Technique to Screen Peanuts for Resistance to the Tobacco Armyworm, *Spodoptera litura* (Lepidoptera: Noctuidae) Under No-Choice Cage Conditions

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ABSTRACT

Peanut is an important oilseed crop in the semi-arid tropics. It is damaged by several insect species, of which tobacco armyworm, *Spodoptera litura* F., is one of the most damaging pests in South and Southeast Asia. Because of uncertainty of *S. litura* infestations under field conditions, we standardized a no-choice cage technique to screen germplasm and breeding material for resistance to this insect. The test plants were infested with different densities of first- and third-instar larvae at 15 d after seedling emergence. Maximum differences in leaf damage rating and percentage loss in plant biomass at 7 d after infestation between JL 24 and ICGV 86031 were observed when the plants were infested with 10 first-instar larvae per plant. When the plants were infested with third-instar larvae, damage evaluation at 48 hr after infestation showed the maximum differences between JL 24 and ICGV 86031. Larval weight was significantly lower on ICGV 86031 as compared to JL 24 at 10, 15, and 20 larvae per plant. At 10 larvae per plant, ICGV 86031, GP-NC 343, and TMV 2 suffered less damage than JL 24. Larval weights in general were lower on FDRS 10 (except at 20 larvae per plant) and ICGV 86031 than on JL 24. Leaf damage rating was significantly and positively correlated with larval weight and loss in plant biomass. Fifteen-d-old peanut seedlings infested with 10 first- or third-instar larvae can be used to evaluate peanut germplasm and segregating breeding material for resistance to leaf defoliators such as *S. litura*.

Key Words: Groundnut, insects, resistance screening technique.

The tobacco armyworm, *Spodoptera litura* (Fabricius) (Noctuidae: Lepidoptera) is a polyphagous pest of several crops in Asia and Oceania, Madagascar, Mauritius, and Columbia (Feakin, 1973; Wightman *et al.*, 1990). Other closely related species such as *S. littoralis* (Biosd.) and *S. frugiperda* (J.E. Smith) cause serious damage to peanut (*Arachis hypogaea* L.) in Africa and the Americas, respectively. In recent years, *S. litura* has emerged as a serious pest of peanut due largely to cropping of groundnut in the postrainy season in the rice fallows in the coastal Andhra Pradesh. As a result, there is a con-

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tinuous availability of host plant throughout the year due to extensive cultivation during the rainy and the postrainy season in contiguous areas (Wightman and Amin, 1988). Heavy infestation, early in the growing season, causes significant reduction in plant biomass and kernel yield (Panchabhavi and Nethradani Raj, 1987; Wightman *et al.*, 1990). The effects of defoliation on seed yields are greater in the postrainy than in the rainy season because of slower plant growth due to the cooler climate (Wightman *et al.*, 1990).

The tobacco armyworm completes its life cycle in 1 mo, and there may be 10 to 12 generations per year in south-central India. As a result, populations continue to increase over the crop growing season and can cause extensive defoliation. Of the several methods employed for pest management in peanut, host plant resistance is the most economic and relevant means of controlling *S. litura* under subsistence farming conditions in the semi-arid tropics. Differences in genotypic susceptibility to *S. litura* have been reported by Wightman and Amin (1988) and Wightman *et al.* (1990). Southeastern Runner 56-15 was reported to possess resistance to *S. frugiperda* (Hammons, 1970).

Because of variation in *S. litura* infestations in space and time, it is difficult to identify reliable and stable sources of resistance under natural infestations. To overcome this problem, it is important to develop multi- or no-choice screening techniques where the test cultivars can be subjected to an uniform insect pressure at the most susceptible stage of the crop (Smith, 1989; Smith *et al.*, 1994). Caging insects with test plants is one of the most dependable methods of screening for insect resistance (Sharma *et al.*, 1992). In this method, considerable control is exercised to maintain uniform insect pressure on the test entries and to infest the test plants at the same phenological stage. This also prevents insects from migrating away from the test plants and excludes natural enemies. Therefore, we standardized a no-choice cage technique to screen peanuts for resistance to *S. litura* under greenhouse conditions.

