Resistance in Vegetative and Reproductive Stages of a Soybean Breeding Line to Three Defoliating Pests (Lepidoptera: Noctuidae)

BRIAN A. NAULT,[1] J. N. ALL,[2] AND H. R. BOERMA[3]

Department of Entomology, University of Georgia, Athens, Georgia 30602

J. Econ. Entomol. 85(4): 1507–1515 (1992)

ABSTRACT Resistance in certain genotypes of soybean, *Glycine max* (L.) Merr., has been shown to adversely affect insect behavior and development in laboratory feeding experiments involving excised leaf material. This study investigated effects of resistance in various developmental stages of intact soybean plants on feeding behavior and physiology of corn earworm, *Helicoverpa zea* (Boddie); soybean looper, *Pseudoplusia includens* Walker; and velvetbean caterpillar, *Anticarsia gemmatalis* Hübner. Soybean genotypes 'Cobb' (susceptible) and 'GatIR81-296' (resistant) were grown in the greenhouse and used in choice and no-choice experiments. Choice tests showed the presence of resistance in 'GatIR81-296', and sequential observations of behavior indicated that the insects were inhibited by constitutive factors and components associated with damaged plants. No-choice tests indicated that resistance occurred in vegetative and reproductive stages of 'GatIR81-296' and resulted in reduced feeding and number of larvae on plants, smaller larvae and pupae, and slower larval development compared with the insects on 'Cobb'. Resistance in 'GatIR81-296' was attenuated in intact reproductive stage plants (R2-R3) compared with vegetative stage (V3-V4) ones.

KEY WORDS Insecta, host plant resistance, maturity, defoliating pests

RESISTANCE IN SOYBEAN to defoliating insects was identified in the early 1970s (Van Duyn et al. 1971) and prompted research on the physiological and behavioral mechanisms of insect response to the inhibitory factors within these resistant lines (Hatchett et al. 1979, Beach & Todd 1988a). Resistance in certain soybean genotypes has been reported to be antixenosis, antibiosis, or a combination of the two (Elden et al. 1974, Beach et al. 1985, Smith 1985) and has been attributed to biochemical endogenous factors present in the plant. Additionally, Kogan & Paxton (1983) and Chiang et al. (1986, 1987) reported higher levels of resistance induced by insect feeding or other stress factors. Most of these studies used excised leaf material rather than whole plants. Previously injured foliage of soybean and other plant species alters larval feeding behavior and development in specific situations (Greene & Ryan 1972, Carroll & Hoffman 1980, Reynolds & Smith 1985), demonstrating a need for additional research in this area.

Insect research with soybean has used foliage of various ages. The stage of plant development has been reported to influence soybean resistance (McWilliams & Beland 1977, Reynolds & Smith 1985, unpublished data), and this may have influenced results on insect development and behavior.

Resistance in the breeding line 'GatIR81-296' to defoliating pests has been extensively studied (Beach & Todd 1987, 1988a, 1988b; Beach et al. 1985), yet these studies have not compared 'GatIR81-296' resistance at different phenologies. This study was designed to identify resistance in various developmental stages of soybean to three insect pests on intact plants using choice and no-choice bioassays.

Materials and Methods

Insect behavioral and physiological studies were performed in a greenhouse at the University of Georgia, Athens. These studies were composed of choice and no-choice tests on potted vegetative or reproductive-stage plants. Two soybean genotypes were used: 'Cobb' (susceptible) and 'GatIR81-296' (an insect-resistant breeding line derived from 'GaSoy 17' × 'PI 229358') (Beach & Todd 1987). All plants were grown under a 14:10 (L:D) photoperiod supplemented with fluorescent light. Test insects included: corn earworm, *Helicoverpa zea* (Boddie), acquired from the Insect Biology and Population Management Research Laboratory, USDA–ARS, Tifton, Ga.; and soybean looper,

[1] Current address: Department of Entomology, North Carolina State University, Box 7630, Raleigh, N.C. 27695.

[2] Department of Entomology, University of Georgia, Athens, Ga. 30602.

[3] Department of Agronomy, University of Georgia, Athens, Ga. 30602.

0022-0493/92/1507–1515$02.00/0

Pseudoplusia includens (Walker), and velvetbean caterpillar, *Anticarsia gemmatalis* Hübner, both received from USDA-ARS rearing facilities in Stoneville, Miss.

Choice Test 1. Feeding preferences of all three species for vegetative-stage 'Cobb' and 'GatIR81-296' were compared. Three seeds were planted in 0.5-liter polystyrene foam cups containing a methylbromide-treated soil and sand mixture. Plants were thinned to one plant per cup after 10 d. Each cup had three holes (8 mm diameter) in the bottom for water access. All cups were placed in stainless steel trays (4.9 m long, 1.2 m wide, 8 cm high). Plants were watered from above until plants reached the V3-V4 stage of development (Fehr & Caviness 1977). Seedlings were fertilized with 12, 5, and 10 g/liter of N, P, and K, respectively, 7 d after planting.

Ninety cups were arranged in a randomized complete block design with five replications for each test insect (three cups per experimental unit × two genotypes × three insect species × five replications). Each replication consisted of three 'Cobb' and three 'GatIR81-296' plants which were close enough together for their leaves to intermingle but not to touch leaves from adjacent replicates. When the plants were infested, the trays were filled with water, which was maintained at a height of 3–4 cm.

Three neonates of *H. zea, P. includens,* or *A. gemmatalis* were placed on the uppermost unfolded leaf of each plant in their respective replicates. Feeding preference was measured by counting the number of larvae on each plant at 24-h intervals for 12 d and visually estimating percentage leaf area removed every other day.

Choice Test 2. *Helicoverpa zea* and *A. gemmatalis* feeding preferences were compared when given damaged 'Cobb' and 'GatIR81-296' plants. The criteria for concluding that the larval behavior exhibited in this test were constitutive or induced were based on discussions by Chiang et al. (1987). Larval preference for an undamaged 'Cobb' rather than an undamaged 'GatIR81-296' within 24 h of infestation was used as a criterion for determining if constitutive resistance occurred in the soybean. Larval preference for one of the undamaged genotypes after 4–5 d or preference for a damaged genotype after 24 h was used as a criterion for induced resistance.

