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Pigeonpea (*Cajanus cajan*) responses to *Helicoverpa armigera* herbivory and simulated herbivory at different crop stages

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ABSTRACT

The ability of a plant to tolerate herbivory depends on the characteristics of the herbivore, the plant itself, and the environment. Understanding how crop plants tolerate pest herbivory is fundamental to development integrated pest management strategies. Pigeonpea (*Cajanus cajan)* is a major multi-purpose pulse crop cultivated throughout the tropics and subtropics. Pigeonpea yields are heavily constrained by insect pests that attack the crop during its reproductive stages. In this study we investigated the response of pigeonpea to herbivory at two stages of crop development: peak flowering and late podding. We compared how plants respond to herbivory by larvae of pigeonpea's key pest *Helicoverpa armigera* (Lepidoptera: Noctuidae) and to simulated *H. armigera* herbivory. Across two semi-field experiments we obtained slightly different results, suggesting that environmental conditions affected the plant responses to herbivory. At flowering, plants tolerated actual and simulated herbivory in the first experiment. But in the second, while plants tolerated simulated herbivory, actual herbivory resulted in yield loss. At podding, all herbivory treatments caused direct yield loss in the first experiment but in the second experiment simulated herbivory did not, although it increased the number damaged (i.e. unmarketable) seeds. Flowering plants were able to tolerate herbivory by redirecting yield to side branches and increasing mean seed weight, whereas there appear to be no tolerance mechanisms available for pigeonpea plants at the late podding stage. Future research should investigate how environmental factors influence tolerance expression, and such investigations will underpin the development of economic thresholds for *H. armigera* in pigeonpea.

1. Introduction

Understanding how crop plants respond to feeding from arthropod pests is a fundamental component of integrated pest management, as it underlies the development of economic thresholds (Pedigo et al., 1986; Ramsden et al., 2017). Improving our understanding of the mechanisms by which plants tolerate pest damage also presents the opportunity to develop crop varieties that are less susceptible to pests (Mitchell et al., 2016; Peterson et al., 2017). Developing tolerance-based management strategies is not straightforward, as tolerance is complex and determined by interactions between numerous intrinsic and extrinsic factors such as the timing of pest infestation, pest feeding mode, interactions with other above- or below-ground herbivores, and environmental conditions (Strauss and Agrawal, 1999; Tiffin, 2000; Wise and Abrahamson, 2007).

The timing of pest attack (i.e. the crop stage attacked) has a strong effect on the ability of a plant to tolerate herbivory (Bardner and Fletcher, 1974; Ramsden et al., 2017; Trumble et al., 1993). Pest managers often assume that crops at the seedling stage are highly susceptible to herbivory, but as they grow their tolerance increases. Tolerance then decreases as plants enter the reproductive stages and start to develop and fill yield-forming organs (i.e. grains or fruits) with assimilates and once yield-forming organs are fully developed, tolerance again increases (Bardner and Fletcher, 1974; Trumble et al., 1993). Although this pattern is widely recognised, the reality is more complicated. For instance, tolerance is strongly influenced by what plant parts are being attacked (i.e. source or sink organs) and whether the yield is source or sink limited (Ramsden et al., 2017). A plant is source-limited when its yield is constrained by the supply of photosynthates, whereas a plant is sink-limited when its yield is constrained by the ability of the yield-forming organs to store photosynthates.

Simulated herbivory is widely employed in studies examining plant response to insect herbivores (Baldwin, 1990; Waterman et al., 2019).

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From a practical perspective, simulating herbivory may be a more effective way of gaining an understanding of the relationship between damage and yield loss. As a simulated approach doesn't involve the complexity of using live herbivores it enables experimenters to impose damage at controlled levels (Hjältén, 2008; Lehtilä, 2003; Tiffin and Inouye, 2000). However, simulated herbivory may not accurately reflect the impact of herbivores if it fails to replicate their spatial and temporal feeding patterns (Volp et al., 2024a) and it is unlikely to elicit the complex phytohormonal responses induced by herbivore saliva and associated microbes (Waterman et al., 2019). Therefore, it is important to experimentally investigate the applicability of simulated herbivory techniques to understand how closely their effects mimic those of pests of interest.

