Mechanism of Resistance in Wild *Gossypium* species to *Helicoverpa armigera* (Hübner)

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ABSTRACT

Eight wild species of Gossypium were evaluated to determine the mechanism of resistance to Helicoverpa armigera (Hübner). Wild species, viz. Gossypium anomalum, G. raimondii, G. harknessii, G. arboreum, G. palmari, G. davidsonii, G. armourianum and G. stocksii and the susceptible control MCU 9 and TCHB 213 were included in the study. Oviposition studies revealed that none of the wild species was preferred for oviposition both under free choice and no choice test. The lowest number of eggs was laid on G. harknessii, G. raimondii, G. stocksii and the largest MCU 9 and TCHB 213. Trichome density did not show linear relationship with oviposition. G. raimondii, G. anomalum, G. davidsonii, G. armourianum and G. stocksii were least preferred for feeding by H. armigera larvae and adversely affected the larval survival, weight gain, pupation, weight and size of the pupae, adult emergence and sex ratio exhibiting antibiotic effect on the growth and development of H. armigera. Tannin, gossypol and total phenols in the leaves and fruiting bodies of wild species were negatively correlated with weight, survival of larvae and pupation of H. armigera.

Introduction

Cotton (*Gossypium* spp.) is the chief source of natural fiber in India, constituting eighty-five per cent of the raw material for the textile industry. Of the important constraints in cotton production, the insect pests problem is the major one. Cotton has a reputation of being a highly susceptible host plant requiring prolonged protection with insecticides as insect pests cause damage right from seedling to maturity. Cotton occupies only five per cent of the total cropped area, but consumes about 53 per cent of pesticides in India (Basu, 1994).

Helicoverpa armigera (Hübner) has become a serious threat to the cotton production in the Peninsular India and widely distributed throughout Africa, Middle East, Southern Europe, Central and South East Asia, New Zealand and many Eastern Pacific Islands (Fitt, 1989) and Eastern and Northern Australia (Fitt, 1994).

Cultivar resistance has been increasingly recognized as the most desirable and economic control tactic in the management of *H. armigera*. The potential value of wild species of *Gossypium* as a source of resistance to the bollworms has been reported by many authors (Narayanan *et al.*, 1985; Kothandaraman, *et al.*, 1988; Tayyab, 1990; Mohite, 1995). Some of these genotypes were studied to determine their mechanism of resistance to *H. armigera*.

Material and Methods

The wild species of *Gossypium*, viz. *G. anomalum, G. raimondii, G. harknessi, G. arboreum, G. palmari, G. davidsonii* and *G. armourianum* and *G. stocksii* maintained at the Department of Cotton, School of Genetics, Tamil Nadu Agricultural University and the Central Institute of Cotton Research Regional Station, Coimbatore, were utilized for the present study.

Oviposition preference

Oviposition preference of *H. armigera* on eight wild species of *Gossypium* and two susceptible checks MCU 9 and TCHB 2.3 was studied under laboratory conditions employing free-choice and no-choice tests.

(a) Free choice test: Branches about 45 cm long twigs with squares, flowers and bolls were detached from the plant and its cut end was placed in a 500 ml conical flask of water. These were kept in 120x90x90 cm insect cages. A sufficient number of newly emerged moths in a ratio of 1:2 (male:female) were released in the cage at 17.00 hours in the evening for oviposition. The moths were provided with 10 per cent sucrose solution for feeding. The number of eggs laid on each of the wild species was recorded on the third day after the release of the moths. There were three replications of the experiment.

(b) No Choice Test: The conical flasks with the branches were covered individually with 30x60 cm polyethylene film cages and one pair of moth was released in each cage. The number of eggs laid on each wild species was recorded on the third day after release. There were three replications.

Estimation of trichomes

The trichome density of third fully opened leaf selected randomly of all the genotypes included in the study was estimated as per the procedure followed by Maite

et al. (1980).

