Published May, 1982

Catechin and Condensed Tannin Contents of Leaves and Bolls of Cotton in Relation to Irrigation and Boll Load[1]

Gene Guinn and Marie P. Eidenbock[2]

ABSTRACT

Water deficit has been shown to decrease insect numbers in cotton, especially *Lygus hesperus* Knight and *Heliothis* spp. Condensed tannins have antibiotic activity and have been shown to decrease the feeding, growth rate, and survival of *Heliothis* spp. Condensed tannins are produced from various catechins, and their concentration has been reported to increase in some plants with various stresses, including drought. Therefore, we measured the catechin and tannin contents of leaves and 4-day-old bolls in cotton during irrigation cycles and after the final irrigation. We obtained no evidence that water deficit increases the concentration of extractable catechin or tannin in leaves or bolls. Therefore, other factors must be responsible for the suppression of insect activity in dryland cotton or in irrigated cotton during the dry part of the irrigation cycle. Partial defruiting did increase the catechin and tannin contents of leaves and bolls, suggesting that loss of fruit may cause biochemical changes that contribute to insect resistance.

Additional index words: ***Tannin content, Catechin content, Cotton, Gossypium hirsutum*** **L., Insect resistance in cotton.**

MOISTURE status affects insect numbers in cotton (*Gossypium hirsutum* L.). Leigh et al. (4) found greater numbers of *Lygus hesperus* Knight and other plant bugs in cotton that was abundantly irrigated than in cotton that was not. Norman et al. (6) reported on production costs and yields of irrigated and dryland cotton in the lower Rio Grande Valley of Texas. Lower insect populations made a considerable savings possible in insecticide cost in the dryland cotton. Slosser (9) found that irrigation timing made a difference in ovipositional activity and larval survival of *Heliothis zea* (Boddie). Irrigations applied during peak ovipositional activity increased the number of eggs laid and increased larval survival.

Among the possible factors that may affect insect survival are changes in the biochemical composition of the plants. Polyphenolic compounds, such as tannins, combine with proteins and reduce the activity of digestive enzymes in herbivores including insects (10). Chan et al. (2), Schuster (7), and Schuster and Lane (8) reported that tannin increases the resistance of cotton to bollworm, *Heliothis* spp. Feeding, growth rate, and survival were decreased by high tannin content. Lane and Schuster (3) also reported evidence that tannin is a factor in resistance to spider mites.

Some stresses, including drought, have been reported to increase the biosynthesis of phenolic compounds in some plants (5). Proanthocyanidin tannins are the most widely distributed tannins in vascular plants and are formed by the condensation of such flavan-3ols as catechin, epicatechin, or the corresponding gallocatechins with flavan-3,4-diols (10). Therefore, we investigated the possible effects of irrigation on the catechin and condensed tannin contents of leaves and young bolls.

MATERIALS AND METHODS

Experiments were conducted during the summers of 1979 and 1980 at the Univ. of Arizona Cotton Research Center, Phoenix. The soil is an Avondale clay loam (a member of the fine-loamy, mixed, hyperthermic family of Typic Torrifluvents). Preplant irrigations of about 25 cm were applied on 16 Mar. 1979, and on 28 Mar. 1980 to wet the soil to a depth of at least 160 cm.

In 1979, 'Deltapine 61' was planted on 10 April in rows 1 m apart in a randomized complete block with four replications. Although three dates (29 May, and 5 and 14 June) for the first post-emergence irrigation were used as a treatment variable in another aspect of the field experiment, differences had largely disappeared by the time leaves and bolls were harvested for this test. A comparison of the data indicated that the date of first irrigation did not affect subsequent catechin and tannin contents. Therefore, dates of first irrigation were disregarded. Plots were 9 rows wide by 25 m long. Twenty flowers were tagged in each of the 12 plots on the day of anthesis for harvest 4 days later. Bolls were harvested from center rows in each plot twice weekly. Primary leaves, about 4 or 5 nodes below the apex, were also harvested from each plot twice weekly, but at different times during the season. At least five bolls or five leaves were harvested for each sample. Young bolls were harvested because they are a preferred feeding site for *Heliothis* spp. Leaves were also sampled because they may be affected more by water deficit than bolls, or they may be affected sooner during a period of drought. Leaves are a feeding site for spider mites (*Tetranychus* spp.) and, as recently shown by Lane and Schuster (3), tannins appear to confer resistance to mites.

