

Antixenosis mechanism of resistance in pigeonpea to the pod borer, *Helicoverpa armigera*

D. A. Kumari^{1,2}, D. J. Reddy² and H. C. Sharma¹

¹International Crops Research Institute for the Semi-Arid Tropics, Patancheru Andhra Pradesh, India;

²Department of Entomology, Acharya N G Ranga Agricultural University, Rajendranagar, Hyderabad, Andhra Pradesh, India

Ms. received: September 17, 2005; accepted: October 31, 2005

Abstract: The noctuid pod borer, *Helicoverpa armigera*, is one of the most important pests of pigeonpea, and plant resistance is an important component for minimizing the extent of losses caused by this pest. To develop insect-resistant cultivars, it is important to understand the contributions of different components of resistance, and therefore, we studied the antixenosis mechanism of resistance to *H. armigera* in a diverse array of pigeonpea genotypes under no-choice, dual-choice, and multi-choice conditions. Antixenosis for oviposition was observed in case of ICPL 187-1, ICP 7203-1, ICPL 88039, T 21, ICPL 84060, and ICPL 332 under no-choice, dual-choice and multi-choice conditions. However, the number of eggs laid on ICPL 88039, T 21 and ICP 7203-1 did not differ significantly from those on ICPL 87 under dual-choice conditions. The susceptible check, ICPL 87 was highly preferred for oviposition. The genotypes ICP 7203-1, ICPL 187-1, T 21, ICPL 332, and ICPL 84060 can be used as sources of non-preference mechanism of resistance in pigeonpea improvement programs to breed for resistance to *H. armigera*.

Key words: *Helicoverpa armigera*, antixenosis, pigeonpea, resistance mechanisms

1 Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is one of the major grain legumes in the semiarid tropics (SAT) (NENE et al., 1990). Its productivity has remained static over the past several decades because of heavy damage by insect pests. More than 150 insect species feed on this crop, of which the pod borer, *Helicoverpa armigera* (Hubner) (Lep., Noctuidae) is the most damaging pest worldwide (SHARMA, 2001). At times, it causes complete crop loss (REED and LATEEF, 1990; YELSHETTY and SIDDE GOWDA, 1998; SHANOWER et al., 1999). In the SAT, *H. armigera* has been reported to cause loss of US\$ 325 million annually (ICRISAT, 1992). *Helicoverpa armigera* damage is particularly severe in the medium-maturity cultivars grown in south-central India. A number of pigeonpea genotypes have been reported to be resistant to *H. armigera* (LATEEF and PIMBERT, 1990; SHARMA et al., 2001). Genotypes with indeterminate growth habit, in general, suffer less damage than the determinate types (REED and LATEEF, 1990).

Oviposition non-preference is one of the important components of resistance to *H. armigera* in pigeonpea (SHARMA et al., 2001). Most of the eggs in pigeonpea are laid on flowers, flower buds, pods and sparingly on the leaves (mostly during the vegetative phase). Under field conditions, greater number of eggs and larvae have been recorded on ICPL 270 than on LRG

30, ICPL 332, and ICPL 84060 (VENUGOPAL RAO et al., 1991). More eggs were recorded on floral parts and new pods when compared with that on the foliage, and the larval density was more on top leaves, flowers and pods compared with the middle and lower parts. SISON et al. (1993) recorded maximum oviposition on the susceptible check, ICPL 87 under multi-choice conditions (more than twice the number of eggs laid on ICPL 88023, ICPL 86015, and ICPL 87101). Under no-choice conditions, maximum oviposition was recorded on ICPL 87 and lowest on ICPL 86005 and ICPL 87101. It is important to characterize different sources of resistance for expression of antixenosis component of resistance to *H. armigera* under multi-choice and no-choice conditions to develop appropriate strategies to breed for resistance to this pest. Therefore, we studied the antixenosis component of resistance to *H. armigera* in a diverse array of pigeonpea genotypes under laboratory and field conditions.

