Influence of Soybean Planting Date and Leaf Age on Resistance to Corn Earworm (Lepidoptera: Noctuidae)

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ABSTRACT Soybean, *Glycine max* (L.) Merr., resistance to insects has been shown to vary with planting date and leaf age. This study determined effects soybean genotype, planting date, and leaf age had on feeding behavior of corn earworm, *Helicoverpa zea* (Boddie). Soybean genotypes 'Cobb' (susceptible) and 'GatIR81-296' (resistant) were planted early (29 May) and late (28 June) in 1989 in the field. Trifoliolate leaf samples from the top, middle, and bottom one-third of the plant were sampled four times throughout the season. Leaves were arranged in a free-choice fashion and presented to third-instar *H. zea* in the laboratory. Leaf area removed was calculated with a computer-based leaf area analysis system. Larvae preferred 'Cobb' over 'GatIR81-296' in each sampling date. Leaves from the late planting had greater leaf area removed on the first two sampling dates compared with leaves from the early planting (15.8 versus 11.7 and 34.5 versus 22.9 cm^2), but leaves from the early planting had more leaf area removed compared with leaves from the late planting on the last two sampling dates (30.9 versus 22.7 and 21.3 versus 16.2 cm^2). Thus, *H. zea* exhibited a bimodal feeding preference for early- and late-planted soybean. Older leaves (middle or bottom one-third) of all plants were typically preferred over younger ones (top one-third) throughout the season. Because resistance may vary during plant development, an accurate identification of soybean resistance should be done only with genotypes at a similar stage of development.

KEY WORDS Insecta, *Helicoverpa zea*, planting date, leaf age

HOST PLANT RESISTANCE in certain soybean genotypes to defoliating insects has been identified (Van Duyn et al. 1971, Hatchett et al. 1979, Beach & Todd 1987). These studies have linked resistance in these genotypes to a reduction in defoliation and to adverse effects on insect behavior and physiology. Other studies have dealt with resistance in soybean that varies with plant developmental stage and leaf age (McWilliams & Beland 1977, Reynolds & Smith 1985), but a direct comparison of ontogenic differences has not been reported.

Soybean genotypes that differ in maturity often are evaluated in the same experiment for insect resistance by subjecting field-grown plants to natural insect infestations (Hatchett et al. 1976, Jones & Sullivan 1978, Joshi 1981). Common difficulties in these experiments include low natural infestations, nonuniform infestations, and insect preference for a specific phenological age (Smith 1989). Plant ontogeny affects the level of resistance expressed late in the season to Mexican bean beetle, *Epilachna varivestis* Mulsant (Elden et al. 1974), and may present problems in proper characterization of levels of resistance among genotypes to other defoliating insects.

Soybean resistance can also be evaluated by allowing a controlled number of insects to discriminately feed on plant material in the laboratory or greenhouse (Rufener et al. 1987, All et al. 1989). These artificial techniques eliminate commonly encountered problems in the field and correlate well with field screening (All et al. 1989). Objectives for this study were to determine effects that soybean genotype, planting date, and leaf age had on feeding of *Helicoverpa zea* (Boddie) in the laboratory.

Materials and Methods

Experiments were planted at the University of Georgia Plant Sciences Farm in Oconee County, Georgia, on 29 May and 28 June 1989. Each experiment consisted of two soybean genotypes ('Cobb' and 'GatIR81-296') arranged in a randomized complete block design with four replications. A plot consisted of two rows of a genotype that were 9.1 m long with 0.8 m spacing between rows.

'Cobb' is a susceptible cultivar whereas 'GatIR81-296' is an insect-resistant breeding line, derived from 'GaSoy 17' × 'PI 229358', with multiple insect resistance (Beach & Todd 1987). Samples of trifoliolate leaves were taken from

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early planted (29 May) and late planted (28 June) soybean plants on 28 July, 11 August, 13 September, and 12 October 1989. One plant was randomly chosen from three of the four plots, whereas one plant was randomly chosen from each row of the fourth plot, for a total of five samples per sampling date per soybean genotype. One trifoliolate leaf was excised from the top one-third (excluding the uppermost fully expanded trifoliolate leaf), middle one-third, and bottom one-third (excluding chlorotic leaves) of each plant. Stage of soybean development was recorded during each sampling date according to methods of Fehr & Caviness (1977).

