Philipp. Ent. 9 (2): 186 - 200 ISSN 0048-3753 OCTOBER 1993

BIOLOGY, FEEDING BEHAVIOR AND DAMAGE OF THE COTTON LEAFHOPPER [*AMRASCA BIGUTTULA* (ISHIDA)] ON SUSCEPTIBLE AND RESISTANT VARIETIES OF OKRA [*ABELMOSCHUS ESCULENTUS* (L.) MOENCH.][1]

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ABSTRACT

Given a choice, significantly more adult leafhoppers [*Amrasca biguttula* (Ishida)] preferred to feed and oviposit on the susceptible okra variety Smooth Green (SG), than on the resistant accessions (Acc. 9 and Acc. 12). The daily leafhopper survival taken for 7 days and the percentage of nymphs becoming adults, fecundity and longevity were significantly better also on SG than on either resistant variety. However, the total developmental periods (egg laying to adult emergence) on the varieties studied were not significantly different.

The damage symptom was most readily exhibited by SG, followed by Acc. 9 and Acc. 12 under similar leafhopper pressure, regardless of infestation level. A pressure of 10 to 20 nymphs per leaf allowed a more detailed comparison of damage symptom progression on the leaves, but 40 nymphs per leaf enabled faster identification of the most tolerant accession within a shorter time.

Antixenosis, antibiosis and tolerance were the identified mechanisms of resistance of Acc. 9 and Acc. 12 and at a higher level in the latter. These resistant accessions are recommended for use as parental sources of cotton leafhopper resistance in future crop improvement program in okra.

Key words: cotton leafhopper, *Amrasca biguttula* (Ishida), okra, *Abelmoschus esculentus* (L.) Moench., host plant resistance, antixenosis, antibiosis, tolerance.

INTRODUCTION

Okra is a vegetable known for its appetizing pods. It has high vitamin C content (Orda, citing Sistrunk *et al.*, 1964); its fibers can be used in paper manufacture (Beattie, 1940); its seed, as a source of oil (Orda citing Miller, 1964) or a substitute for coffee (Encyclopedia Britannica, 1983). It is very popular in Southern USA where it is used largely as a soup thickener. As per record of 1984, okra has also been exported from this country to Japan (Umandal, 1988).

One of the more serious pests of this crop is the cotton leafhopper, *Amrasca biguttula* (Ishida), a sucking insect which also attacks other malvaceous and solanaceous crops. Long-term control of this pest may be possible through a well-planned pest management program, usually utilizing a combination of genetic, cultural, chemical and biological control methods. According to Guzman (1987) and Maxwell and Jennings (1980), a resistant variety can provide a foundatiom on which to build an integrated control system and may be most productive when used in adjunct with cultural, chemical and biological control methods.

Selection of resistant crop varieties or clones is the first step in the development of insect resistance as a method of pest control. This has already been done for okra both locally and abroad. In 1988, a total of 221 okra accessions were evaluated for leafhopper resistance at the Institute of Plant Breeding, UPLB. Sixty-eight varieties were tested in the dry season and 153 in the rainy season. Five entries from the first group (Accessions 9, 12, 14, 39 and 63) and one in the second group (Acc. 269) were rated resistant. The commercial variety Smooth Green (SG), on the other hand, showed very high susceptibility to leafhopper.

In 1989, field evaluation of the more promising okra germplasm selected in the previous year, and some new entries was done again. Unfortunately, all the identified more resistant okra accessions do not have the desirable horticultural features concerning fruit size, shape and color, fruiting earliness and plant type (Annual Report, IPB, 1989), suggesting the need for crop improvement. Some basic information on the nature of resistance as suggested by the biology, feeding behavior and damage of the cotton leafhopper on susceptible and resistant varieties will facilitate the determination of the plant factors responsible for resistance and their combination with acceptable horticultural qualities in improved okra varieties.

This study was conducted to determine the resistance mechanisms of okra Acc. 9 and Acc. 12 to the cotton leafhopper based on the biology, feeding behavior and damage of the pest. Painter (1951) categorized the general mechanisms into: 1) lesser attraction to pests for use as food, shelter or oviposition site (antixenosis or non-preference); 2) adverse effects on survival, growth, development and reproduction of pests (antibiosis); and 3) greater ability to withstand pest feeding and colonization (tolerance).

