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Vertical and temporal distribution of *Helicoverpa zea* (Lepidoptera: Noctuidae) larvae in determinate and indeterminate soybean

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Abstract

Most oviposition by *Helicoverpa zea* (Boddie) occurs near the top of the canopy in soybean, *Glycine max* (L.) Merr, and larval abundance is influenced by the growth habit of plants. However, the vertical distribution of larvae within the canopy is not as well known. We evaluated the vertical distribution of *H. zea* larvae in determinate and indeterminate varieties, hypothesizing that larval distribution in the canopy would vary between these two growth habits and over time. We tested this hypothesis in a naturally infested replicated field experiment and two experimentally manipulated cage experiments. In the field experiment, flowering time was synchronized between the varieties by manipulating planting date, while infestation timing was manipulated in the cage experiments. Larvae were recovered using destructive sampling of individual soybean plants, and their vertical distribution by instar was recorded from three sampling points over time in each experiment. While larval population growth and development varied between the determinate and indeterminate varieties within and among experiments, we found little evidence that larvae have preference for different vertical locations in the canopy. This study lends support to the hypothesis that larval movement and location within soybean canopies do not result entirely from oviposition location and nutritional requirements.

Introduction

Helicoverpa zea (Boddie) is one of the costliest and widely distributed insect pests of US soybean, *Glycine max* (L.) Merr., especially in the southern USA (Musser *et al.*, 2018, 2019). Abundance of *H. zea* in soybean is influenced by different cultural practices, which can have an impact on the growth habit of soybean plants (Ablett *et al.*, 1991). *H. zea* larval populations are generally greater in soybean where plants do not overlap between adjacent rows (open canopy) compared to soybean where plants on adjacent rows are overlapping (closed canopy) (Sprenkel *et al.*, 1979; Bradley and van Duyn, 1980; Alston *et al.*, 1991). However, planting date, maturity, or row spacing is confounded in many of these studies. For example, threshold levels of *H. zea* were reached in 2.8% of the fields with a closed canopy compared with 22% of those with an open canopy in a 1973 survey of 1500 soybean fields in North Carolina (Bradley and van Duyn, 1980). Nonetheless, the authors recognized that this survey potentially confounded effects such as planting date and maturity group and, in general, noted there were fewer *H. zea* in early planted and early maturing soybeans compared with later planted and later maturing soybeans. Bradley *et al.* (1986) proposed three hypotheses relevant to soybean canopy closure and *H. zea* abundance: (1) canopy closure is collinear with other variables that are more important to explain abundance, (2) closed canopies obscure visual ovipositional cues, and (3) closed canopies are more favorable to predators, parasites, or entomopathogens (Mayse, 1978, 1984). Later it was discovered that oviposition is generally greater in closed canopies (Terry *et al.*, 1987), lending support against the second hypothesis. Again, this study confounded planting date and canopy closure. In another study, row spacing was used to manipulate canopy closure, and the predation portion of the third hypothesis was disproven. In that study, 70% of *H. zea* eggs were consumed within 24 h of being in the field independent of canopy closure (Anderson and Yeargan, 1998). Therefore, the hypothesis that canopy closure is collinear with other variables is the most likely of the three.

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From past research, it is clear that *H. zea* larvae are generally more abundant in late-planted soybean with open canopies, but it is unclear how important canopy closure is to larval mortality. Furthermore, the growth habit of soybean (including canopy closure) varies with plant spacing, environmental conditions, and variety (Ablett *et al.*, 1991). One overlooked factor influencing the abundance and spatial distribution of *H. zea* in different soybean canopies is the availability of different microhabitats in determinate (vegetative growth ends when flowering begins) and indeterminate (vegetative growth continues after flowering begins) soybean plants. An experiment that held all other factors constant, while only manipulating whether the plant was determinate or indeterminate would be ideal to study the influence of the termination of soybean growth on the abundance and spatial distribution of *H. zea*. However, varieties are not available to test this hypothesis. While varieties can be found that are close together in maturity, generally an indeterminate soybean variety will initiate blooming prior to a determinate soybean variety (Parvez *et al.*, 1989). Furthermore, *H. zea* prefers to oviposit in soybean during the R2 (full flowering) stage (Hillhouse and Pitre, 1976). Therefore, field experiments investigating the impact of a single factor, such as soybean variety, growth habit, row spacing, or planting date on *H. zea* are difficult. In order to isolate a single factor, other factors cannot usually be held constant. For example, in a field experiment investigating the impact of different indeterminate and determinate varieties on abundance of *H. zea*, *H. zea* females will likely prefer to oviposit in the variety that is currently flowering. Hence, in this experiment, ovipositional choice would be more reflective of plant phenology at the time of oviposition rather than variety.