Soybean plants were grown in the greenhouse as described previously. When plants reached the V2 stage, some were damaged (one-third artificially and one-third by insect feeding) before testing. The artificially damaged plants were punctured with an insect pin, creating 16 small holes for the first 2 d and with a hole-puncher for the next 4 d (total of 12 large holes, 1 cm diameter). Meanwhile, *H. zea* and *A. gemmatalis* neonates (three per plant) were placed on one-third of the plants and allowed to feed for 6 d before they were removed. The remaining third of the plants were left undamaged. Percentage of pretreatment defoliation averaged 5% for artificially damaged plants and 16.9% for insect-damaged plants. All plants (now V3-V4) were arranged in a randomized complete block design with four replications and 12 treatments. The treatment design was a split-plot with six treatment combinations as main plots (two insect species × three defoliation treatments) and the two soybean genotypes as subplots. Foliage from two 'Cobb' and two 'GatIR81-296' plants in each main plot intermingled, but foliage among main plots were separated. These plants were infested with either three neonate (young) or three third-instar (old) *H. zea* or *A. gemmatalis*. Third instars were maintained on a soybean flour-based diet for 6 d before infestation. Data were recorded daily by counting number of larvae on each plant and by visually estimating percentage leaf area removed every other day.

No-Choice Test 1. Larval development of all three species was evaluated when confined to vegetative stage (V3-V4) 'Cobb' and 'GatIR81-296'. The soybean plants were grown as described previously. In addition, each cup was placed in the center of a plastic pot (30.5 cm diameter, 30.5 cm high) with 5 cm of sand in the bottom. Sixty pots were arranged in a randomized complete block design with 10 replications for each test insect (two genotypes × three insect species × 10 replications). Each experimental unit (one plant) was isolated from all others.

Three neonates of *H. zea, P. includens,* or *A. gemmatalis* were placed on the uppermost unfolded leaf of each plant. Larvae on each plant (represented as number larvae remaining/original number larvae × 100%) were counted at 24-h intervals for 12 d.

One larva was removed from each plant and weighed, then returned to the same plant daily, beginning on the 4th d. Larval weights were measured with an electronic balance (A30; Mettler Instrument Company, Hightstown, N.J.). *H. zea, P. includens,* and *A. gemmatalis* head capsule widths were also measured and categorized into a specific larval instar using the following scale: first (<0.83 mm), second (0.84–1.33 mm), third (1.34–1.78 mm), fourth (1.79–2.48 mm), fifth (2.49–2.78 mm), and sixth (>2.79 mm).

No-Choice Test 2. *Helicoverpa zea* larval development was investigated when confined to reproductive stage (R2-R3) 'Cobb' and 'GatIR81-296' plants. Soybean seeds were grown in black plastic pots (15.2 cm diameter, 16.5 cm high) using the same procedure described previously. When plants reached later vegetative stages, they were supported with bamboo stakes, and tests were initiated when they developed to R2-R3.

Eight 'Cobb' and eight 'GatIR81-296' plants, with each plant isolated from one another, were

arranged in a randomized complete block. Five *H. zea* neonates were placed near flowers of each plant. Number of larvae per plant, larval weights, and head capsule widths were recorded daily, beginning on the fourth day of the test. Data were calculated as percentage larvae per plant on both genotypes. Larval head capsule widths were measured and categorized into specific larval instar stages using methods described in no-choice test 1.

No-Choice Test 3. Final larval mortality and pupal development of *H. zea, P. includens,* and *A. gemmatalis* reared on vegetative-stage (V3–V4) or reproductive-stage (R2–R3) 'Cobb' and 'GatIR81-296' plants were compared to determine the influence of plant age on insect physiology. Soybean seeds were planted in black plastic pots (15.2 cm diameter, 16.5 cm high) in the manner described previously. In addition, an extra layer of sand (5 cm) was added to the top of the previously existing soil and sand mixture for a pupation site. Soybean seeds were planted 34 d apart to obtain two different developmental stages (V3–V4 and R2–R3). There were 60 vegetative stage (two genotypes × three insect species × 10 replications) and 24 reproductive stage (two genotypes × three insect species × four replications) plants which were arranged in a completely randomized design in stainless steel trays. Each plant was infested by placing five neonates of *H. zea, P. includens,* and *A. gemmatalis* on the uppermost unfolded leaf of V3–V4 plants and near flowers of R2–R3 plants. Screen wire cages were used to completely enclose each plant to prevent larvae from escaping and to avoid disturbing larvae during their development. Cages enclosing V3–V4 plants were 61 cm high, 28 cm diameter at the top, and 15.2 cm diameter at the bottom, and cages enclosing R2–R3 plants were 91 cm high, 30.5 cm diameter at the top, and 15.2 cm diameter at the bottom.

Pupae were recovered by sifting through the top layer of sand. Final larval mortality was determined by the number of pupae recovered minus the original number of neonates placed on each plant. Pupal weights were measured with an electronic balance, and pupal lengths and widths were measured with a hand caliper. Pupae were placed in round plastic containers (26.7 cm diameter, 10.1 cm high) which were kept in incubators maintained at 27.0 °C and a 14:10 (L:D) photoperiod. The number of emerging adults was recorded over 3 wk.

Leaf Area Analysis. On the final day of observations during choice test 1 and no-choice test 1, a visual estimation of percentage leaf area removed from the soybean plants was recorded. Additionally, all fully expanded trifoliolate leaves were removed and photocopied. These copies were analyzed with a computer-based leaf digitation system which calculated the "actual" percentage leaf area of the plant (Hargrove & Crossley 1988). Before analysis in choice test 2, pretreatment defoliation estimates were subtracted from final estimates (percentage leaf area removed).

