BIOCHEMICAL ASPECTS OF INDUCED RESISTANCE IN COTTON TO THE COTTON BOLLWORM

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Abstract

Induced resistance in cotton plants to herbivory by the cotton bollworm (*Helicoverpa zea*) was studied. Induced resistance was indicated by decreased larval growth of *H. zea* when larvae fed on previously damaged foliage or squares compared to the undamaged controls. Herbivory caused a significant increase in several primary gene products including peroxidase, ascorbate oxidase, lipoxygenase and diamine oxidase in foliage or squares. The exogenous application of methyl jasmonate on cotton plants also elicited resistance against *H. zea*. Our findings suggest that enhanced resistance to the bollworm may be achieved via genetic amplification of several defensive genes and/or by application of chemical elicitors of resistance.

Introduction

Research on the phytochemical basis of arthropod resistance in cotton has been primarily focused on constitutive factors. These factors include condensed tannins, flavonoids, and terpenoid aldehydes such as gossypol (Chan et al., 1978a,b; Zummo et al., 1983, 1984; Hedin et al., 1992).

Induced resistance to arthropods has also been well documented in cotton using spider mites and some lepidopterans (Karban and Carey, 1983; Karban, 1986a,b; Karban, 1988), but a phytochemical basis for induced resistance has not been reported. The current study was initiated to determine if prior feeding by *H. zea* [1] induces *H. zea* resistance in cotton foliage and squares, [2] induces the accumulation of defensive phytochemicals (e.g. gossypol, tannins, etc.), and [3] induces the formation of primary gene products implicated in arthropod resistance.

Materials and Methods

Induction of Resistance and Related Phytochemicals

Eggs of *H. zea* were obtained from the University of Arkansas Insect Rearing Facility. Larvae were maintained on artificial diet until used in the experiment (Chippendale, 1970). Cotton (*Gossypium hirsutum*) seeds (cv. Deltapine 50) were grown in both greenhouse and field.

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To determine if *H. zea* feeding induces resistance, a single fourth instar larva was placed on each of 40 four-node stage plants in greenhouse. Plants were placed individually in screen cages to prevent larval escape. Forty control plants were identically treated except that larvae were excluded. Larvae were starved for 24 h prior to infesting the plants. After 72 h, two terminal fully-expanded leaflets from each damaged plant and the same-positioned two leaflets from each control plant were excised. The 72-h period was chosen because preliminary experiments indicated that resistance to *H. zea* was not induced at 24 to 48 h post damage.

The excised leaves from each plant were placed in a 500-ml clear plastic container (Fabri-Kal Co., Kalamazoo, MI) with two layers of moist filter paper (Whatman No. 1) in the bottom. A newly-molted fourth instar or ten neonates were placed in each container. Leaves from 20 damaged and 20 control plants were used for the single fourth-instar treatment and the leaves from the other plants were used for the ten-neonate treatment. Containers were randomly placed in an incubator at 28 C. Each fourth instar was weighed at the beginning of the test and again after 48 h. There were no significant differences between the initial weights of the larvae used in the treatment and control containers. The neonates were weighed 96 h after being placed in the containers.

To evaluate the induced phytochemical responses in cotton foliage to feeding by *H. zea*, 20 damaged and 20 control plants as described above were used. Fully-expanded terminal foliage from each of 10 damaged and 10 control plants was individually assayed for lipoxygenase (LOX), peroxidase (POD), ascorbate oxidase (AOX), diamine oxidase (DAO) and H_2O_2. The terminal foliage of the remaining plants was excised and immediately placed in plastic bags which were held on dry ice. After being fully frozen, the foliage was freeze-dried and then ground to powder for assays of lipid peroxides and phenolic compounds.

In a second test, conducted in the field, sixteen 15-node stage plants were used: eight control and eight treated plants. Each plant was enclosed in an organdy-covered 1 m^3 cage. Plants were damaged by placing eight fourth instar *H. zea* on each treatment plant. After five days, the original larvae had pupated or died. Five newly-molted fourth instars were weighed individually and placed for three days on each of the control or treatment plants. The larvae were then collected and weighed individually. Because of the movement of larvae on plants in their respective cages and the inability to identify original individuals, the larval relative growth rate (RGR) was calculated using the mean initial weight rather than individual weights.

Squares were also used for bioassay because they represent the preferred food source for later instars of *H. zea*. Three damaged squares from each of eight treated plants and three

same-positioned squares from each of eight control plants were excised. Each square was placed individually in a 500-ml container as described above with a newly-molted fourth instar *H. zea* for 48 h in a 28 C incubator. The RGR was then computed.

Two fresh squares from each of the eight damaged or eight control plants were assayed for LOX, POD, AOX and DAO. Another two squares from each of the eight damaged or eight control plants were freeze-dried, ground to powder and pooled together for treatment or control group. The powder was assayed for phenolics and lipid peroxides.