Soybean canopies can vary depending on row spacing, height, and environmental conditions that influence canopy coverage. For example, double-cropped soybeans will sometimes be planted late on wider rows and will never become a closed canopy. However, the canopy may have closed if the rows were narrower. Similarly, even if row spacing is held constant, environmental conditions can allow soybeans to grow very short or very tall. Therefore, canopy architecture could potentially bias sampling methods that vary in efficiency in sampling different portions of the canopy. Generally, soybean is sampled using a sweep net or beat sheet (Zeiss and Klubertanz, 1993) and there is generally good agreement between results for beat-sheet and sweep-net sampling for *H. zea* larvae, which would suggest a more even vertical distribution (Dieghan *et al.*, 1985; Studebaker *et al.*, 1991). Despite this, *H. zea* is thought to preferentially oviposit in the top of the plant on the abaxial side of leaves (Hillhouse and Pitre, 1976), especially when the proportional area of available plant surface is taken into account (Terry *et al.*, 1987). Similarly, both *Helicoverpa armigera* (Hübner) and *Helicoverpa punctigera* (Wallengren) prefer to oviposit in the top 20 cm of the canopy on the abaxial side of leaves (Duffield and Dillon, 2005). However, in one study, the majority of *H. zea* larvae were found near the middle part of the plant and they were especially concentrated in this area during the blooming stage of soybean (Pitre and Hillhouse, 1981). Hence, following hatching, larvae may move down in the canopy to suitable food sources as they develop.

Our goal in this study was to evaluate the vertical distribution of *H. zea* larvae over time in a determinate variety and an indeterminate variety in a naturally infested replicated field experiment and two experimentally manipulated cage experiments. In the field experiment, flowering time was synchronized between the varieties by manipulating planting dates, while infestation timing was manipulated in the cage experiments. Our hypothesis was that vertical distribution of larvae would be different between determinate and indeterminate varieties because of growth habit. Furthermore, we hypothesized that this distribution would change over time.

Methods

Natural infestation study

An indeterminate (AG4533, Bayer Crop Science, St. Louis, MO) and determinate (AG5533; Bayer Crop Science) soybean variety was planted at Plymouth, NC, in a randomized complete block design with four replications. Each variety was planted on a date intended to synchronize the period when soybean would be most attractive to oviposition by *H. zea* (the flowering stages, but especially GS R2). Hence, the indeterminate variety was planted on 17 June 2015, while the determinate variety was planted on 27 May 2015. Plot sizes were four rows wide (93 cm row spacing) by 12.2 m long. Acephate (Orthene 97, 1.05 kg ai ha^{-1}) was applied during mid-May prior to oviposition to reduce the abundance of natural enemies of *H. zea* eggs and larvae. Plants were monitored once each week using a 1-m long beat sheet from single rows (alternating rows one and four on each sampling date) until neonate larvae were observed in the experiment (30 July 2015).

Beat sheet samples were taken in a similar fashion from rows one and four on 3, 10 and 17 August. By the last sampling date, most larvae had completed development. Also on these dates, 25 plants were removed from rows two and three, alternating rows when plants were removed at each sampling event. Plants were selected at random, clipped at the base using scissors and taken to a table adjacent to the experiment where they were divided into three equal vertical sections by height: (1) upper, (2) middle, and (3) lower. Growth stage was recorded at each sampling event. Plant parts were kept in containers with 30% aqueous ethanol, and sections were examined for the presence of any larvae that might have avoided being dislodged. These containers were then transported to the laboratory, and *H. zea* larvae were quantified to instar using measurements of head-capsule width (Hardwick, 1965). Fourth and fifth instar larvae ($n = 20$) were chosen at random, and their mandibles were examined to determine the proportion of *H. zea* to *Chloridea virescens* (F.) that might have been present in the experiment.

