Detached Leaf Assay to Screen for Host Plant Resistance to *Helicoverpa armigera*

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ABSTRACT The noctuid *Helicoverpa armigera* (Hübner) is a major insect pest of chickpea *Cicer arietinum* L., pigeonpea *Cajanus cajan* (L.) Millsp., peanut *Arachis hypogaea* L., and cotton *Gossypium* spp., and host plant resistance is an important component for managing this pest in different crops. Because of variations in insect density and staggered flowering of the test material, it is difficult to identify cultivars with stable resistance to *H. armigera* across seasons and locations. To overcome these problems, we standardized the detached leaf assay to screen for resistance to this pest in chickpea, pigeonpea, peanut, and cotton under uniform insect pressure under laboratory conditions. Terminal branch (three to four fully expanded leaves) of chickpea, first fully expanded leaf of cotton, trifoliate of pigeonpea, or quadrifoliate of peanut, embedded in 3% agar-agar in a plastic cup/jar of appropriate size (250–500-ml capacity) infested with 10–20 neonate larvae can be used to screen for resistance to *H. armigera*. This technique keeps the leaves in a turgid condition for ≈1 wk. The experiments can be terminated when the larvae have caused >80% leaf damage in the susceptible check or when differences in leaf feeding between the resistant and susceptible checks are maximum. Detached leaf assay can be used as a rapid screening technique to evaluate germplasm, segregating breeding materials, and mapping populations for resistance to *H. armigera* in a short span of time with minimal cost, and under uniform insect infestation. It also provides useful information on antibiosis component of resistance to the target insect pest.

KEY WORDS *Helicoverpa*, transgenics, resistance screening, detached leaf assay

THE NOCUTID *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is one of the most important constraints to crop production in Asia, Africa, Australia, and Mediterranean Europe (IIE 1993). It is a polyphagous pest and attacks >200 crop species, including cotton, chickpea, pigeonpea, sunflower, sorghum, peanut, tobacco, maize, and a range of vegetables, fruit crops, and tree species (Fitt 1989, Matthews 1999, Sharma 2001). Crop production in many countries, especially in the semiarid tropics (SAT), is severely threatened by the increasing difficulties in controlling the damage by *H. armigera.* It has developed a high level of resistance to many of the commonly used insecticides (McCaffery et al. 1989, Kranthi et al. 2002). In the SAT, it causes an estimated loss of >U.S. $2 billion, despite application of insecticides costing >$500 million annually (Sharma 2001). In addition to the huge direct economic losses, there are several indirect costs resulting from the deleterious effects of pesticides on the environment. Therefore, it has become necessary to devise a suite of environmentally safe pest management tactics to contain the damage caused by this pest. It is in this context that host plant resistance, including transgenics, assumes a central role for the management of this difficult to control pest. The identification and utilization of cultivars resistant/tolerant to *H. armigera* would provide an equitable, environmentally sound, and sustainable pest management tool. Although several genotypes with less susceptibility to *H. armigera* have been identified in different crops (Sharma 2001, Sharma and Ortiz 2002), the resistance genes have not been transferred into high-yielding cultivars with desirable plant and grain characteristics, and adaptation to different agroclimatic conditions.

There are large differences in the flowering times of different genotypes, whereas the *H. armigera* abundance varies over space and time (Sharma et al. 2003). Rarely is a researcher able to grow a set of genotypes in the field and accurately evaluate insect damage to identify the genotypes with resistance to the target insect pests. Either there are insufficient insect numbers to cause adequate damage or insects occur at an inappropriate phenological stage of the crop. *H. armigera* infestations may be too high, which result in complete damage to the crop, or too low, which does not allow proper assessment of genotypic reaction to *H. armigera* infestation. The onset of insect infestation

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also varies over the crop-growing season, resulting in differential crop response to damage by *H. armigera.* Because of variation in insect pressure and onset of insect infestation, it is difficult to get reliable results under natural infestation over seasons and locations.

Field evaluations also are influenced by the nontarget insects such as *Melanagromyza obtusa* Malloch, *Maruca vitrata* (Geyer), pod-sucking bugs *Clavigralla* spp., and the wasp *Tanaostigmodes cajanini* La Salle in pigeonpea; jassids *Amrasca biguttula biguttula* Ishida; pink bollworm, *Pectinophora gossypiella* (Saunders); and *Earias vittella* (F.) in cotton; and *Spodoptera litura* (F.), leaf miner *Aproarema modicella* Meyer, and jassid *Amrasca* sp. in peanut. As a result, it becomes difficult to achieve dependable screening of the test material under natural infestation. Therefore, it is important to develop techniques to screen the test material at the most susceptible stage of the crop under optimum level of insect infestation. Several procedures have been used to obtain adequate insect pressure for resistance screening under field/greenhouse conditions (Smith et al. 1994, Sharma et al. 1997). The objective of all these techniques is to have an optimum insect density to damage ratio that allows the researcher to observe maximum differences between the resistant and susceptible genotypes.