Materials and Methods

Insect Culture. The nuclear insect culture was collected from farmers' fields in Medak district, Andhra Pradesh, India. Field-collected larvae were reared individually in 15-mL glass vials in the laboratory on peanut leaves. The pupae were kept in moistened sand. Upon emergence, the moths were released in a 30 x 30 x 30-cm screened cage for oviposition and fed on 10% honey solution. The moths laid eggs on blotting paper strips (30 x 10 cm) which were changed daily. The egg masses were sterilized in 2% sodium hypochlorite solution. The emerging larvae were reared on artificial diet [amount of ingredients used for 450 g of diet included water (360 mL), chickpea flour (43.84 g), yeast (3.2 g), sorbic acid (0.4 g), Vitamin E (0.46 g), methyl para-hydroxy benzoate (0.64 g), ascorbic acid (1.04 g), sorghum leaf powder (16.0 g), agar-agar (4.08 g), and formaldehyde (0.32 mL)] used to rear the spotted stem borer (Taneja and Leuschner, 1985; Sharma *et al.*, 1992). First- to third-instar larvae were reared in groups of 100 larvae in a plastic jar of 1 L capacity. Later-instar larvae were reared individually in six-cell well (4 cm diam.; 2 cm deep). The culture was maintained in the laboratory throughout the year, and the neonate or third-instar larvae were used for experiments as needed.

Plants. Peanut plants were raised in the greenhouse in plastic pots (30 cm diameter, 30 cm deep). The pots were filled with a potting mixture of soil (Alfisols), sand, and farmyard manure (2:1:1). Five seeds were sown in each pot at 7-cm depth, and plants were watered as needed. Two seedlings with similar growth were retained in each pot 10 d after seedling emergence. The greenhouse was cooled by desert coolers to maintain the temperature at 28 ± 5 C and relative humidity >65%.

The effects of defoliation by the fourth-instar larvae on pod yield are greater in the crop infested between 10 (seedling stage) to 30 d (flowering stage) after seedling emergence (28 and 18% loss in pod yield, respectively) than in the crop infested at 50 to 70 d after seedling emergence (13 and 14% loss, respectively) (Wightman *et al.*, 1990). There is a progressive reduction in loss in pod yield as the time of infestation advances from 10 to 70 d after seedling emergence. However, *S. frugiperda* has been reported to consume more leaf tissue on 67- to 92-d-old plants than on 45- to 70-d-old plants or the 92- to 120-d-old plants (Barfield *et al.*, 1980), even though the larvae in general prefer younger leaves than the older leaves (Garner and Lynch, 1981; Lynch *et al.*, 1981). Therefore, we screened peanut seedlings for resistance to *S. litura* at 15 d after seedling emergence.

Insect Infestation. To study insect density damage relationships, the plants were infested at 15 d after seedling emergence. One plant in each pot was covered with a plastic jar cage (11-cm diam., and 26 cm height) with two wire mesh-screened windows (4-cm diam.) on the sides (Fig. 1). The top of the plastic jar cage was covered with the lid fitted with a wire mesh screen. The first- or third-instar larvae were counted in the laboratory, placed in 25-mL plastic cups, and taken to the greenhouse for infestation. Larvae were released inside the cage (as indicated in each experiment), and the lower end (up to 2 cm) of the cage was pushed into the soil. Cages were removed after completion of the experiment and observations were recorded on plant damage and insect survival as indicated below. The effect of caging on plant growth, if any, was uniform across treatments and hence would not influence the conclusions drawn from these experiments.

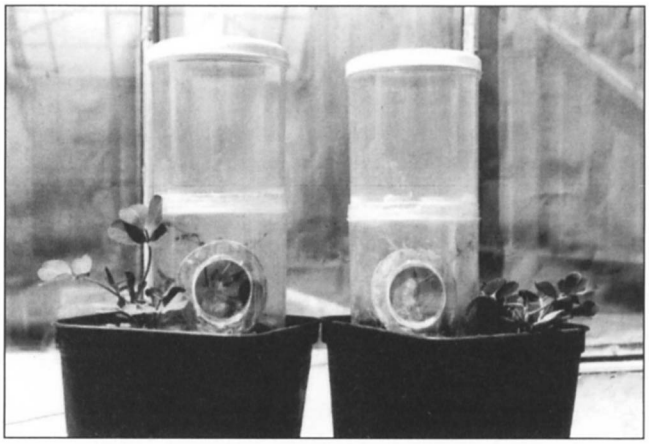

Fig. 1. Wire mesh-screened cage used for screening peanuts for resistance to *Spodoptera litura*.