Statistical Analyses. In both choice tests 1 and 2, the number of larvae on 'Cobb' and 'GatIR81-296' were compared by analysis of variance (ANOVA) and either a single-degree-of-freedom F test (SAS Institute 1985) or a protected LSD ($P = 0.05$) (Carmer et al. 1969).

Data represented as percentage larvae per plant were transformed by arcsine to remove the interdependence of means and variances (Steel & Torrie 1980) in no-choice tests 1 and 2. These means and larval weights were compared by ANOVA and an F test (df = 1, $P = 0.05$). Insect development was compared using Chi-square contingency table analysis ($P = 0.05$) in no-choice tests 1 and 2. Final larval mortality was compared using Chi-square contingency table analysis ($P = 0.05$) in no-choice test 3. Pupal weights, lengths, and widths were compared by PROC GLM and an F test (df = 1, $P = 0.05$) only in no-choice test 3.

Actual percentage leaf area removed on both genotypes was compared by ANOVA and by an F test (df = 1, $P = 0.05$). Visual estimations of percentage leaf area removed and the actual percentage leaf area removed were compared using a paired t test ($P = 0.05$) in choice test 1 and no-choice test 1.

Results and Discussion

Choice Test 1. There were higher numbers of *H. zea* on 'Cobb' than on 'GatIR81-296' throughout their development (Fig. 1), and the numbers were significantly different on day 5 ($F = 4.87$; df = 1, 4; $P = 0.09$) and day 6 ($F = 10.82$; df = 1, 4; $P \leq 0.05$). Numbers of *H. zea* increased on both 'Cobb' and 'GatIR81-296' during the first 4 d. This was an observational error due to difficulty in detecting neonates on the plants because they typically feed inside folded leaves. Previous experiments demonstrated that prying these tender leaves apart with a probe or forceps either disrupts, injures, or kills young larvae and damages the plant tissues. Greater numbers of *P. includens* were observed on 'Cobb' than on 'GatIR81-296' (Fig. 1), and the differences were significant on day 4 through day 10 ($F \geq 7.52$; df = 1, 4; $P \leq 0.05$) . There was a greater number of *A. gemmatalis* on 'Cobb' than on 'GatIR81-296' on days 1, 2, 4, 5, 6, 7, and 8 ($F \geq 7.50$; df = 1, 4; $P \leq 0.05$) and day 3 ($F = 5.51$; df = 1, 4; $P = 0.08$) (Fig. 1).

Actual percentage leaf area removed was calculated using a computer-based digitation process. *H. zea, P. includens,* and *A. gemmatalis* defoliated 'Cobb' more than 'GatIR81-296' (33.6 versus 18.9%), (22.7 versus 5.9%), and (72.9 versus 49.0%), respectively. Comparisons between

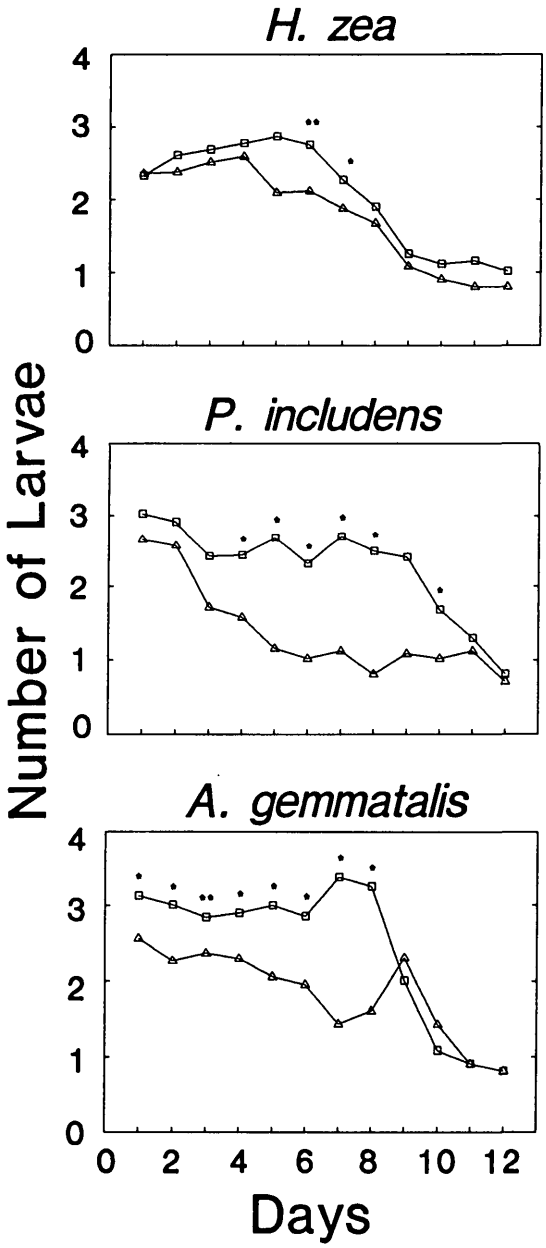

Fig. 1. Number of *H. zea*, *P. includens*, and *A. gemmatalis* larvae observed on two vegetative-stage (V3–V4) soybean genotypes, 'Cobb' (□), and 'GatIR81-296' (△), choice test 1. Means followed by one or two asterisks on a certain day are significantly different ($P \leq 0.05$, df = 1) and ($P \leq 0.10$, df = 1) (*F* test), respectively.

visual estimates of percentage leaf area removed and actual percentage leaf area removed (33.2 versus 33.8%) were not significantly different ($t = 0.53$, df = 89, $P > 0.05$). Visually estimating percentage leaf area removed requires considerably less time than using the digitation method.

Choice Test 2. Because the tests were conducted on intact plants instead of excised leaves, it was decided that larval movement from one genotype to another within 24 h indicated that constitutive factors within foliage produced the preferential behavior. If larval movement occurred after 24 h on previously undamaged plants or if larvae quickly moved from predamaged plants (<24 h), induced resistance was indicated. Kogan & Paxton (1983) reported that induced resistance in soybean to the Mexican bean beetle, *Epilachna varivestis* Mulsant, occurred after cotyledon leaf disks were exposed to UV light for 48 h. Chiang et al. (1987) discovered that prior *E. varivestis* feeding on soybean leaflets induced higher levels of resistance to subsequent *E. varivestis* herbivory on other leaflets of the same leaf at both 12 and 24 h after the treatment (prior feeding).