Cage study experiment 1

An indeterminate (AG46X6, Bayer Crop Science) and determinate (AG5533; Bayer Crop Science) variety were planted at Stoneville, MS, in a randomized complete block design with four replications on 11 May 2017. Individual plots were four rows wide (1 m row spacing) by 12.2 m long. Once each variety reached R2 (30 June for indeterminate variety and 6 July for determinate variety), one 1.8 m^3 cage using Amber Lumite® screen (7.9 openings in 1 cm^2 mesh; BioQuip Products, Inc., Rancho Dominguez, CA) was placed over ca. 90 plants in each plot. These cages were immediately infested with using 12–15 pairs of *H. zea* newly emerged moths in a 1:1 male to female ratio. These moths were obtained from a laboratory-reared colony originating from a collection of late instar larvae from non-Bt corn ears during the same season and reared for one generation.

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Larvae were reared individually on Stonefly Heliothis Diet (Ward's Natural Science, Rochester, NY) in 36 ml plastic cups (Bio-Serv®, Frenchtown, NJ). Rearing conditions were 25°C, 75–80% relative humidity, and 16:8 (L:D) photoperiod. Ten individual plants in each plot were sampled on 20, 25, and 27 July for the indeterminate variety and 20, 25, and 28 July for the determinate variety. Soybeans were R2–R4 for the determinate variety and R2–R3 for the indeterminate variety on 20 July, R3 on 25 July, and R3–R4 on 27 and 28 July. Cages were removed immediately prior to sampling and replaced as soon as plants were collected. The same procedure as described in the field experiment was used to divide the plant into vertical sections and to dislodge the larvae. Larval instar was determined by visual estimation using the same qualifications for instar as described previously, but head capsule diameter was not measured.

Cage study experiment 2

The same indeterminate variety (AG46X6) used in the previous cage experiment was planted using the same plot size in four replications in Stoneville, MS, on 1 May 2018. Cages and infestations were placed and made as described previously at the R2 growth stage (29 June). Cages were removed, and five plants were sampled similar to the cage study in experiment 1 on 16 July, and ten plants per plot were sampled on 19 and 24 July. Soybean plants were in the R3, R3, and R3–R4 growth stages on 16, 19, and 24 July, respectively. The same procedure as described in the field experiment was used to divide the plant into vertical sections and to dislodge the larvae. Larval instar was determined by visual estimation using the same qualifications for instar as described previously, but head capsule diameter was not measured.

Statistical analyses

For all studies, individual generalized linear mixed models were constructed (PROC GLIMMIX; SAS Institute, 2011) for the dependent variables of total larvae number, mean head capsule size, mean instar, and number of each individual instar. In the natural infestation experiment, instars were recorded up to L6, while L5 and L6 instars were recorded together in the cage study experiments. Fixed variables in the natural infestation experiment and cage study experiment 1 included vertical section, maturity group, date, and their interactions. Random variables included replication, replication × variety, replication × variety nested within date, and the repeated subject. Fixed variables in cage study experiment 2 included vertical section, date, and their interaction. Random variables included replication, replication × vertical section, replication × date, and the repeated subject. The logit link function was used, compound symmetry was selected for the covariance structure with the residuals as an overdispersion parameter, and the proper transformations (square root or square root + 1) and distributions (Gaussian or log-normal) were chosen using Pearson graphs and fit statistics (Littell *et al.*, 2006). Mean separations were analyzed using Tukey's honest significant differences test, and degrees of freedom were adjusted using Kenward and Roger (1997) degrees of freedom approximation. When interactions were significant for date, the SLICE function was used to isolate the main effect during each sampling date. Furthermore, when interactions were only marginally significant, there were occasions when Tukey's honest significant differences test did not indicate that the interaction means should separate. In this case, the significant main effect or effects driving the interaction was analyzed using mean separations and presented. Finally, all data were converted to number of larvae per 100 plants for reporting in the results.