Pigeonpea (*Cajanus cajan*) is one of the world's most important grain legume crops with an annual global yield of around 5 million tonnes (FAO, 2025). The crop is cultivated by smallholder farmers in Asia, Africa, and Latin America, and is an important protein source for the developing world (Mula and Saxena, 2010). A major constraint to global pigeonpea production is the 'cotton bollworm' or 'pod-borer' *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Shanower et al., 1999; Volp et al., 2025). In pigeonpea crops, *H. armigera* typically causes yield loss by feeding on the reproductive yield-forming organs (Shanower et al., 1999; Volp et al., 2025). Reproductive development in pigeonpea begins with bud initiation on racemes, buds then expand to form flowers which remain open for several days. Following fertilisation, pods form and expand as seeds fill with assimilates before they harden and then dry. *Helicoverpa armigera* typically oviposits into flowering crops, with early instar larvae establishing at flowers/buds and transitioning to pod-feeding as larvae develop (Volp et al., 2024a, 2024b).

Pigeonpea is renowned for its substantial capacity to tolerate damage to its reproductive organs, particularly flowers and small pods (Sheldrake et al., 1979; Tayo, 1980). Under non-stressed conditions only a small proportion (<20%) of pigeonpea flowers will convert to pods, partially explaining why plants can compensate for the loss of floral structures (Lawn and Troedson, 1990; Pandey and Singh, 1981; Sheldrake et al., 1979; Togun and Tayo, 1990). Sheldrake et al. (1979) demonstrated that pigeonpea plants may tolerate flower and small pod removal for up to five weeks post-flowering by extending their flowering period and setting pods from later-formed flowers on racemes. However, pigeonpea plants are less capable of compensating for the removal of developing pods (Tayo, 1980). Most studies conducted to date on pigeonpea examining yield response to damage have used simulated herbivory (Table 1), which may not completely reflect the nature and severity of damage caused by *H. armigera* (Chauhan et al., 1992). Studies have typically enacted simulated herbivory by completely removing reproductive structures, whereas *H. armigera* larvae feed on plant organs gradually over time and move from one structure to another without completely destroying them (Rogers and Brier, 2010; Zalucki et al., 1986). Chauhan et al. (1992) demonstrated that when whole pigeonpea pods were physically removed, the plants compensated better than when pods were damaged using methods more closely resembling herbivore feeding (i.e. pod clipping and stapling), suggesting that when damaged pods remain attached to a plant, compensation may be inhibited compared with when pods are completely removed.

Given the lack of accurate economic thresholds for *H. armigera* in pigeonpea, the paucity of quality data linking *H. armigera* feeding to yield loss in pigeonpea, and the uncertainty around appropriate techniques to generate such data (Volp et al., 2025), we investigated i) how crop stage affects pigeonpea tolerance to *H. armigera* feeding, and ii) if simulated herbivory is an appropriate substitute for *H. armigera* herbivory in such studies.

2. Methods

2.1. Plants

We conducted two experiments using a single short-duration determinate pigeonpea cultivar (ICPL 86012). In both experiments plants were grown under semi-field conditions in a growth tunnel (6m × 12m) at the Queensland Department of Primary Industries (QDPI) in Toowoomba, Australia (−27.534137, 151.929201). The growth tunnel was

Table 1
Experimental studies conducted on pigeonpea examining yield response to physical damage to plant reproductive structures (flowers and pods). Although these studies are highly relevant to *H. armigera* threshold development, they have all been conducted by plant physiologists examining yield formation and source-sink relationships in pigeonpea. Although there is a plethora of 'open field screening' studies that report on *H. armigera* infestations and yield loss in pigeonpea (Volp et al., 2025), we were unable to find any studies that quantified yield response to controlled *H. armigera* populations without other pest's present and in conjunction with pest-free control treatments.