MATERIALS AND METHODS

Preparation of the Test plants

The materials used were okra Acc. 9 and Acc. 12 which showed the highest level of field resistance* among the materials tested at IPB in 1988 and 1989, and the commercial variety Smooth Green as the susceptible check for comparison. All seeds were obtained from the said Institute. Staggered planting (36 pots per batch) was done to ensure a continuous availability of the test plants. The recommended cultural management practices for okra were adopted except insecticide application.

Preference Tests

Adult Preference. Three plants each of SG and Acc. 9 were placed at opposite ends of a large mosquito net with a divider that can be folded up. Each

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* Although the highest, the resistance level should be considered only "moderate" for practical purposes.

plant per variety served as 1 replication. At least 200 field-collected adults were released at the middle of the set-up and leafhopper counts were taken at 12, 24, 48 and 73 hours after introduction. The divider was released before each count and folded up again afterwards to allow free choice of the hoppers between the paired hosts offered. Two trials were made. The same procedure was followed for SG and Acc. 12 pairing.

Ovipositional Preference. After the last observation in the adult preference test (72 hours), all the test plants were separately confined in smaller mosquito nets. Ovipositional preference was gauged by the total number of 1st instar nymphs counted on each plant for 5 days starting a week after exposure to the hoppers.

Antibiosis Tests

Newly-hatched nymphs from the ovipositional preference test were transferred to undetached leaves of the same accessions where they hatched earlier. To each leaf were confined 10 nymphs using nylon mesh nets. A total of 100 individuals per okra variety were observed for daily leafhopper survival, adult emergence, and longevity. Fecundity of 50 females per variety was also determined.

Tolerance Tests

Leaves of each test variety were again caged individually with nylon mesh nets and subjected to varying levels of nymphal infestation, namely, 10, 20, 30, and 40 first to fourth instar nymphs. Three replications per treatment were made. The damage symptom progression on the leaves was monitored by assigning the damage ratings each day until the susceptible check variety SG exhibited the highest numerical rating following this scale: 1 - no damage; 2- slight crinkling and curling of leaf with a little yellowing at periphery; 3- much crinkling, curling, browning and yellowing; and 4- curling, crinkling, extreme discoloration and drying.

RESULTS AND DISCUSSION

Leafhopper Preference

Adult Preference. When given a free choice between two okra varieties, the leafhopper adults showed significantly greater preference for the susceptible variety Smooth Green (SG), than for either of the resistant accessions, Acc. 9 and Acc. 12 (Table 1 and Fig. 1).

According to Maxwell and Jennings (1980), orientation to potential host plants and the discrimination of host from non-host require a highly developed sensory system. Specific plant "odors" are seldom single compounds, but are usually complexes of several volatile substances. The dominant compound in such complexes is usually called the "token stimulus" because it signals the presence of a host plant.

In the present study, this "token stimulus" may not have been emitted in adequate amount by the resistant varieties (Acc. 9 and Acc. 12) as in the susceptible variety SG. Furthermore, the adult leafhoppers may have encountered some repellents from the two resistant hosts. Feeny (in Levin, 1976) referred to chemicals in plants that repel a potential attacker as qualitative defense. Alkaloids, mustard oil glucosides and other unusual plant products afford qualitative protection. These substances are also known as secondary plant substances or "allelochemics" (Whittaker in Maxwell and Jennings, 1980).

The specific plant factors contributory to the observed non-preference for the resistant accessions were not determined in the present study. However, various authors have mentioned that hairiness, thickness of leaf surface, hair density on midrib and lamina, thickness of palisade cells, toughness and compactness of leaf veins confer resistance to plants against leafhoppers (Adordionisio citing Batra and Gupta, Parnell, Sikka *et al.*, Yadava *et al.*, Afsal and Ghani, and Ayengan, 1979).

