried, silylated, and separated by GLC (8). Arabinose was used as an internal standard.

RESULTS AND DISCUSSION

Bolls from droughted plants produced more ethylene after the plants wilted than those from control plants (Table I). Despite reports that water stress decreases both photosynthesis and translocation, I found no consistent evidence that drought decreased the sugar content of young cotton bolls. Bolls from droughted plants contained about the same concentrations of fructose, glucose, and sucrose as bolls from control plants (Table I). Some factor other than decreased sugar content must have increased ethylene evolution by bolls on droughted plants. The drought treatment caused a very small, but statistically significant, decline in moisture content of bolls.

Partial desiccation of detached bolls also increased their ethylene production, but only if bolls were kept in the desiccator longer than 4 hr (Table II). Although 4 hr in the desiccator caused a decline of 1.3% in moisture content, it did not increase the rate of ethylene evolution. Whether this was because a greater loss of water was required to stimulate ethylene production or more time was required for the stimulus to act can not be determined from the data. Bolls on droughted plants produced much more ethylene than those on control plants after a decline of only 0.9% in moisture content of the bolls (Table I, test 2), but the stress developed over a longer time than with detached bolls. Detached control bolls may have absorbed some moisture from the saturated atmosphere in which they were stored. If so, this would have increased the difference in moisture content between control and desiccated bolls. Desiccation for 16 and 24 hr caused large increases in rate of ethylene evolution and, of course, caused the loss of more moisture than occurred with a shorter time in the desiccator (Table II).

If the stimulus that increases ethylene production is proportional to the amount of desiccation, I expected young bolls to show a greater response to a desiccating treatment than older bolls because their smaller size would facilitate water loss. Young bolls did lose more water than older bolls, but the stimulation of ethylene production was about the same for all bolls of the four ages tested (Table III). Bracts lost much more water and showed a much greater increase in ethylene evolution than the rest of the boll (Table IV). Although partial desiccation caused large increases in ethylene evolution by bracts, it also increased ethylene evolution by the rest of the boll so that no more than 32% of the total ethylene produced by bolls came from their bracts. The sum of ethylene produced by separated bracts and bolls was greater than that produced by whole bolls, presumably because of the wounding that occurred when bracts were removed (*cf.* Tables II and IV). The wound response appeared to be greater with partially desiccated than with control bolls.

Partial desiccation obviously stimulated ethylene production by detached cotton bolls, but other factors may also have been involved. The rate of ethylene evolution by control bolls increased with time after detachment, although not as much as was observed with desiccated bolls (Tables II and IV). This increase in ethylene evolution by detached control bolls may have been caused by the depletion of some essential substance that is normally supplied by the plant. This "starvation" effect apparently did not include the bracts (Table IV). Because of the increase in ethylene evolution by detached control bolls, the difference in rates between detached control and desiccated bolls was not as great as the difference between bolls on control and droughted plants. Absolute rates of ethylene evolution, however, were comparable for bolls on droughted plants and detached bolls after 24 hr in a desiccator (Tables I and II). Whether drought affected the translocation of an essential substance into developing bolls in intact plants is open to speculation.

A possible role of ABA in stimulating ethylene production in bolls subjected to water deficit cannot be excluded. Wilting causes a very rapid increase in ABA content of leaves of many

Table II. *Effect of Time in Desiccator on Moisture Content and Ethylene Evolution by Young Cotton Bolls*

Cotton bolls were detached from plants 4 days after anthesis (4.5 days for the 16-hr treatment) and stored in a humid atmosphere (control) or in a desiccator over silica gel (desiccated) for 2, 4, 16, or 24 hr before ethylene was collected and measured.

Treatment	Time in Storage (hr)			
	2	4	16	24
		nl/boll·hr		
Ethylene				
Control	0.56 ± 0.04	1.11 ± 0.12	1.64 ± 0.16	4.21 ± 0.28
Desiccated	0.59 ± 0.13	0.95 ± 0.05	5.67 ± 0.77	9.68 ± 2.46
Ratio D/C	1.05	0.86	3.44	2.30
		% (w/w)		
Moisture content of bolls				
Control	82.1 ± 0.5	80.3 ± 0.3	81.0 ± 0.4	82.2 ± 0.3
Desiccated	82.0 ± 0.4	79.0 ± 0.4	77.6 ± 0.2	76.2 ± 0.4
Difference	0.1	1.3	3.4	6.0
		nl/kg·hr		
Ethylene				
Control	347 ± 37	675 ± 48	840 ± 76	2482 ± 147
Desiccated	396 ± 109	748 ± 50	3690 ± 472	6690 ± 1794

Table I. *Sugar Content, Moisture Content, and Ethylene Evolution by 4-day-old Cotton Bolls from Control and Droughted Plants*

Watering was discontinued in the drought treatment on October 25 in test 1 and on November 8 in test 2.