Study	Damage method	Damage level	Damage timing	Experiment type	Yield response
Sheldrake et al. (1979)	Physical flower and pod removal.	100%	Commencing at flowering for up to 5 weeks post-flowering.	Field experiments.	No yield loss across most cultivars and experiments (except for a single determinate cultivar in one out of two seasons).
Tayo (1980)	Physical pod removal.	100%	After 1-, 2-, or 3-weeks post-flowering.	Pot and field experiments.	Pod removal 1- and 2-weeks post-flowering resulted in compensation or over-compensation. Pod removal 3 weeks after flowering caused yield loss.
Pandey and Singh (1981)	Physical flower removal.	Pot experiment – 30% and 60%. Field experiment – 40% and 80%.	Commencing at flowering, conducted at 3d intervals until maturity.	Pot and field experiments.	No significant yield loss from the flower removal treatments in the glasshouse experiment or the field experiment
Thirathon (1986)	Physical pod removal.	All pods removed except for either 5, 10, 15, or 20 pods retained per plant.	Established 2 weeks post-flowering and maintained for either 2 or 4 weeks.	Field experiment.	Plants could not compensate for pod removal – seed yields highly correlated to number of pods retained on plants.
Chauhan et al. (1992)	Physical removal of pods, pod clipping (cutting the distal half of each pod) and pod stapling (inserting metal staples into central locules of pods).	50% and 100% in manual removal treatments. 100% of pods damaged in the clipping and stapling treatments.	Treatments imposed once at 10d post 50% flowering, then again 10d later.	Field experiments.	Pod clipping/stapling had a more severe impact on yield than pod removal. In the drier season, damaged treatments had a limited effect. But in the wetter season, damage had a strong effect.
Lopez et al. (1994)	Physical flower and pod removal.	All nodes damaged underneath the top 3 nodes.	At flowering and podfill or just podfill.	Field experiment.	Seed yield of top 3 nodes was increased by damage at flowering and podfill (overcompensation), but not podfill only (compensation).

covered in a screen mesh that reduces incoming photosynthetically active solar radiation by approximately 30% (quantified with a Spectrum™ Solar Electric Quantum Meter).

Pigeonpea seeds were inoculated with commercial Group J rhizobium inoculant (NoduleN™, New Edge Microbials) and hand-planted in 50 cm wide rows at a density of 25 seeds per m^2. Two weeks after seedling emergence, plants were thinned to a density of 20 plants per m^2. Experiment 1 was planted on 16/12/20 and Experiment 2 was planted on 20/12/22. In both experiments the growth tunnel was irrigated with trickle tape immediately after planting to promote germination and seedling establishment. After germination, plants were irrigated as required to ensure that they did not show any visual symptoms of drought stress (e.g. wilting or leaf drop).

2.2. Insects

Helicoverpa armigera larvae used in experiments were obtained from a QDPI laboratory culture. The culture was established from insects collected from various field crops from South-East Queensland in 2020 and the colony was regularly supplemented with field-collected insects to minimise inbreeding.

Moths were kept in 5-litre plastic buckets and supplied with 10% sucrose solution using a cotton wick in 70 mL plastic containers. An 18 cm hole was cut in the bucket lid and the edges of the lid were used to secure a nappy liner (bamboo rayon) which was used as an oviposition substrate. Eggs were removed daily, washed in 1% sodium hypochlorite solution, and collected onto filter paper using vacuum filtration. Filter paper was allowed to air dry and then placed in Petri dishes (90 mm diameter), which were sealed with parafilm, until neonates hatched. Neonate larvae were placed in groups onto soybean flour-based artificial diet (ingredients provided in (Volp et al., 2023)), in 500 mL rectangular plastic containers. Upon reaching the third instar, larvae were transferred to fresh diet in 32-well plastic trays (12 mL per well) until they developed to pupae. Pupae were washed in 1% sodium hypochlorite, air-dried, and placed in 500 mL containers until eclosion. The colony was maintained in a controlled temperature room at 25 ± 2 °C under 12:12 L:D. All experiments used diet-reared fourth instar larvae.

