Yield Response of Soybeans to Defoliation by the Mexican Bean Beetle (Coleoptera: Coccinellidae)

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ABSTRACT This study measured the yield response of soybeans, *Glycine max* (L.) Merrill, to different levels of defoliation produced by Mexican bean beetle, *Epilachna varivestis* Mulsant. *E. varivestis* were allowed to feed on caged soybeans to produce continuous progressive defoliation over different consecutive growth stages. At appropriate growth stages, cages were removed, plant samples for defoliation measurements obtained, and the *E. varivestis* destroyed. At soybean maturity, counts of plants per plot, pods per plant, weight per seed, and plot yields were obtained. Yields were converted to a percentage of yield loss and regressions fitted to the relationship between percentage yield loss and defoliation. The percentage of yield loss was influenced by the level of defoliation, the growth stages over which defoliation occurred, and an undefined factor associated with different years, possibly environmental factors or planting date. Generally, defoliations which began at growth stages before the full seed stage had a significant effect on yield. The continuous progressive *E. varivestis* defoliations produced linear relationships between defoliation and percentage yield loss. The duration and level of accumulated defoliation by the beginning or full seed growth stage determined the slope of the regression. A decrease in either of the two factors reduced the slope. The yield component affected by *E. varivestis* defoliation in 1981–1983 was weight per seed, which decreased as defoliation increased.

KEY WORDS Insecta, defoliation, yield, Mexican bean beetles

MEXICAN BEAN BEETLE, *Epilachna varivestis* Mulsant, is a sporadic pest of soybeans, *Glycine max* (L.) Merrill, in Indiana. Indiana's economic injury levels for *E. varivestis* are derived from the levels for the green cloverworm, *Plathypena scabra* (F.), on soybeans in Iowa (Stone & Pedigo 1972). Stone and Pedigo's economic injury levels are in turn based on yield and defoliation information from a study by Kalton et al. (1949), who used artificial defoliation (picked leaves) at single growth stages of soybeans to simulate insect damage. The use of data from Kalton et al. (1949) to make management decisions for the *E. varivestis* may lead to incorrect decisions. One source of error may be the difference between damage caused by *E. varivestis* feeding behavior and damage caused by artificial defoliation. Studies by Poston et al. (1976) found that some artificially produced damage does not affect net photosynthesis to the same degree as equal amounts of damage produced by an insect or other artificial means. The effects on plant yield of *E. varivestis* damage and how this damage compares with artificially produced damage is unknown.

Another source of error may be the duration of the damage. In Indiana, the major damage from *E. varivestis* is produced by the second generation that begins in late July or early August. Soybeans at this time of the year are usually in growth stages R1 (beginning pod) to R5 (beginning seed), depending on planting dates and the variety planted (growth stages according to Fehr and Caviness [1977]). The defoliation that occurs as a result of an *E. varivestis* infestation is spread over several consecutive growth stages. Todd & Morgan (1972) and Thomas et al. (1978) used artificial defoliation applied to multiple consecutive growth stages of soybeans and found that such defoliation has a greater effect on yield than similar levels of defoliation applied to a single growth stage.

To develop more current yield information relative to *E. varivestis* damage, yield response to different levels of defoliation produced by *E. varivestis* caged on small plots was measured. The time and duration of the defoliations were adjusted to measure yield response to early and late infestations of *E. varivestis*. Because research by Fehr et al. (1981) showed that the critical stage for soybean defoliation occurs during the R5 or R5.5 growth stage, our experiments were designed to study the effects of defoliations started before R5 and ended at R6 (full seed) growth stage, and to study later defoliations covering R5–R6, R6–R7 (beginning maturity), and R7 growth stages.