Date	Treatment	Concn of Sugars in Bolls			Moisture in Bolls	Ethylene Evolution
		Fructose	Glucose	Sucrose		
		mg/g dry weight			*% (w/w)*	*nl/kg·hr*
Test 1						
10/30	Control	16.9 ± 1.3	35.6 ± 2.6	27.2 ± 3.3	82.1 ± 0.1	403 ± 33
10/30	Drought	17.2 ± 1.9	35.8 ± 1.8	21.2 ± 1.6	81.8 ± 0.3	348 ± 60
10/31	Control	15.4 ± 1.7	28.4 ± 2.1	25.3 ± 6.5	82.1 ± 0.2	730 ± 182
10/31[1]	Drought	12.6 ± 0.8	29.8 ± 2.0	24.4 ± 1.8	80.8 ± 0.2	774 ± 145
11/1	Control	12.0 ± 1.0	32.0 ± 2.0	21.7 ± 2.2	82.1 ± 0.2	730 ± 98
11/1	Drought	9.8 ± 0.7	33.0 ± 1.9	19.4 ± 1.7	80.4 ± 0.2	6755 ± 1762
Test 2						
11/12	Control	16.8 ± 2.3	40.2 ± 2.7	20.0 ± 0.8	80.2 ± 0.3	396 ± 25
11/12[1]	Drought	17.5 ± 1.8	40.7 ± 1.3	25.7 ± 1.5	80.3 ± 0.2	735 ± 189
11/13	Control	24.9 ± 1.6	49.8 ± 5.3	21.7 ± 2.0	80.2 ± 0.4	340 ± 46
11/13	Drought	16.6 ± 0.5	41.8 ± 1.3	23.7 ± 2.3	79.3 ± 0.4	6682 ± 1059
11/14	Control	13.8 ± 2.0	44.3 ± 3.8	24.3 ± 2.0	80.1 ± 0.2	534 ± 119
11/14	Drought	13.3 ± 0.6	49.0 ± 2.5	23.8 ± 1.9	78.9 ± 0.2	7572 ± 1521

[1] First day of wilting.

Table III. *Effects of Boll Age and Partial Desiccation on Ethylene Evolution, Moisture Contents, and Fresh Weights*

Bolls were harvested between 4:00 and 4:30 PM and stored overnight in a humid atmosphere (control) or in a desiccator over silica gel (desiccated) prior to ethylene measurements.

Treatment	Boll Age (days)			
	1.5	2.5	3.5	4.5
			nl/boll·hr	
Ethylene evolution				
Control	0.62 ± 0.04	1.18 ± 0.11	1.32 ± 0.25	1.03 ± 0.08
Desiccated	3.40 ± 0.37	5.12 ± 0.69	5.59 ± 0.51	5.44 ± 0.72
Ratio D/C	5.48	4.34	4.23	5.28
			% of fresh wt	
Moisture contents				
Control	78.8 ± 0.8	80.3 ± 0.5	80.9 ± 0.4	81.2 ± 0.2
Desiccated	73.1 ± 0.5	76.0 ± 0.5	76.8 ± 0.3	78.2 ± 0.3
Difference	5.7	4.3	4.1	3.0
			g	
Boll fresh wt				
Control	1.19	1.43	1.80	2.21
Desiccated	0.93	1.16	1.37	1.87

Table IV. *Effect of Time in Desiccator on Ethylene Evolution and Moisture Contents of Bolls and Bracts*

Bolls for the 16-hr test were harvested between 4:00 and 4:30 when they were 4.5 days old, and bolls for the 24-hr test were harvested between 8:00 and 8:30 when they were 4 days old. Bracts were removed after the desiccating treatment.

Treatment	Time in Desiccator			
	16 hr		24 hr	
	Bolls	Bracts	Bolls	Bracts
			nl/boll·hr	
Ethylene				
Control	1.76 ± 0.19	0.37 ± 0.03	6.30 ± 0.56	0.38 ± 0.03
Desiccated	11.54 ± 1.05	5.38 ± 0.34	17.20 ± 1.46	4.46 ± 0.74
Ratio D/C	6.56	14.54	2.73	11.74
			% evolved by bracts	
Total ethylene				
Control	17.4		5.7	
Desiccated	31.8		20.6	
			% of fresh wt	
Moisture content				
Control	83.0 ± 0.23	80.7 ± 0.74	81.8 ± 0.29	80.2 ± 0.5
Desiccated	81.1 ± 0.24	72.1 ± 1.24	79.2 ± 0.22	64.8 ± 1.0
Difference	1.9	8.6	2.6	15.4
			μl/kg·hr	
Ethylene				
Control	1.27 ± 0.16	0.80 ± 0.05	4.90 ± 0.47	0.90 ± 0.07
Desiccated	8.12 ± 1.12	18.10 ± 1.15	16.89 ± 1.11	20.37 ± 3.14

plants including cotton (16). Cracker and Abeles (4) reported that ABA stimulated ethylene production by cotton and bean explants, but the stimulation was not great and was evident for cotton only at the highest concentration of ABA tested, 0.5 mM. In at least one case, ABA inhibited rather than promoted ethylene production (16). Although wilting causes a dramatic increase in ABA content of leaves, the effect of water deficit on ABA content of bolls has not been reported. Therefore, convincing experimental evidence in support of a role of ABA in stimulating ethylene evolution by young cotton bolls is not yet available.

Because the sensitivity of organs to ethylene varies with age, condition, and the levels of other hormones (11, 12, 17), it is difficult to establish a threshold value at which ethylene will definitely cause abscission (13). The increase in ethylene evolution by bolls on water-stressed plants is probably a causal factor in the increased rates of boll abscission that sometimes occur when cotton plants are subjected to drought.

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