Effect of Infestation Levels on Leaf Feeding and Insect Survival. In the first experiment, plants of the peanut cultivar JL 24 (early maturity cultivar) and ICGV 86031 (moderately resistant to *S. litura*) (Wightman *et al.*, 1990) were infested with 10, 20, 30, and 40 first-instar larvae per plant 15 d after seedling emergence (R1 stage, i.e., before initiation of flowering). One plant was infested with the larvae inside the cage while the other plant outside the cage was left as an uninfested control. Three replications were used in a randomized complete block design. Observations were recorded 7 d after infestation. The numbers of larvae surviving on each plant were recorded, and the larvae were placed in plastic cups. The larval weights were recorded 4 hr after termination of the experiment. The plants were visually rated for leaf feeding on a 1 to 9 damage scale (1 = <10% leaf area damaged, 2 = 11 to 20%, 3 = 21 to 30%, 4 = 31 to 40%, 5 = 41 to 50%, 6 = 51 to 60%, 7 = 61 to 70%, 8 = 71 to 80%, and 9 >80% leaf area damaged). After recording the leaf damage rating, the plants were excised at the base, and the fresh weight of the infested and the uninfested plant in each pot was recorded separately. The loss in plant biomass due to insect feeding was computed in relation to the biomass of the uninfested control plant in each pot.

In another experiment, plants of JL 24 and ICGV 86031 were infested with 5, 10, 15, and 20 third-instar larvae for a rapid screening for resistance to *S. litura*. There were three replications in a randomized complete block design. Leaf feeding observations were recorded on a 1 to 9 visual rating scale as described above at 24, 48 and 72 hr after infestation. After 72 hr, when the larvae had consumed over 80% of the leaf area in the susceptible cultivar JL 24, the numbers of surviving larvae were recorded on each plant. Larvae were removed from the plants and placed in 25-mL plastic cups. The weights of surviving larvae were recorded 4 hr after removing them from the plants. The plants were then excised at the base, and the weight of the infested and uninfested plants in each pot recorded. The loss in plant biomass as a result of insect feeding was computed in relation to the biomass of the uninfested control plant in each pot.

Relative Susceptibility of Peanut Genotypes to S. litura. Six peanut genotypes [JL 24 and TMV 2 (commercial cultivars); Robut 33-1, FDRS 10, and ICG 86031 (improved cultivars with less susceptibility to *S. litura*); and GP-NC 343 (a cultivar with multiple resistance to insects)] were screened for resistance/susceptibility to *S. litura* beginning 15 d after seedling emergence. The plants were infested with 10, 20, 30, and 40 first-instar larvae per plant inside the cages as previously described. Another plant with similar growth in the same pot served as an uninfested control. The cages were removed at 7 d after infestation. Leaf feeding ratings, number of surviving larvae, larval weights, fresh weight of infested and uninfested plants, and percentage loss in plant biomass were recorded as previously described.

Statistical Analysis. Data were subjected to analysis of variance, and means were separated by using least significant difference (LSD) where F-values were significant at $P \leq 0.05$ using GENSTAT release 5.0. The data for leaf damage rating, larval survival and weight, and percentage loss in plant biomass were subjected to correlation analysis to determine the association between different parameters used to assess genotypic resistance to *S. litura*.

Results and Discussion

Effect of Infestation Levels with First-Instar larvae on Leaf Feeding and Insect Survival. There were significant ($P \leq 0.05$, df = 6) differences in the leaf damage rating between JL 24 and ICGV 86031 when the plants were infested with 10 first-instar larvae per plant (Fig. 2a). Differences in the relative susceptibility of the cultivars tested decreased as the insect infestation level increased. Loss in plant biomass increased with an increase in level of infestation. Greatest loss in plant biomass was observed in plants infested with 40 larvae per plant (Fig. 2b). Maximum differences in plant biomass loss were recorded in plants infested with 10 larvae per plant. However, the differences were not significant at $P \leq 0.05$ (df = 6). Percentage larval survival was significantly ($P \leq 0.05$) lower on ICGV 86031 than on JL 24 when the plants were infested with 10 larvae per plant (Fig. 2c). The larval weights were significantly ($P \leq 0.05$, df = 6) lower on ICGV 86031 than on JL 24 when the plants were infested with 20, 30, and 40 larvae per plant. The weight of the surviving larvae decreased as the infestation level increased from 10 to 40 larvae per plant.