Leaves were taken to the laboratory and placed in a free-choice feeding arrangement in round plastic containers (26.7 cm diameter, 10.1 cm high, with a 7.6 by 7.6 cm hole in the lid covered with cheese cloth). Leaves were laid on metal screening in the container with their petioles pointing downward. Petioles were submerged in 5-ml glass vials containing water capped with cotton. One early- and one late-planted 'Cobb' and 'GatIR81-296' trifoliolate leaf (total of four leaves) were randomly placed in each container. Leaves collected from the top, middle, and bottom one-third of the plant were kept in separate containers, which were arranged in a randomized complete block design each with five replications. This test was conducted under fluorescent lights on a 14:10 (L:D) photoperiod.

Bioassays were performed immediately after each leaf sample was taken during the season. This method prevented having to preserve leaves for long periods of time; however, this required testing of *H. zea* larvae at various times during the season. To ensure *H. zea* genetic uniformity, the insects were obtained from a large culture that has been maintained for 22 yr at the Insect Biology and Population Management Research Laboratory, USDA–ARS, Tifton, Ga. *H. zea* neonates were placed on pinto bean-based diet and permitted to feed for 5 d before they were placed on soybean leaves. Two third instars were placed on each of the four trifoliolate leaves in a container. These larvae were allowed to feed for 48 h before they were removed.

Data Analysis. Insect feeding damage on leaves was calculated as leaf area removed using a computerized leaf-area analysis system developed by Hargrove & Crossley (1988). Data were combined across the early- and late-planted experiments, assuming replication and planting date as random effects and genotype and leaf age as fixed effects in the statistical model. Data were analyzed by analysis of variance (ANOVA) (SAS Institute 1985). Differences between genotypes and planting dates were compared using an F test (df = 1, $P \leq 0.05$) whereas differences among leaf ages were compared using a protected LSD ($P \leq 0.05$) (Carmer et al. 1969).

Table 1. Developmental stages of early and late-planted soybean from which foliage was used in feeding preference assays for *H. zea*

Sampling date	Early planted	Late planted
28 July	V10[a]	V5
11 Aug.	R2–R3	R1
13 Sept.	R6	R5
12 Oct.	R6	R6

[a] Developmental stage corresponded to soybean growth designations established by Fehr & Caviness (1977). Planting dates were 29 May (early planting) and 28 June (late planting) of 1989. Average date of stage R8 was 28 October for the early planting and 31 October for the late planting.

Results and Discussion

Variable effects that natural pest populations often cause during resistance screening were eliminated by presenting excised field-grown soybean foliage to a constant number of *H. zea* in the laboratory. The methodology used to assay *H. zea* feeding behavior appeared effective for determining larval preference for different age foliage. Placing leaf petioles in water enabled foliage to remain turgid throughout the bioassay. Third instars moved freely throughout the container to select the most palatable foliage. *H. zea* feeding never exceeded 74% leaf area removed from a single trifoliolate leaf within the 48-h bioassay and larvae were not forced to feed on less desirable foliage.

Late-planted soybean developed at a faster rate than early-planted ones (Table 1). Photoperiod is the major controlling factor in soybean plant development and caused a shorter developmental time from planting to harvest maturity (R8) for the late-planted soybean compared with early-planted ones (124 versus 152 d) (Parker & Borthwick 1950). Although 'Cobb' is classed as a maturity group VIII and 'GatIR81-296' as a maturity group VII, 'Cobb' averaged only 3 and 0 d later in maturity than 'GatIR81-296' in the early and late planting, respectively.

Soybean genotype, planting date, and leaf age significantly affected *H. zea* feeding preference (Table 2). Even though significant interactions between one or more of these variables were discovered for a given sampling date, they did not occur consistently throughout the season.

There was greater feeding (leaf area removed) on 'Cobb' leaves when averaged across planting dates and leaf ages compared with 'GatIR81-296' in each sampling date (Fig. 1). Differences in magnitude between leaf area removed from 'Cobb' and 'GatIR81-296' on each sampling date declined after 13 September. Because sampling dates cannot be directly compared, these results may indicate that resistance declines in 'GatIR81-296' with maturity, that acceptability of 'Cobb' declines with maturity, or both.

Mean leaf area removed from the early planting was compared with the late planting (Fig. 2).