Effect of Exogenous MeJA and MeSA on Cotton Foliar Resistance

To evaluate the effect of exogenous application of signal compound methyl jasmonate (MeJA) or methyl salicylate (MeSA) on cotton resistance to *H. zea*, a test was conducted in the field. Ninety cotton plants were grown in pots as described above. At the 4-node stage, the potted plants were divided into 3 groups separated by a distance of 5 m. Group 1 was sprayed with 100 µM MeJA in 0.1% ethanol, group 2 was sprayed with 100 µM MeSA, and group 3 was sprayed with 0.1% ethanol as a control. The sprays were conducted until runoff on 3 consecutive evenings. Twenty-four hours following the final application, a single upper-most fully-expanded leaf from each plant was excised. Thirty excised leaves from each group were individually placed in a plastic container with water-moistened filter paper as described above. In each group, a newly molted 3rd instar was placed in each of 20 containers, and 7 neonates were placed in each of 10 containers. Containers were randomly placed in a growth chamber at 28 C. Each 3rd instar was weighed at the beginning of the test and again after 48 h and 64 h. The RGR was computed for each interval. The neonates were weighed 96 h after being placed in the containers. There were no significant differences between the initial weights of larvae used in treatment or the control.

Results

Induced Resistance in Foliage and Squares due to Herbivory

Resistance was significantly induced in foliage and squares by *H. zea* larval feeding (Tables 1 and 2). The RGR of fourth instars was reduced by 11.2% when they fed on excised foliage from previously damaged plants, 37.6% on squares excised from damaged plants, and 24.0% on damaged intact plants compared to larvae on the respective controls. The larval weight of neonates was decreased 61% when larvae fed on damaged foliage compared to control foliage. The larval survivorship was not significantly affected by treatment.

Oxidative Responses in Foliage and Squares to Herbivory

Larval *H. zea* feeding increased the levels of several defensive proteins in cotton foliage and squares (Tables 3 and 4). Foliar POD activities increased by 2-fold, AOX by 2-fold and DAO by 1-fold compared with the control foliage. However, the feeding did not significantly increase the activity of foliar LOX. The feeding also significantly increased activities of the assayed oxidases in squares including POD by 4-fold, AOX by 2-fold, LOX by 2-fold, and DAO by 1-fold.

Feeding by *H. zea* also resulted in significant increase in foliar hydrogen peroxide by 62% compared to controls (Table 3). However, foliar lipid peroxides were not significantly changed by the herbivory (Table 3). In damaged squares, a 45% increase in lipid peroxides occurred compared to the control treatment (Table 4).

Changes in Phenolics in Foliage and Squares due to Herbivory

Larval *H. zea* feeding on cotton altered the levels of several foliar phenolic compounds, including condensed tannins which decreased 31% compared with the control foliage. Total flavonoids and terpene aldehydes were decreased very slightly (<5%) by herbivory (Table 5). Conversely, herbivory raised levels of chlorogenic acid by 59.6%, and rutin by 10.7%.

Changes in phenolic compounds also occurred in cotton squares following the herbivory (Table 6). Larger decreases in terpene aldehydes (35.4%), rutin (15.4%), and a small loss (5.9%) of total flavonoids, were observed in wounded squares compared to the control squares. In contrast, an increase in chlorogenic acid (28.9%) was found in damaged squares compared to the control treatment. Condensed tannins in squares were relatively unaffected by treatment.

Effect of Exogenous MeJA and MeSA on Cotton Foliar Resistance

Application of exogenous MeJA decreased neonate *H. zea* growth by 35.5%, 3rd *H. zea* RGR by 7.4% at 48 h, and by 7.7% at 64 h compared to the respective controls (Table 7 and 8). However, applying exogenous MeSA did not affect the weight gain or survivorship of neonates after a 96-h feeding period (Table 7). The effect of MeSA on third instar *H. zea* growth rate was also insignificant ($P>0.05$) at both 48- and 64-h feeding periods (Table 8).

Discussion

Previous feeding damage on cotton foliage and squares induced resistance to *H. zea* when measured in excised leaves and squares or intact plants (Table 1 and 2). Reduced growth rates may enhance larvae susceptibility to Bt, pathogenic virus, parasitoids and predators.

Correlated with induced resistance is a significant induction of several primary gene products in cotton. These gene products do not include condensed tannins, total flavonoids or gossypol but instead are defensive proteins (Table 3,4,5 and 6). Resistant cultivars may be developed by conventional breeding for higher levels of these gene products or by using biotechnology to increase the expression of these resistance genes.

Another means to enhance plant resistance against insects is to identify the plant signal pathways that are used to recognize herbivore attack and subsequently increase the production of natural resistance factors. Cotton already produces these gene products in response to insect attack but well below their maximal levels. Foliar application of MeJA, a natural chemical that is harmless to environment, markedly increases the resistance against *H. zea* (Table 7 and 8). Jasmonate is naturally released from damaged plant tissues and is an important signal eliciting the synthesis of several plant defensive materials such as proteinase inhibitors, chalcone synthase, proline-rich cell wall proteins, phenylalanine ammonia lyase, and alkaloids. Understanding these signaling systems may provide a novel approach to insect pest management.