2.3. Herbivory x phenological stage treatments

In both experiments, plants were exposed to one of two herbivory treatments (feeding by fourth instar *H. armigera* larvae or simulated herbivory) and compared with an undamaged control. Herbivory treatments were enacted at one of two distinct phenological stages, peak flowering and late podding. Plants in the flowering treatment were infested one week after crops reached 50% flowering, which was 68 days after sowing (DAS) in Experiment 1 and 77 DAS in Experiment 2. Similarly, plants in the podding treatment were exposed to the treatments at 90 DAS in Experiment 1 and 110 DAS in Experiment 2. Single plants were used for a replicate and there were at least 2 buffer plants between experimental plants within a row. Each treatment (injury type x crop stage) was replicated 18 times in Experiment 1 and 20 times in Experiment 2.

In the treatments and the undamaged control, the top 8 nodes of a pigeonpea plant were covered with organza mesh bags (28 cm × 13 cm). In the determinate cultivar used (ICPL 86012) most of the reproductive structures are produced on the top 8 nodes of the plant and were therefore contained within the bags. For the *H. armigera* herbivory treatment, a single 4th instar *H. armigera* larva (previously starved for 4h and then weighed on a microbalance (HR-250AZ™; A&D)) was placed in the bag and allowed to feed *ad libitum*.

To ensure equal exposure periods of *H. armigera* larval feeding across crop stages and experiments, exposure time was controlled using degree days (°Cd). For each treatment and the undamaged control, bags were left on the plants for approximately 60°Cd. We calculated degree days using 11.3 °C as the lower development threshold for *H. armigera* larvae (Jallow and Matsumura, 2001). Temperature was measured with a TinyTag™ within a Stevenson screen placed at canopy height in the growth tunnel. When 60°Cd was reached, larvae were removed, their stage of development was recorded, and they were returned to the laboratory where they were re-weighed. Larval relative growth rates (RGR) were calculated using Equation (1):

$$RGR = \frac{\ln(wt_1) - \ln(wt_0)}{t_1 - t_0} \tag{1}$$

Where, wt_0 = larval weight after 4h starvation, wt_1 = larval weight at the end of the assay, t_0 = beginning of the experiment, and t_1 = end of the experiment measured in °Cd.

While removing larvae from plants, we examined the racemes within the cage for herbivory and recorded the number of damaged reproductive structures (bud initials, buds, flowers, and pods). We observed that from the fourth instar onwards, *H. armigera* larvae typically chew large holes in floral structures (bud initials, buds, and flowers), usually attacking the petals but ignoring the calyxes. When larvae feed on small pods, they either eat the entire structure (leaving the base of the pod and the calyx), or chew holes in expanding pods. When larvae feed on large pods, they chew a single hole through the pod wall and feed on the internal seed. We recorded the numbers of damaged structures and the different types of damage. For each structure x type of damage combination, we calculated the mean number structures damaged and those calculated values were then used as the damage level for the simulated herbivory treatment at that crop stage (Table 2).

As the level of damage from the *H. armigera* treatment had to be recorded so that a similar damaged could be inflicted in the simulated treatment, simulated herbivory was inflicted 60°Cd (4 to 9 calendar days, depending on weather conditions) later than the actual herbivory treatment (i.e. at the end of the bagged period). Pigeonpea plants retain buds and flowers for several days and pod development occurs over a period of weeks (Mahendraraj, 2022; Reddy, 1990), so we expected the delay of 60°Cd to have a minimal effect on plant response. Simulated herbivory was inflicted with sharp pliers (Precision oblique cutters, Toledo™). Floral structures (bud initials, buds, flowers, and spent flowers) were damaged by severing through the calyx and ovary, expanding pods were damaged by severing the base of the pod, and large pods (only present in the late podding treatment) were damaged by 'boring' into seeds with the end of the pliers.