2 Materials and Methods

2.1 Plants

The antixenosis mechanism of resistance to the pod borer, *H. armigera* was studied on a diverse array of 12

pigeonpea genotypes under laboratory conditions. The agronomic attributes of the test genotypes are given in table 1. The test genotypes were of determinate, semi-determinate, and indeterminate growth habit, and of short, medium and late maturity. The plant height ranged from 78 to 170 cm. Flower colour was yellow, red and yellow red (yellow petals with red streaks); while the pods were of green, green purple (purple streaks in green background) or purple green (green streaks in purple background) colouration. The leaves and pods were hairy (trichomed). ICPL 87 and ICPL 332 were used as susceptible and resistant checks, respectively. The material was grown on deep black Vertisols (deep black clay soils) during the rainy season (June to December). The seeds were sown on ridges 75 cm apart. Each genotype was planted in four-row plots, 4 m long. There were three replications in a randomized complete block design. The test material was sown twice at an interval of 25 days so that inflorescences of the test genotypes were available for conducting the experiments on antixenosis for oviposition at the same time. Normal agronomic practices were followed for raising the crop. No insecticide was applied in the test plots.

2.2 Insect culture

The moths of *H. armigera* were obtained from the laboratory culture maintained at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. Nearly half of the insect colony was replaced with the field-collected insects twice a year to maintain the genetic diversity of the colony. The larvae were reared on chickpea-based artificial diet (ARMES et al., 1992) at $27 \pm 1^\circ\text{C}$, 60–85% relative humidity (RH), and 12 h photophase. The adults were released in a cage ($30 \times 30 \times 30$ cm) for oviposition and provided with nappy liners (5×20 cm) as an oviposition substrate. The adults were fed on 10% sucrose solution on absorbent cotton. Eggs laid on the nappy liners were sterilized with 1% sodium hypochlorite, and transferred into 250-ml cups lined with 2–3-mm-thick layer of artificial diet. After 5 days, the larvae were transferred to six-cell-well plates, each cell having 10 ml of diet. The pupae were collected from the cell-wells, sterilized in sodium hypochlorite solution, and placed in vermiculite in a 1000-ml plastic jar for adult emergence. Adult moths of same age (2 days) were used for studies on antixenosis mechanism of resistance in pigeonpea.

2.3 Antixenosis for oviposition

Antixenosis for oviposition was studied under no-choice, dual-choice, and multi-choice conditions under laboratory conditions ($27 \pm 1^\circ\text{C}$, $65 \pm 5\%$ RH, and a photoperiod of 12 h). The inflorescences (30 cm long) for studying antixenosis for oviposition were brought from the field and placed in a 100-ml conical flask containing 50 ml of water. The conical flask was plugged with absorbent cotton to hold the pigeonpea inflorescences in an upright position. The plant material was thoroughly examined for the presence of eggs or larvae before using them for oviposition preference studies.

2.4 Antixenosis for oviposition under no-choice conditions

Under no-choice conditions, only one genotype was offered to the *H. armigera* females for oviposition in a cage ($30 \times 30 \times 30$ cm) fitted with glass pans on the two sides, and wire-mesh screened windows on the other two sides. Five inflorescences were placed in a conical flask inside the cage, and five pairs of 2-day-old moths were released inside each cage. Each of these no-choice tests in a cage constituted a replication. There were five replications in a completely randomized design. The moths were provided with sucrose solution in a cotton swab for feeding, which was changed daily. The moths were allowed to oviposit on the inflorescences for 3 days. To avoid predation by the ants, Tangle foot® (The Tanglefoot Company, Grand Rapids, MI, USA) glue was smeared on all the four legs of the cages. Observations were recorded on the number of eggs laid on the inflorescences placed in a cage. Data were subjected to analysis of variance in a completely randomized design using Genstat 6.0.

2.5 Antixenosis for oviposition under dual-choice condition

Non-preference for oviposition under dual-choice conditions was studied by keeping the inflorescences of a test variety and the susceptible check (ICPL 87) inside the wooden cage. Five inflorescences of the test variety and the susceptible check were kept in two conical flasks separately at the opposite corners of the cage. Five pairs of 2-day-old moths were released inside the cage, and provided with 10% sucrose on a cotton swab. The moths were allowed to oviposit on the test genotypes for three consecutive days. Each dual-choice test in a cage constituted a replication. There were five replications in a completely randomized design. Data were recorded on

Table 1. Agronomic characteristics of 12 pigeonpea genotypes used to study antixenosis mechanism of resistance to the pod borer, *Helicoverpa armigera*