Insects reared on artificial diet or natural hosts in the laboratory can be used to screen the test material in the laboratory or greenhouse or under field conditions (Sharma et al. 1988, Smith 1989, Smith et al. 1994). Eggs or the first instars can be spread uniformly in the test material by hand or by mixing them with an inert carrier. Infestation with the first instars has been found to give better results with *Heliothis virescens* (F.), because it overcomes the problems associated with egg viability (Hall et al. 1980). Manual infestation with newly hatched larvae is effective, but at times is cumbersome and time-consuming. Mixing of neonate larvae with an inert carrier and dispensing the larvae into the plants with an applicator has been used effectively on several crops with several species of insects (Mihm 1982, Sharma et al. 1992, Smith et al. 1994). Use of field infestations or cage-screening techniques is also cumbersome because large number of insects or cages may be required to complete the screening process. The insects also are exposed to the influence of several biotic and abiotic mortality factors under field conditions. Therefore, to overcome some of these problems and to screen a large number of lines rapidly, we standardized the detached leaf assay to screen for host plant resistance to *H. armigera* in cotton *Gossypium* spp., peanut *Arachis hypogaea* L., chickpea *Cicer arietinum* L., and pigeonpea *Cajanus cajan* (L.) Millsp.

Materials and Methods

The plants grown in greenhouse were used in the bioassays conducted in the laboratory under similar environmental conditions (27 ± 2°C, 65–75% RH, and a photoperiod of 12:12 [L:D] h) at the International Crops Research Institute for the Semiarid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. The experiments were standardized with detached leaves (first fully expanded leaf in cotton, trifoliate in pigeonpea, quadrifoliate of peanut, and a terminal branch with three to four fully expanded leaves and a terminal bud in chickpea) by using different densities of neonate larvae of *H. armigera.*

Test Material. For chickpea, five genotypes (*desi* types: ICC 506, resistant; Annigeri, landrace cultivar; ICC 3137, susceptible germplasm line; ICCC 37, a commercial susceptible cultivar; and L 550, susceptible *kabuli* type cultivar) (Sharma et al. 2003a) were used to standardize the detached leaf assay with the neonate larvae of *H. armigera.* For pigeonpea, five genotypes (ICP 187-1, germplasm source of resistance; ICP 7203-1, germplasm source of resistance; ICPL 332, improved moderately resistant cultivar; ICPL 84060, improved moderately resistant cultivar; and ICPL 87, susceptible high-yielding cultivar) (Sharma 2001) were used for standardizing the screening technique. For peanut, six genotypes (NCAc 343, source of multiple resistance to insects; Robut 33-1, local landrace with less susceptibility to insects; ICGS 86031, improved line with resistance to leaf feeding by *Spodoptera litura* (F.); FDRS 10, high-yielding cultivar with resistance to leaf diseases and less susceptible to leaf feeding by insects; JL 24 and TMV 2, high-yielding commercial cultivars) (Sharma et al. 2003b) were used for standardizing the screening technique. For cotton, transgenic and nontransgenic versions of the hybrid Mech 162; L 604, a commercial *Gossypium hirsutum* cultivar; and Aravinda, *G. arboreum* bollworm-resistant cultivar, were used to standardize the detached leaf assay.

Plants. The plants were raised on a sterilized mixture of black soil (Vertisols), sand, and farmyard manure (2:1:1). The soil was filled into medium-sized pots (30 cm in diameter, 30 cm in depth). The seeds were sown 5 cm below the soil surface and watered when required. For chickpea, 10 seeds were sown in each pot, and five plants were retained at 10 d after seedling emergence, whereas for pigeonpea, peanut and cotton, five plants were sown in each pot, and three plants were retained in each pot. The plants were fertilized with diammonium phosphate (DAP) at 20 g per pot at 15 d after seedling emergence. There were five pots for each genotype. The plants were raised in the greenhouse, which was cooled by desert coolers (27 ± 5°C and 65–90% RH). There was no pesticide application on the test plants. Leaf samples were taken for bioassay at 30–45 d after seedling emergence.

Insects. The *H. armigera* culture was raised in the laboratory on an artificial diet (Armes et al. 1992). Field-collected larvae of *H. armigera* were reared in the laboratory on the natural host for one generation before being introgressed into the laboratory culture to avoid contamination with the nuclear polyhedrosis virus, bacteria, or fungi. The *H. armigera* neonates were reared in groups of 200–250 in 200-ml plastic cups having a 2–3-mm layer of artificial diet on the bottom and the sides for 5 d. After 5 d, the larvae were