Insect species, larval age, and soybean genotype all significantly affected the number of larvae observed on plants during days 1–6 ($F \geq 19.12$; df = 1, 36; $P \leq 0.05$), days 2–5 ($F \geq 4.67$; df = 1, 36; $P \leq 0.05$), and days 1–6 ($F \geq 20.54$; df = 1, 36; $P \leq 0.05$), respectively. There were significant interactions within defoliation on day 3 ($F = 4.01$; df = 2, 36; $P \leq 0.05$), genotype × species on days 5 and 6 ($F \geq 15.68$; df = 1, 36; $P \leq 0.05$), and genotype × age on day 5 ($F = 6.22$; df = 1, 36; $P \leq 0.05$), but they did not occur consistently throughout the experiment. A greater number of *A. gemmatalis* were seen on 'Cobb' and 'GatIR81-296' than on *H. zea* each day (Fig. 2A). *A. gemmatalis* is "less" polyphagous than *H. zea* and will feed voraciously on soybean, whereas *H. zea* were observed wandering from soybean plants. This type of behavior by *H. zea* was likely because of their search for unfolded leaves, flowers, and pods, which they prefer rather than open leaves (Eckel et al. 1992).

There were more young (initially placed on plants as neonates) *H. zea* and *A. gemmatalis* larvae on 'Cobb' and 'GatIR81-296' than old (initially placed on plants as third instars) larvae each day (Fig. 2B). Observations during this study noted greater activity in old larvae than in young larvae. Old larvae often moved from the foliage and ultimately drowned in the water-filled trays. Terry et al. (1989) found that young *H. zea* larvae tended not to move from their original location on a soybean plant, especially during the host's younger phenological stages.

Greater numbers of *H. zea* and *A. gemmatalis* larvae were seen on 'Cobb' than on 'GatIR81-296' plants within 24 h and each succeeding day (Fig. 2C). These results indicate that constitutive resistance occurs in intact 'GatIR81-296' plants; both species preferred 'Cobb' rather than 'GatIR81-296'.

Insect species, larval age, soybean genotype, and defoliation all affected the percentage leaf area removed from the plants by *H. zea* and *A. gemmatalis* each day (Table 1). All interactions of main effects were from changes in magnitude of the treatments but did not change the ranks of

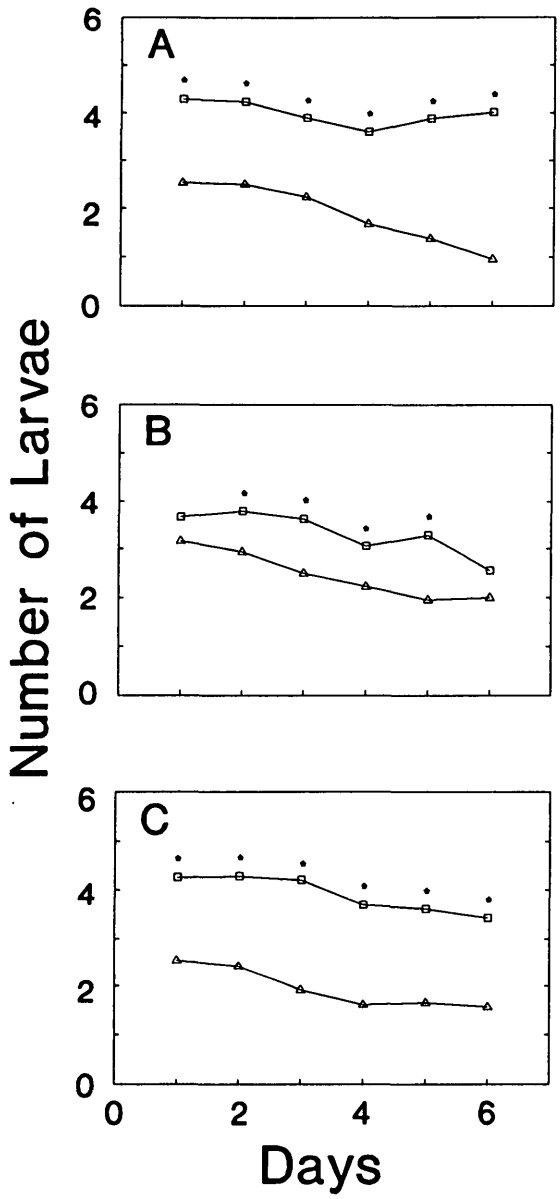

Fig. 2. (A) Number of *H. zea* (△) and *A. gemmatalis* (□) larvae (young and old) observed on all soybeans (undamaged, artificially damaged, and insect damaged 'Cobb' and 'GatIR81-296'). (B) Number of young (first instars) (□) or old (third instars) (△) larvae (*H. zea* and *A. gemmatalis*) observed on all plants. (C) Number of larvae (young and old *H. zea* and *A. gemmatalis*) observed on all 'Cobb' (□) or 'GatIR81-296' (△) plants (choice test 2). Means followed by one or two asterisks on a certain day are significantly different ($P \leq 0.05$, df = 1) and ($P < 0.10$) (*F* test), respectively.

treatments. There was greater percentage leaf area removed on all plants by *A. gemmatalis* than by *H. zea* each day. There was more percentage leaf area removed on all plants by old larvae (*H. zea* and *A. gemmatalis*) than by young larvae each day. Additionally, there was greater percentage leaf area removed on all 'Cobb' plants by *H. zea* and *A. gemmatalis* than on 'GatIR81-296' plants each day. These results provide further evidence that there is constitutive resistance in intact 'GatIR81-296' plants.