Results

Natural infestation study

H. zea were the only larvae recovered in the study. The interaction between maturity group and sampling date (Table 1) was significant for the mean total number of larvae ($F = 9.60$, d.f. = 2, 12, $P = 0.0032$) and first instar larval numbers ($F = 8.05$, d.f. = 2, 12, $P = 0.0156$). The interaction between maturity group and sampling date was significant for fourth instar larval numbers ($F = 4.81$, d.f. = 2, 12, $P = 0.0283$), but the SLICE function did not detect differences in maturity group at each sampling date using the mean separation procedure. The main effect of maturity group was not significant ($F = 3.40$, d.f. = 1, 3, $P = 0.1622$), but the effect of sampling date (Table 2) was significant ($F = 10.49$, d.f. = 2, 11, $P = 0.0023$). The effect of sampling date (Table 2) was significant for mean head capsule diameter ($F = 7.42$, d.f. = 2, 11, $P = 0.0091$) and third instar larval numbers ($F = 8.05$, d.f. = 4, 208, $P = 0.0386$). Although mean larval instars were different among sampling dates ($F = 4.30$, d.f. = 2, 11, $P = 0.0417$), they did not separate using the mean separation procedure.

The interaction between vertical section, maturity group, and sampling date was significant for second instar larval numbers ($F = 8.05$, d.f. = 4, 208, $P = 0.0386$). The SLICE function indicated that the vertical section and maturity group interaction was significant during the first sampling date only. On the first sampling date, more second instars were found in the middle (4.72 ± 1.85 SE) and lower (4.33 ± 1.67) portion of the determinate variety compared with the lower portion of the indeterminate variety (0.53 ± 0.36). There were no significant effects for numbers of fifth instars. Finally, the interaction between vertical section and sampling date was significant for sixth instar larval numbers ($F = 2.99$, d.f. = 4, 208, $P = 0.0198$). The SLICE function indicated differences among vertical section during the first sampling date only, with an average of 5.6 ± 2.02 larvae recovered from the middle portion of the vertical section compared with 1.07 ± 0.60 in the upper portion and 1.11 ± 0.62 in the lower portion.

Cage study experiment 1

The effect of sampling date (Table 2) was significant for mean total number of larvae ($F = 21.1$, d.f. = 2, 16, $P < 0.0001$), second instar larval numbers ($F = 21.72$, d.f. = 2, 16, $P < 0.0001$), and fifth and sixth instar larval numbers ($F = 6.29$, d.f. = 2, 16, $P = 0.0096$). The interaction between vertical section and sampling date was significant for mean larval instar ($F = 2.68$, d.f. = 4, 102, $P = 0.0359$), but the SLICE function did not detect differences in vertical section at each sampling date using the mean separation procedure. The main effect of vertical section was not significant ($F = 1.62$, d.f. = 2, 102, $P = 0.2028$), but the effect of sampling date (Table 2) was significant ($F = 107.68$, d.f. = 2, 15, $P < 0.0001$). The interaction of vertical section and maturity group was also significant for mean larval instar ($F = 4.77$, d.f. = 2, 102, $P = 0.0105$). Instars were larger for all three vertical section locations in the indeterminate variety (mean instar stage of 3.87 ± 0.20, 3.56 ± 0.21, and 3.19 ± 0.18 in the lower, middle, and upper vertical sections, respectively) compared with all

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Table 1. Mean *H. zea* larvae ± standard error across three sampling dates (from GS R2 to R5) for the significant effect of the interaction of growth habit (determinate or indeterminate) with sampling date, in two experiments