Painter (1951) reported that hairiness of legumes and cotton, which cause differences in the intensities of light reflected from the leaves, may have caused the lower number of leafhoppers found on them. Pathak (in Adordionisio, 1979) reported that leafhoppers fed equally on the resistant and susceptible plants. However, they cannot stay or make "sustained feeding" in the resistant variety. According to Beck (in Bergonia, 1983), this is because successful host plant utilization by an insect unquestionably depends on the existence of a proper "fit" between the biological characteristics of the insect and the host plant. Therefore, even if the physical and chemical cues needed in the host selection behavior of the cotton leafhopper were present in the resistant varieties, the required "fitness" between the leafhoppers' biological characteristics and that of the resistant accessions may have been very slight.

Ovipositional Preference. The less preferred varieties for feeding were also less preferred for oviposition (Table 2 and Fig. 2).

Field-collected test insects were used instead of laboratory-reared hoppers since the present study is a verification of the field resistance observed earlier. Ovipositional preference was based on the number of newly-hatched nymphs and not on the number of eggs actually laid. This is because hopper eggs are minute, opaque and laid singly inside leaf vein tissues, hence, they may be crushed or missed during dissection.

The plant factors responsible for ovipositional non-preference were not determined in this study. However, the findings of Caasi-Lit and Bernardo (1990) on eggplant resistance to the cotton leafhopper are very relevant. They found significant negative correlations between trichome characteristics (trichome length and number of branches per trichome) and ovipositional preference of the same leafhopper, *A. biguttula* (Ishida).

According to Maxwell and Jennings (1980) thick cell walls, tough tissues, and the accumulation of surface waxes on plant parts can also adversely affect the oviposition of the pests. Toughness of cuticle of leaf veins has been considered as an important factor preventing the entry of the ovipositor into the plant tissues during egg-laying (Bernardo and Taylo, 1990 citing Afzal and Abbas, 1943).

Antibiosis

Observations for one week on daily leafhopper survival (Table 3 and Fig.

3) revealed that significantly more deaths occurred on the resistant hosts than on the susceptible variety SG. Thus, fewer nymphs reached the adult stage on Acc. 9 and Acc. 12 than on SG (Table 4). However, the differences among the 3 varieties, although statistically different, were not very marked.

The nymphal period (ranging from 6.6. to 7.2 days) observed on okra in this study (Table 4), was very close to the findings of Bhalani and Patel (1981) on the same host (8 days) in India. However, it was markedly shorter than that observed by Cendaña and Baltazar (1947) on cotton (13 to 15 days). Apparently, the nymphal period of the insect is affected by the host on which it is reared.

The incidence of deaths occurred throughout the nymphal period. This suggests that the cause of death of hoppers on the resistant varieties may be largely nutrient deficiency, rather than the presence of toxic materials in the plants. The other indications of nutritional deficiency of the food taken from the two resistant accessions were reduced fecundity and shorter adult life (Table 4). The deficiency can be due to absence of some essential food nutrients or inadequacy in amount. It was possible also that the amount of food taken from the resistant varieties, compared to that from the susceptible host, was lesser due to feeding impediments. The resistant accessions may had various anatomic-morphological characteristics that inhibited leafhopper feeding. Maxwell and Jennings (1980) reported that silica in plants may bring about the abrasion of the cuticle of insects feeding on them. Proliferation of wounded host tissues may also impede further insect feeding after the intitial injury to the plants.

Singh and associates (1972) concluded that the higher content of minerals (especially silicon, iron and magnesium) in resistant strains of cotton (*Gossypium hirsutum* L.) increases the osmotic pressure of the cell sap, thereby adversely affecting the feeding ability of the cicadellid, *Amrasca devastans* (Distant). Jayarij (1967) suggested that the possible operational mechanism of a pronounced antibiosis observed in castor varieties against the leafhopper *Empoasca flavescens* (F.) was related to the excessive accumulation of total carbohydrates, especially sucrose, glucose and fructose and the lower total nitrogen and free amino acid contents in the resistant varieties. Caasi-Lit and Bernardo (1990) mentioned the possibility that antibiotic effects of the resistant eggplant variety PI-381272-2 on *Amrasca biguttula* (Ishida) was due to the higher alkaloid content.