In the *H. armigera* herbivory treatment, some replicates were removed from analyses because larvae died, disappeared, or failed to develop during the infestation period: one replicate at flowering and four at podding (Experiment 1) and five at podding (Experiment 2). One control replicate from Experiment 1 at flowering and one simulated herbivory replicate from Experiment 2 at podding were also removed due to abnormal plant growth.

In Experiment 1 cowpea aphid (*Aphis craccivora*) infested the pigeonpea plants and they were successfully controlled by releasing a predatory ladybird (*Harmonia conformis*) sourced from a local biocontrol agent supplier (Bugs for Bugs™). In Experiment 2, both *A. craccivora* and cotton aphid (*Aphis gossypii*) infested plants and *H. conformis* releases proved unsuccessful in managing the aphids. Therefore, we treated plants with primicarb (Aphidex™' 2.4g a.i./L) at 52 and 97 DAS and then with sulfoxaflor (Transform™; 0.5g a.i./L) at 98 DAS, as the second pirimicarb application was ineffective against *A. gossypii*. Insecticide applications and natural enemy releases occurred outside of the experimental periods, no other pests or natural enemies were present on experimental plants during the bagged periods, and neither insecticide used has any documented activity against lepidoptera.

2.4. Plant traits at harvest

At physiological maturity, plants were desiccated by spraying them with glyphosate (Roundup Max™; 11 mL/L) using a knapsack sprayer.

Table 2
Level of simulated herbivory inflicted per plant across experiments and treatments. Values represent the number of structures damaged. Annotations describe the types of feeding damage by *H. armigera* observed in this experiment, which was replicated in the simulated herbivory treatment.

	Crop Stage						
	Flowering (Structures damaged)					Podding (Structures damaged)	
Experiment	Bud initials[a]	Buds[b]	Flowers[b]	Small pods (entire)[c]	Small pod (holes)[d]	Seeds[e]	Pods[e]
1	1	2	4	6	1	6	4
2	0	1	8	1	2	4	3

[a] Larvae chew a single small hole in the side of the structure.
[b] Larvae feed on petals, ignoring the calyx.
[c] Larvae remove the entire pod, leaving only the pod base and calyx.
[d] Larvae create a single feeding hole though which they damage a developing seed.
[e] Larvae chew a feeding hole though the pod wall and feed on a developing seed, sometimes feeding on multiple seeds per pod.

Stems were cut at ground level and returned to the laboratory where we recorded morphometric measurements (plant height, basal stem diameter, and branch count) and yield data. We also recorded the number of pod-bearing racemes and the total number of pods on each plant. During processing, samples were divided into mainstem and branches and separated into biomass components – stems, seeds, husks and dried at 70 °C for 72h before weighing to record plant biomass. Seeds were weighed for yield and then counted with a seed counter (Contador™) for total seed counts and to calculate mean seed weight of undamaged seeds.

2.5. Statistical analysis

When examining pigeonpea at the single-plant level, there can be substantial variation in biomass and yield. We found basal stem diameter (as a proxy for plant size) is strongly correlated with single plant yield (see Results). Therefore, to control for plant size variation when analysing plant response variables (yield, yield components, and other morphological measurements) we conducted ANCOVAs using basal stem diameter as a covariate. When there were significant treatment effects, we used Fisher's protected LSD test for multiple comparisons. For the number and weight of damaged seeds we used Kruskal-Wallis tests, as data would not meet ANCOVA assumptions. For larval performance data, we examined RGRs across crop stage treatments within experiments; for Experiment 1 we used ANOVA but for Experiment 2 we used a Kruskal-Wallis test, as data did not meet ANOVA assumptions. All analyses were performed in R version 3.6.2 (R Core Team, 2019), post-hoc analyses were performed with the package 'agricolae' (De Mendiburu, 2020), and graphs were made with the package 'ggplot2' (Wickham et al., 2016).