Materials and Methods

'Williams 82' soybeans were planted near Bedford, Ind., at the Feldun Purdue Agricultural Center from 1981 to 1983. Plots were planted in 76.2 cm rows at approximately 9 seeds per 30.5 cm according to normal agronomic practices of the region. The experimental design was a split-split plot. Six replications served as the whole plot treatments, and split plot treatments were the plant growth stages infested. Six treatments were arranged as a randomized complete block within each growth stage split. In 1981, to achieve a range of defoliations, six treatments were applied within a growth-stage split consisting of five cages infested with different levels of field-collected *E. varivestis* adults and larvae and one cage with no *E. varivestis*. In 1982 and 1983, five cages were used per growth-stage split; one of the five did not contain any *E. varivestis*. The sixth plot was left uncaged and kept free of insect defoliation by using insecticides. The yields from the plot with no cage and the cage without *E. varivestis* were compared to test cage effects on yield. At appropriate soybean growth stages, cages constructed of 2.5- by 7.6-cm pine frames (2.1 × 1.5 × 1.2 m) and covered on the sides and top with nylon mesh (tulle), were set over 2.1 m of two adjacent soybean rows. The bottom perimeters of all cages were sealed with loose soil to contain the beetles. Different levels of defoliation by field-collected *E. varivestis* adults and larvae were allowed to occur over a period of time (Table 1) after which the cages were removed, plant samples for defoliation measurements were taken, and the beetles were removed by spraying with an insecticide. Plots to be infested later in the season, plots receiving zero levels of infestation, and plots infested after cage removal were sprayed with Sevin 80S (Rhone-Poulenc, Monmouth, N.J.) at 0.56 kg (AI)/ha as needed to keep defoliation to a minimum until infestation or harvest. Defoliators other than *E. varivestis*, if present, were removed by hand from the plot before the cages were infested.

Defoliation measurements were made on three plants selected from the northern row of each cage at the time cages were removed. Plants were consistently removed from the same row to reduce the possibility of sampling from the row used to determine yield. The plants nearest 0.6, 1.2, and 1.8 m from the west end of a cage were cut below the cotyledon node, placed in a plastic bag, moved to a laboratory where leaflets were removed, and placed in plant presses along with plant information and identification. Area and percentage of defoliation of the dried leaves were measured using a technique developed by Nolting & Edwards (1985).

Counts of pods per plant were taken at soybean maturity on two plants per plot in 1981 and three plants per plot in 1982 and 1983. These samples were obtained from the northern row at approximately 0.6 and 1.2 m from the east cage end in 1981, and 0.6, 1.2, and 1.8 m in 1982 and 1983. The 2.1 m of the southern row were harvested by hand, the numbers of plants were counted, and the seeds were removed by a stationary mechanical harvester. Seeds were weighed, measured for percentage of moisture, and plot yields were converted

Table 1. Date and growth stage of soybeans when cages were infested and removed, and range of defoliations achieved

Infested		Removed		Range of defoliation	
Date	Growth stage[a]	Date	Growth stage[a]	Min %	Max %
		1981			
28 July	R2	5 Sept.	R5	4	62
6 Aug.	R3	20 Sept.	R5	3	97
29 Aug.	R5	20 Sept.	R5	5	38
		1982			
23 June	R1	29 July	R5	1	52
1 July	R2	9 Aug.	R5	2	47
29 July	R5	28 Aug.	R6	5	95
16 Aug.	R6	6 Sept.	R7	7	72
31 Aug.	R7	6 Sept.	R7	12	88
		1983			
20 July	V9	10 Aug.	R5	1	14
28 July	R3	16 Aug.	R5	1	13
6 Aug.	R4	30 Aug.	R5	1	21
12 Aug.	R5	9 Sept.	R5	1	39

[a] Growth stages according to Fehr & Caviness (1977).

to kg/ha at 14% moisture. In 1981 and 1982, a random sample of 100 seeds selected from the harvested seeds was dried to 0% moisture, weighed, and yields were converted to kg/ha at 14% moisture. In 1983, all of the seeds from the plants selected for pod counts were saved for weight per seed analysis.

Plot yield data (kg/ha) were converted to percentage yield loss (PYL) with the following formula:

$$PYL = (Y - Y_0)/Y_0 \times 100$$

where PYL = percentage yield loss, Y = yield value being converted to PYL, and Y_0 = average yield observed at 0% defoliation. The presence of some defoliation in all plots caused by combinations of wind, rain, or insects made it necessary to estimate Y_0. The Y_0 value for each growth stage tested in a year was obtained from the intercept estimate of a regression model fit to the 36 data points from each growth stage. The data were fit with a linear model:

$$Y = B_0 + B_1X_1$$

and a polynomial model:

$$Y = B_0 + B_1X_1 + B_{11}X_1^2$$

where Y = yield (kg/ha) and X_1 = percentage of defoliation.