Table 1. Percentage leaf area removed from undamaged, artificially damaged, and insect-damaged (V2–V3) 'Cobb' and 'GatIR81-296' soybean plants, choice test II

Main effect	% Leaf area removed on d		
	2	4	6
Insect species			
A. gemmatalis	2.9*	3.9*	10.5*
H. zea	1.7	2.3	4.1
Age of larvae			
Old (third instar)	3.0*	4.8*	11.1*
Young (first instar)	1.6	1.3	3.5
Genotype			
'Cobb'	2.7*	4.3*	11.6*
'GatIR81-296'	1.9	1.8	3.1
Defoliation[a]			
Undamaged	1.6b	3.2b	10.3a
Insect-damaged	4.8a	4.8a	6.9b
Artificially damaged	0.5b	1.2c	4.7b

[a] Means followed by an asterisk or a different letter on a certain day are significantly different ($P \leq 0.05$; df = 1; *F* test or a protected LSD).

There was more percentage leaf area removed from insect-damaged plants than from undamaged or artificially damaged plants on days 2 and 4 (Table 1). By day 6, undamaged plants had greater percentage leaf area removed compared with artificially or insect-damaged plants, indicating that resistance may be induced in the damaged treatments after the sixth day of the study. Past studies by Smith (1985) and Chiang et al. (1987) found less herbivory on damaged foliage of leaf disks than on undamaged ones.

No-Choice Test 1. There were numerically more *H. zea*, *P. includens*, and *A. gemmatalis* larvae on 'Cobb' than on 'GatIR81-296' during the test (Fig. 3); and these results are similar to findings by Beach & Todd (1988a), who conducted experiments with excised leaves of 'GatIR81-296'.

By day 12, all three insect species had produced higher defoliation on 'Cobb' than on 'GatIR81-296', but the differences were significant only with *H. zea*. Comparisons between estimated percentage leaf area removed (taken visually) and actual percentage leaf area removed (35.2 versus 32.0) were not significantly different ($t = 1.88$, df = 54, $P > 0.05$). These results are similar to those in choice test 1.

Weights of *H. zea*, *P. includens*, and *A. gemmatalis* that fed on 'Cobb' were significantly greater than those on 'GatIR81-296' from day 4 through day 8, day 7 through day 10, and day 4 through day 8, respectively (Fig. 4). At the termination of this study, *H. zea* larvae that developed on 'Cobb' reached the third (40%) and fourth instars (60%), whereas those larvae that developed on 'GatIR81-296' varied from first

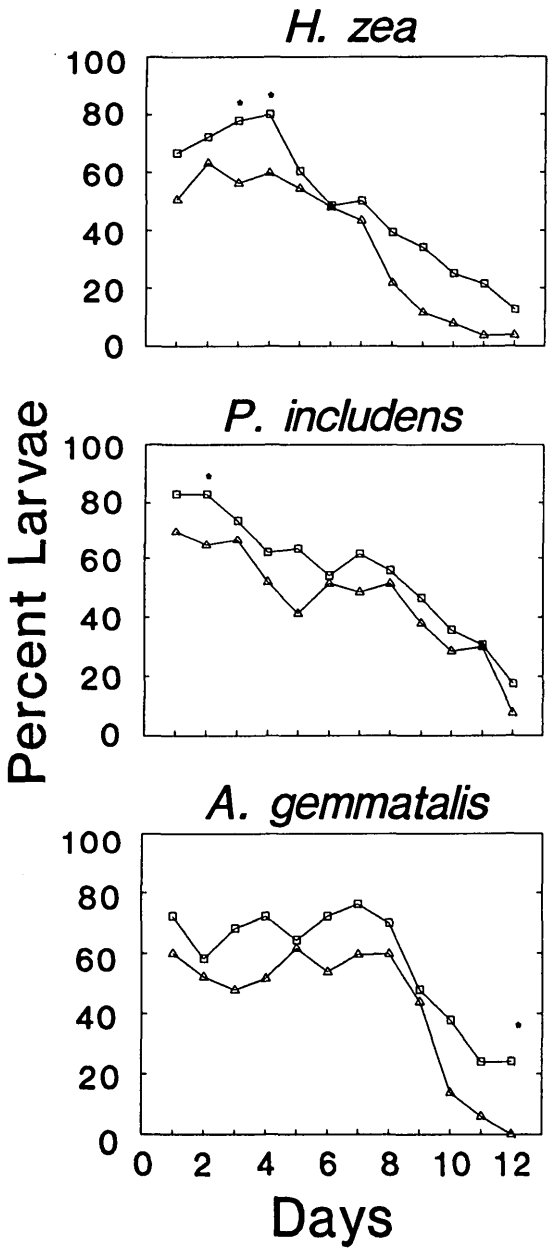

Fig. 3. Percentage of *H. zea*, *P. includens*, and *A. gemmatalis* larvae per V3–V4 'Cobb' (□) or 'GatIR81-296' (△) plant, no-choice test 1. Data were transformed by an arcsine function and analyzed, but the original data points are presented. Means followed by an asterisk on a certain day are significantly different ($P \leq 0.05$, df = 1) (*F* test).