3. Results

Pigeonpea plant size strongly influences individual plant yield, and we used basal stem diameter as a proxy for plant size. Basal stem diameter was correlated with plant yield in the controls of both Experiment 1 ($t_{33} = 10.438$; $P < 0.001$; R-squared = 0.7605) and Experiment 2 ($t_{38} = 4.408$; $P < 0.001$; R-squared = 0.3209).

3.1. Experiment 1

3.1.1. Flowering

At flowering, neither *H. armigera* herbivory nor simulated herbivory affected seed yield ($F_{2,48} = 1.605$; $P = 0.21$; Fig. 1). However, pigeonpea plants altered some of their yield components in response to herbivory (Fig. 2), including the number of pods per plant ($F_{2,48} = 6.857$; $P = 0.002$) and mean seed weight ($F_{2,48} = 6.92$; $P = 0.002$); plants in the simulated damage treatment had the fewest pods but the largest seeds.

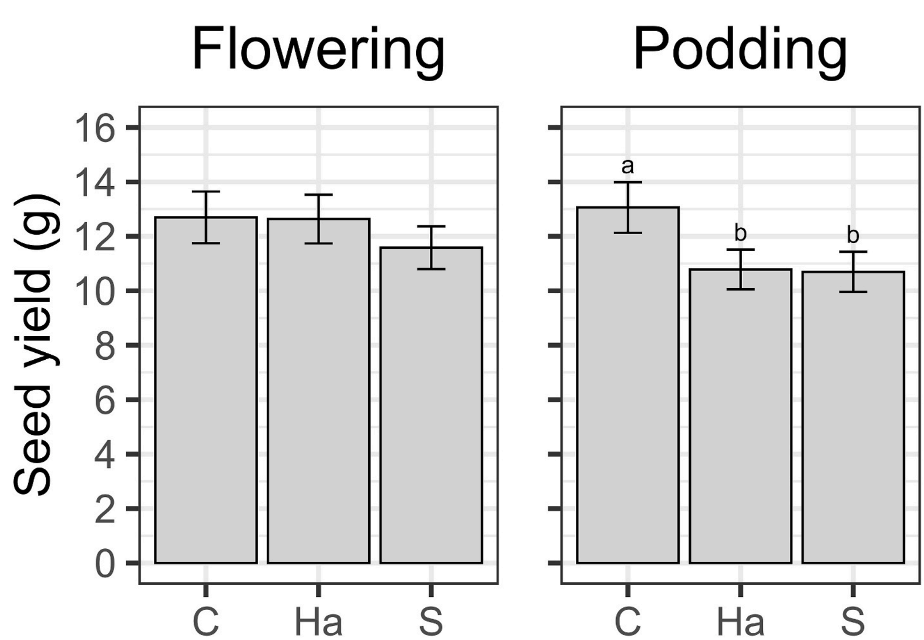

Fig. 1. Seed yield per plant when injured in the flowering and podding crop stages in Experiment 1. Bars represent means and error bars are standard errors. Different letters indicate significant differences (P < 0.05) between means (among injury treatments, within a crop stage) according to Fisher's protected LSD test. C = control (no herbivory), Ha = *H. armigera* larva (actual herbivory), and S = simulated herbivory.