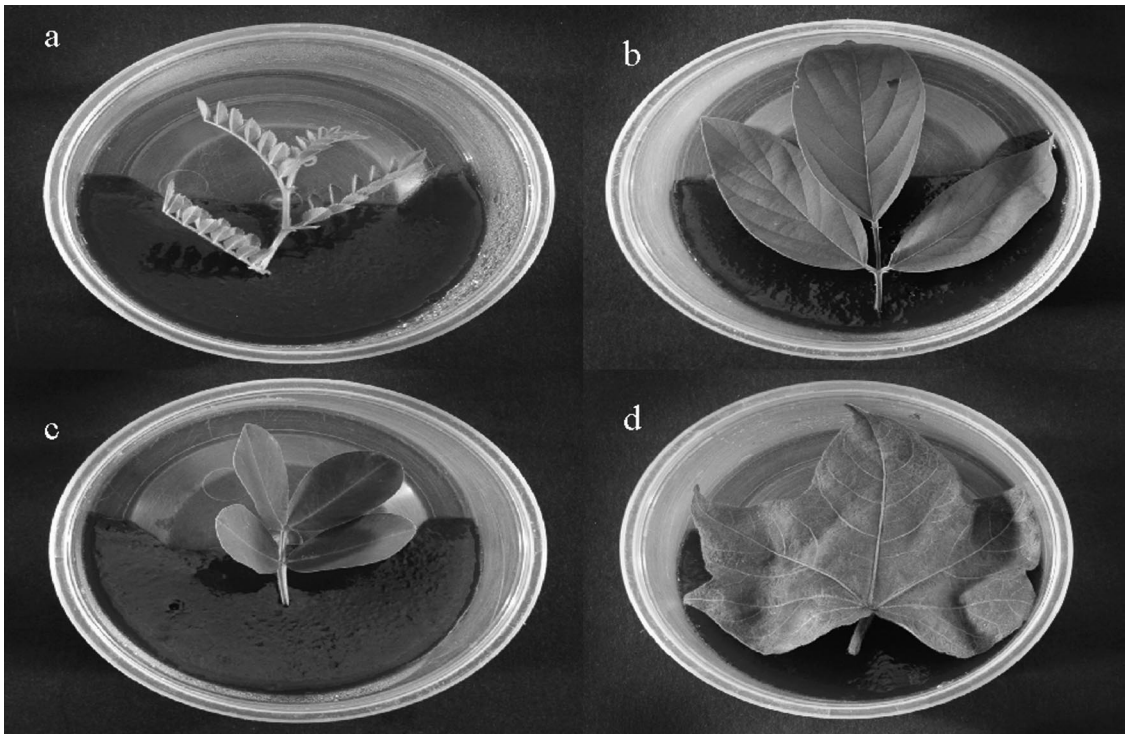

Fig. 1. Detached leaves of chickpea (a), pigeonpea (b), peanut (c), and cotton (d) embedded in agar-agar for bioassay against the neonate larvae of *H. armigera*.

transferred individually to six-cell well plates (each cell well 3.5 cm in diameter, 2.0 cm in depth) to avoid cannibalism. Each cell well had sufficient amount of diet (7 ml) to support larval development until pupation. The pupae were removed from cell wells, sterilized with 2% sodium hypochlorite solution, and kept in groups of 50 in plastic jars containing vermiculite. Upon emergence, 10 pairs of adults were released inside an oviposition cage (30 by 30 by 30 cm). Adults were provided with 10% sucrose or honey solution on a cotton swab for feeding. Diaper liners, which have a rough surface for the females to lay eggs, were hung inside the cage as an oviposition substrate. The liners were removed daily, and the eggs were sterilized in 2% sodium hypochlorite solution. The liners were dried under a table fan and then placed inside the plastic cups with diet. After egg hatching, the larvae moved to the artificial diet, and the liners were removed after 4 d. Neonate larvae were used for infesting the test plants under laboratory conditions.

Insect Density × Damage Relationships of *H. armigera* Larvae in Different Crops. The early instars of *H. armigera* (1–5 d old) largely feed on the leaves and flowers in chickpea, whereas the older larvae (third instar onward) feed on the leaves (if the pods are not available) and the pods. However, for peanut, the larvae largely feed on terminal leaves. For pigeonpea and cotton, most of the damage is caused to reproductive structures. The larvae feed on the leaves when the reproductive structures are not available. Therefore, the genotypic resistance to *H. armigera* was evaluated against the neonates at the vegetative stage.

Terminal branches (three to four fully expanded leaves and a bud) for chickpea, first fully expanded trifoliate for pigeonpea, quadrifoliate for peanut, and the first fully expanded leaf in case of cotton were used to standardize the leaf assay to screen for resistance to *H. armigera.* The leaves/terminal branches were cut with scissors and immediately planted in a slanting manner into 3% agar-agar medium in a 250-ml plastic cup (Fig. 1). There were five replications for each genotype in a completely randomized design. Bioassays were conducted with neonate larvae (5, 10, 15, and 20 larvae per leaf) of *H. armigera.* The bioassay cups were kept in the laboratory at 27 ± 2°C, 65–75% RH, and a photoperiod of 12:12 [L:D] h. The experiments were terminated when >80% of the leaf area was consumed in the susceptible control or when there were maximum differences between the resistant and susceptible checks (generally at 5–6 d after releasing the larvae on the leaves). The plants were scored for leaf feeding visually on a 1–9 scale (1, <10% and 9, >80% leaf area/pods damaged). The number of larvae surviving after the feeding period was recorded, and larvae were placed in 25-ml plastic cups individually. The weights of larvae were recorded at 4 h after separating them from the food. The data are expressed as percentage of larval survival and mean weight of the larvae.

Table 1. Expression of resistance to *H. armigera* across four infestation levels in chickpea by using detached leaf assay during flowering stage (ICRISAT, Patancheru, 2003)

