Simulated Corn Earworm, *Helicoverpa zea,* Injury in an Indeterminate Soybean Cultivar at Various Growth Stages under Non-Irrigated Conditions in the Southern United States †

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Abstract: Soybean is considered one of the most valuable crops in the United States of America. *Helicoverpa zea* (Boddie) is among several insect pests which are associated with soybean, damaging leaves when infestations occur during the vegetative stages, and flowers and pods during the reproductive stages, which can directly impact yield. Artificial fruit removal is a method used to understand insect damage and to adjust action levels for control. The objective of this work was to evaluate the impact of five levels of fruit removal (0%, 25%, 50%, 75% and 100%) at four stages (R2, R3, R4 and R5) on maturity and yield of soybean. These methods were used to simulate *H. zea* damage under controlled conditions in non-irrigated environments, during 2016 and 2017. There was a significant interaction between fruit removal timing and fruit removal level for the percentage of non-senesced main stems and abscised leaves. For soybean yield, there was no significant interaction between fruit removal timing and fruit removal level. Plots that received fruit removal treatments at R5 had significantly lower soybean yields compared to plots that received damage at other growth stages and the nontreated control. Plots with 100% fruit removal had significantly lower yields compared to plots that received any of the other fruit removal treatments. These data demonstrate that indeterminate midmaturity group IV soybeans that are commonly grown in the midsouthern region of the United States may be able to compensate for even severe levels of fruit loss early during the reproductive portion of the growing season if favorable growing conditions occur.

Keywords: *Helicoverpa zea*; *Glycine max*; artificial removal

1. Introduction

Soybean (*Glycine max* (L.) Merrill) represents one of the world's main crops when considering the oil and protein content [1]. In terms of planted area and commodity value, soybean is considered one of the most valuable commodities in the southeastern region of the United States of America [2]. In 2017, the United States sowing area was more than 36 million hectares, production of about 120 million tons,

and productivity of 3299 kg/ha, making it the largest soybean producer in the world. Soybean is an important component in animal feed in the USA, due to the high protein content present in the seed [3]. Most farmers in the state of Mississippi have adopted early soybean production, which includes early planting (beginning of April to early May) and earlier maturing (maturity group IV and V) varieties with indeterminate growth habits [4].

Several insect pests, which feed at different stages of development and cause damage to leaves, stem, roots, and pods, are associated with soybean [5–7]. *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), also known as "New World bollworm" and corn earworm, is an important insect pest, being able to damage several crops of economic interest [8–11]. Although soybean is not the main host, the damage caused by *H. zea* to soybean has increased over time, especially in the southeastern region of the United States [2,12–16]. In relation to losses in yield and cost of control, *H. zea* has been considered one of the most economically important pests for soybean producing regions of the southeastern region of the United States [2,14]. In 2016, damage caused by *H. zea* resulted in a loss of approximately $108 million (US), in contrast during 2017, losses increased to more than $160 million (US) (economic losses plus cost of control) [14]. One of the causes for the increased cost of control of *H. zea* may be due to the increase in area planted to corn over the years, which resulted in an increase in the number of moths migrating to soybean and cotton [17,18].

Historically, in Mississippi, *H. zea* has been a pest during the reproductive stages of soybean development [18]. When *H. zea* adults that developed on corn as larvae emerge, corn is typically no longer attractive. However, cotton and soybean are beginning to flower and are attractive as oviposition hosts [2,16,19,20]. Infestations occur during the reproductive phase of the soybean, between stages R1 and R3, and caterpillars feeding on it can result in delayed maturity of the plants, damaged grains, reduction of seed numbers per pod and abscission of pods, and ultimately lower yield [16,19,21].

The impact of the *H. zea* caterpillar on soybean yield depends on the size of the caterpillars, the plant development stage, the time of damage, and the ability of the plant to compensate for damage [16,22]. Soybean plants have the ability to compensate for these damaged reproductive tissues [3,23,24], through the production of more pods or through greater weight of the individual seed [23]. Artificial removal of pods in the reproductive stages of the soybean has been used to understand the damage caused by *H. zea* and to adjust action levels for control [24–26]. The objective of this work was to evaluate the impact of flower and pod removal on the maturation and yield of the soybean plant, in order to simulate the damage of *H. zea* in different reproductive stages of development under non-irrigated conditions that may limit yield potential.