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Table 2. Significance of main effects and interactions related to *H. zea* feeding on soybean on four sampling dates

Sources of variation	df	Sampling date							
		28 July		11 August		13 September		12 October	
		MS	*F*	MS	*F*	MS	*F*	MS	*F*
Genotype	1	2,888.71	47.33**	25,066.39	151.82**	20,800.77	49.50**	4,913.43	51.84**
Planting date[a]	1	249.13	4.08**	2,011.10	12.18**	1,008.93	2.40 NS	393.83	4.16**
Genotype × Planting date	1	30.76	0.50 NS	1,316.67	7.97**	498.82	1.19 NS	8.51	0.09 NS
Leaf age[b]	2	502.45	8.23**	478.09	2.90 NS	1,833.46	4.36**	373.67	3.94**
Leaf age × Planting date	2	46.86	0.77 NS	201.04	1.22 NS	766.56	1.82 NS	800.24	8.44**
Leaf age × Genotype	2	191.02	3.13 NS	418.91	2.54 NS	927.06	2.21	270.10	2.85 NS
Leaf age × Planting date × Genotype	2	21.43	0.35 NS	521.56	3.16 NS	234.46	0.56 NS	438.60	4.63**

NS, no significant difference ($P \geq 0.05$); **, significant difference ($P \leq 0.05$) based on an *F* test.
[a] 29 May versus 28 June planting dates.
[b] Trifoliolate leaf located on top, middle, and bottom one-third of the plant.

Leaves from younger (late-planted) plants were preferred more than older (early-planted) plants on the first sampling date and the second sampling date. Larvae preferred older foliage (early-planted) later in the season. Leaves from the early-planted experiment had greater leaf area removed on the third sampling date and fourth sampling date compared with late-planted ones. These results indicate that deterrents or nutrients or both decline in soybean foliage during its later stages of development, affecting feeding behavior. Leaf area removed from foliage collected from early- and late-planted 'Cobb' and 'GatIR81-296' was separated into the top, middle, and bottom one-third of the plant. On the first sampling date, the youngest leaves (top third) had the least leaf area removed (Fig. 3). On the remaining sampling dates, the youngest leaves had lower leaf area removed than leaves

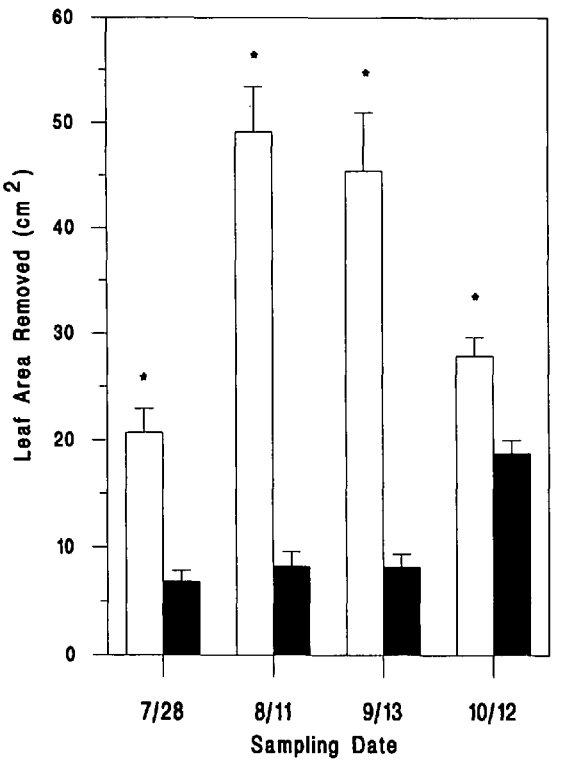

Fig. 1. Feeding (leaf area removed) on soybean genotypes 'Cobb' (□), and 'GatIR81-296' (■), by *H. zea* on all trifoliolate leaves in early- and late-planted experiments in a free-choice bioassay in the laboratory. SEM is represented above each bar. Asterisks signify a significant difference ($P \leq 0.05$; df = 1) (*F* test).

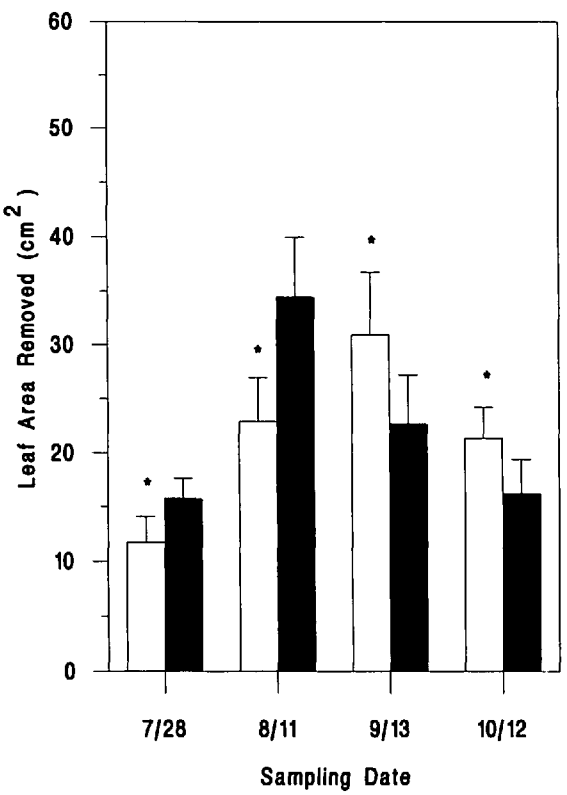

Fig. 2. Feeding (leaf area removed) on all trifoliolate leaves from early- (□), and late-planted (■), experiments selected from the top, middle, and bottom third of the plant by *H. zea* in a free-choice bioassay in the laboratory. SEM is represented above each bar. Asterisks signify a significant difference ($P \leq 0.05$; df = 1) (*F* test).