Tolerance

In tolerance studies, there is a need to determine the appropriate infestation level which would clearly demonstrate the differing abilities of plants to withstand pest attack. This is because at a very high infestation level, even the resistant varieties may succumb readily and tolerance may not be detected. On the other hand, if the infestation level is very low, even the susceptible variety may appear resistant to the pest being studied.

The results indicate that any of the four infestation levels (10, 20, 30 and 40 nymphs) can be used for differentiating tolerance limits since they all gave similar rankings of the three varieties. Acc. 12 was always the most tolerant, followed by Acc. 9 and then by SG (Table 5). But if a detailed documentation of damage progression on the susceptible variety is desired also, less than 30 nymphs per plant should be used. Further studies can determine the ideal infestation level for damage progression documentation on okra. However, if mere separation of the more tolerant materials is desired in the shortest time possible, 40 nymphs per leaf is the most practical to use among the four levels tested (Table 6).

Various morphological and physiological factors may have conditioned the tolerance exhibited by the two accessions. They may have had harder tissues and/or ability to inactivate the enzymes or toxic materials injected by the leafhoppers. Maxwell and Jennings (1980) stated that various anatomical adaptations of non-specialized organs and protective structures of plants may cause various effects, promoting resistance to insect pests.

The damage of cotton leafhopper is readily noticeable and localized. Thus, it was more practical to use plant parts (leaves) instead of whole plants for tolerance tests in this study.

CONCLUSION, IMPLICATION AND RECOMMENDATION

Based on the results of all the tests conducted, it can be concluded that the mechanisms or resistance of okra Acc. 9 and Acc. 12 are non-preference, antibiosis and tolerance. The information generated in this study would facilitate the determination of the plant factors responsible for the resistance observed in okra Acc. 9 and Acc. 12. However, even if these plant factors have not yet been identified, either of the resistant accessions can already be used as parent materials in future breeding work.

The different mechanisms of resistance are usually governed by different plant factors. Thus, even upon identification, it may not be feasible to incorporate all of the factors in an improved okra variety in a reasonably short time. It is therefore necessary that breeders be made aware of the most important plant factors to be considered in future crop improvement work. This means that the relative importance of the mechanisms identified in the two accessions should be evaluated for prioritization purposes.

For instance, if *A. biguttula* is discovered to be a vector of systemic organisms causing very destructive diseases of okra, the most important mechanism of resistance then would be non-preference to reduce feeding incidence and consequential pathogen transmission.

In multiple cropping systems where okra may be grown along with several other crops, the hoppers may not be confined to okra for feeding and reproduction since they are very mobile and they may infest the other hosts present with okra plants. Thus, even if antibiosis is present in the okra variety planted, this may not markedly affect the population of the leafhoppers in the area. A high population may still be found on the crop as a result of transfer from other hosts. Under these situations, the tolerance mechanism will be more advantageous to have than non-preference or antibiosis.

On the other hand, a high level of antibiosis is desirable in large-scale, monoculture, and continuous growing of the crop, since the leafhoppers will then be confined to feeding largely on the resistant plants. This can eventually reduce the population of the pest through increased mortality, abnormal development, reduced fecundity and shortened adult life. Needless to say, a combination of the three resistance mechanisms should be the ultimate goal in okra breeding for cotton leafhopper resistance.

Follow-up studies should focus on the identification of specific morphological features and biochemical components of okra Acc. 9 and Acc. 12 responsible for the resistance to cotton leafhopper observed in the field, and related to the biological mechanisms determined by the present study.

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Received 27 June 1993.