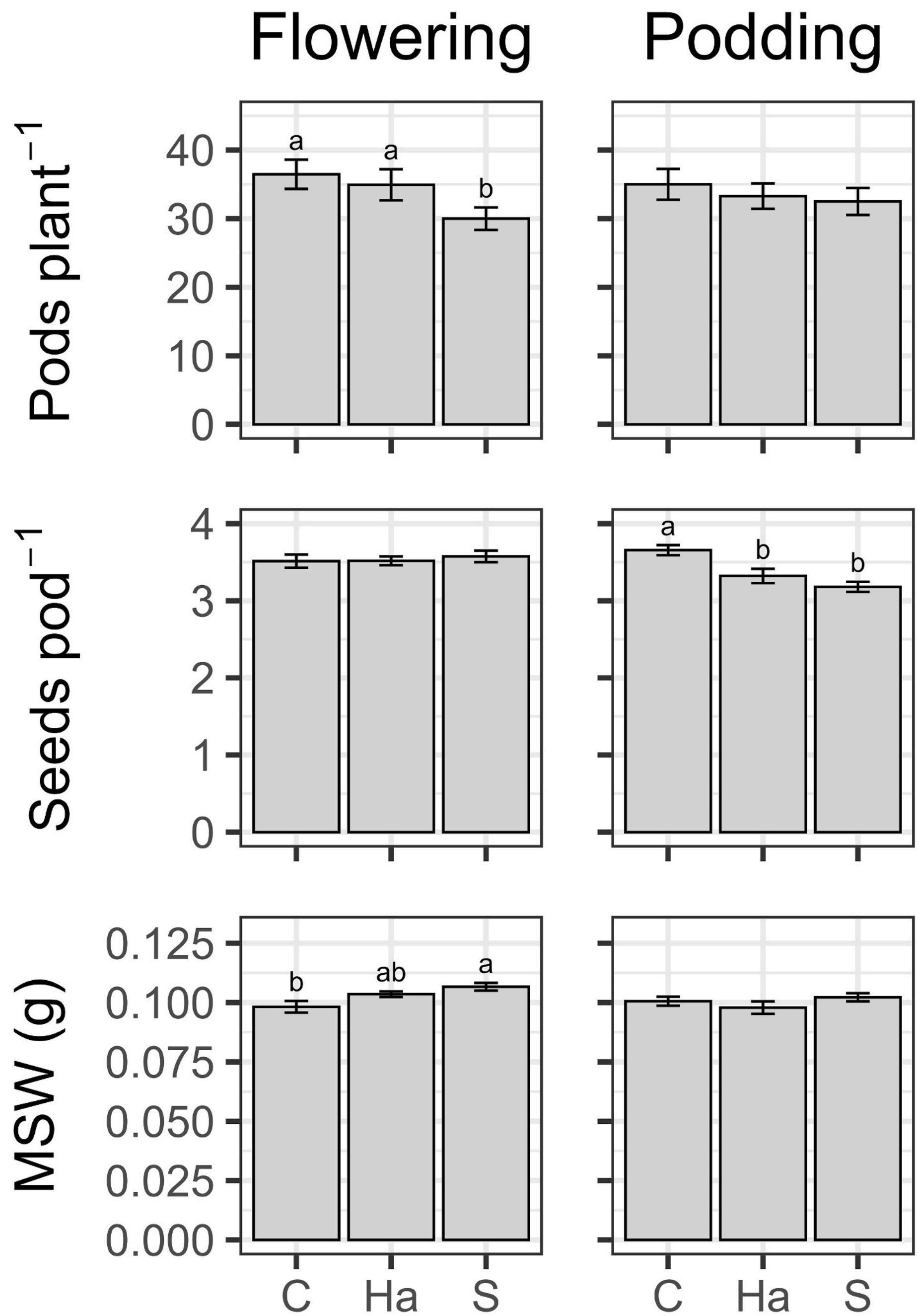

Fig. 2. Yield components (pods per plant, seeds per pod, and mean seed weight (MSW)) for plants injured in the flowering and podding crop stages in Experiment 1. Bars represent means and error bars are standard errors. Different letters indicate significant differences (P < 0.05) between means (among injury treatments, within a crop stage) according to Fisher's protected LSD test. C = control (no herbivory), Ha = *H. armigera* larva (actual herbivory), and S = simulated herbivory.

Herbivory treatments did not affect seed number per pod ($F_{2,48} = 0.232$; $P = 0.79$) and we did not find any damaged seeds from plants damaged

Table 3
Mean (±SE) yield and growth characteristics taken from plant samples at harvest maturity in Experiment 1. Seed yield and its components are displayed in Figs. 1 and 2. Within a stage, different letters indicate significant differences (P < 0.05) between means according to Fisher's protected LSD test.