Effect of Infestation with Third-Instar Larvae on Leaf Feeding and Larval Survival. The differences in leaf feeding at different intervals were significant ($P \leq 0.05$, df = 6) when the plants were infested with 10 third-instar larvae per plant (Fig. 3a,b,c). Differences in leaf feeding between the cultivars tested were not apparent at other infestation levels. There were no significant differences ($P \leq 0.05$, df = 6) in percentage loss in plant biomass (Fig. 4a) and larval survival (Fig. 4b) between the two cultivars tested. However, the differences between the two cultivars were greatest for loss in plant biomass when infested with 20 larvae per plant. Larval weights were significantly lower ($P \leq 0.05$, df = 6) in larvae fed on ICGV 86031 than those fed on JL 24 in plants infested with 10, 15, and 20 larvae per plant (Fig. 4c). However, the differences in larval weights between the two cultivars were not apparent at five larvae per plant.

There was a significant ($P \leq 0.05$* and 0.01**) and positive correlation between larval survival and leaf damage rating ($r = 0.84$**) and leaf damage rating and percentage loss in plant biomass ($r = 0.75$*). Positive, but nonsignificant, correlations were observed between larval survival and percentage loss in plant biomass ($r = 0.61$), larval survival and larval weight ($r = 0.31$), leaf damage rating and larval weight ($r = 0.41$), and larval weight and percentage loss in plant biomass ($r = 0.51$).

Relative Susceptibility of Peanut Genotypes to S. litura. Differences in leaf damage rating were significant ($P \leq 0.01$, df = 10) when the plants were infested with 40 larvae per plant. At 10 and 20 larvae per plant, the differences between the genotypes tested were significant at $P \leq 0.10$. In plants infested with 10 larvae per plant, ICGV 86031, GP-NC 343, and TMV 2 suffered relatively less leaf damage than JL 24 (Table 1). The differences in genotypic susceptibility to *S. litura* de-