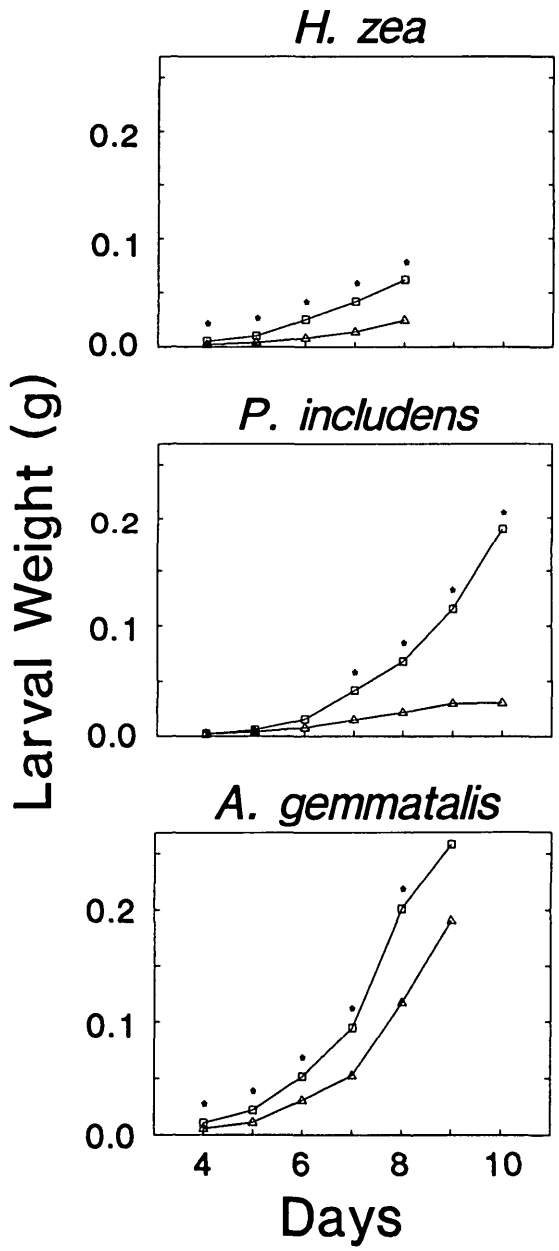

Fig. 4. Larval weights of *H. zea*, *P. includens*, and *A. gemmatalis* that fed on vegetative stage (V3–V4) 'Cobb' (□) and 'GatIR81-296' (△) plants, no-choice test 1. Means followed by an asterisk on a certain day are significantly different ($P \leq 0.05$, df = 1) (*F* test).

(10%), second (30%), third (30%), and fourth instars (30%) ($\chi^2 = 4.20$, df = 3, $P > 0.05$). *P. includens* larvae that consumed 'Cobb' foliage were in the second (29%), third (14%), and fourth instars (57%), whereas larvae that developed on 'GatIR81-296' were in the second (50%), third (40%), and fourth instars (10%) ($X^2 = 4.88$, df = 2, $P > 0.05$). *A. gemmatalis* larvae that consumed 'Cobb' were in the fourth (13%), fifth (74%), and sixth instars (13%), whereas larvae that developed on 'GatIR81-296' were in the third (11%), fourth (22%), fifth (56%), and sixth instars (11%) ($\chi^2 = 3.00$, df = 3, $P > 0.05$). Although all three species that fed on 'Cobb' developed faster and more uniformly than did larvae on 'GatIR81-296', these differences were nonsignificant.

No-Choice Test 2. There were numerically (although nonsignificant) more *H. zea* larvae on

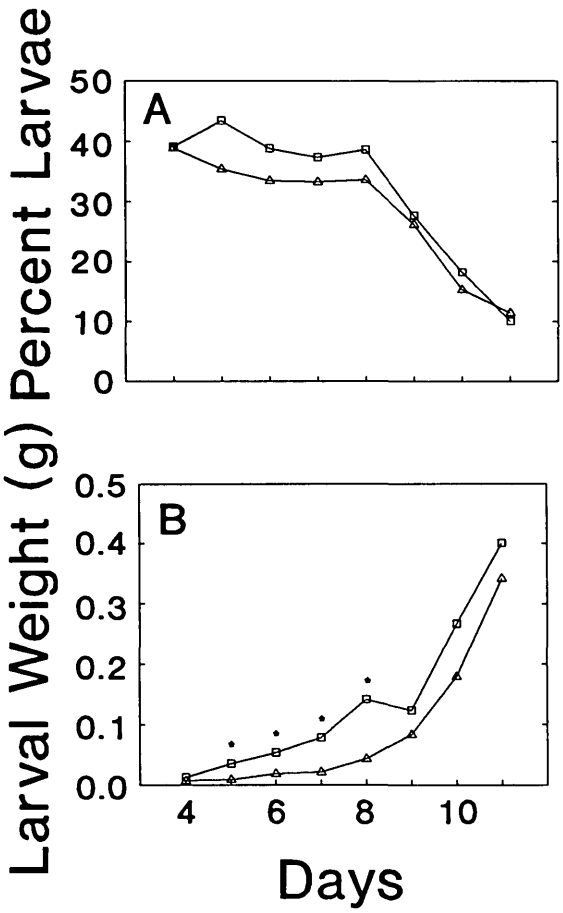

Fig. 5. (A) Percentage of *H. zea* larvae per R2–R3 'Cobb' (□) or 'GatIR81-296' (△) plant. Data were transformed by an arcsine function and analyzed, but the original data points are presented. (B) Larval weights of *H. zea* that fed on reproductive stage (R2–R3) 'Cobb' (□) and 'GatIR81-296' (△) plants, no-choice test 2. Means followed by an asterisk on a certain day are significantly different ($P \leq 0.05$, df = 1) (F test).

days 4–10 on R2–R3 'Cobb' than on 'GatIR81-296' (Fig. 5A). These observations corresponded to results in no-choice test 1, where fewer numbers of *H. zea* were found on 'GatIR81-296' than on 'Cobb' and are similar to findings by Beach et al. (1985), who conducted tests with excised leaves from genotypes of reproductive stage (R1–R5) resistant plants.

Weights of larvae confined on 'Cobb' were significantly higher than those on 'GatIR81-296' from day 5 through day 8 (Fig. 5B). Results were similar to those of no-choice test 1, where resistance was observed in V3–V4 'GatIR81-296'. On day 8, *H. zea* larvae that developed on 'Cobb' were in the third (14%) and fourth instar (86%), whereas those larvae that fed on 'GatIR81-296' were in the second (29%) and third instar (71%) ($\chi^2 = 10.60$, df = 2, $P \leq 0.05$). *H. zea* that fed on 'Cobb' developed more rapidly and more uniformly than those on 'GatIR81-296'. These results were observed in no-choice test 1, where *H. zea* development was reduced (although not significantly) on young (V3–V4) 'GatIR81-296' plants compared with 'Cobb'.