		Mean larval number/100 plants			Mean first instar number/100 plants		
Experiment	Growth habit	1st sampling period[a]	2nd sampling period[a]	3rd sampling period[a]	1st sampling period[a]	2nd sampling period[a]	3rd sampling period[a]
Natural infestation	Determinate	16.11 ± 2.65a	6.78 ± 1.49a	0.55 ± 0.21a	1.67 ± 0.43a	0.44 ± 0.27a	0.00 ± 0.00a
	Indeterminate	6.43 ± 1.14b	4.61 ± 1.17a	2.56 ± 0.67a	0.43 ± 0.19b	0.35 ± 0.17a	0.33 ± 0.19a
		Mean first instar number/100 plants			Mean third instar number/100 plants		
Experiment	Growth habit	1st sampling period[b]	2nd sampling period[b]	3rd sampling period[b]	1st sampling period[b]	2nd sampling period[b]	3rd sampling period[b]
Cage study 1	Determinate	38.50 ± 12.30a	0.50 ± 0.89a	0.17 ± 0.17a	0.50 ± 0.29a	12.17 ± 2.89a	3.50 ± 1.28a
	Indeterminate	1.67 ± 0.89b	0.00 ± 0.00a	0.00 ± 0.00a	15.50 ± 3.72b	1.33 ± 0.50b	0.00 ± 0.00a

The effect of date was held constant using the SLICE function in SAS. Therefore, letter groupings correspond to a single variable and a single sampling date within a column.
[a]First sampling period (GS R2–R3), second sampling period (R3), third sampling period (R3–R4).
[b]First sampling period (GS R2–R4 determinate; R2–R3 indeterminate), second sampling period (R3–R4 determinate; R3 indeterminate), third sampling period (R4 determinate; R3–R4 indeterminate).

three vertical section locations in the determinate variety. In the determinate variety, larvae were larger in the middle vertical section (2.85 ± 0.19) compared with the lower (2.30 ± 0.19) and upper (2.34 ± 0.21) vertical sections.

The interaction between maturity group and sampling date (Table 1) was significant for first instar larval numbers (F = 8.05, d.f. = 2, 12, P = 0.0156) and third instar larval numbers (F = 35.87, d.f. = 2, 16, P < 0.0001). Finally, the interaction between maturity group and sampling date was significant for fourth instar larval numbers (F = 4.98, d.f. = 2, 16, P = 0.0209), but the SLICE function did not detect differences in maturity group at each sampling date using the mean separation procedure. Moreover, the main effects of maturity group (F = 0.34, d.f. = 1, 4, P = 0.5793) and sampling date (F = 1.06, d.f. = 2, 16, P = 0.3699) were not significant.

Cage study experiment 2

There were no significant effects for mean total number of larvae. However, the effect of sampling date (Table 2) was significant for mean larval instars (3.00; F = 12.43, d.f. = 2, 6, P = 0.00073), third instar larval numbers (F = 7.61, d.f. = 2, 6, P = 0.0226), and fourth instar larval numbers (F = 5.32, d.f. = 2, 6, P = 0.0468). There were no significant effects for first, second, and fifth and sixth instar larval numbers.

Discussion

Our goal was to evaluate the vertical distribution of *H. zea* larvae over time in determinate and indeterminate soybeans in a naturally infested field experiment and two artificially infested cage experiments. We found little evidence that larvae have a preference for different vertical locations in the canopy, despite reports of preferential oviposition by *H. zea* and related species near the top of soybean plants on the abaxial side of leaves (Hillhouse and Pitre, 1976; Terry *et al.*, 1987; Duffield and Dillon, 2005) and a greenhouse study that found more larvae in the middle of the plant (Pitre and Hillhouse, 1981). We did not sample plants to record where eggs were vertically oviposited in the canopy. However, the results of all three experiments were generally similar. Previous studies have shown that a majority of first instars placed on a mature trifoliate will feed elsewhere or spin down toward the ground using silk (Terry *et al.*, 1989). Therefore, first instar larvae are likely moving from their ovipositional site soon after eclosion toward feeding sites throughout the canopy.