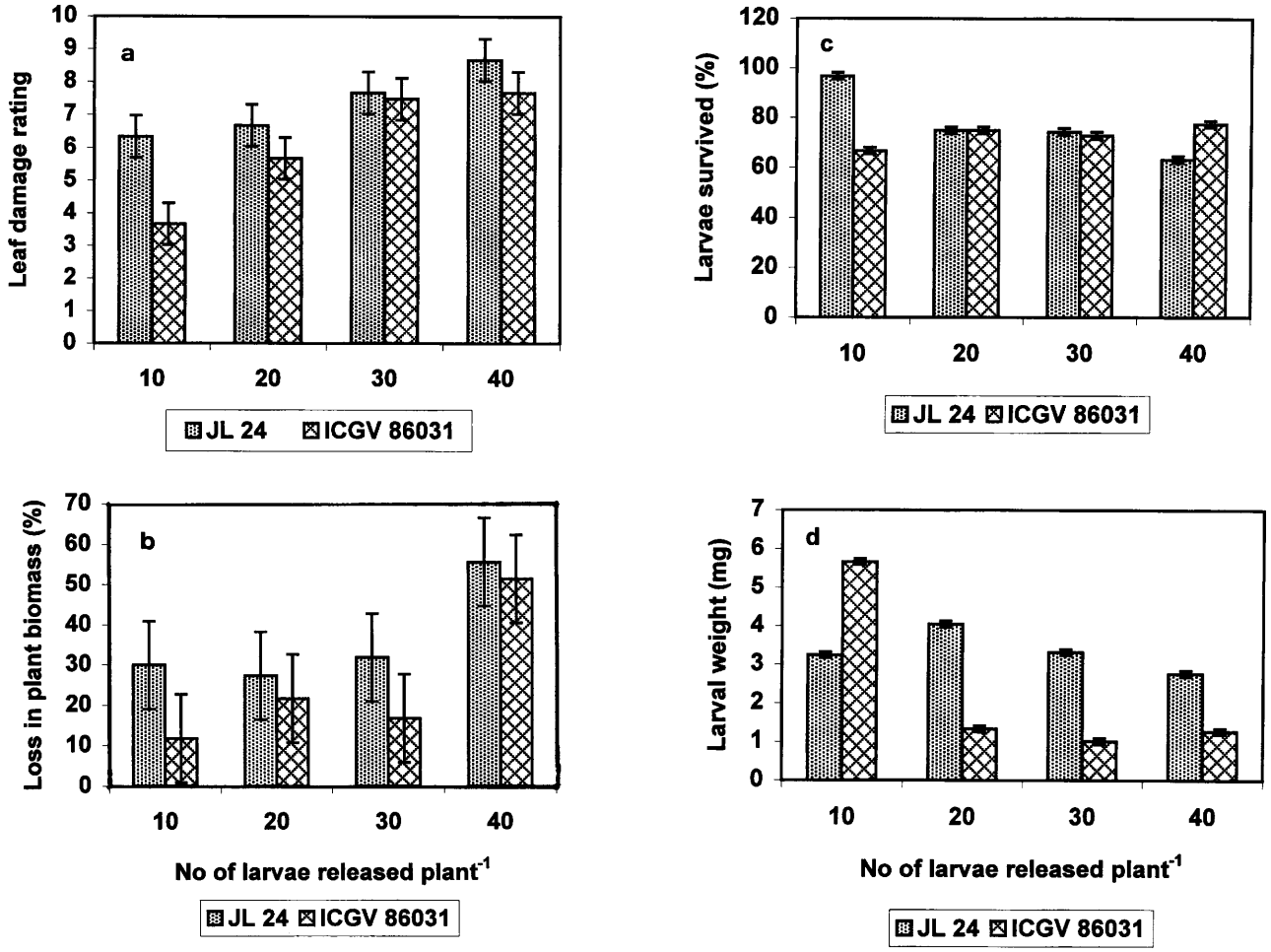

Fig. 2. Effect of different infestation levels with the first-instar larvae of *Spodoptera litura* on (a) leaf damage rating (1 = < 10% leaf area consumed and 9 > = 80% leaf area consumed), (b) % loss in plant biomass, (c) larval survival, and (d) larval weight. Differences between the two genotypes tested were significant (P ≤ 0.05, df = 6) for leaf feeding and larval survival at 10 larvae per plant and the larval weight at all infestation levels.

Table 1. Screening of six peanut genotypes at varying levels of *Spodoptera* litura infestation under no-choice cage conditions in the greenhouse (ICRISAT, Patancheru, 2000 rainy season).

	Damage rating (DR)				Larvae survival				Larval weight				Loss in seedling weight			
Ent	10[a]	20	30	40	10	20	30	40	10	20	30	40	10	20	30	40
	1-9				%				mg				%			
JL 24	5.9	3.9	7.0	5.8	63.3	69.0	73.7	65.6	5.8	6.7	5.8	26.1	4.6	13.5	21.7	
TMV 2	2.5	5.3	6.7	6.5	80.0	75.0	56.7	79.5	4.4	3.5	3.8	3.9	6.5	22.5	31.9	15.4
Robut 33-1	3.7	5.3	6.3	8.0	76.7	78.3	79.5	60.0	6.9	5.3	4.2	5.4	11.8	17.1	21.1	22.3
FDRS 10	3.9	3.7	6.7	6.7	33.3	69.9	63.3	71.7	1.1	4.7	3.1	3.0	21.7	17.8	17.7	23.1
ICGS 86031	3.0	6.3	6.7	6.3	73.3	79.0	74.4	64.2	3.6	2.7	3.6	3.1	29.3	14.7	12.9	15.7
GP-NC 343	2.7	3.7	5.5	8.0	63.3	75.0	73.7	58.3	3.4	4.1	4.8	3.8	10.2	10.5	27.0	14.1
Mean	3.6	4.7	6.5	6.9	65.0	74.4	70.2	66.6	4.1	4.3	4.4	4.2	17.6	14.5	20.7	18.7
SE ±	0.77	0.68	0.34	0.38	8.98	9.44	5.64	6.13	0.73	0.84	0.49	0.37	5.12	6.12	10.62	14.1
LSD (P < 0.05)	NS	NS	NS	1.19	28.24	NS	17.73	NS	2.29	NS	1.54	1.16	NS	NS	NS	NS
F-test	2.67	2.73	2.40	5.96	3.57	0.20	2.26	1.66	7.08	1.85	6.83	12.86	3.39	1.49	0.51	0.45
F probability*	0.1	0.09	0.13	0.01	0.05	NS	0.15	NS	0.01	NS	0.01	0.001	0.07	NS	NS	NS

[a]No. of larvae released per plant. Damage rating = 1 = < 10% leaf area consumed, and 9 = > 80% leaf area consumed.