Genotype	Leaf feeding score				Mean	Larval survival (%)				Mean	Larval wt (mg/larva)				Mean
	5*	10	15	20		5	10	15	20		5	10	15	20	
Annigeri	1.9	4.0	7.9	7.9	5.4	84.0	84.3	96.0	83.0	86.8	3.952	3.961	3.268	3.069	3.6
ICC 3137	1.6	3.0	6.7	7.9	4.8	88.0	64.0	90.8	77.0	80.0	4.331	4.706	4.012	4.492	4.4
ICC 506	2.3	2.5	4.0	4.9	3.4	88.0	74.0	66.7	64.0	73.2	5.911	4.522	4.343	4.597	4.8
ICCC 37	3.2	6.6	7.4	8.3	6.4	84.0	86.0	76.0	76.0	80.5	8.908	7.786	5.790	5.560	7.0
L 550	4.3	7.4	8.0	8.4	7.0	96.0	82.0	82.7	77.0	84.4	9.615	6.384	6.114	4.312	6.6
Mean	2.7	4.7	6.8	7.5	5.4	88.0	78.1	82.4	75.4	81.0	6.543	5.472	4.705	4.406	5.3
LSD for comparing															
Genotype (G)	0.62 (df = 4, $Fp < 0.01$)					7.41 (df = 4, $Fp < 0.006$)					1.04 (df = 40, $Fp < 0.01$)				
Infestation level (L)	0.56 (df = 3, $Fp < 0.01$)					6.63 (df = 3, $Fp < 0.002$)					0.93 (df = 3, $Fp < 0.01$)				
G × L	1.25 (df = 12, $Fp < 0.01$)					14.82 (df = 12, $Fp < 0.020$)					2.08 (df = 12, $Fp < 0.081$)				

* Number of larvae released per leaf.

Field Screening. The test material also was evaluated for resistance to *H. armigera* under natural infestation in the field. Cotton, pigeonpea, and chickpea crops were raised on deep black Vertisols, whereas peanut was raised on red laterite Alfisols. The seeds were sown on ridges 75 cm apart, and the plants were thinned to a spacing of 30 cm between the plants at 15 d after seedling emergence. Each plot measured four rows, 2 m in length. The experiments were planted in a randomized complete block design, and there were three replications. Normal agronomic practices were followed for raising the crop. There was no insecticide application in this trial. Chickpea and peanut were evaluated for leaf damage by *H. armigera* during the vegetative stage (45 d after seedling emergence) visually on a 1–9 scale (1, <10% leaf area damaged; 9, >80% leaf area damaged). Pigeonpea was rated for *H. armigera* damage on a 1–9 scale (1, <10% pods damaged; 9, >80% pods damaged) at maturity. In cotton, data are recorded on percentage of bolls with bollworm damage in a sample of five plants selected at random in each plot.

Statistical Analysis. Data were subjected to analysis of variance by using GENSTAT release 5.0. The data on detached leaf assays were analyzed by factorial analysis with genotypes as the main treatment, and the infestation levels as the subtreatment. The data for the field experiments were analyzed using a randomized complete block design. The significance of differences between the treatments was measured by F test at $P = 0.05$, whereas the treatment means were compared using the least significant difference (LSD) at $P = 0.05$.

Results

Insect Density × Damage Relationships of *H. armigera* Larvae in Chickpea. There were significant differences in leaf feeding ($Fp < 0.01$, df = 4, LSD = 0.62) between genotypes, and the infestation levels ($Fp < 0.01$, df = 3, LSD = 0.56) (Table 1). Mean leaf damage rating (DR) varied from 3.4 in ICC 506–7.0 in L 550 across infestation levels. Maximum differences in leaf damage between the genotypes tested were observed when infested with 10 neonates per branch (2.5 in ICC 506 and 7.4 in L 550). The relative susceptibility of genotypes followed the same pattern both in the field conditions and in detached leaf assay with 10 larvae per branch. The interaction effects between the genotypes and infestation levels were also significant ($Fp < 0.01$, df = 12, LSD = 1.25). There was a progressive increase in leaf feeding (DR = 2.7–7.5) with an increase in larval density from five to 20 larvae per branch. ICC 506 suffered significantly less damage than L 550 at all infestation levels. At 10 larvae per branch, Annigeri and ICC 3137 also sustained less damage than ICCC 37 and L 550. Under natural infestation in the field, ICC 506 sustained significantly less leaf feeding damage than ICC 3137, whereas Annigeri and ICCC 37 showed moderate susceptibility to *H. armigera* (Fig. 2). In the detached leaf assay, ICC 506 was most resistant, whereas ICCC 37, ICC 3137, and Annigeri showed a susceptible reaction when infested with 10–20 larvae per branch. The relative susceptibility of the resistant and susceptible checks was similar under field conditions and in the detached leaf assay. However, there was a slight difference in the reaction of Annigeri and ICCC 37 under field conditions, which takes into account the oviposition nonpreference and recovery resistance, in addition to the antifeedant and antibiosis components assessed in the detached leaf assay.

Differences in larval survival between genotypes ($Fp < 0.01$, df = 4, LSD = 7.41) and infestation levels

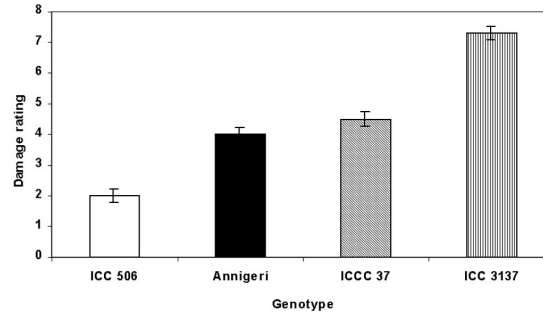

Fig. 2. Leaf feeding by *H. armigera* in four chickpea genotypes under field conditions. Damage rating (1, <10% leaf area damaged; 9, >80% leaf area damaged).