Analyses of these two models show that the intercept value from the linear model is the best substitute for Y_0 in the transformation of yield (kg/ha) to PYL. The polynomial models did not provide a significantly better fit of the data, and the polynomial intercepts were more variable than those from the linear model (Table 2). In addition, estimated parameters from four of the polynomials were intuitively incorrect, indicating yield increases at higher levels of defoliation.

Table 2. Parameter estimates for linear and polynomial models fit to defoliation-yield data[a]

Growth stage[b]	$Y = B_0 + B_1X_1$				$Y = B_0 + B_1X_1 + B_{11}X_1^2$				
	b_0	b_1	r^2	CV[c]	b_0	b_1	b_{11}	r^2	CV
					1981				
R2–R5	3,235.0	−18.30	0.49	12.0	2,975.7	3.88	−0.34	0.54	11.5
R3–R5	3,376.7	−16.84	0.73	13.7	3,369.2	−16.18	−0.006	0.73	13.9
R5–R5	2,996.3	−7.45	0.01	14.0	3,728.5	−106.67	2.66	0.28	12.1
					1982				
R1–R5	3,468.5	−28.39	0.31	24.2	3,252.9	−5.89	−0.38	0.35	24.0
R2–R5	3,375.7	−26.07	0.32	19.0	3,706.9	−67.55	0.87	0.37	18.6
R5–R6	2,747.2	−11.38	0.15	31.7	2,682.9	−6.99	−0.04	0.15	32.4
R6–R7	2,720.8	3.23	0.02	14.3	2,605.8	11.74	−0.11	0.03	14.6
R7–R7	2,992.9	−2.02	0.00	18.8	3,136.7	−9.42	0.07	0.01	19.1
					1983				
V9–R5	2,638.1	−3.86	0.00	13.0	2,603.8	12.24	−1.25	0.00	13.2
R3–R5	2,484.7	−8.23	0.01	12.4	2,379.6	36.6	−3.28	0.03	12.5
R4–R5	2,341.9	−25.6	0.31	12.0	2,365.6	−33.12	0.36	0.31	12.2
R5–R5	2,431.6	−17.6	0.31	10.8	2,388.0	−10.1	−0.22	0.31	10.9

[a] Parameter estimate for 1982 growth stages R5–R6, R6–R7, and R7–R7 are based on 24, 19, and 28 data points, respectively; all others based on 36 observations.
[b] Growth stages according to Fehr & Caviness (1977).
[c] CV, coefficient of variation.

The transformations used to convert yields from weight per unit area to percentage of yield loss did not change the position of the data points relative to each other; therefore, the linear model was still the appropriate model for describing the relationship between percentage of yield loss and defoliation. In addition, because Y_0 represents 0% yield loss, and 0% yield loss is located at the origin of the PYL-defoliation plot, no intercept is required to describe the PYL model. Since the transformation to PYL did not change the position of the points relative to each other, the slopes for the PYL model could have been derived from the linear models of Table 2 by solving for the x intercept and then dividing 100 (yield loss at 0% defoliation) by the x intercept. However, it was necessary to fit the no-intercept model to obtain new values for some of the descriptive statistics (r^2, coefficient of variation).

Regression lines for those defoliations that significantly affected the percentage of yield loss were compared with other significant regression lines within the same year and with any significant regression lines of similar defoliations from other years.

Yields (kg/ha) were regressed on the yield components, plants per plot, pods per plant, weight per seed, and in 1983 only, seeds per plant. The yield components, plants per plot, pods per plant, weight per seed, and in 1983 only, seeds per plant were regressed on the percentage of defoliation of the plot.

General linear tests were used for all model comparisons (Neter & Wasserman 1974). Regressions or differences between regressions reported as significant have a probability of a greater $F < 0.05$.

Results and Discussion

The 1981 planting was delayed until 21 June by an extended period of wet weather. The delay limited the number of periods of defoliation to three. Planting in 1982 took place on 10 May, and we had five periods of defoliation under conditions favorable for soybean and *E. varivestis* growth and development. Plots were planted on 9 June in 1983. Above-normal temperatures from June through September resulted in heavy *E. varivestis* mortality and low population levels by mid-July. Heavy *E. varivestis* mortality made it difficult to obtain desired levels of defoliation; four periods of defoliation were completed.