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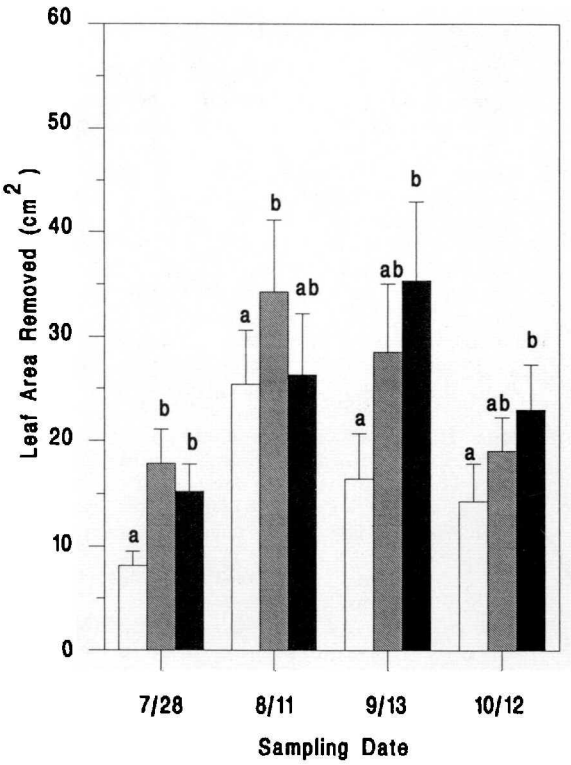

Fig. 3. Feeding (leaf area removed) on the top (▭), middle (▨), and bottom third (■) of all trifoliolate leaves from early- and late-planted experiments by *H. zea* in a free-choice bioassay in the laboratory. SEM is represented above each bar. Means followed by the same letter at one sampling date are not significantly different ($P \geq 0.05$; protected LSD).

from either the middle or bottom one-third of the plant. These results indicate that deterrents or nutrients or both decline in soybean leaves during its development. This is in contrast to results of Moscardi et al. (1981), who reported that the velvetbean caterpillar, *Anticarsia gemmatalis* Hübner, consumed more leaf material (fresh leaf weight) in early stages of plant development than in later ones.

These results demonstrate that feeding preference by *H. zea* on a resistant and a susceptible soybean genotype is affected by planting date and leaf age, which result in differences in plant ontogeny. The preference factor(s) could involve feeding deterrents (Kogan 1972, Norris & Kogan 1980) that are produced at different levels in the genotypes during the season. The experiment results do not preclude involvement of fluctuating quantities of a feeding stimulant(s) in the plants that are mediated by the environment. Beland et al. (1970) reported that resistance of *Melilotus infesta* Guss. plants to the sweetclover weevil, *Sitona cylindricollis* Fahraeus, is influenced by the ratio of a deterrent and a stimulant in the leaves, which is mediated by plant phenology. They related nitrate content (higher in young than mature leaves) with weevil preference for mature leaves. Hanaway & Weber (1971) determined that nitrogen, potassium, and phosphorous decreased in soybean leaves with increasing plant age. Thus, insects must feed more to ingest acceptable amounts of nutrients, which may partially explain the greater amounts of leaf area removed from the early-planted soybean late in the season and from trifoliolate leaves from the bottom third of the plants.

A general concern about larval choice tests conducted in the laboratory is that larvae will not make host choices to an appreciable degree compared with the ovipositing female. Therefore, laboratory preference tests can only offer insight into plant resistance to insects. In this study, larval feeding preferences are simply being used as a tool to answer basic questions that involve developmental changes in soybean resistance, which may be explored later in the field. Results of these investigations have implications for design of field experiments to evaluate genotypic resistance to defoliating insects. Our results indicate that resistance will vary during plant development. Thus, an accurate assessment and ranking of soybean genotypes for resistance can be achieved by comparing genotypes of similar maturity. This can be accomplished by including genotypes of the same maturity in the field experiment or including resistant and susceptible standards of various maturities represented by the test genotypes.

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