Table 2. Expression of resistance to *H. armigera* across four infestation levels in pigeonpea by using detached leaf assay (ICRISAT, Patancheru, 2003)

Genotype	Leaf feeding score				Mean	Larval survival (%)				Mean	Larval wt (mg/larva)				Mean
	5*	10	15	20		5	10	15	20		5	10	15	20	
ICP 187-1	1.4	3.1	5.0	7.2	4.2	68.0	78.0	65.3	80.7	72.8	0.608	0.756	0.582	0.730	0.669
ICP 7203-1	2.5	4.1	6.0	5.9	4.6	80.0	64.0	81.0	57.2	70.6	0.550	0.703	0.480	0.555	0.572
ICPL 332	2.4	3.3	6.2	5.8	4.4	84.0	74.0	72.0	72.0	75.5	0.817	0.629	0.905	0.655	0.752
ICPL 84060	2.2	5.0	5.8	7.1	5.0	88.0	74.0	81.3	69.0	78.1	0.758	0.598	0.460	0.684	0.625
ICPL 87	2.6	4.1	6.7	7.3	5.2	60.0	85.0	78.7	73.0	74.2	0.458	0.311	0.379	0.429	0.394
Mean	2.2	3.9	5.9	6.7	4.7	76.0	75.0	75.7	70.2	74.2	0.638	0.599	0.561	0.611	0.602
LSD for comparing															
Genotype (G)	0.66 (df = 4, $Fp < 0.020$)					8.62 (df = 4, $Fp < 0.497$)					0.160 (df = 4, $Fp < 0.01$)				
Infestation level (L)	0.59 (df = 3, $Fp < 0.01$)					7.71 (df = 3, $Fp < 0.414$)					0.140 (df = 3, $Fp < 0.752$)				
G × L	1.32 (df = 12, $Fp < 0.078$)					17.25 (df = 12, $Fp < 0.009$)					0.320 (df = 12, $Fp < 0.492$)				

* Number of larvae released per leaf.

($Fp < 0.01$, df = 3, LSD = 6.63) were significant. Larval survival was lower in ICC 506 (73.2%) than on Annigeri (86.8%) across infestation levels. The interaction effects ($Fp = 0.02$, df = 12, LSD = 14.82) between the genotypes and infestation levels were also significant. Larval survival was greater at five larvae than at 20 larvae per branch. The decrease in larval survival with an increase in larval density might be because of decreased food supply and cannibalism among the larvae. Differences in larval weights between the genotypes ($Fp < 0.01$, df = 4, LSD = 1.04) and infestation levels ($Fp < 0.01$, df = 3, LSD = 0.93) were significant. The interaction effects were nonsignificant, suggesting that antibiosis effects of genotypes on the *H. armigera* larvae were independent of the larval density, and thus larval weight can be used as a reliable criterion to assess genotypic resistance to this insect in chickpea. Larval weights were significantly lower on Annigeri, ICC 3137, and ICC 506 compared with those on ICCC 37 and L 550 at five and 10 larvae per branch. The differences in larval weights across genotypes narrowed down with an increase in larval density. There was a progressive decrease in larval weights with an increase in larval density, possibly because of decreased food supply.

Insect Density × Damage Relationships of *H. armigera* Larvae in Pigeonpea. There were significant differences in leaf feeding between the genotypes ($Fp = 0.020$, df = 4, LSD = 0.66) and infestation levels ($Fp < 0.01$, df = 3, LSD = 0.59), whereas the interaction effects were nonsignificant. Mean leaf damage rating across infestation levels varied from 4.2 to 5.2 (Table 2). Maximum differences in leaf damage between the genotypes tested were observed at 10 (DR = 3.1 in ICPL 187-1 and 5.0 in ICPL 84060) and 15 (5.0 in ICP 187-1 and 6.7 in ICPL 87) larvae per trifoliate. There was a progressive increase in leaf feeding with an increase in larval density from five to 20 larvae per trifoliate (DR = 2.2–6.7). The genotype ICP 187-1 suffered numerically less damage than ICPL 87 at 10 and 15 larvae per trifoliate. The differences in leaf feeding among the genotypes tested were not as large as for chickpea. Pod damage under natural infestation in the field was significantly lower on ICP 187-1, ICPL 86040, and ICPL 332 compared with ICPL 87 (Fig. 3). Leaf feeding damage ratings of the genotypes tested did not show the same trend as the pod damage ratings under natural infestation. Some of these differences may be related to the time of onset of insect infestations, insect density, and the influence of other components of resistance not taken into account in the detached leaf assay. Such an outcome is not unexpected because *H. armigera* larvae rarely feed on the pigeonpea leaves under natural conditions, and hence it may not be proper to use detached leaf assay to evaluate pigeonpea genotypes for resistance to *H. armigera.*

The interaction effects between the genotypes and infestation levels for larval survival were significant ($Fp = 0.009$, df = 12, LSD = 17.25), whereas the differences in larval survival between the genotypes and the infestation levels were nonsignificant. Therefore, larval survival on detached leaves cannot be used as a dependable criterion to measure genotypic resistance to *H. armigera.* Differences in larval weight between the genotypes tested were significant ($Fp < 0.01$, df = 4, LSD = 0.160), whereas the differences between infestation levels and interaction effects were nonsignificant. Larval weights were significantly lower in the larvae fed on the leaves of ICPL 87 compared with those fed on the leaves of ICPL 332, ICPL 84060, ICP 187-1, and ICP 7203-1.