2. Materials and Methods

Experiments were conducted during 2016 and 2017 at the Mississippi State University R. R. Foil Plant and Soil Sciences Farm, Starkville, MS and during 2017 at Delta Research and Extension Center. During 2016 and 2017 experiments were conducted at the Delta Research and Extension Center on a Bosket very fine, sandy loam soil (fine-loamy, mixed, active, thermic Mollic Hapludalfs) and a Tunica clay soil (clayey over loamy, smectitic over mixed, superactive, nonacid, thermic Vertic Epiaquepts). Methods were similar to Adams et al. [18] with some modifications. An indeterminate variety with a maturation group (MG) IV (Asgrow 4632, Monsanto Company, St. Louis, MO, USA) was used. This variety was chosen because it was utilized extensively by Mississippi soybean growers and represented a typical soybean variety planted in Mississippi and other portions of the midsouthern U.S. Soybeans were planted into raised conventional tilled beds with a 0.97 m row spacing in Starkville (305,915 seeds per ha) and 1.02 m row spacing in Stoneville (290,619 seeds per ha). Soybeans were planted in Stoneville, MS, on 5 April 2016 for the silt loam trial and on 8 April 2016 for the clay trial. During 2017, soybeans were planted in Stoneville on 2 May 2017 for the silt loam and clay trials. Plot size during 2016 was 4 rows by 1.5 m, and 4 rows by 3.05 m during 2017. Treatments were imposed on the center 2 rows of each plot. The seed was treated with a commercial premix of imidacloprid, pyraclostrobin, metalaxyl and fluxapyroxad (Acceleron, Monsanto Company, St. Louis,

MO, USA) to minimize the impact of early season insect pests and seedling diseases. Weed and disease pests were managed according to Mississippi State University Extension Service recommendations [7]. Additionally, all experiments were conducted under non-irrigated conditions.

Insect pest populations were monitored weekly and insecticides were applied when published thresholds were reached to minimize the confounding insect damage [7]. The experimental design was a randomized complete block with a complete factorial arrangement of treatments with four replications. Factor A was considered the development phase of the plant (reproductive growth stage) at the time of the removal of the flowers and pods (removal timing) and included the growth stages of R2 (flower in the upper two nodes), R3 ($^3/_{16}$-inch-long pod in upper four nodes), R4 ($^3/_4$-inch-long pod in upper four nodes) and R5 (visible seeds in pod of upper four nodes) according to Fehr and Caviness [27]. Factor B was the percentage of flowers and pods (0%, 25%, 50%, 75% or 100%) removed at each of the previously mentioned growth stages. Once the soybean plots reached the determined development stage (R2, R3, R4 or R5), the artificial removal of the flowers and pods was performed by hand according to predetermined percentages. Plots were established for each growth stage and fruit removal level combination. During the 2016 season, soybean growth between the R3 and R5 growth stages was rapid and the R4 fruit removal timing was not included in the experiment.

Twenty five percent flower/pod removal was accomplished by removing one flower or pod and leaving the next three; 50% removal was accomplished by removing a flower or pod and leaving the next flower or pod. For 75% removal, three flowers or pods out of every four were removed, and for 100% removal, all flowers or pods on the plant were removed. Fruit removal treatments were initiated at the lowest node on the plant and proceeded to the terminal.

The impact of loss of flowers or pods on plant maturity was determined by visually estimating the percentage of abscised leaves in each plot on 15 September 2017 in Starkville and 14 and 18 September 2017, in Stoneville, when the untreated plot (no fruit removal) were 15 days from harvest. The percentage of non-senesced main stems (main stems that remained green) in each plot was determined on 13 and 14 September for the silt loam and clay soil experiments at Stoneville during 2016 and on 20 September 2017, and on 18 September in Starkville during 2017. The total number of non-senesced main stems and the total number of plants on the center 2 rows of each plot was counted. These data were used to determine the percentage of non-senesced main stems. Once plants had reached maturity and a harvestable moisture, each plot was machine-harvested with a Kincaid 8XP plot combine with a weigh system, and seed weights and moisture were determined. Seed yields were corrected to 13% moisture and converted to kg/ha.