Genotype	Growth habit	Duration	Plant height (cm)	Flower colour	Pod colour	Leaf/pod hairiness
ICPL 187-1	Semi-determinate	SD	136	Yellow	Green purple	Pubescent
ICP 7203-1	Semi-determinate	SD	140	Yellow	Green	Pubescent
ICPL 88039	Semi-determinate	SD	110	Yellow	Green purple	Pubescent
ICPL 98001	Determinate	SD	90	Yellow	Green purple	Pubescent
ICPL 98008	Semi-determinate	SD	170	Yellow	Green purple	Pubescent
ICPL 87091	Determinate	SD	66	Red/yellow	Green purple	Pubescent
T 21	Semi-determinate	SD	140	Yellow	Green purple	Pubescent
ICPL 84060	Semi-determinate	MD	160	Yellow	Green purple	Pubescent
ICPL 87119	Semi-determinate	LD	159	Yellow	Green purple	Pubescent
ICP 7035	Semi-determinate	LD	142	Red	Purple	Pubescent
ICPL 87	Determinate	SD	78	Yellow	Green purple	Pubescent
ICPL 332	Indeterminate	MD	176	Yellow	Purple green	Pubescent

SD, short duration (105–120 days to maturity); MD, medium duration (120–140 days to maturity); and LD, late duration (> 140 days to maturity).

the number of eggs laid on each genotype. Significance of differences between the two test genotypes was compared by paired *t*-test at 'P 0.05' using Genstat 6.0.

2.6 Antixenosis for oviposition under multi-choice conditions

Antixenosis for oviposition under multi-choice conditions was studied by keeping all the 10 test genotypes inside a wooden cage (80 × 70 × 60 cm) placed inside a growth chamber (26° C during the day and 20° C during night, and 12 h photophase). Inflorescences of the test genotypes were brought from the field and kept in conical flasks filled with water. Conical flasks containing the inflorescences of test genotypes were placed inside the wooden cage. There were three replications for each genotype in a randomized complete block design inside the cage. Thirty pairs of moths were released inside the cage. Moths were provided with 10% sucrose solution on a cotton swab. The moths were allowed to oviposit on the test genotypes for three consecutive days. Observations were recorded on the number of eggs laid on each genotype. Data were subjected to analysis of variance. The significance of differences between the genotypes was judged by F-test, while the treatment means were compared by least significant difference at 'P 0.05' using Genstat 6.0.

3 Results

3.1 Antixenosis for oviposition under no-choice conditions

The *H. armigera* females laid 97–381 eggs, and there were significant differences in numbers of eggs laid on

the pigeonpea genotypes tested (fig. 1a). The genotypes ICPL 187-1, ICP 7203-1, ICPL 88039, ICPL 98001, ICPL 98008, T 21, ICPL 84060, and ICPL 332 were significantly less preferred for oviposition (97–176 eggs per female) when compared with the susceptible check, ICPL 87 (381 eggs per female). Moderate levels of oviposition were recorded on ICPL 87091, ICPL 87119, and ICP 7035. These genotypes had < 23% to 31% less eggs when compared with the eggs laid on the susceptible check, ICPL 87.

3.2 Antixenosis for oviposition under multi-choice conditions

Under the multi-choice conditions, the *H. armigera* females laid 91.7 (ICPL 332) to 272.3 (ICPL 87) eggs per female on different pigeonpea genotypes (fig. 1b). Oviposition was lower (< 173 eggs per female) on ICPL 187-1, ICP 7203-1, ICPL 88039, ICPL 98001, T 21, ICPL 84060, ICPL 87119, and ICPL 332 when compared with that on susceptible check, ICPL 87 (272.3 eggs per female). These genotypes received < 22.4% to 41.2% less eggs when compared with the susceptible check, ICPL 87.

3.3 Antixenosis for oviposition under dual-choice conditions

Under dual-choice conditions, significantly less number of eggs (37.27–56.53 eggs per female) were laid on ICPL 187-1, ICPL 84060, ICPL 87119, and ICPL 332