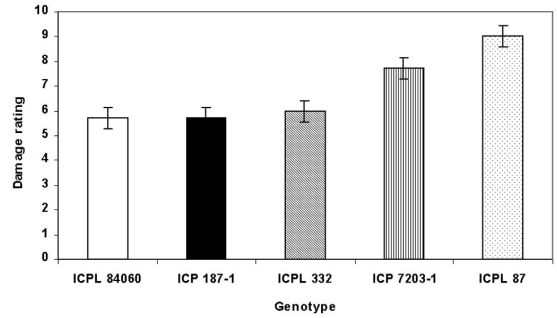

Fig. 3. Pod damage by *H. armigera* in five pigeonpea genotypes under field conditions. Damage rating (1, <10% pods damaged; 9, >80% pods damaged).

Table 3. Expression of resistance to *H. armigera* across four infestation levels in peanut by using detached leaf assay (ICRISAT, Patancheru, 2003)

Genotype	Leaf feeding score				Mean	Larval survival (%)				Mean	Larval wt (mg/larva)				Mean
	5*	10	15	20		5	10	15	20		5	10	15	20	
FDRS 10	1.4	4.0	5.5	6.8	4.4	40.8	50.0	64.0	68.0	55.7	0.909	0.623	0.704	0.701	0.734
ICGV 86031	2.0	2.2	3.4	4.4	3.0	84.0	42.0	44.0	37.0	51.8	0.647	0.395	0.361	0.315	0.430
JL 24	3.8	5.9	6.6	7.4	5.9	84.0	78.0	74.7	75.0	77.9	0.839	0.819	0.837	0.732	0.807
NCAc 343	1.2	2.6	3.0	3.1	2.5	55.9	62.0	40.8	30.5	47.3	0.390	0.336	0.326	0.346	0.350
ROBUT 33-1	1.2	3.9	5.2	6.2	4.1	45.1	50.0	54.5	65.0	53.6	0.323	0.425	0.308	0.416	0.368
TMV 2	3.8	4.4	5.0	7.7	5.2	92.0	78.0	68.0	82.0	80.0	0.935	0.639	0.544	0.695	0.703
Mean	2.2	3.8	4.8	5.9	4.2	67.0	60.0	57.7	59.6	61.0	0.674	0.540	0.513	0.534	0.565
LSD for comparing															
Genotype (G)	0.75 (df = 5, $Fp < 0.001$)					12.15 (df = 5, $Fp < 0.01$)					0.18 (df = 5, $Fp < 0.01$)				
Infestation level (L)	0.61 (df = 3, $Fp < 0.001$)					9.92 (df = 3, $Fp < 0.270$)					0.15 (df = 3, $Fp < 0.140$)				
G × L	1.50 (df = 15, $Fp = 0.029$)					24.29 (df = 15, $Fp < 0.005$)					0.37 (df = 15, $Fp < 0.950$)				

* Number of larvae released per leaf.

Insect Density × Damage Relationships of *H. armigera* Larvae in Peanut. Leaf damage rating varied from 2.5 in NCAc 343 to 5.9 in JL 24 across infestation levels (Table 3). There were significant differences in leaf feeding between genotypes ($Fp < 0.001$, df = 5, LSD = 0.75) and infestation levels ($Fp < 0.001$, df = 3, LSD = 0.61). The interaction effects were also significant ($Fp = 0.029$, df = 15, LSD = 1.50), suggesting that the level of insect damage in the resistant lines increases with insect density. Maximum differences in leaf feeding between the genotypes tested were observed at 20 larvae per quadrifoliate (DR = 3.1 in NCAc 343 compared with 7.4 in JL 24). There was a progressive increase in leaf feeding with an increase in larval density from five (DR = 2.2) to 20 (DR = 5.9) larvae per quadrifoliate. ICGS 86031 and NCAc 343 suffered significantly lower leaf damage than JL 24 and TMV 2 across infestation levels. The relative reactions of the resistant and the susceptible genotypes under field conditions and in the detached leaf assay were similar because the *H. armigera* larvae largely feed on the tender leaves of peanut under field conditions. NCAc 343 experienced the least and TMV 2 the most leaf damage in the field (Fig. 4) and detached leaf assay (Table 3). The genotypes Robut 33-1, FDRS 10, and Robut 33-1 showed moderate levels of susceptibility to *H. armigera* at 15 and 20 larvae per leaf in the detached leaf assay as well as under field conditions.

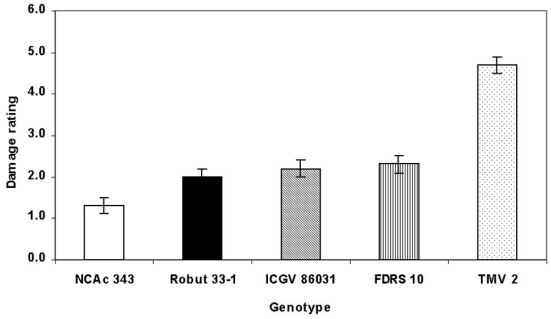

Fig. 4. Leaf feeding by *H. armigera* in five peanut genotypes under field conditions. Damage rating (1, <10% leaf area damaged; 9, >80% leaf area damaged).

However, the differences in susceptibility in relation to TMV 2 were greater in the field compared with the detached leaf assay, which may be because of insect distribution, oviposition nonpreference, and recovery resistance.