Crop maturity and yield data were analyzed with generalized linear mixed model analysis of variance. Fruit removal timing, fruit removal level, and the interaction between removal timing and level of fruit removal were considered fixed effects. Control plots for each growth stage were identical (no fruiting structure removal), therefore data for the nondamaged plots were pooled by replication in an analysis for a randomized complete block design with incomplete factorial treatment arrangement. Site-year and rep and site-year nested within year by location were designated as random effects [28]. Site-years were considered environmental or random effects; this allowed inferences to be made over a range of environments [28,29]. Fisher's protected LSD test ($p \leq 0.05$) was used for mean comparison. All the analyses were performed using the statistical software PROC GLIMMIX SAS (Institute Inc., 2011, Cary, NC, USA).

3. Results

3.1. Fruit Removal Plant Stage and Abscised Leaves

A significant interaction between fruit removal timing and fruit removal level was not observed for abscission of leaves (F = 3.61; df = 9; 168; p = 0.0004). Fruit removal below 100% at the R2 growth stage did not significantly impact natural leaf abscission compared with the nontreated plots, whereas plants that received 100% fruit removal retained significantly more leaves (50.8 ± 6.2%) than plants that

incurred 25%, 50% and 75% of fruit removal at R2 (Table 1). There were no significant differences in natural leaf senescence and abscission between the nontreated control plants and plants that received fruit removal treatments at R3 growth stage. Plants that received fruit removal of 75% and 100% at R4 growth stage, did not show a significant impact on natural leaf abscission compared with the nontreated plants. Plants that received removal of 25 and 50% of fruiting structures retained significantly more leaves (66.2 ± 6.7% and 66.6 ± 5.3%, respectively) than the nontreated control plants. At the R5 growth stage, there were no significant differences in natural leaf abscission between the nontreated control plants and plants that received fruit removal below 100%. Plants that received fruit removal of 100% retained significantly more leaves (65.0 ± 7.9%) than the nontreated control plants (Table 1).

Table 1. Effect of fruit removal plant stage on mean percentage of normally abscised leaves during 2017.

Growth Stage	Removal Level (%)	Mean Percentage Abscised Leaves (±SE) [a]
Nontreated control	0	79.1a (1.5)
R2	25	72.0abc (1.9)
	50	78.7a (1.9)
	75	77.0ab (2.2)
	100	50.8d (6.2)
R3	25	74.2abc (5.8)
	50	77.9ab (3.9)
	75	77.0ab (2.8)
	100	80.0a (1.7)
R4	25	66.2bc (6.7)
	50	66.6bc (5.3)
	75	70.0abc (5.8)
	100	75.0abc (4.1)
R5	25	80.0a (2.8)
	50	77.9ab (3.4)
	75	71.2abc (4.8)
	100	65.0c (7.9)

[a] Means within a column followed by the same letter are not significantly different according to Fisher's protected LSD test (α = 0.05).

3.2. Fruit Removal Plant Stage and Nonsenesced Main Stems

Results similar to those for percentage of abscission of leaves were observed for natural senescence as measured by nonsenesced main stems. A significant interaction between fruit removal timing and fruit removal level was observed for a percentage of nonsenesced main stems (F = 3.04; df = 9; 254.5; p = 0.0018). The percentage of nonsenesced main stems ranged from 70.2 (±8.1) to 31.2 (±5.7) %. At the R2 growth stage, the removal of 25% (48.5 ± 6.3%), 50% (40.1 ± 7.1%) and 75% (47.5 ± 6.7%) did not significantly impact the percentage of nonsenesced main stems compared with the nontreated plants, whereas the removal of 100% of fruit resulted in a significantly higher percentage of nonsenesced main stems (70.2 ± 8.1%) compared with all other fruit removal level and timing treatments. At R3 through R5 growth stages no fruit removal levels impacted the percentage of nonsenesced main stems (Table 2).

Table 2. Effect of the interaction of fruit removal plant stage and fruit removal level on percentage of nonsenesced main stem during 2016 and 2017.