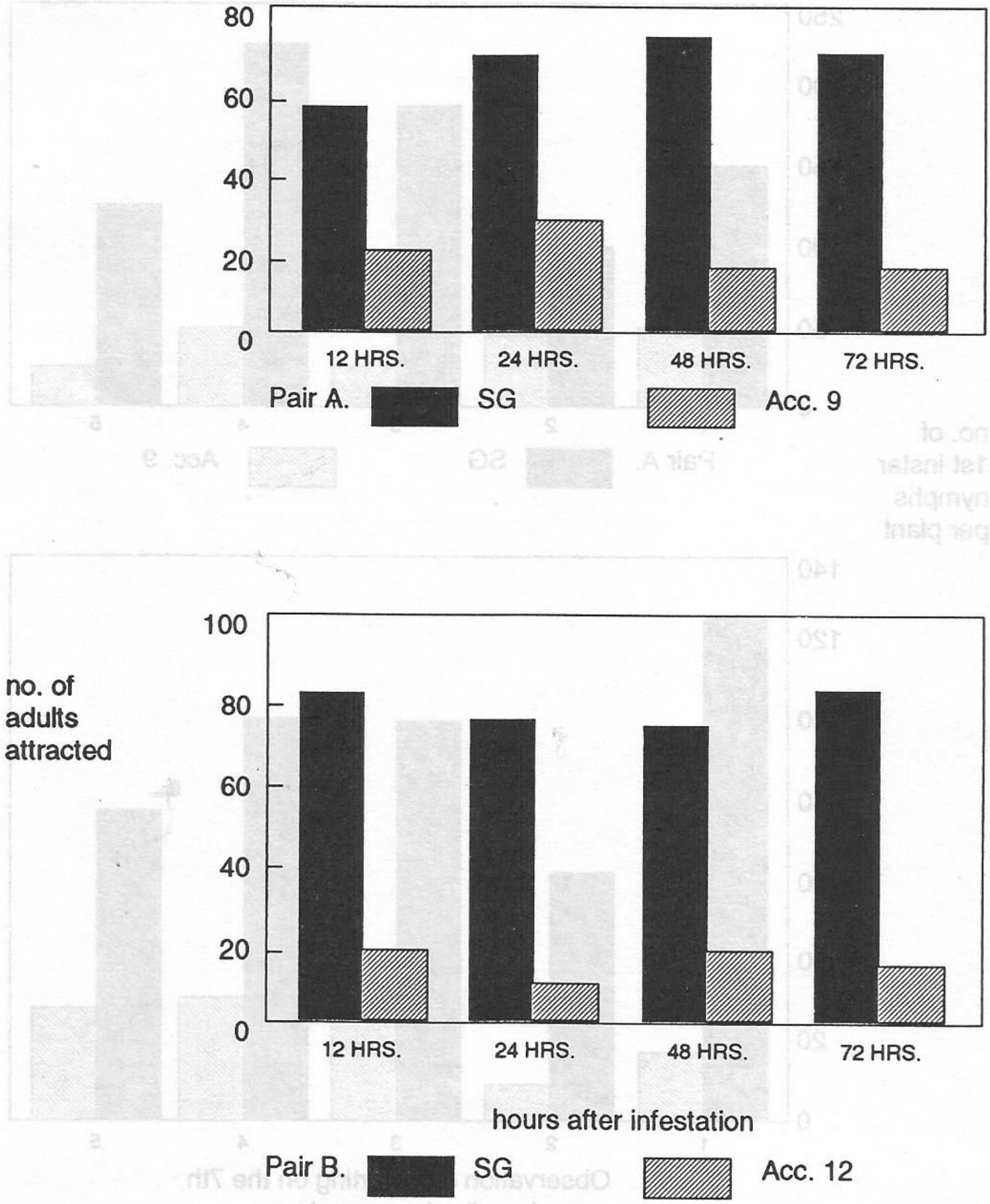

Figure 1. **Host preference of cotton leafhopper adults for paired okra varieties: A. Smooth Green or SG (susceptible) and Acc. 9 (resistant), B. SG (susceptible) and Acc. 12 (resistant).**