*df = 10. NS = F-test nonsignificant at P < 0.050.

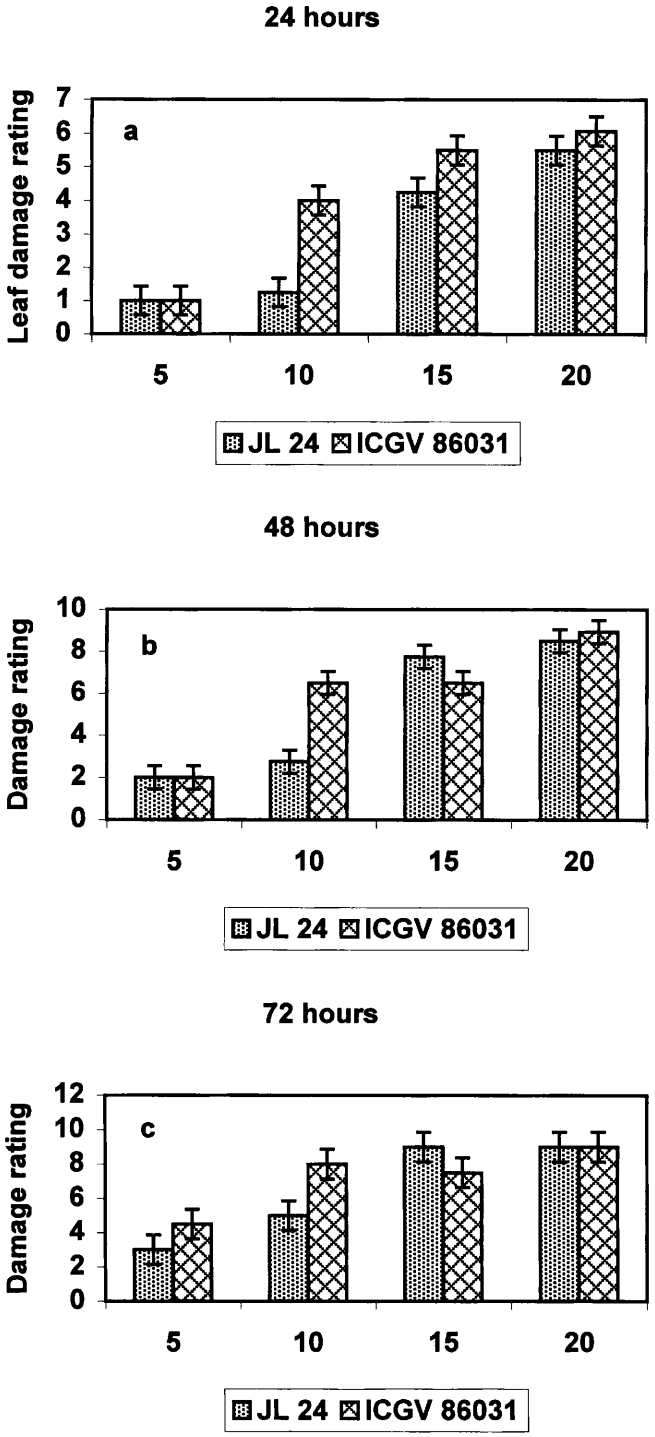

Fig. 3. Effect of different infestation levels with the third-instar larvae of *Spodoptera litura* on (a) leaf damage rating (1 = < 10% leaf area consumed and 9 = > 80% leaf area consumed) at 24 hr, (b) 48 hr, and (c) 72 hr after infestation. Differences in leaf damage rating between the two genotypes tested were significant ($P \leq 0.05$, df = 6) when the plants were infested with 10 larvae per plant.