Growth Stage	Removal Level (%)	Mean Percentage Nonsenesced Main Stems (±SE) [a]
Nontreated control	-	46.8bcd (3.9)
R2	25	48.5bc (6.3)
	50	40.1bcd (7.1)
	75	47.5bcd (6.7)
	100	70.2a (8.1)
R3	25	48.2bc (8.2)
	50	47.6bc (7.6)
	75	36.0cd (6.7)
	100	31.3d (5.7)
R4	25	45.6bcd (11.3)
	50	46.8bcd (8.7)
	75	55.9ab (11.0)
	100	55.1ab (7.1)
R5	25	42.9bcd (7.1)
	50	41.4bcd (8.3)
	75	45.4bcd (8.8)
	100	53.9b (8.6)

[a] Means within a column followed by the same letter are not significantly different according to Fisher's protected LSD test ($\alpha = 0.05$).

3.3. Fruit Removal Plant Stage, Fruit Removal Level and Soybean Yield

There was no significant interaction observed between the fruit removal timing and the fruit removal level for soybean yield (F = 0.88 df = 9; 250.6; *p* = 0.55). There were significant main effects of fruit removal timing (F = 5.13; df = 3; 63.39; *p* < 0.01) and fruit removal level (F = 12.74; df = 3; 250.4; *p* ≤ 0.01) for soybean yield. There were no significant differences in soybean yields between the nontreated control plots and plots that received fruit removal treatments at the R2, R3 and R4 growth stages. Plants that received fruit removal treatments at R5 had significantly reduced soybean yield compared with the nontreated control (Table 3).

Table 3. Effect of the fruit removal plant stage on soybean yield during the 2016 and 2017 seasons.

Growth Stage	Mean Yield in kg/ha (±SE) [a]
Nontreated control	4227.5a (103.5)
R2	3989.9a (105.4)
R3	4035.1a (108.7)
R4	3976.9a (99.5)
R5	3644.4b (120.4)

[a] Means within a column followed by the same letter are not significantly different according to Fisher's protected LSD test ($\alpha = 0.05$).

Fruit removal of 25% did not result in significant differences when compared with the nontreated plants (Table 4). The plants that received fruit removal of 50%, 75% and 100% had significantly lower yields when compared to the control, with 100% fruit removal resulting in the greatest yield reduction.

Table 4. Effect of the fruit removal level on soybean yield during the 2016 and 2017 seasons.

Removal Level (%)	Mean Yield in kg/ha (±SE) [a]
Nontreated control	4227.6a (103.5)
25	4059.9ab (99.5)
50	4008.9b (101.5)
75	4010.6b (111.7)
100	3566.9c (133.8)

[a] Means within a column followed by the same letter are not significantly different according to Fisher's protected LSD test (α = 0.05).

4. Discussion and Conclusions

The timing of fruit initiation is important in soybean development. Any physiological stress during this period can have an impact on the final yield since the growth rate of the fruit affects the senescence time and the death of the plant [30]. In the current experiments, the interaction of fruit removal timing and fruit removal level on the percentage of non-senesced main stems and on percentage of normally abscised leaves was observed. When grain has reached a harvestable moisture content, the presence of non-senesced leaves and main stems becomes a problem for farmers [18] that can potentially reduce yield and increase seed loss due to a decrease in harvest efficiency. A decline in photosynthesis and the loss of leaf protein characterize the senescence of soybean leaves. The visual symptom of leaf yellowing is widely used as an index of plant and leaf senescence [31–33]. The maturation of soybean and abscission of leaves are a natural process. Late planting of cultivars may lead to failures in natural senescence and the retention of green leaves in response to stress from adverse conditions [34]. Sosa-Gomez and Moscardi [35] associated the phenomenon of leaf retention with several factors, such as drought during flowering and/or the pod development period, excess moisture during the maturation period, nutrient deficiency [36,37] and insect pests [38] can interfere in the formation and filling of pods. Leaf retention can also be associated with the absence of pods on the plant [39]. Because it is rarely possible to achieve defined fruit removal from natural insect infestations, simulated or artificial damage is commonly used to establish injury−yield loss relationships [40]. Injured flowers may abort or remain in place, resulting in a reduced number of seeds per pod, subsequently reducing yield [21,41,42].