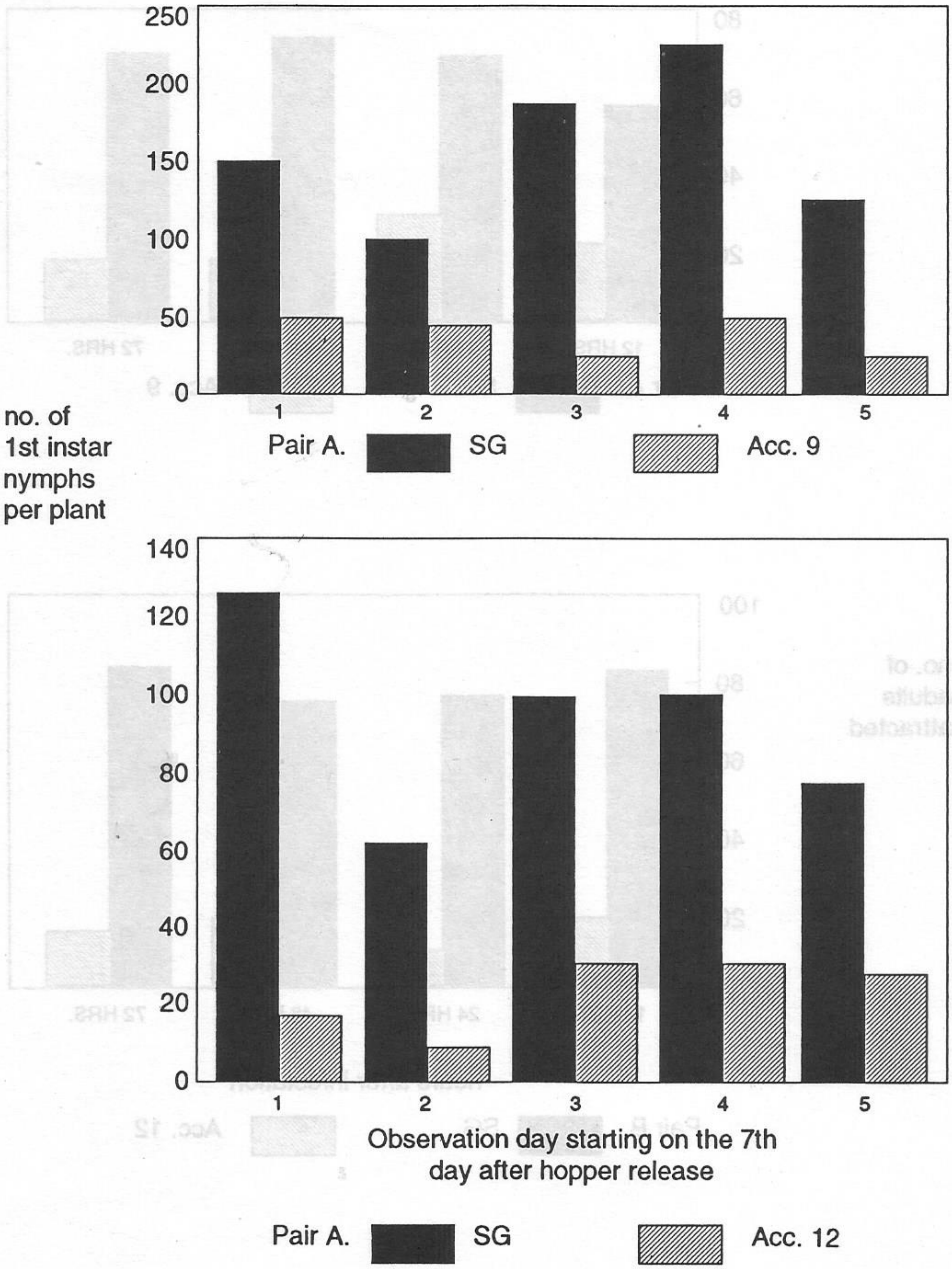

Figure 2. Ovipositional preference of the cotton leafhopper for paired okra varieties: A. Smooth Green or SG (susceptible) and Acc. 9 (resistant), B. SG (susceptible) and Acc. 12 (resistant).