Preference for feeding

In order to study the feeding preference of *H. armigera* larvae, the branches of wild and cultivated varieties (approx. 45 cm long) with squares, flowers and bolls were kept in a conical flask of water. These flasks were placed a cage, equi-distance from each other. Twenty-five third instar larvae were released at a centre after starving for four hours. The number of larvae found attached and feeding on each wild species at five min., one, two and six hours after release were counted. Each treatment had three replications arranged in a complete randomized block design (CRBB).

Assessment of antibiosis

Antibiosis to *H. armigera* was studied under laboratory conditions following the methods of Oliver *et al.* (1967) and Parrott *et al.* (1978). Third instar larvae were weighed individually and placed on the plant parts (leaves, squares and bolls) of wild species of *Gossypium* in a plastic container. The food was changed every two days. Six days after release, the larvae were re-weighed and the net weight gained for each larva was calculated. Observations were also made on pupal size and weight and the sex ratio of adults.

Phytochemical analysis

The estimation of phytochemicals of wild species in leaves and fruiting part was carried out following the established procedures indicated for tannin and gossypol (Sadasivan and Manickam, 1992) and total phenols (Bray and Thorpe, 1954).

Results

Oviposition preference

The wild species of *Gossypium* were less preferred for oviposition compared to susceptible checks under free choice tests (Table 1). The mean number of eggs laid on wild species ranged from 0 to 8.67 per plant. Of these *G. armourianum* was not preferred for oviposition, while *G. arboreum* recorded a mean of only one egg per plant which was comparable to *G. stocksii, G. harknessii* and *G. davidsonii*. The mean number of eggs laid per plant on TCHB 213 was 22.33 and on MCU 9, 28.00.

Under no choice test conditions, relatively more eggs were laid per plant on the different genotype than under free choice test conditions. MCU 9 and TCHB 213 were most preferred, recording 34.67 and 31.00 eggs per plant, respectively. Under no choice condition, preference for wild species *G. harknessii, G. raimondii, G. stocksii* and *G. anomalum* was significantly below the other species, while *G. davidsonii, G. arboreum* and *G. armourianum* were equally preferred for oviposition.

There was no linear relationship between trichome density and oviposition preference (r = 0.091).

Preference for feeding

Within five minutes of release, significantly more larvae were observed on *G. palmari*, MCU 9 and *G. raimondii*. *G. harknessii* and *G. davidsonii* were less preferred for feeding (Table 2). One hour after release, 68.0 and 60.0 per cent larvae were found feeding on *G. palmari* and TCHB 213, respectively. Two hours after release, significantly more, i.e. 64.0 and 60.00 percent of larvae were found feeding on MCU 9 and TCHB 213, respectively. Preference of *G. palmari* was also significantly reduced. At six hours after release, the wild species were significantly less preferred by larvae for feeding compared to cultivated species.

Assessment of antibiosis

The larval survival was 100 per cent on cultivated MCU 9 and TCHB 213, while in wild species, it ranged from 53.3 per cent on *G. raimondii* to 73.3 per cent on *G. harknessii, G. arboreum, G. davidsonii* and *G. armourianum* (Table 3).

The weight of third instar larvae six days after feeding was also higher on cultivated varieties of cotton (0.104 to 0.189 g) than on wild species. The larvae reared on *G. raimondii* and *G. anomalum* recorded the lowest weight gain of 0.104 and 0.106 gms., respectively.

The pupation rate was 90 per cent on cultivated cotton, while in wild species, it ranged from 26.7 per cent on *G. raimondii* to 60.4 percent in *G. anomalum*. The weight and size of the pupae was also higher when larvae were reared on cultivated cotton than on wild species. The adult emergence was ranged from 20 per cent in *G. raimondii* to 76.67 percent in MCU 9. The sex ratio of insect reared on *G. raimondii* was 1:0.5 and 1:1.7 on *G. anomalum*.

There were no linear relationship between the weight gain of larva and tannin or gossypol content (Table 4). However, highly significant negative correlations were observed between per cent larvae pupating and tannin, gossypol and phenol content.