Only three significant effects of larval vertical distribution within the canopy were observed in all experiments. Two of these were in the natural infestation study, and one was in the first cage experiment. In the natural infestation study, there was an interaction of vertical section with maturity group for second instars. Within maturity group, however, were no differences in distribution of second instars. In contrast to the results of other instars, five times as many sixth instars were found in the middle of the canopy compared with the upper and lower portion of the canopy. This single result mirrors the findings of Pitre and Hillhouse (1981), who found that an average of 69% of the larvae was located in the middle third of the plant during R2, but that the percentage in this area dropped to 52% by R5 and R6. Finally, in our first cage experiment, mean head capsule size was larger in the middle portion of the canopy compared with upper and lower canopies in the determinate variety. Past studies have supported the hypothesis that development of *H. zea* in

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Table 2. Mean *H. zea* larvae ± standard error for the significant effect of sampling date in each of three experiments

Experiment	Mean	Sampling period		
		First[a]	Second[b]	Third[c]
Natural infestation	Head capsule[d]	0.75 ± 0.05b	1.20 ± 0.12a	1.32 ± 0.20a
	Third instar[e]	3.71 ± 0.62a	0.88 ± 0.21b	0.15 ± 0.09b
	Fourth instar[e]	7.44 ± 1.35a	3.05 ± 0.71b	0.88 ± 0.36b
Cage study 1	Larval number[e]	42.83 ± 8.00a	16.41 ± 3.54b	6.67 ± 1.64b
	Larval instar	2.30 ± 0.10a	3.32 ± 0.14b	4.00 ± 0.17c
	Second instar[e]	13.00 ± 2.35a	5.33 ± 1.89b	0.67 ± 0.26b
	Fifth instar[e]	0.08 ± 0.08b	0.92 ± 0.29ab	1.75 ± 0.47a
Cage study 2	Larval instar	1.52 ± 0.11b	2.65 ± .17a	2.99 ± 0.19a
	Third instar[e]	2.08 ± 0.89b	14.17 ± 3.09a	6.25 ± 1.80ab
	Fourth instar[e]	0.00 ± 0.00b	7.50 ± 1.75a	5.00 ± 2.49ab

Letter groupings correspond to a single variable across a single row.
[a]First sampling period (GS R2–R3), second sampling period (R3), third sampling period (R3–R4).
[b]First sampling period (R2–R4 determinate; R2–R3 indeterminate), second sampling period (R3–R4 determinate; R3 indeterminate), third sampling period (R4 determinate; R3–R4 indeterminate).
[c]First sampling period (R3), second sampling period (R3), and third sampling period (R3–R4).
[d]Size in mm.
[e]Number per 100 plants.

soybeans tracks crop phenology. Results of these studies have indicated that adults preferentially oviposit in plants that are blooming, and the resulting small larvae consume leaves and flowers. These larvae develop and are later able to penetrate pods and consume seed (Hillhouse and Pitre, 1976; McWilliams, 1983; Terry *et al.*, 1989; Eckel *et al.*, 1992; Suits *et al.*, 2017). In our studies, mean instar tended to increase over time as it presumably did in the Pitre and Hillhouse (1981) study (although they did not quantify larval growth over time after 10 days). However, in contrast to Pitre and Hillhouse (1981), who saw the greatest differences in vertical distribution early when larvae were smaller, in our study, vertical distribution rarely varied early; it tended to be when larvae were larger. We also want to caution against a direct comparison, because Pitre and Hillhouse (1981) used eggs and larvae placed on potted plants of a determinate variety in a greenhouse.

Our hypothesis is that larvae of *H. zea* move extensively throughout the canopy, but do not do so entirely for nutrition. Clearly, nutrition plays a role in larval distribution. For example, smaller instars are found most often on blooms and leaves (Eckel *et al.*, 1992; Reisig *et al.*, 2020) and cannot feed on pods (McWilliams, 1983). Furthermore, second instars, when given a choice, will feed on leaves, flowers, and pods, but, when fed a single tissue type, will only survive to pupation on leaves and flowers (Suits *et al.*, 2017). In our studies, we did not quantify egg oviposition location. However, previous studies have found that eggs are deposited on leaves in the top of the canopy in this species and related species (Hillhouse and Pitre, 1976; Terry *et al.*, 1987; Duffield and Dillon, 2005). If this were also true in our study, then many of the first instar larvae found in the middle and bottom portions of the canopy likely moved there from the top of the canopy. This agrees with previous findings in soybeans (Terry *et al.*, 1989) and cotton (Braswell *et al.*, 2019), where first instars of *H. zea* were found to have extensive dispersal. If larvae moved primarily for nutritional reasons, we hypothesized that smaller larvae would be found lower in the canopy in the indeterminate variety compared with the determinate variety. This is because the lower portion of the indeterminate variety, which starts to flower and form pods from the bottom of the plant, would have more tissue types available for consumption than the top, compared with the determinate variety which flowers and forms pods more synchronously throughout the canopy. In the determinate variety, smaller larvae should have had all the tissue types needed for development near the location where the eggs were oviposited (presumably in the top, based on previous studies). However, with the exception of sixth instars in the natural infestation study, larvae were distributed vertically throughout the canopy in both the determinate and indeterminate varieties. Furthermore, sixth instars could have been moving to drop to the soil and pupate.