Table 2. Comparison of final percentage larval mortality of *P. includens* and *A. gemmatalis* when reared on either V3–V4 or R2–R3 stage 'Cobb' and 'GatIR81-296' plants, no-choice test III

Species	Genotype	% Final larval mortality			
		Stage			
		V3–V4	R2–R3	df	χ^{2a}
P. includens	Cobb	80	45	1	19.66*
	GatIR81-296	88	70	1	15.52*
A. gemmatalis	Cobb	70	25	1	22.50*
	GatIR81-296	84	40	1	23.92*

[a] *, Significant difference using χ^2 contingency table analysis ($P \leq 0.05$).

No-Choice Test 3. High *H. zea* mortality occurred before pupation, resulting in lack of data for statistical analysis for this insect species. *P. includens* and *A. gemmatalis* final larval mortality was significantly greater when larvae were reared on V3–V4 stage 'Cobb' and 'GatIR81-296' plants compared with R2–R3 stage plants (Table 2). Although larval mortality was high in this study, these results indicate that V3–V4 stage soybean is less acceptable than R2–R3 stage plants. This trend was observed in both susceptible and resistant genotypes.

Pseudoplusia includens pupal weights of larvae that fed on 'Cobb' averaged 14% greater than ones on 'GatIR81-296' (Table 3). *P. includens* pupal lengths averaged 4% greater from larvae that fed on 'Cobb' as compared with 'GatIR81-296', but *P. includens* pupal widths were equal. *A. gemmatalis* pupal weights, lengths, and widths from larvae that fed on 'Cobb' were greater than ones on 'GatIR81-296'. These results indicate that resistance occurs in both vegetative and reproductive developmental stages of intact 'GatIR81-296' plants and corresponds to results of research conducted with excised leaves (Beach & Todd 1988b).

Pseudoplusia includens and *A. gemmatalis* pupae that developed from larvae that fed on reproductive stage plants had greater weights, lengths, and widths than those confined on vegetative stage plants (Table 3). These results indicate that vegetative stage foliage contains a higher level of inhibitory components that adversely affect pupal development in comparison with reproductive stage foliage. Nault et al. (1992) reported that third-instar *H. zea* preferred older reproductive stage soybean foliage rather than younger reproductive stage foliage, collected from the field late in the season, when given a choice in a laboratory feeding bioassay.

Helicoverpa zea, *P. includens*, and *A. gemmatalis* larvae that fed on either genotype and

Table 3. Comparison of *P. includens* and *A. gemmatalis* pupal characteristics on V3–V4 and R2–R3 'Cobb' and 'GatIR81-296' plants, no-choice test III

Species	Genotype or stage	Pupa		
		Wt, g	Length, mm	Width, mm
	Genotype			
P. includens	Cobb	0.1922**	17.3*	4.5
	GatIR81-296	0.1682	16.6	4.5
A. gemmatalis	Cobb	0.2451*	17.7*	5.2*
	GatIR81-296	0.2150	16.9	5.0
	Stage			
P. includens	R2–R3	0.1948**	17.6**	4.7**
	V3–V4	0.1685	16.4	4.3
A. gemmatalis	R2–R3	0.2461**	17.9**	5.3*
	V3–V4	0.2229	17.0	5.0

[a] *, **, Means are significantly different ($P \leq 0.05$ and $P \leq 0.10$, df = 1, respectively; F test).

survived to pupate had a high probability of emergence (90, 95, and 87% on 'Cobb', respectively; all three species at 100% on 'GatIR81-296'). These results are similar to those reported by Hatchett et al. (1976), who found virtually no difference in adult emergence when larvae were fed resistant foliage.

The experiments with plants in V2–V3 and R2–R3 stages of development were designed to compare and contrast the effectiveness of soybean resistance during plant growth. Our secondary objective was to evaluate results of authors who have conducted insect feeding studies using excised leaves (Beach et al. 1985, Beach & Todd 1987). Excising foliage disrupts plant physiology and could influence insect behavior and biology on soybean. Overall, the results of our tests on intact plants were similar to those with excised leaves and together demonstrate that constitutive resistance factors in soybean produce deleterious behavioral and physiological reactions in insects.

Kogan's (1982) definitions of antixenosis and antibiosis describe distinct mechanisms in which plants resist feeding by insects. Applying these strict terms to our study, these mechanisms would be responsible for the resistance of 'GatIR81-296' to *H. zea*, *P. includens*, and *A. gemmatalis*. Increased feeding and higher larval counts on 'Cobb' than on 'GatIR81-296' when given a choice would indicate antixenosis in 'GatIR81-296'. Although these choice tests do not reflect realistic field situations in which only one cultivar is planted, it is important to observe larval behavioral responses on resistant plants. An increase in larval movement may make larvae more vulnerable to predation and parasitism (Feeny 1976). Smaller larvae and pupae, and a slower larval development when larvae were isolated on 'GatIR81-296' compared with 'Cobb' in our study, would indicate antibiosis. Hatchett et al. (1976), Beach et al. (1985), and others reported antibiosis to various insects from experiments using excised foliage of soybean.

Smith (1989) suggested that antibiosis and antixenosis are not mutually exclusive with respect to the underlying mechanisms of resistance. For example, in choice test 1, a greater number of larvae and percentage leaf area removed were observed on 'Cobb', which could have been partially from larvae becoming malnourished by feeding on 'GatIR81-296' and then moving to 'Cobb'. Conversely in no-choice tests (1–3), the lower percentage of larvae surviving and lower larval and pupal weights of individuals reared on 'GatIR81-296' may have been partially attributed to lack of feeding by the insect.

Insect resistance in soybean declined with plant maturity. This was evident when pupal weights, lengths, and widths of larvae that fed on reproductive stage (R2–R3) plants were greater and final larval mortality was lower than those fed vegetative stage (V3–V4) plants. Current procedures that screen many vegetative stage soybean genotypes (V2–V3) for insect resistance in the greenhouse select the most promising 30%, and screen these potentially resistant genotypes in their reproductive stage (R2–R5) in the field (All et al. 1989). Studies of plant resistance and their relationship to plant ontogeny may augment breeding efficiency for insect resistance.