Discussion

Host plant resistance is a key component of the integrated insect pest management of cotton. The wild species play a major role, especially in breeding for resistance and cytoplasmic male sterility. The wild species of *Gossypium* have been reported as a source of resistance to bollworm by many workers (Narayanan *et al.*, 1985; Altman *et al.*, 1990; Wang and Lin, 1992; Tayyab *et al.*, 1994), but the exact mechanism of resistance has not been studied so far.

The wild species *G. harknessi* and *G. armourianum* with glabrous leaves were not preferred for oviposition by *H. armigera*. The findings are in agreement with those reported by Lukefahr *et al.* (1971) and Ramnath *et al.* (1992), who stated that glabrous cottons do not provide favourable oviposition site and prove effective

in reducing *H. armigera* oviposition on cotton under free choice and no choice conditions (Benedict *et al.* (1988):). The wild species of *Gossypium* with dense pubescence were not preferred for oviposition. Sivasubramanian (1991) reported that the dense pubescence in wild species *G. anomalum, G. raimondii* and *G. davidsonii* might interfere with larval movement, resulting in failure of larvae to reach the target feeding site. The presence of resistance in wild species of *Gossypium* due to dense pubescence has also been reported by many workers (Santhanam *et al.*, 1964; Narayanan *et al.*, 1988; Sivasubramanian and Uthamasamy, 1995; Narayanan and Singh, 1994).

Among the wild species, *G. raimondii* and *G. palmari* were initially preferred for feeding. This may be due to the size, shape and colour of the flower that may be responsible for attracting the larvae for feeding. The feeding preference of *H. armigera* larvae six hours after release revealed that wild species are not preferred for feeding, probably because of the chemical constituents of the plant. Even the presence of extraneous chemicals on host plants also prevents feeding on crops (Lukefahr *et al.*, 1971; Maxwell *et al.*, 1972). It is assumed that volatile compound produced by the plants attract the insects to the host and stimulate a behavioural response such as feeding (Hedin *et al.*, 1974).

The wild species of *Gossypium* adversely affected the larval survival, weight of larva, pupation, weight and size of the pupa, adult emergence and sex ratio. A high significantly negative correlation between biochemical constituents and larval survival and pupation indicated the antibiotic activity of wild species. Higher quantities of the biochemical constituents gossypol, tannin and total phenols were detected in wild species of *Gossypium*. The role of gossypol in imparting antibiosis to *Helicoverpa* spp. has already been documented by Chan *et al.*, (1978), Kay *et al.*, (1979) and Thomson (1957). Not much work has been done on the assessment of antibiosis in wild species of *Gossypium*.

Wild *Gossypium* species have not been utilized in crop improvement because of several barriers that operate at various levels and prevent successful gene transfer from wild into cultivated cotton (Pundir, 1972; Gill and Bajaj, 1986). There are strong possibilities that genes conferring useful resistance to *H. armigera* from the wild species could be incorporated through biotechnological methods.

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Table 1. Preference of *H. armigera* for oviposition among wild species of *Gossypium.*

Species	Number of Eggs laid		Trichome density
	No-choice	Free-choice	(No./cm2)
G. anomalum	6.00 (7.62)	8.67 (3.03)	137.33
G. raimondii	5.00 (6.69)	8.33 (2.87)	132.00
G. harknessii	4.67 (6.66)	2.33 (1.00)	0.00
G. arboreum	8.67 (8.98)	1.00 (1.10)	52.00
(Red *arboreum*)			
G. palmari	10.67 (8.98)	4.66 (2.22)	72.00
G. davidsonii	8.00 (8.66)	3.00 (1.86)	149.00
G. armourianum	9.00 (9.20)	0.00 (0.71)	3.40
G. stocksii	5.33 (7.14)	1.00 (1.10)	32.66
TCHB 213	31.00 (15.79)	22.33 (4.77)	98.67
MCU 9	34.67 (17.74)	28.00 (5.33)	99.00
SD (P=0.05)	1.01**	0.88**	

*Mean of three replications.
** Significant at 1%. Figures in parenthesis √X + 0.5 transformations.