In the present study, the removal of 100% of the flowers and pods at the R2 growth stage, 75 and 100% at R3 growth stage and 100% removal at R4 growth stage caused a higher percentage of green stems. McAlister and Krober [43], when assessing the response of soybean cultivars to the removal of leaves and pods observed that plants with 40% and 80% removal had lower percentages of maturation when compared to untreated plots. Delay in plant maturity allowed some stressed plants to compensate for fruit loss [23]. Hicks and Pendleton [39] when evaluating the effect of floral bud removal on performance in an indeterminate "Wayne" soybean, observed that plants that had 0 to 60 floral buds removed per plant at random over all nodes showed failures in the senescence of the vegetative parts of the soybean. The authors also report that the protein content increased, and the oil content decreased with increasing numbers of flower buds removed. Tayo [44] when determining the effects of different levels of fruit removal on soybean performance, concluded that fruit removal led to lower accumulation of dry matter, higher leaf area and late maturation of plants as the percentage of removal increased. The author also reported that the number of mature pods per plant was lower when all fruit were removed after three weeks of development. In an attempt to compensate for damage, plants may produce new flowers and pods within a short time, depending on the level of stress, leading to delayed maturity [23].

Depending on the level, fruit removal may affect yield by reducing the weight of the soybean seed [39,44]. In this experiment, there was no significant interaction observed between fruit removal timing and fruit removal level for soybean yield. When the removal levels were applied at the R5 growth stage, yield was significantly reduced relative to the control. Rocha et al. [45] evaluated

the effect of two levels of fruit removal (50% and 100%) at four growth stages (R4, R5, R5.5 and R6), and reported that both levels of fruit removal reduced the number of pods per plant compared to the control, being more pronounced when the pods were removed in the R5 and R5.5 stages. Adams et al. [18] evaluated the impact of two levels of fruit removal (50% and 100%) on different stages of soybean development and they reported a significant reduction in yield and crop value when both removal levels were applied at the R5.5 stage of plant development, when compared to untreated plots. Evaluating the ability of soybean to tolerate five pod removal levels (0%, 10%, 20%, 30%, 40% and 80%) at different growth stages of soybean, Smith and Bass [46] found that when 80% of pods were removed at R5 stage, yield was significantly lower when compared to the untreated plots. The 50%, 75%, and 100% fruiting structure removal, regardless of growth stage, resulted in a mean yield reduction when compared to the control treatment. Rocha et al. [45] reported that 100% pod removal at R6 did not allow the plants to compensate for damage, resulting in fewer pods per plant. Thomas et al. [22] evaluated the influence of different levels of depodding on the yield of soybeans; the authors observed that yield was reduced when high levels of removal (100%) were applied at the R5 growth stage. When levels of pod removal occurred in the early stages of development (R2, R3 and R4), yields did not differ statistically when compared to the control treatment, demonstrating the ability of plants to compensate for damage when it occurs in the early growth stages. McAlister and Krober [43] evaluated the response of 10%, 20%, 30%, 40% and 80% removal pod of 'Hawkeye' and 'Lincoln' soybeans and reported that, during the initial reproductive stages of soybean, they produced almost the same number of pods when compared to plants without treatment.

This may have occurred because soybean plants usually lose 30% to 85% of flower buds naturally and compensation of fruit loss occurs during the early reproductive stages without causing any delay in plant maturation [18,47,48]. Delayed leaf senescence is a compensatory response of the plant when damaged by an insect. When unfavorable conditions exist, soybean plants could recover from fruit or tissue loss, and this may influence yield recovery [49–51]. These data demonstrated that soybean, regardless of the cultivar used, may be able to compensate for even severe levels of fruit loss early during the reproductive portion of the growing season if favorable growing conditions occur. The data also illustrate that soybean is more sensitive to the type of damage caused by *H. zea* during the R5 growth stage when compared to earlier reproductive growth stages. The results found in this study were only tested on a single cultivar. While similar results on different cultivars of similar maturity groups would generally be expected, it is beyond the scope of the data to conclude with certainty that a similar response would be observed. However, for soybean cultivars from the same maturation group, the inferences obtained with the cultivar of the present study can be extrapolated. Finally, these data demonstrate that under adequate to optimal growing conditions recommendations for managing fruit feeding insects should be similar for the non-irrigated soybean. Soybeans planted before and at the end of the optimal planting period responded similarly to fruit loss as soybeans planted at an optimum time in other studies. However, these results may not apply to extremely late-planted soybeans.

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