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Table 1. Effect of previous herbivory of cotton plants on fourth instar *H. zea* relative growth rate[1]

Larval Diet	Control	Damaged
Excised foliage (mg day-1 mg-1)	0.501 (0.009) a	0.445 (0.010) b
Excised squares (mg day-1 mg-1)	0.497 (0.012) a	0.310 (0.025) b
Intact plants (mg day-1 mg-1)	0.250 (0.018) a	0.190 (0.017) b

[1]Fourth instars fed on excised foliage or squares for 48 h in laboratory and fed on intact plants for 72 h in field.
Means in the same row followed by different letter were significantly different at LSD.05, and numbers in parentheses are standard errors.

Table 2. Effect of previous herbivory of cotton foliage on neonate *H. zea* growth

Treatment	Larval Weight[1] (mg)	Survivorship (%)
Control	9.2 (0.55) a	80 a
Damaged	3.6 (0.21) b	83 a

[1]Larval weight was measured at 96 h after neonate feeding on excised foliage in laboratory.
Means in the same column followed by different letter were significantly different at LSD.05, and numbers in parentheses are standard errors.

Table 3. Effect of herbivory by *H. zea* on oxidative status in cotton terminal foliage

Phytochemical	Control	Damaged
AOX (nmol min-1 g-1 fresh weight)	101.0 (11.55) a	205.0 (15.65) b
DAO (mol min-1 g-1 fresh weight)	1.89 (0.127) a	2.30 (0.092) b
LOX (nmol min -1 g-1 fresh weight)	11.2 (0.52) a	13.8 (1.78) a
POD (OD min-1 g-1 fresh weight)	82.0 (7.00) a	180.0 (11.98) b
H_2O_2 (μmol g^{-1} fresh weight)	1.49 (0.12) a	2.41 (0.35) b
Lipid peroxides (nmol g-1 dry weight)	3450.5 (54.0) a	3543.4 (41.1) a

Means in the same row followed by different letter were significantly different at LSD.05, and numbers in parentheses are standard errors.

Table 4. Effect of herbivory by *H. zea* on oxidative status in cotton squares

Phytochemical	Control	Damaged
AOX (nmol min-1 g-1 fresh weight)	49.0 (8.87) a	82.0 (8.05) b
DAO (mol min-1 g-1 fresh weight)	1.50 (0.11) a	2.18 (0.24) b
LOX (nmol min -1 g-1 fresh weight)	112.2 (18.04) a	187.0 (17.13) b
POD (OD min-1 g-1 fresh weight)	0.26 (0.05) a	0.92 (0.14) b
Lipid peroxides (nmol g-1 dry weight)	1548.3 (91.0) a	2244.3 (97.0) b

Means in the same row followed by different letter were significantly different at LSD.05, and numbers in parentheses are standard errors.

Table 5. Effect of herbivory by *H. zea* on phenolic compounds in cotton terminal foliage

Phenolic	Control	Damaged
Condensed tannins (mg g-1 dry weight)	10.2 (0.08) a	7.0 (0.09) b
Total flavonoids (mg g-1 dry weight)	43.2 (0.18) a	42.1 (0.07) b
Chlorogenic acid (µg g-1 dry weight)	996.0 (43.8) a	1590.1 (56.2) b
Rutin (µg g^{-1} dry weight)	1094.3 (19.7) a	1211.4 (37.3) b
Terpene aldehydes (mg g-1 dry weight)	2.7 (0.02) a	2.6 (0.02) b

Means in the same row followed by different letter were significantly different at LSD.05, and numbers in parentheses are standard errors.

Table 6. Effect of herbivory by *H. zea* on phenolic compounds in cotton squares

Phenolic	Control	Damaged
Condensed tannins (mg g-1 dry weight)	132.8 (0.25) a	132.2 (0.54) a
Total flavonoids (mg g-1 dry weight)	18.5 (0.17) a	17.4 (0.18) b
Chlorogenic acid (µg g-1 dry weight)	2144.5 (27.7) a	2763.2 (106.4) b
Rutin (µg g-1 dry weight)	3458.9 (12.5) a	2926.3 (18.7) b
Terpene aldehydes (mg g-1 dry weight)	8.2 (0.26) a	5.3 (0.12) b

Means in the same row followed by different letter were significantly different at LSD.05, and numbers in parentheses are standard errors.

Table 7. Effect of application of MeJA and MeSA to cotton plants on neonate *H. zea* growth

	Larval Weight[1] (mg)	Survivorship (%)
Control	3.52 (0.27) a	79 a
MeJA	2.27 (0.19) b	79 a
MeSA	3.37 (0.19) a	76 a

Larval weight was measured at 96 h after feeding on excised foliage in the laboratory.
Means in the same column followed by different letter were significantly different at LSD.05, and numbers in parentheses are standard errors.

Table 8. Effect of application of MeJA and MeSA to cotton on third *instar H. zea* growth rate

	RGR (mg day^{-1} mg^{-1} larva)	
	at 48 h	at 64 h
Control	0.635 (0.008) a	0.529 (0.007) a
MeJA	0.588 (0.057) b	0.488 (0.052) b
MeSA	0.620 (0.010) a	0.516 (0.043) ab

Means in the same column followed by different letter were significantly different at LSD.05, and numbers in parentheses are standard errors.