Table 2. Distribution of larvae on wild species of *Gossypium.*

Species	5 min		1 h		2 h		6 h	
	Number	%	Number	%	Number	%	Number	%
G. anomalum	0.33	4.0	0.66	8.0	1.33	16.0	1.33	16.0
	(0.88)		(1.00)		(1.29)		(1.34)	
G. raimondii	2.66	32.0	1.67	20.0	1.00	12.0	0.00	0.0
	(1.72)		(1.44)		(1.71)		(0.71)	
G. harknessii	1.00	12.0	1.67	20.0	1.67	20.0	1.00	16.0
	(1.22)		(1.44)		(1.14)		(1.27)	
G. arboreum	1.67	20.0	1.67	20.0	2.00	24.0	1.33	16.0
(Red *arboreum*)	(1.46)		(1.46)		(1.58)		(1.68)	
G. palmari	4.33	52.0	5.67	68.0	3.67	44.0	2.33	28.0
	(2.19)		(2.45)		(2.03)		(1.76)	
G. davidsonii	1.33	16.0	1.00	12.0	1.00	12.0	2.67	32.0
	(1.29)		(1.17)		(1.17)		(2.60)	
***G.** armourianum*	1.67	20.0	1.67	20.0	1.33	16.0	1.33	16.0
	(1.39)		(1.39)		(1.29)		(1.29)	
G. stocksii	1.33	20.0	2.33	20.0	1.33	16.0	0.33	4.0
	(1.29)		(1.67)		(1.34)		(0.88)	
TCHB 213	2.67	32.0	5.00	60.0	5.00	60.0	6.33	76.0
	(1.77)		(2.34)		(2.34)		(2.60)	
MCU 9	3.00	36.0	3.00	36.0	5.33	64.0	8.00	96.0
	(1.86)		(1.86)		(2.41)		(2.90)	
CD (P=0.05)	0.63*		0.68**		0.61**		0.57**	

Figures in parentheses are √ X+0.05 transformation
*Significant at 5% level. **Significant at 1%level.

Table 3. Effect of *Gossypium* species on the development of *H. armigera*.

Species	Larvae after six days		Pupae			Adult	Sex
	survival-(%)	weight gms.	Pupation (%)	weight (mg)	length (mm)	Emergence (%)	Ratio (M:F)
G.anomalum	66.67	0.106±0.03	60.40	76.8±4.53	11.0	26.67	1:1.7
G.ramondii	53.33	0.104±0.04	26.67	117.4±2.94	11.0	20.00	1:0.5
G.harknessii	73.33	0.178±0.06	53.33	150.2±2.79	13.6	40.00	1:1.4
G.arboreum	66.67	0.184±0.03	53.33	182.0±2.76	13.4	40.00	1:1.0
G.palmari	66.67	0.164±0.02	60.00	138.4±2.06	12.8	46.67	1:1.33
G.davidsonii	73.33	0.160±0.03	46.67	148.2±1.83	12.2	33.33	1:1.5
G.armourianum	73.33	0.189±0.03	60.00	170.0±3.41	12.0	46.67	1:0.75
G.stocksii	66.67	0.112±0.01	40.00	95.0±2.91	11.0	26.67	1:1.6
TCHB 213	100.00	0.215±0.02	90.00	259.6±3.26	15.6	73.33	1:1.2
MCU 9	100.00	0.210±0.02	90.00	267.6±2.25	15.4	76.67	1:1.6

Table 4. Correlation coefficients between *H. armigera* larval survival and weight, pupation and phytochemicals in *Gossypium.*

Determination	Weight of larvae	Survival (%)	Pupation (%)	Tannin	Gossypol	Phenols
Survival (%)	-0.09	---	---	---	---	---
Pupation (%)	-0.39	0.32	---	---	---	---
Tannin	-0.36	-0.47	-0.83**	---	---	---
Gossypol	-0.35	-0.57	-0.89**	-0.95*	---	---
Phenols	-0.42	-0.50	-0.87**	0.98**	0.97**	---

*, **Correlation coefficients are significantly different from zero at the 0.05 and 0.01 level of probability, respectively