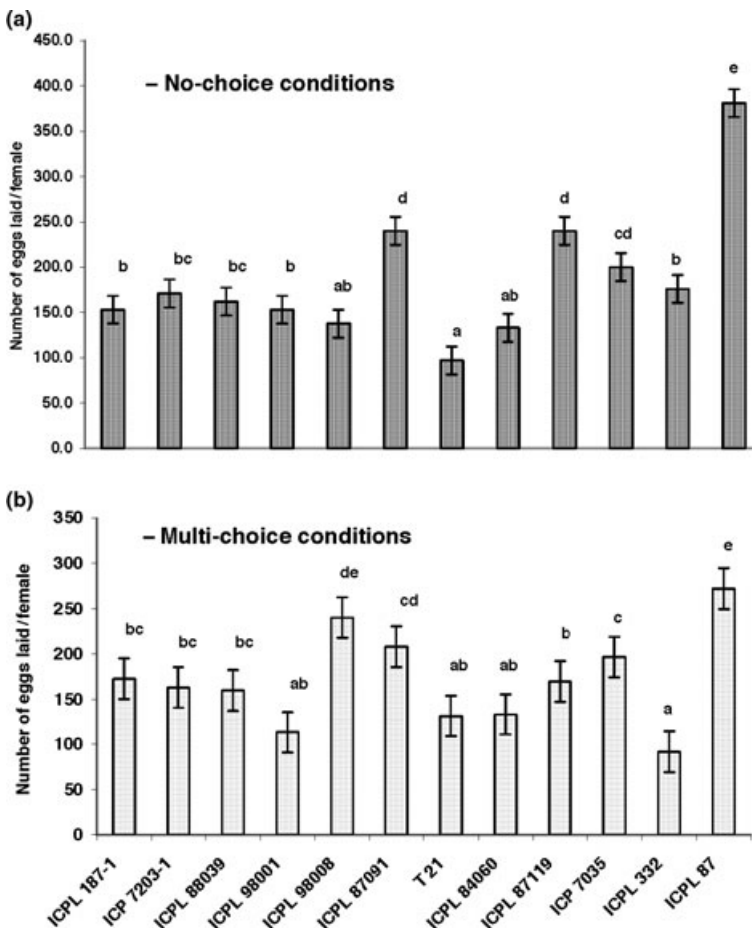
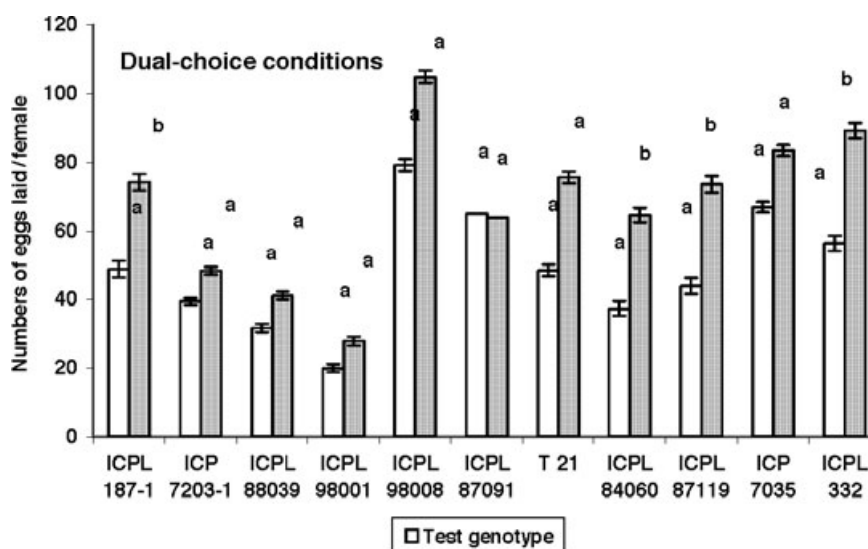


Fig. 1. Oviposition by *Helicoverpa armigera* females on 12 pigeonpea genotypes under no-choice (a) and multi-choice (b) conditions. The bars with the same letter are not significantly different at $P < 0.05$

Fig. 2. Oviposition by *Helicoverpa armigera* females on 12 pigeonpea genotypes under dual-choice conditions (in relation to susceptible check, ICPL 87 – black bars) under laboratory conditions. The genotypes with the same letter within a pair are not significantly different from ICPL 87 at $P < 0.05$



than the susceptible check, ICPL 87 (64.67–89.33 eggs per female) (fig. 2). Low oviposition was also recorded on some other genotypes as well, but these did not differ significantly from ICPL 87.