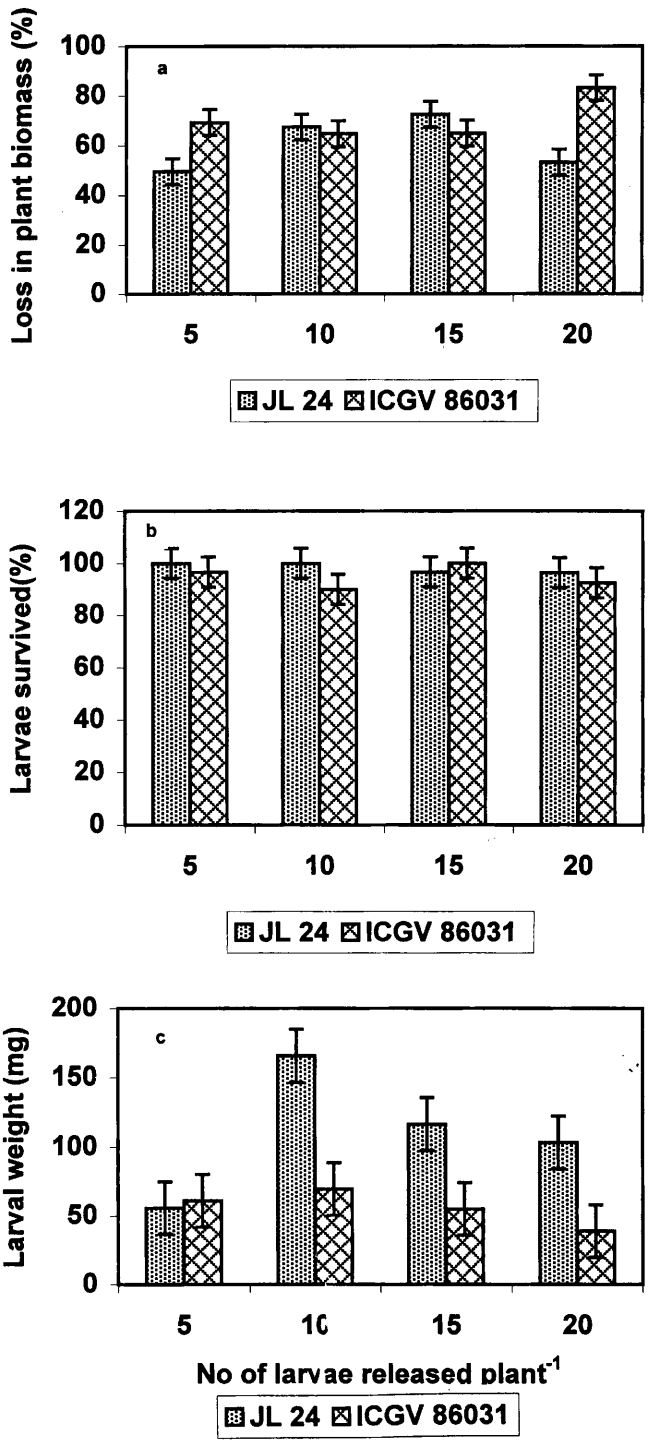

Fig. 4. Effect of different infestation levels with the third-instar larvae of *Spodoptera litura* on (a) loss in plant biomass, (b) larval survival, and (c) larval weight. Differences in loss in plant biomass were significant at 20 larvae per plant and the larval weights at 10, 15, and 20 larvae per plant ($P \leq 0.05$, df = 6).

creased as the infestation level increased. There was a progressive increase in the mean leaf damage rating as the infestation levels increased from 10 to 40 larvae per plant. Differences in larval survival were significant when the genotypes were infested with 10 larvae per plant. The weights of the surviving larvae on different genotypes were significantly different (except at 20 larvae per plant). Larval weights in general were lower on FDRS 10 (except at 20 larvae per plant), GP-NC 343 (except at 30 larvae per plant) and ICGV 86031 than on JL 24. The

weights of the surviving larvae decreased with an increase in infestation levels above 20 larvae per plant possibly because of competition for food. At this infestation level, the larval survival was lower on FDRS 10 (33%), GP-NC 343 (69%), and JL 24 (63%) as compared to TMV 2 (80%). Differences in percentage loss in plant biomass were nonsignificant because leaf feeding by the larvae formed a small fraction of the total biomass of the plant and hence plant biomass at the seedling stage may not be a reliable measure of resistance/susceptibility to *S. litura*.