In 1980, 'Deltapine 70' was planted on 14 April in rows 1 m apart at the same location as 1979, but in an adjacent field. Each plot was 12 rows wide by 26 m long; 8 m sections of rows 4 and 5 were sampled in each of six plots. To determine the effects of partial defruiting, all visible flowers were removed from row 5 for 4 days of each week. Primary leaves, 4 to 5 nodes below the apex, and 4-day-old bolls were harvested once each week. Each sample contained at least five bolls or leaves (up to 20 bolls).

Midday leaf water potentials were determined periodically during both summers. Three primary leaves 4 to 5 nodes below the apex were randomly sampled between 1230 and 1500 hours in each plot. The leaves were immediately sealed in plastic Ziploc[3] bags and placed in a humid styrofoam chest for transport to the laboratory. Leaf water potentials were determined with a pressure chamber (11).

Attempts were made to determine soil moisture gravimetrically in 1979, but dry soil became too hard to sample. Access tubes were installed in each plot in 1980 and soil moisture was estimated at 20-cm increments with a neutron moisture meter to a depth of 160 cm. Field capacity of the soil was 28% (v:v basis) and the cotton plants were unable to extract water below 11%. Volumetric water content and available soil moisture to a depth of 160 cm are shown in Table 4. Infrequent rains temporarily

[1]Contribution of the USDA, SEA-AR, and the Univ. of Arizona. Arizona Agric. Exp. Stn. Journal Paper No. 3428. Received 22 June 1981.

[2]Plant physiologist and biological lab technician, respectively, USDA, SEA-AR, Western Cotton Research Laboratory, 4135 E. Broadway, Phoenix, AZ 85040.

Table 1. Catechin and tannin contents of leaves in 1979 as influenced by irrigation.† Temperatures are means for 3 days before each harvest.

Date	Days after irrigation	Leaf water potential	Air temp. Max.	Air temp. Min.	Catechin	Tannin
	no.	bars	— C —		— mg/g dry wt. —	
18 June	4		39.1	23.3	4.4±0.2	31.1±2.7
21 June	7		35.2	16.7	5.7±0.7	26.2±2.1
11 June	13		37.1	20.2	5.7±0.6	19.9±5.7
12 June	14	−22.4				
14 June	17		41.5	24.1	4.3±0.4	15.5±0.6
15 August	2	−17.6				
16 August	3		34.4	20.4	7.1±0.4	27.8±1.5
20 August	7		34.6	19.3	9.6±0.5	32.2±2.7
22 August	9		34.6	18.9	8.3±0.6	34.5±2.3
24 August	11		38.7	17.4	8.3±0.6	29.9±2.3
9 September	27	−27.4				
12 September	30		41.5	24.6	6.2±0.3	27.0±1.6

† Data are means of 9 to 12 samples per harvest. Standard errors of the means are shown.

Table 2. Catechin and tannin contents of 4-day-old bolls in 1979 as influenced by irrigation.† Temperatures shown are means for 3 days before each harvest.

Date	Days after irrigation	Leaf water potential	Air temp. Max.	Air temp. Min.	Catechin	Tannin
	no.	bars	— C —		— mg/g dry wt. —	
3 July	19	−24.2	41.5	31.5	35.0±1.2	77.3±1.1
6 July	3	−18.4	40.2	20.2	38.8±1.4	81.5±3.2
10 July	7		43.9	23.2	34.3±1.4	76.0±2.1
13 July	10		43.3	20.4	31.0±1.4	63.2±3.6
17 July	14		42.9	25.6	27.1±1.0	60.2±2.1
18 July	15	−19.4				

† Data are means of 12 samples per harvest. Standard errors of the means are shown.

increased the humidity but were not adequate to cause any significant effect on soil moisture content. Rainfalls of 0.1, 0.7, 1.9, and 0.9 cm were recorded on 17 and 29 July, 12 and 14 Aug. 1979. Rainfalls of 0.3, 1.27, and 0.5 cm were recorded on 21 and 25 July, and 10 August, respectively, in 1980.

Air temperatures were recorded continuously. Average maximum and minimum temperatures were determined for the 3-day period before each harvest and are shown in Tables 1, 2, and 3.