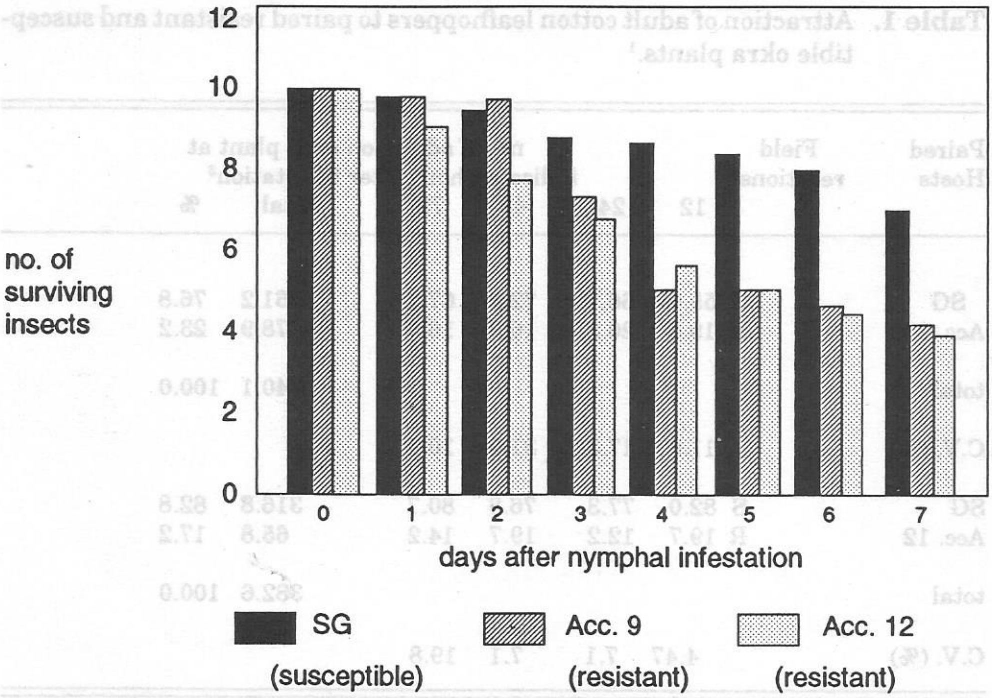

Figure 3. **Daily leafhopper survival on susceptible and resistant varieties of okra.**

Table 1. Attraction of adult cotton leafhoppers to paired resistant and susceptible okra plants.[1]

Paired Hosts	Field reactions[2]	no. of adults on each plant at indicated hour after infestation[3]					
		12	24	48	72	Total	%
SG	S	55.0	66.7	72.3	67.2	261.2	76.8
Acc. 9	R	19.5	26.8	16.3	16.3	78.9	23.2
total						340.1	100.0
C.V. (%)		11.9	17.3	31.1	10.1		
SG	S	82.0	77.3	76.8	80.7	316.8	82.8
Acc. 12	R	19.7	12.2	19.7	14.2	65.8	17.2
total						382.6	100.0
C.V. (%)		4.47	7.1	7.1	19.8		

[1] Figures are averages of 3 replications and 2 trials; at least 200 adults released per trial.

[2] Based on 1988 and 1989 field evaluations, IPB, UPLB; S=susceptible, R=resistant.

[3] Difference in values between the paired varieties, highly significantly at 5% level ANOVA.

Table 2. Ovipositional preference of cotton leafhoppers for resistant and susceptible okra plants.[1]

Paired Hosts	Field reaction[2]	no. of 1st instar nymphs collected at indicated day of observation[3]					
		1	2	3	4	5	total
SG	S	150.8	97	184	222	119	772.8
Acc. 9	R	51.7	47	31	48	32	208.5
C.V. (%)							12.38
SG	S	126.5	63	99	102	79.2	470.0
Acc. 12	R	16.7	7.5	33	35	28.5	120.3
C.V. (%)							40.1

[1] Data are averages for 3 replications and 2 trials; at least 200 adults per trial.

[2] Based on 1988 and 1989 field evaluations, IPB, UPLB; S=susceptible, R=resistant.

[3] Counting of newly hatched nymphs per plant started on the 7th day after release of adult hoppers; the nymphs were removed after counting; difference in values between varieties, significant at 5% level ANOVA.