Peanut genotypes, ICGV 86031 and ICGV 86535 have been reported to be less susceptible to fourth-instar larvae of *S. litura* at different crop growth stages, and reduction in pod yield is greater in the susceptible cultivar TMV 2 than in the relatively resistant cultivar ICGV 86031 (Wightman *et al.*, 1990). However, ICGV 86031 suffered greater damage than JL 24 when infested with third-instar larvae, even though larval weights and larval survival were lower on ICGV 86031 than on JL 24.

Differences in leaf damage rating and loss in plant biomass were greatest when the plants were infested with 10 larvae per plant. Lower larval weights on ICGV 86031 (at infestation levels above 10 larvae per plant) than on JL 24 may be because of greater feeding at the higher infestation levels, resulting in increased production of secondary plant substances. Induction of secondary plant metabolites as a result of insect feeding has been observed in several crops (Sharma and Agarwal, 1983; Ebel, 1986; Sharma and Norris, 1991). Therefore, visual leaf damage rating of peanut plants infested with 10 first- or third-instar larvae can be used in evaluating peanut germplasm and segregating breeding material for resistance to leaf defoliators such as *S. litura*.

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Literature Cited

Barfield, S.S., J.W. Smith Jr., C. Carlysle, and E.R. Mitchell. 1980. Impact of peanut phenology on select population parameters of fall armyworm. Environ. Entomol. 9:381-384.

Ebel, J. 1986. Phytoalexin synthesis: The biochemical analysis of the induction process. Ann. Rev. Phytopath. 24:235-264.

Feakin, S.D. 1973. Pest Control in Groundnuts. PANS Manual No. 2. Center for Overseas Pest Research, Foreign and Commonwealth Office, Overseas Development Administration, London, UK.

Garner, J.W., and R.E. Lynch. 1981. Fall armyworm leaf consumption and development on Florunner peanuts. J. Econ. Entomol. 74:191-193.

Hammons, R. O. 1970. Registration of Southeastern Runner 56-15 peanuts. Crop Sci. 10:27.

Lynch, R.E., W.D. Branch, and J.W. Garner. 1981. Resistance of *Arachis* species to the fall armyworm, *Spodoptera frugiperda*. Peanut Sci. 8:106-109.

Panchabhavi, K.S., and C.R. Nethradani Raj. 1987. Yield of groundnut as affected by varying larval density of *Spodoptera litura*. Indian J. Agric. Sci. 57:525-527.

Sharma, H.C., and R.A. Agarwal. 1983. Role of some chemical components and leaf hairs in varietal resistance in cotton to jassid, *Amrasca biguttula buguttula* Ishida. J. Ent. Res. 7:145-149.

Sharma, H.C., and D.M. Norris. 1991. Chemical basis of resistance in soybean to cabbage looper, *Trichoplusia ni*. J. Sci. Fd. Agric. 55:353-364.

Sharma, H.C., S.L. Taneja, K. Leuschner, and K.F. Nwanze. 1992. Techniques to screen sorghums for resistance to insects. ICRISAT Info. Bull. No. 32, ICRISAT Center, Patancheru 502 324, Andhra Pradesh, India.

Smith, C.M. 1989. Plant Resistance to Insects. John Wiley and Sons, New York.

Smith, C.M., Z.R. Khan, and M.D. Pathak. 1994. Techniques for Evaluating Insect Resistance in Crop Plants. CRC Press, Inc., Boca Raton, FL.

Taneja, S.L., and K. Leuschner. 1985. Methods of rearing, infestation, and evaluation for *Chilo partellus* resistance in sorghum. Proc. Int. Sorghum Entomology Workshop, 15-21 July 1984, Texas A&M Univ., College Station and ICRISAT Center, Patancheru 502 324, Andhra Pradesh, India.

Wightman, J.A., and P.W. Amin. 1988. Groundnut pests and their control in semi-arid tropics. Trop. Pest Mgmt. 34:218-226.

Wightman, J.A., K.M. Dick, G.V. Ranga Rao, T.G. Shanower, and C.G. Gold. 1990. Pests of groundnut in the semi-arid tropics, pp. 243-322. *In* S.R. Singh (ed.) Insect Pests of Legumes. Longman and Sons Ltd., New York.