Larval survival was significantly lower on FDRS 10, ICGS 86031, NCAc 343, and Robut 33-1 compared with that on JL 24 and TMV 2 across infestation levels. Differences in larval survival between the genotypes ($Fp < 0.01$, df = 5, LSD = 12.15) were significant but nonsignificant between the infestation levels. The interaction effects were also significant ($Fp < 0.01$, df = 15, LSD = 24.29). There were significant differences in larval weights between the genotypes tested ($Fp < 0.01$, df = 5, LSD = 0.18). Larval weights were significantly lower in the larvae fed on the leaves of ICGS 86031, NCAc 343, and Robut 33-1 (0.350–0.430 mg per larva) compared with those fed on the leaves of FDRS 10, JL 24, and TMV 2 (0.703–0.807 mg per larva). The differences between the infestation levels and the interaction effects were nonsignificant. Larval weights can be used as a reliable parameter in evaluating peanut genotypes for resistance to *H. armigera.*

Insect Density × Damage Relationships of *H. armigera* Larvae in Cotton. First fully expanded leaf infested with 10, 15, and 20 neonate larvae of *H. armigera* resulted in large differences in leaf feeding between the resistant and susceptible genotypes (transgenic and nontransgenic versions of the hybrid Mech 162) (Table 4). There were significant differences in leaf feeding ($Fp < 0.001$, df = 3, LSD = 0.62) between genotypes and infestation levels ($Fp < 0.001$, df = 3, LSD = 0.62). The interaction effects were nonsignificant. Lowest leaf feeding was recorded in transgenic hybrid Mech 162 with a DR = 4.3 compared with a DR = 8.1 in the nontransgenic Mech 162 leaves infested with 20 larvae. Leaf feeding was also lower on the leaves of L 604 (*G. hirsutum*) and Aravinda (*G. arboreum*) across infestation levels than in nontransgenic hybrid Mech 162. Based on *H. armigera* damage to the bolls under natural infestation in the field, transgenic hybrid Mech 162 showed a resistant reaction (25% boll damage), whereas the nontransgenic hybrid Mech 162 and L 604 showed a susceptible reaction

Table 4. Expression of resistance to *H. armigera* across four infestation levels in cotton by using detached leaf assay (ICRISAT, Patancheru, 2003)

Genotype	Leaf feeding score				Mean	Larval survival (%)				Mean	Larval wt (mg per larva)				Mean
	5*	10	15	20		5	10	15	20		5	10	15	20	
Mech 162	1.6	2.6	4.0	4.3	3.1	68.0	75.3	76.0	60.0	69.8	0.909	0.609	1.059	0.770	0.837
L 604	2.4	4.1	5.7	5.4	4.4	92.0	86.0	89.3	86.0	88.3	1.824	1.789	1.965	1.213	1.698
Aravinda	3.2	4.7	5.6	6.8	5.1	80.0	92.0	76.0	84.0	83.0	1.695	1.099	0.927	0.820	1.135
Nontransgenic Mech 162	3.3	5.6	6.6	8.1	5.9	100.0	90.0	96.0	94.0	95.0	3.111	4.105	3.168	2.954	3.335
Mean	2.6	4.3	5.5	6.2	4.6	85.0	85.8	84.3	81.0	84.0	1.885	1.901	1.780	1.439	1.751
LSD for comparing															
Genotype (G)	0.62 (df = 3, $Fp < 0.001$)					9.09 (df = 3, $Fp < 0.01$)					0.41 (df = 3, $Fp < 0.01$)				
Infestation level (L)	0.62 (df = 3, $Fp < 0.001$)					9.09 (df = 3, $Fp < 0.729$)					0.41 (df = 3, $Fp < 0.092$)				
G × L	1.23 (df = 9, $Fp < 0.338$)					18.17 (df = 9, $Fp < 0.536$)					0.83 (df = 9, $Fp < 0.195$)				

* Number of larvae released per leaf.

(49.5–59.8% boll damage) (Fig. 5). In the detached leaf assay, transgenic hybrid Mech 162 suffered significantly less damage than the nontransgenic hybrid Mech 162 when infested with 15 or 20 larvae per leaf. The *G. arboreum* 'Aravinda' showed moderate susceptibility under both conditions. The *G. hirsutum* 'L 604' showed a moderate susceptibility in the detached leaf assay, but it was highly susceptible under field conditions, which may be because of the differences in insect distribution, density, and oviposition nonpreference.

There were significant differences in larval survival (69.8% on transgenic and 95% on nontransgenic versions of the hybrid Mech 162) and larval weight (0.84 mg per larva on transgenic and 3.34 mg per larva on nontransgenic cotton) across infestation levels. There were significant differences in larval survival between the genotypes ($Fp < 0.01$, df = 3, LSD = 9.09), whereas such differences between infestation levels and the interaction effects were nonsignificant. Therefore, larval survival and larval weights can be used as reliable criteria to evaluate cotton genotypes for resistance to *H. armigera.* Differences in larval weights between the genotypes ($Fp < 0.01$, df = 3, LSD = 0.41) were significant. Larval weights on the *G. arboreum*'Aravinda' were not significantly different than those fed on the transgenic hybrid Mech 162. Larval weights on the *G. hirsutum* 'LK 604' were also significantly lower than those on the nontransgenic hybrid Mech 162.