The percentage of yield loss responded linearly to the continuous progressive insect defoliation used in this study (Fig. 1–3; Table 3). The effects of defoliation on yield have been shown to vary over time. Stone & Pedigo (1972) and Thomas et al. (1978) used second-degree polynomials to describe defoliation-yield relationships. Data from Begum & Eden (1965) and Todd & Morgan (1972) also appear to be nonlinear over certain ranges of defoliation (Fig. 4). All of the above-cited research used artificial damage (picking and cutting), and except for Todd & Morgan (1972) and Thomas et al. (1978), defoliated only over a single growth stage. The linear relationship between the percentage of yield loss and defoliation observed in this study may be because of the different durations and methods of defoliation we used.

Defoliations started at growth stages before R6, except the R5–R5 defoliation in 1981, had a significant effect on PYL in 1981 and 1982 (Table 3). The early defoliations in 1983 over growth stages,

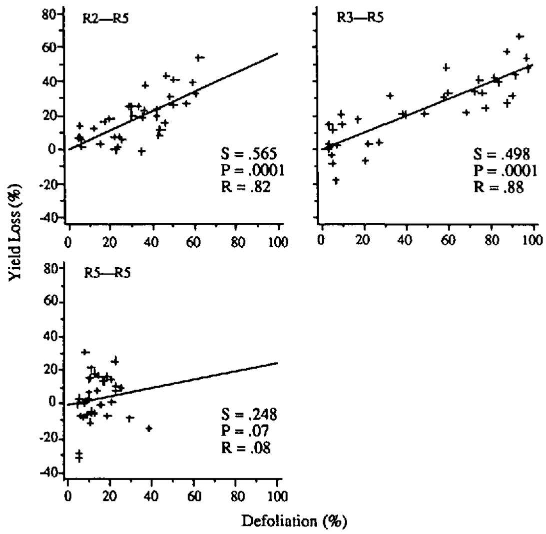

Fig. 1. Plots of percentage yield loss by defoliation for the different soybean growth stages defoliated in 1981. Solid line is derived from equations listed in Table 3.

V9–R5 and R3–R5 did not have a significant effect on PYL. The nonsignificance of these two treatments may be due to low defoliation levels because the maximum percentage defoliation achieved in the 72 plots used in the V9 and R3 defoliations was 14%.

The testing of the relationship between percentage of yield loss and defoliation for later growth stages R6 through R7 was possible in 1982 only. No yield losses were observed for the levels of defoliation achieved for R6–R7 defoliations, or for defoliations occurring during growth stage R7.

The slope of the PYL regression line, for those growth stages within a year that had significant regressions, decreased as the growth stage at which defoliation was begun increased. This indicates that the earlier defoliation was started in the soybean's growth stages the more severe the yield loss. Tests comparing regression lines within a year found significant differences between all 1981 and 1983 regression lines representing significant yield responses to defoliation. No significant differences were found among 1982 lines representing significant yield responses to defoliation. The increased variability in the 1982 data contributed to the lack of significant differences in the 1982 data. The source of the additional variation is not known.

Comparisons of significant regression lines from plots defoliated over similar growth stages in different years showed that all regression lines for R5–R5 or R5–R6 defoliations, including a line fit to the nonsignificant regression of R5–R5 in 1981, were significantly different. The regression line for defoliation beginning at stage R2 in 1981 was also

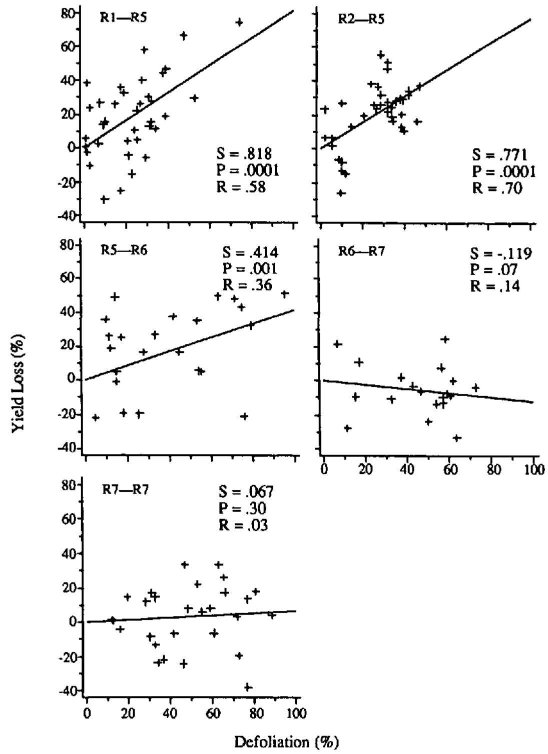

Fig. 2. Plots of percentage yield loss by defoliation for the different soybean growth stages defoliated in 1982. Solid line is derived from equations listed in Table 3.