Harvested leaves and bolls were frozen at −90 C, freeze-dried, and ground to pass a 40 mesh screen. Catechins were extracted with water at 98 C and condensed tannins were extracted with butanol-HC1 (95:5, v:v) at 98 C and determined by the methods of Bell and Stipanovic (1). For standards, commerical (+)-catechin was obtained from Sigma Chemical Co.[3] and tannin was prepared by the method of Chan et al. (2).

RESULTS AND DISCUSSION

1979. Assuming that stress was at a minimum during the first 7 days after irrigation, we found no evidence that catechin or tannin content of leaves increased with stress (Table 1). On the contrary, their concentrations may have decreased with stress. The lowest values were obtained on 14 June (17 days after irrigation) and on 12 September (30 days after the final irrigation). The relatively low values obtained on 16 August may have resulted from a lingering effect of stress that occurred before the 13 August irrigation. Data obtained in 1981 indicate that leaf water potentials do not attain maximum (least negative) values until about 6 days after irrigation. Stress was quite severe by 12 September, but catechin and tannin contents were lower than in August when moisture was still adequate.

Likewise, neither the catechin nor the tannin content of bolls increased with stress (Table 2). Concentrations of both were about the same on 3 July (19 days after the previous irrigation) as they were on 6 and 10 July (3 and 7 days, respectively, after the next irrigation). Furthermore, their concentrations tended to decrease rather than increase with time after irrigation.

1980. Because leaves and bolls were harvested only once per week during 1980, we obtained only two sets of tissues per irrigation cycle. Leaves from previously stressed plants were harvested immediately after irrigation instead of immediately before (only 10 hours, in some cases, between irrigation and harvest). Although the differences were not always significant, catechin content of leaves was higher 8 days after irrigation (following a week of minimum stress) than 1 day after irrigation (following a period of stress) (Table 3). Tannin content of leaves was higher 8 days after irrigation only on 23 July, and was not affected during the later irrigation cycles.

Catechin content of bolls increased as stress developed between 22 and 29 July, but decreased during the next irrigation cycle (Table 3). Tannin content of bolls usually decreased with stress and increased after the stress was relieved by irrigation.

Available soil moisture was considerably depleted by 28 July and 12 August (Table 4) and average temperatures were high during the 3 days preceding each of the harvests on 29 and 30 July and 12 and 13 August (Table 3). Midday leaf water potentials indicated moderate stress on 28 July and more severe stress on 11 August (Table 3). However, bolls that were harvested on 12 August at the end of a period of severe stress had lower concentrations of catechins and tannins than those harvested on other dates (Table 3).

Synthesis of catechin and tannin may have been affected by the availability of fixed carbon. Partial defruiting increased the concentrations of catechin and tannin in leaves and bolls during July and August (Table 3), although differences were not always statistically significant. Reduced competiton by a smaller boll load for photosynthate may have permitted the synthesis of slightly more catechin and tannin in the partially defruited plants. These increases suggest that loss of fruit (e.g., as a result of insect feeding) could cause biochemical changes that contribute to resistance. The extent, if any, of such increased resistance remains to be determined.

We found no evidence either year that catechin or tannin content of either leaves or young bolls increased with water stress. On the contrary, the data indicate that their concentrations may have decreased slightly as stress developed. Therefore, we conclude that neither the concentration of catechins nor the concentration of extractable condensed tannins can account for the decreases in activity of some insects, such as *Heliothis* spp., that have been noted as cotton became stressed between irrigations.

Since this work was completed a paper was published by

[3]Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

Table 3. Catechin and tannin contents of leaves and bolls in 1980 as influenced by irrigation and partial defruiting.† Temperatures are means for 3 days before each harvest.