Acknowledgment

We thank D. Wood, G. Rowan, M. Mitchell, and B. Tanner for their technical assistance and T. All for help with graphics. Comments by J. W. Todd, R. B. Hammond, L. R. Nault, C. S. Eckel, G. G. Kennedy, and an anonymous reviewer on earlier versions of this manuscript are appreciated.

References Cited

All, J. N., H. R. Boerma & J. W. Todd. 1989. Screening soybean genotypes in the greenhouse for resistance to insects. Crop Sci. 29: 1156–1159.

Beach, R. M. & J. W. Todd. 1987. Resistance of the soybean breeding line 'GatIR81-296' to foliar feeding by three *Spodoptera* sp. J. Agric. Entomol. 4: 193–199.

1988a. Foliage consumption and developmental parameters of the soybean looper and the velvetbean caterpillar (Lepidoptera: Noctuidae) reared on susceptible and resistant soybean genotypes. J. Econ. Entomol. 81: 310–316.

1988b. Oviposition preference of the soybean looper (Lepidoptera: Noctuidae) among four soybean genotypes differing in larval resistance. J. Econ. Entomol. 81: 344–348.

Beach, R. M., J. W. Todd & S. H. Baker. 1985. Antibiosis of four insect-resistant soybean genotypes to the soybean looper (Lepidoptera: Noctuidae). Environ. Entomol. 14: 531–534.

Carmer, S. G., W. M. Walker & R. D. Seif. 1969. Practical suggestions on pooling variances for F tests of treatment effects. Agron. J. 61: 334–336.

Carroll, C. R. & C. A. Hoffman. 1980. Chemical feeding deterrent mobilized in response to insect herbivory and counteradaptation by *Epilancha tredecimnotata*. Science 209: 414–416.

Chiang, H. S., D. M. Norris, A. Ciepiela, A. Oosterwyk, P. Shapiro & M. Jackson. 1986. Comparative constitutive resistance in soybean lines to Mexican bean beetle. Entomol. Exp. Appl. 42: 19–26.

Chiang, H. S., D. M. Norris, A. Ciepiela, P. Shapiro & A. Oosterwyk. 1987. Inducible versus constitutive PI227687 soybean resistance to Mexican bean beetle, *Epilachna varivestis*. J. Chem. Ecol. 13: 741–749.

Eckel, C. S., L. I. Terry, J. R. Bradley, Jr., & J. W. Van Duyn. 1992. Changes in within-plant distribution of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) on soybean. Environ. Entomol. 21: 287–293.

Elden, T. C., J. A. Schillinger & A. L. Steinhauer. 1974. Field and laboratory selection for resistance in soybeans to the Mexican bean beetle. Environ. Entomol. 3: 785–788.

Feeny, P. O. 1976. Plant apparency and chemical defense. Recent Advances in Phytochemisty 10: 1–40.

Fehr, W. R. & C. E. Caviness. 1977. Stages of soybean development. Iowa State Univ. Spec. Rep. 80.

Greene, T. R. & C. A. Ryan. 1972. Wound-induced proteinase inhibitor in plant leaves: a possible defense mechanism against insects. Science 175: 776–777.

Hargrove, W. H. & D. A. Crossley. 1988. Video digitizer for the rapid measurement of leaf area lost to herbivorous insects. Ann. Entomol. Soc. Am. 81: 593–598.

Hatchett, J. H., G. L. Beland & E. E. Hartwig. 1976. Leaf feeding resistance to bollworm and tobacco budworm in three soybean plant introductions. Crop Sci. 16: 277–280.

Hatchett, J. H., G. L. Beland & T. C. Kilen. 1979. Identification of multiple insect resistant soybean lines. Crop Sci. 19: 557–559.

Kogan, M. 1982. Plant resistance in pest management, pp. 93–134. *In* R. L. Metcalf & W. H. Luckmann [eds.], Introduction to insect pest management, 2nd ed. Wiley, New York.

Kogan, M. & J. Paxton. 1983. Natural inducers of plant resistance to insects, pp. 153–171. *In* P. A. Hedin [ed.], Plant resistance to insects. American Chemical Society, Washington, D.C.

McWilliams, J. M. & G. L. Beland. 1977. Bollworm: effect of soybean leaf age and pod maturity on development in the laboratory. Ann. Entomol. Soc. Am. 70: 214–216.

Nault, B. A., J. N. All & H. R. Boerma. 1992. Influence of soybean planting date and leaf age on resistance to corn earworm (Lepidoptera: Noctuidae). Environ. Entomol. 21: 264–268.

Reynolds, G. W. & C. M. Smith. 1985. Effects of leaf position, leaf wounding, and plant age of two soybean genotypes on soybean looper (Lepidoptera: Noctuidae) growth. Environ. Entomol. 14: 475–478.

SAS Institute. 1985. SAS user's guide: statistics, version 6 ed. SAS Institute, Cary, N.C.

Smith, C. M. 1985. Expression, mechanisms and chemistry of resistance in soybean, *Glycine max* (L.)(Merr.) to the soybean looper, *Pseudoplusia includens* (Walker). Insect Sci. Appl. 6: 243–248.

1989. Plant resistance to insects: a fundamental approach. Wiley, New York.

Steel, R. H. & C. M. Torrie. 1980. Principles and procedures of statistics, 2nd ed. McGraw-Hill, New York.

Terry, I., J. R. Bradley, Jr., & J. W. Van Duyn. 1989. Establishment of early instar *Heliothis zea* on soybeans. Entomol. Exp. Appl. 51: 233–240.

Van Duyn, J. W., S. G. Turnipseed & J. D. Maxwell. 1971. Resistance in soybeans to the Mexican bean beetle. I. Sources of resistance. Crop Sci. 11: 572–573.

Received for publication 26 June 1991; accepted 11 March 1992.