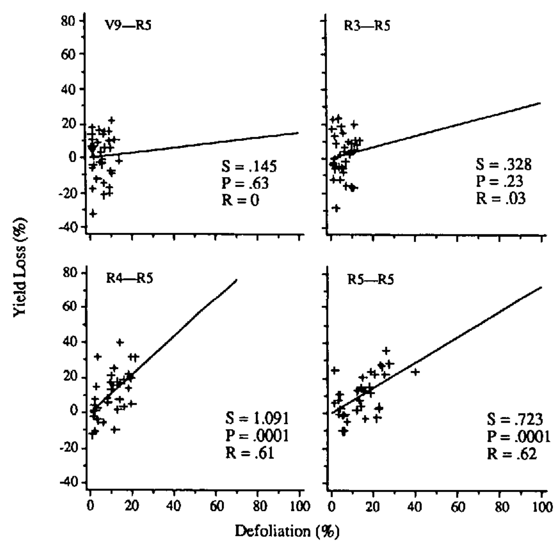

Fig. 3. Plots of percentage yield loss by defoliation for the different soybean growth stages defoliated in 1983. Solid line is derived from equations listed in Table 3.

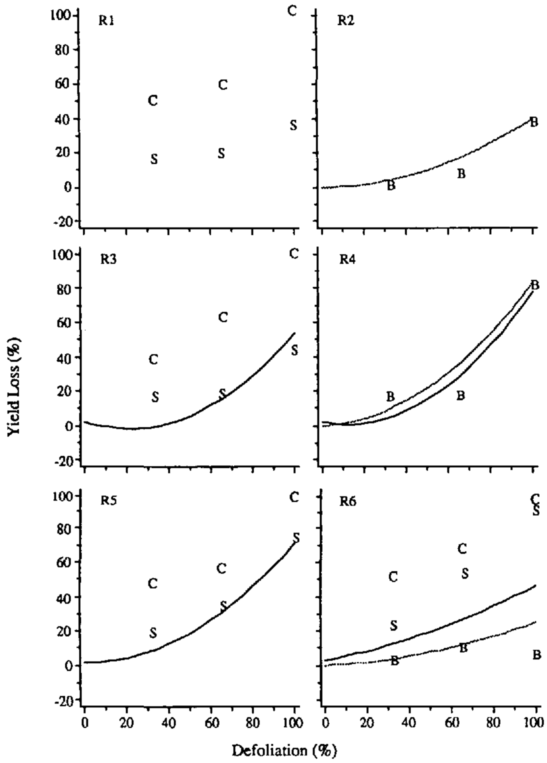

Fig. 4. Plots of data and equations reported in literature for defoliation-yield loss relationships on different growth stages of soybeans. Points plotted as C and S represent yield losses for defoliations occurring over continuous (C) and single (S) growth stages as reported by Todd & Morgan (1972); points plotted as B are single growth stage defoliation studies from Begum & Eden (1965). The solid line is from single growth stage defoliation studies by Thomas et al. (1978). The broken line is from equations reported by Stone & Pedigo (1972) for single growth stage defoliations.

significantly different from regression lines for defoliations beginning at R1 or R2 in 1982. The need for different regression lines to describe defoliation occurring over the same growth stages in different years indicates an interaction of the relationship between defoliation and percentage of yield loss with other variables associated with years; possibly environmental factors or different planting dates. The observed variations among years in yield response to defoliation accumulated over similar growth stages is similar to observations made by Fehr et al. (1981). They found that the critical stage of defoliation shifts from R5 to R5.5 depending on the year.