Lending further support to our hypothesis that larvae do not move exclusively for nutrition, the most prominent difference between the determinate and indeterminate varieties involved the timing of population growth and larval development, rather than a difference in larval distribution within the canopy. This also varied between the two experiments where both varieties were present. For example, first instars were present throughout the sampling period in the natural infestation study on the indeterminate variety, but were nearly four times higher on the first sampling date in the determinate variety than the indeterminate variety. By the second sampling date, numbers were similar, and, while numbers between varieties were not statistically different between varieties on the third sampling date, no first instars were recovered in the determinate variety on the third sampling date. In contrast, first instars were present in the first cage experiment throughout the sampling period in the determinate variety but only recovered from the indeterminate variety on the first sampling and second sampling dates. However, in both experiments, larval population growth and development did not correspond with the development of reproductive structures on the soybean varieties. Furthermore, in the second cage experiment, where an indeterminate variety was used exclusively, there were no differences in vertical location where larvae were recovered

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across the canopy. Finally, in all experiments, larval growth across the population tended to increase over time, with mean head capsule size increasing over time and with larger larvae recovered in later sampling dates.

All three of these experiments were conducted in soybeans with closed canopies, and, as mentioned previously, previous studies have not disproven the hypothesis that canopy closure is collinear with other variables that are more important to explain abundance (Bradley *et al*., 1986). In Anderson and Yeargan's (1998) careful study, it was demonstrated that predation was not associated with *H. zea* larval abundance and canopy closure. In our studies, although an acephate application was made in the natural infestation study prior to oviposition, beneficial arthropods could readily be observed in the field. In contrast, the cage studies would have excluded some, although not all beneficial arthropods, because of the mesh size. The mesh size was likely large enough to allow predators of *H. zea* eggs and small larvae (such as immature *Geocoris* spp. or *Orius* spp.) to pass through, but not predators of larger larvae. Very few predatory arthropods were observed in cages. Hence, while our experiment did not directly test differences on whether or not *H. zea* larval distributions might be influenced by the presence of predators, parasitoids, or entomopathogens, microclimates, etc., conditions were likely very different between the natural infestation study and the cage study. Despite this, our three studies indicated that the vertical distribution of larvae in the canopy was generally equal.

Our data were not designed to test hypotheses regarding sampling. Studies designed to test sampling methods have demonstrated that the precision of the beat sheet and sweep net is equal for sampling larvae of *H. zea* (Dieghan *et al*., 1985; Studebaker *et al*., 1991). The beat sheet is typically used in rows that are wider, because it must be laid on the ground between rows, and a section of the plant canopy must be shaken over the sheet. This is difficult to do as rows narrow. In both wide and narrow rows, a sweep net can be used, but it is more difficult to sample the entire plant. Hence, the beat sheet is a more absolute sampling method, while the sweep net is a more relative sampling method (Zeiss and Klubertanz, 1993). Because *H. zea* larvae are equally distributed vertically throughout the canopy, data from our study support findings that relative sweep-net method can be equilibrated to the more absolute beat-sheet numbers.

In conclusion, we found little support for differences in vertical distribution of *H. zea* larvae in the soybean canopy, even though previous studies have indicated a preference for oviposition in blooming soybeans toward the top of the canopy. Because there were no differences between the indeterminate and determinate varieties, this study lends support to the hypothesis of Reisig *et al*. (2020) that, while larvae of *H. zea* may move for nutritional reasons, there are likely other under-explored and non-mutually exclusive factors that can explain larval movement.

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