Table 3. Daily leafhopper survival on susceptible and resistant varieties of okra.[1]

Variety/ Accession	number of surviving insects at indicated day after infestation							
	0	1	2	3	4	5	6	7
SG	10	9.8A	9.8A	8.6A	8.4A	8.0A	7.5A	6.9A
Acc. 9	10	9.8A	9.5A	7.4A	5.6B	5.2B	4.8B	4.4B
Acc. 12	10	8.8B	7.7B	6.9A	5.3B	5.2B	4.7B	4.2B

[1] 10 Leaves per variety; 10 first instar nymphs per leaf.
[2] Means with the same letter are not significantly different at 5% level DMRT.

Table 4. Development, fecundity and longevity of the cotton leafhoppers on resistant and susceptible okra varieties.[1]

Parameters	Host plants			C.V. values
	Smooth Green (susceptible)	Acc. 9 (resistant)	Acc. 12 (resistant)	
Total developmental period (egg laying to adult emergence)[2]	13.6 A	14.1 A	14.2 A	8.36
Percent nymphs becoming adults[2]	69.0 A	41.0 B	30.0 C	27.3
Adult fecundity[3]	147.1 A	82.8 B	3.4 C	16.92
Adult longevity[2]	8.3 A	4.9 B	2.6 C	22.8

[1] Means with the same letters are not significantly different at 5% level DMRT.

[2] Based on 100 inviduals.

[3] Based on 50 females; values are total number of 1st instar nymphs produced by 5 females in 11 days.

Table 5. Mean damage rating of susceptible and resistant varieties of okra at different levels of nymphal infestation.[1]

No. of leafhopper nymphs per leaf on each okra variety[2]

days after infestation	10			20			30			40		
	SG[3]	Acc. 9	Acc. 12	SG	Acc. 9	Acc. 12	SG	Acc. 9	Acc. 12	SG	Acc. 9	Acc. 12
0	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	2	1	1	2	1	1
2	1	1	1	1	1	1	2	1	1	2	2	1
3	1	1	1	2	1	1	2	2	1	2	2	2
4	1	1	1	2	1	1	2	2	2	2	2	2
5	1	1	1	2	2	2	2	2	2	2	2	2
6	2	1	1	2	2	2	2	2	2	2	2	2
7	2	1	1	2	2	2	2	2	2	3	2	2
8	2	1	1	2	2	2	2	2	2	3	2	2
9	2	1	1	2	2	2	3	2	2	3	2	2
10	2	1	1	2	2	2	3	2	2	3	3	2
11	2	1	1	2	2	2	3	2	2	3	3	2
12	2	2	1	2	2	2	3	3	2	3	3	2
13	2	2	1	2	2	2	3	3	2	3	3	2
14	2	2	2	3	2	2	3	3	2	4	3	2
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16	2	2	2	3	2	2	3	3	2	-	-	-
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19	3	2	2	3	3	2	4	3	3	-	-	-
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25	3	3	3	-	-	-	-	-	-	-	-	-
26	3	3	3	-	-	-	-	-	-	-	-	-
27	3	3	3	-	-	-	-	-	-	-	-	-
28	3	3	3	-	-	-	-	-	-	-	-	-
29	3	3	3	-	-	-	-	-	-	-	-	-
30	4	3	3	-	-	-	-	-	-	-	-	-

[1] one leaf per replication; 3 replications; infested with 1st to 4th instar nymphs.

[2] damage rating: 1=no damage; 4=severely damaged, dried up.

[3] SG or susceptible variety Smooth Green; resistant accessions Acc. 9 and Acc. 12.

Table 6. Number of days that the okra leaves remained at indicated degree of damage when subjected to different leafhopper infestation levels.

Damage Rating	Variety	number of nymphs per leaf 40	30	20	10
1	SG	0	0	2	5
	Acc. 9	1	2	4	11
	Acc. 12	3	3	4	13
2	SG	6	8	13	17
	Acc. 9	9	11	16	22
	Acc. 12	14	16	19	24
3	SG	13	18	24	29
	Acc. 9*	-	-	-	-
	Acc. 12*	-	-	-	-

***Observation was discontinued when the susceptible variety, SG, already had the highest damage rating of 4 (on the 14th day at 40 nymphs per leaf) but Acc. 9 and Acc. 12 still had a damage rating of 3.0**