The results of the regression of yield on the yield components, plants per plot, pods per plant, and weight per seed are presented in Table 3. The slopes for the regression of yield on plants per plot were significant for defoliations of R5–R5 in 1981, R5–R6 in 1981 and 1982, and R4–R5 in 1983. The presence of these significant regressions was fortuitous, because the estimated slopes were negative for the 1981 and 1982 significant regressions, and plots of the data showed that no linear or nonlinear relationship exists between yield and plants per plot. The regression of yield on pods per plant was significant for R3–R5, R4–R5, and R5–R5 defoliations in 1983. Analysis of plots of the data and the r^2 indicated that a weak relationship may exist. The significance of the relationship between yield and pods per plant in 1983 only may be a result of the high temperatures that year. Caviness & Thomas (1980) found that yield losses were a result of reductions in pod numbers for soybeans under water stress. Stronger relationships were found between weight per seed and yield. All defoliations that produced a significant yield loss, except for the R5–R5 defoliation in 1983, had significant regressions of yield on weight per seed. Regression of yield on seeds per plant (seeds per plant taken in 1983 only) for the four growth stages infested in 1983 produced $P > F$ and r^2 as follows: V9–R5 0.63, 0.00; R3–R5 0.007, 0.19; R4–R5 0.009, 0.18; R5–R5 0.004, 0.21. The seeds per plant data reflected what was seen in the pods per plant data.

The regression of the yield components, plants per plot, pods per plant, and weight per seed on percentage defoliation are presented in Table 4. The level of defoliation had no effect on the number of plants per plot. Slopes for the regression of pods per plant on defoliation were significantly different from zero for the R2–R5 defoliation of 1981 and the V9–R5 defoliation of 1983. The slopes for the two regressions were of opposite signs, and analysis of the plotted data and the r^2 for the relationships found no curvilinear or linear relationships for the relationship between defoliation and pods per plant. Based on these observations the significance was again fortuitous. Weight per seed was significantly reduced in all plots which had a significant yield loss from defoliation. Regression of seeds per plant on defoliation for the four growth stages infested in 1983 produced $P > F$ and r^2 as follows: V9–R5 0.017, 0.15; R3–R5 0.51, 0.01; R4–R5 0.52, 0.01; R5–R5 0.24, 0.04. These data indicated that levels of defoliation did not affect seeds per plant.

Based on the regressions of yield on the yield component data and on the regression of yield component data on defoliation, the yield reductions that occurred as a result of defoliation were because of a loss in weight per seed. Reductions in pods per plant were partially responsible for yield losses in 1983; however, the loss from pods per plant was not a function of defoliation levels as were the losses in weight per seed.

t tests between yields of plots receiving a cage and no beetles and yields of uncaged plots kept free of insect defoliation in 1982 and 1983 showed no significant ($P < 0.05$) yield losses between the two types of plots. The total leaf area of plots re-

Table 3. Parameter estimates for PYL–defoliation equations, levels of significance, and r^2 for regressions of yield on yield components

Growth stages defoliated[b]	PYL = defoliation			CV[d]	kg/ha = plants per plot		kg/ha = pods per plant		kg/ha = weight per seed	
	Equation[a,c]	$P > F$	r^2		$P > F$	r^2	$P > F$	r^2	$P > F$	r^2
					1981					
R2–R5	0.565 × X1	0.0001	0.82	52	0.08	0.08	0.30	0.03	0.0001	0.45
R3–R5	0.498 × X1	0.0001	0.88	45	0.36	0.02	0.08	0.08	0.0001	0.80
R5–R5	0.248 × X1	0.07	0.08	373	0.008	0.18	0.08	0.08	0.43	0.01
					1982					
R1–R5	0.818 × X1	0.0001	0.58	104	0.78	0.00	0.88	0.00	0.0001	0.43
R2–R5	0.771 × X1	0.0001	0.70	74	0.32	0.02	0.15	0.05	0.0015	0.25
R5–R6	0.414 × X1	0.001	0.36	162	0.01	0.16	0.84	0.00	0.0001	0.61
R6–R7	−0.119 × X1	0.09	0.14	269	0.60	0.007	0.19	0.04	0.07	0.09
R7–R7	0.067 × X1	0.30	0.03	551	0.36	0.02	0.44	0.01	0.13	0.06
					1983					
V9–R5	0.145 × X1	0.63	0.00	1,472	0.43	0.02	0.59	0.00	0.03	0.12
R3–R5	0.328 × X1	0.23	0.03	583	0.08	0.08	0.01	0.15	0.30	0.03
R4–R5	1.091 × X1	0.0001	0.61	95	0.001	0.26	0.003	0.22	0.005	0.20
R5–R5	0.723 × X1	0.0001	0.62	91	0.80	0.00	0.007	0.19	0.66	0.00