4 Discussion

The physiological state of the *H. armigera* females influences the host plant specificity and propensity for oviposition (MUSTAPHA et al., 1998). The *H. armigera* females used in the present studies were of similar age, and similar choice was provided to the ovipositing females in different experiments. Therefore, the number of eggs laid on a particular genotype was largely the function of physio-chemical cues perceived by the females, as the females are highly selective in the choice of host plants regarding their suitability for survival and development of larvae (HARDWICK, 1965). The *H. armigera* females show distinct preference for different host plants (HARDWICK, 1965; SCHOONHOVEN, 1990), and also for different genotypes of the same host (BUTTER and SINGH, 1996; SHARMA et al., 2001, 2005).

Antixenosis for oviposition under no-, dual-, and multi-choice conditions was observed in case of ICPL 187-1, ICP 7203-1, ICPL 88039, T 21, ICPL 84060, and ICPL 332. However, the number of eggs laid on ICPL 88039, T 21 and ICP 7203-1 did not differ significantly from those laid on ICPL 87 under dual-choice conditions. Antixenosis for oviposition in case of ICPL 98008 was observed only under no-choice conditions. The *H. armigera* females showed moderate levels of preference/non-preference for oviposition towards ICPL 87091, ICPL 87119, and ICP 7035. The susceptible check, ICPL 87 was highly preferred for oviposition under no-, dual-, and multi-choice conditions. Heavy oviposition has earlier been recorded on ICPL 87, both under field (SHARMA et al., 2001) and laboratory conditions (SISON et al., 1993). Egg and larval numbers have also been found to be lower on ICPL 84060 (ICRISAT, 1992). More eggs were laid on genotypes with yellow flowers than those with pink

flowers. Similar results have earlier been reported by LAKSHMIPATHI (2000). Under field conditions, there were 12 eggs per 10 inflorescences on ICPL 332, 29 on ICPL 84060, 39 on ICPL 187-1, and 143 on ICP 7203-1 when compared to 69 eggs on ICPL 87 (SHARMA et al., 2001). Antixenosis for oviposition observed under laboratory conditions is also exhibited under natural conditions in the field. There were significant differences in oviposition on the genotypes reported to be resistant to *H. armigera*. The genotypes such as ICP 7203-1, ICPL 187-1, T 21, ICPL 332, ICPL 84060, and ICPL 88039, which showed antixenosis for oviposition under multi-, dual-, and no-choice conditions, can be used in pigeonpea improvement programmes to breed for resistance to *H. armigera*.

Pigeonpea pods and leaves have a dense covering of glandular trichomes. Exudates from glandular trichomes contain factors that influence oviposition and/or feeding behavior of *H. armigera* (SHARMA et al., 2001; GREEN et al., 2003). The density of pod trichomes has been found to be important in host-plant resistance to insects (PETER et al., 1995). In resistant wild species of pigeonpea, the trichomes are primarily non-glandular, whereas in the cultivated species, they are glandular (ROMEIS et al., 1999). Several chemicals on the pod surface of cultivated pigeonpea, that are absent from the pods of wild species, influence the host plant selection by *H. armigera*. Methanol extracts of *C. cajan* pods have a significant positive stimulant effect on oviposition by *H. armigera*, whereas methanol extracts from *C. scarabaeoides* pods showed no such effects (SHARMA et al., 2001; GREEN et al., 2003). The hexane extracts from the pod surface of ICPL 87 do not elicit oviposition response from *H. armigera* females. GC-MS analysis has revealed nearly 120 compounds in pigeonpea plant headspace volatiles (SHARMA et al., 2001). Six sesquiterpenes have been identified in pigeonpea leaf volatiles that are attractive to the *H. armigera* females (REMBOLD and TOBER, 1985; HARTLIEB and REMBOLD, 1996). Variations in the relative concentrations of headspace leaf volatiles and the nature and density of trichomes may be responsible for the differences in

oviposition preference by the *H. armigera* females towards different pigeonpea genotypes. It is important to be able to manipulate the genes that control the level of synthesis of these chemicals to breed pigeonpeas for resistance to *H. armigera*.

Acknowledgements

The authors are thankful to the staff of entomology for their help in the experiments, and to Dr M.K. Dhillon for reviewing the manuscript.

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Author's address: H. C. Sharma (corresponding author), International Crops Research Institute for the Semi-Arid Tropics, Patancheru – 502 324, Andhra Pradesh, India. E-mail: h.sharma@cgiar.org