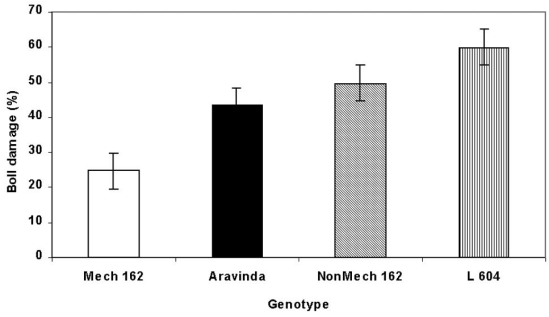

Fig. 5. Percentage of damage in bolls by *H. armigera* in four cotton genotypes under field conditions.

Discussion

Screening for resistance to *H. armigera* under natural conditions is a long-term process because of variations in insect population in space and time. As a result, it is difficult to identify stable sources of resistance under natural infestation (Sharma et al. 1997). Therefore, development and standardization of techniques to screen for resistance to insect pests is the key for an effective insect resistance breeding program, marker-assisted selection, and development of transgenic plants with resistance to insects. Genotypic reactions to feeding by *H. armigera* are diverse; therefore, careful consideration should be given to use the insect density that results in maximum differences between the resistant and susceptible genotypes. Percentage of damage to bolls/pods is the most common parameter used for determining genotypic resistance or susceptibility to *H. armigera* under field conditions (Sharma et al. 2003). However, this criterion often leads to variable results due to variations in insect population and the stage at which the crop is infested. In addition, the damage to foliage, flowers, and small pods, which are devoured by the larvae, is not reflected in percentage of pod damage. At times, the pods or bolls sampled for recording insect damage may be from the second flush, which might have escaped insect damage. To overcome these problems, the test material can be evaluated for resistance to the target insect by using the detached leaf assay under uniform insect pressure at the seedling, flowering, or pod developmental stages.

In chickpea, maximum differences in leaf damage were observed at 10 larvae per branch. Leaf damage and larval weights were significantly lower on ICC 506 compared with those on L 550, and these two can be used as reliable criteria to screen for resistance to this insect in chickpea. The detached leaf assay not only gives an idea of the relative feeding by the larvae on different genotypes but also provides useful information on antibiosis component of resistance in terms of larval weight. For pigeonpea, maximum differences in leaf damage were observed at 20 larvae per trifoliate. However, differences in leaf feeding among the genotypes tested were not as large or as consistent as for chickpea. This may be because of the typical insect–

host plant interactions of *H. armigera* in pigeonpea: the larvae rarely feed on the leaves of pigeonpea under natural conditions, whereas leaf feeding by the young larvae is common for chickpea. Larval weights were significantly lower in the larvae fed on the leaves of ICPL 87 (although this genotype is most susceptible to pod borer at the podding stage under field conditions) compared with those fed on the leaves of ICPL 332, which is resistant to *H. armigera* at the podding stage (Lateef and Sachan 1990). Some of these differences may be because of the differences in relative susceptibility of leaves and pods of different genotypes, and the oviposition nonpreference as an additional component of resistance under field conditions. Thus, the detached leaf assay did not seem to be a proper test to screen for resistance to *H. armigera* in pigeonpea. In peanut, maximum differences in leaf damage were observed at 20 larvae per leaf. Larval survival and larval weights were significantly lower on FDRS 10, ICGS 86031, NCAc 343, and Robut 33-1 compared with that on JL 24 and TMV 2. For cotton, leaves infested with 20 neonate larvae of *H. armigera* resulted in maximum differences in leaf feeding between the transgenic and the nontransgenic hybrids. Leaf feeding, larval survival, and larval weights can be used as criteria to screen for resistance to *H. armigera.*

One to three terminal leaves embedded in moist filter paper have previously been used to evaluate excised leaves of potato for resistance to green peach aphid, *Myzus persicae* (Sulzer), and the results were highly correlated with genotypic reactions under field conditions (Sams et al. 1975). Thomas et al. (1966) compared the reactions of attached versus excised leaves of alfalfa for resistance to spotted alfalfa aphid, *Therioaphis maculata* (Buckton). They reported that nymphal survival was greater on excised leaves than on intact leaves, but the differences in survival varied across genotypes. The results suggested that excised leaves tended to underestimate the resistance levels of the plant population tested. Similar observation also has been reported by Hackerrot and Harvey (1959). In some cases, excised leaves also have been associated with induced resistance, which is not representative of the plant organ (van Emden and Bashford 1976). Olsen and Daly (2000) used the detached leaf assay to evaluate transgenic cotton resistance to *H. armigera.* The relationship between insect reaction to the excised leaves and the field performance of a genotype depends on insect–host plant relationships, plant part preferred by the insect, and induced resistance. In addition, the relative susceptibility of the test genotypes in the field and in the detached leaf assay will be influenced by the relative importance of nonpreference for oviposition and feeding, antibiosis, and tolerance. Therefore, care should be exercised to see that the results of excised leaf assays are not totally different than those under field conditions. However, where the nonpreference for feeding and antibiosis are important components of resistance, this technique can be used effectively for rapid and large-scale screening of germplasm, breeding material, and mapping populations under uniform insect pressure and optimum environmental conditions. It also provides useful information on antifeedant and antibiosis components of resistance.

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