[a] PYL = defoliation equations for 1982 growth stages R5–R6, R6–R7, and R7–R7 are based on 24, 19, and 28 data points, respectively. The kg/ha = weight per seed equation for R6–R7 in 1982 is based on 35 data points. All other regressions for 1981–1983 are based on 36 data points.
[b] Growth stages according to Fehr & Caviness (1977).
[c] X1, percentage defoliation.
[d] CV, coefficient of variation.

ceiving no cage were compared with the total leaf area of plots receiving a cage and no beetles for the R1–R5 treatment of 1982 and the V9–R5 treatment of 1983. These were the treatments which received use of a cage over the longest period of time in each of the years. Analysis of variance indicated no significant ($P < 0.05$) differences in leaf area between plots caged with no beetles and uncaged plots. Thus, the type of cage used in the study did not affect yields or leaf area of the plots.

Table 4. Levels of significance and r^2 for regressions of yield components on defoliation

Growth stages defoliated[b]	Plants per plot = defoliation[a]		Pods per plant = defoliation[a]		Weight per seed = defoliation[a]	
	$P > F$	r^2	$P > F$	r^2	$P > F$	r^2
			1981			
R2–R5	0.95	0.00	0.02	0.14	0.0001	0.57
R3–R5	0.98	0.00	0.24	0.03	0.0001	0.87
R5–R5	0.48	0.01	0.06	0.09	0.27	0.03
			1982			
R1–R5	0.05	0.10	0.08	0.08	0.0001	0.51
R2–R5	0.84	0.00	0.12	0.06	0.0001	0.37
R5–R6	0.51	0.01	0.71	0.00	0.0001	0.66
R6–R7	0.43	0.03	0.57	0.01	0.05	0.21
R7–R7	0.67	0.00	0.12	0.08	0.71	0.005
			1983			
V9–R5	0.49	0.01	0.04	0.10	0.02	0.14
R3–R5	0.76	0.00	0.40	0.02	0.05	0.10
R4–R5	0.25	0.03	0.37	0.02	0.03	0.12
R5–R5	0.70	0.00	0.26	0.03	0.01	0.15

[a] Plants per plot, pods per plant, and weight per seed = defoliation equations for R5–R6, R6–R7, and R7–R7 in 1982 are based on 24, 19, and 28 data points, respectively. The weight per seed = defoliation equation for R6–R7 in 1982 is based on 18 data points. All other regressions for 1981, 1982, and 1983 are based on 36 data points.
[b] Growth stages according to Fehr & Caviness (1977).

Results of this research indicate that continuous progressive *E. varivestis* defoliation started before growth stage R6 has a potential to reduce yields. The yield reductions are a function of the growth stages infested, the level of defoliation accumulated by the R6 growth stage and unidentified factors associated with variation between years, possibly, environmental factors, or differences in planting date. The most obvious difference in environmental factors between the three years of this study was the amount of precipitation. Studies by Todd & Mullinix (1984) found that increases in precipitation at R4 increased soybean yield only at the higher combined populations of two insect pests. When precipitation increased during R5 and R6, yield increased only when the populations of the two insect species were low. Overall, they observed a linear relationship between increased defoliation and declining yield regardless of rainfall levels. Caviness & Thomas (1980) report similar observations from their study. They concluded that percentage of reduction in yield from defoliation is similar for soybeans grown with adequate moisture or under drought stress. Although these studies tend to discount precipitation as a possible cause, other environmental conditions such as temperature and humidity, which are associated with a wet or dry year, may produce different effects in combination with precipitation. The variation between years in yield response to defoliation, which occurs over similar stages of defoliation, indicates a need to investigate the causal mechanisms involved and incorporate these into future yield-defoliation studies.

The defoliation-yield responses for infestations beginning in the reproductive stages of plant growth and continuing until R6 are linear for the levels of defoliation achieved in this study, and the slopes of the relationships decrease as the number of reproductive growth stages infested before R6 decrease. Yield reductions from defoliations beginning at R6 or later growth stages are unlikely. Fehr et al. (1977) found that yield reductions occur with 100% defoliation at R6 and R7; however, field populations of *E. varivestis* generally do not reach levels high enough to cause such levels of defoliation. The yield component affected by *E. varivestis* defoliation in 1983 was weight per seed, which decreased as defoliation increased. Not all yield components were measured in 1981 and 1982; however, weight per seed decreased in these years as defoliation increased.

Acknowledgment

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