Date	Days after irrigation	Leaf water potential	Air temp.		Catechin		Tannin	
			Max.	Min.	Control	Defruited	Control	Defruited
	no.	bars	C		mg/g dry wt.			
					Leaves			
16 July	1		43.2	25.0	4.1±0.3	6.4±0.5	24.6±1.8	28.5±2.1
17 July	2	−18.8						
23 July	8		41.5	27.1	6.1±0.1	9.2±0.6	30.2±1.0	39.1±1.8
28 July	13	−24.8						
30 July	1		44.1	28.9	5.9±0.5	8.2±0.3	26.4±1.5	31.5±1.3
31 July	2	−19.4						
6 August	8		41.7	28.2	6.7±0.7	9.4±0.3	26.4±1.5	35.4±2.6
7 August	9	−25.4						
11 August	13	−29.8						
13 August	1		42.8	28.2	4.8±0.4	8.0±0.8	24.6±1.3	28.0±1.9
14 August	2	−22.4						
20 August	8		39.6	22.8	6.8±1.1	9.0±0.8	23.6±1.8	28.3±2.3
					Bolls			
17 July	2	−18.8						
22 July	7		41.1	27.4	20.8±1.2	26.2±0.5	65.7±3.6	79.7±4.2
28 July	13	−24.8						
29 July	14		44.4	27.2	28.6±0.6	30.9±1.6	70.5±3.7	65.3±4.5
31 July	2	−19.4						
5 August	7		40.7	28.3	23.1±2.1	29.3±1.8	67.9±1.5	79.9±2.9
11 August	13	−29.8						
12 August	14		43.5	28.3	20.6±4.6	25.4±1.7	37.2±1.8	54.7±1.5
14 August	2	−22.4						
19 August	7		40.0	23.7	41.1±1.0	46.1±2.9	50.3±3.9	61.3±2.6

† Data are averages of six samples per harvest. Standard errors of the means are shown.

Table 4. Soil moisture contents in the top 160 cm of soil in 1980.†

Date	Volumetric water content	Available water remaining
	%	%
14 July	21±0.6	60±4.5
16 July	27±0.7	95±3.4
28 July	18±0.8	44±4.7
30 July	25±1.1	81±6.3
12 August	17±1.2	38±7.0
14 August	24±1.2	76±7.3
16 September	13±0.6	9±3.4

† Data are averages of six replications and standard errors of the means are shown. Field capacity of this soil is 28% on a volumetric basis and the available range is 11 to 28%.

Lane and Schuster (3) in which they pointed out that concentration of tannin is only one of several factors that may affect resistance. Other factors such as kind of polymer (e.g., procyanidin, prodelphinidin, or mixed), molecular weight, and astringency may also affect antibiotic activity.

ACKNOWLEDGMENT

We thank R. L. McDonald for making the soil moisture measurements.

REFERENCES

1. Bell, A. A., and R. D. Stipanovic. 1972. Chemistry and nature of fungitoxic compounds in diseased cotton. p. 87–88. *In* Proc. Beltwide Cotton Production Research Conf. National Cotton Council, Memphis, TN.
2. Chan, B. G., A. C. Waiss, Jr., and M. J. Lukefahr. 1978. Condensed tannin, an antibiotic chemical from *Gossypium hirsutum*. J. Insect Physiol. 24:113–118.
3. Lane, H. C., and M. F. Schuster. 1981. Condensed tannins of cotton leaves. Phytochemistry 20:425–427.
4. Leigh, T. F., D. W. Grimes, W. L. Dickens, and C. E. Jackson. 1974. Planting pattern, plant population, irrigation, and insect interactions in cotton. Environ. Entomol. 3:492–496.
5. McKey, D. 1979. The distribution of secondary compounds within plants. p. 56–133 *In* G. A. Rosenthal and D. H. Janzen (ed.) Herbivores, their interaction with secondary plant metabolites. Academic Press, New York.
6. Norman, J. W., Jr., J. L. Henson, D. A. Wolfenbarger, J. A. Harding, E. V. Gage, R. D. Parker, Jr., and G. Collins. 1979. Effects of water management practices on economics of insect control on cotton, lower Rio Grande Valley, Texas. J. Econ. Entomol. 72:367–370.
7. Schuster, M. F. 1979. New sources of high tannin resistance to *Heliothis* in upland cotton resulting in feeding deterrence. p. 85–87. *In* Proc. Beltwide Cotton Prod. Res. Conf. National Cotton Council, Memphis, TN.
8. ----, and H. C. Lane. 1980. Evaluation of high-tannin cotton lines for resistance to bollworms. p. 83–84. *In* Proc. Beltwide Cotton Prod. Res. Conf. National Cotton Council, Memphis, TN.
9. Slosser, J. E. 1980. Irrigation timing for bollworm management in cotton. J. Econ. Entomol. 73:346–349.
10. Swain, T. 1979. Tannins and lignins. p. 657–682. *In* G. A. Rosenthal and D. H. Janzen (ed.) Herbivores, their interaction with secondary plant metabolites. Academic Press, New York.
11. Waring, R. H., and B. D. Cleary. 1967. Plant moisture stress evaluation by pressure bomb. Science 155:1248–1254.