	Flowering			Podding		
Plant characteristic	Control	*Helicoverpa armigera*	Simulated herbivory	Control	*Helicoverpa armigera*	Simulated herbivory
Pods per raceme	2.5 ± 7.6a	2.3 ± 9.1 ab	2.2 ± 5.1 b	2.3 ± 0.1	2.3 ± 0.1	2.2 ± 0.1
Racemes per plant	15 ± 0.9	14.8 ± 0.8	13.8 ± 0.7	15.5 ± 0.7	14.4 ± 0.5	15.0 ± 0.6
Yield allocated to mainstem (%)	43.7 ± 2.8a	38.4 ± 3.1 ab	34.5 ± 2.4b	40.8 ± 2.3	42.6 ± 3.1	38.5 ± 2.4
Harvest index	0.46 ± 0.01	0.45 ± 0.01	0.44 ± 0.004	0.45 ± 0.01 a	0.42 ± 0.01 b	0.43 ± 0.01 ab
Total plant biomass (g)	27.8 ± 2.0	28.3 ± 2.1	26.1 ± 1.7	25.8 ± 2.0 a	25.5 ± 1.4 b	24.8 ± 1.6 b
Plant height (cm)	89.9 ± 1.3	93.6 ± 1.5	90.6 ± 1.1	91.3 ± 1.5	89.2 ± 1.9	89.7 ± 1.3
Branch count	6.9 ± 0.3	7.0 ± 0.5	7.1 ± 0.4	7.4 ± 0.4	6.9 ± 0.5	7.3 ± 0.4

at flowering.

Herbivory also affected the number of pods per raceme ($F_{2,48}$ = 3.464; P = 0.039; Table 3), with simulated herbivory plants having the fewest, but the number of pod-bearing racemes per plant was not affected by the herbivory treatments ($F_{2,48}$ = 1.501; P = 0.233). Herbivory affected the proportion of yield located on the mainstem ($F_{2,48}$ = 4.784; P = 0.013; Table 3), with the simulated herbivory treatment resulting in greater allocation to branches. Finally, treatments did not differ in harvest index ($F_{2,48}$ = 0.943; P = 0.3965), total plant biomass ($F_{2,48}$ = 1.67; P = 0.198), plant height ($F_{2,48}$ = 2.991; P = 0.0597), or branch count ($F_{2,48}$ = 0.083; P = 0.921).

3.1.2. Podding

At podding, both *H. armigera* herbivory and simulated herbivory treatments decreased yield ($F_{2,46}$ = 7.62; P = 0.001; Fig. 1). Both herbivory treatments decreased the number of undamaged seeds per pod ($F_{2,46}$ = 12.35; P < 0.001), but neither treatment affected the number of pods per plant ($F_{2,46}$ = 0.879; P = 0.42) nor mean seed weight ($F_{2,46}$ = 1.37; P = 0.26). Both herbivory treatments affected damaged seed count (χ^2 = 32.53; df = 2; P < 0.001; Table A1) and the total weight of damaged seeds (χ^2 = 32.478; df = 2; P < 0.001; Table A1). Only plants subjected to the herbivory treatments had damaged seeds – 11/14 replicates in the *H. armigera* treatment and 17/18 replicates in the simulated herbivory treatment.

At the podding stage herbivory did not change the number of pods per raceme ($F_{2,46}$ = 1.076; P = 0.35) or the number of pod-bearing racemes per plant ($F_{2,46}$ = 1.359; P = 0.27; Table 3). Herbivory did not alter the distribution of yield between mainstem and branches ($F_{2,46}$ = 0.81; P = 0.45) but it did affect harvest index ($F_{2,46}$ = 3.80; P = 0.0298) and plant biomass ($F_{2,46}$ = 6.8; P = 0.0026). Herbivory treatments had no impact on plant height ($F_{2,46}$ = 0.698; P = 0.503) or the number of branches ($F_{2,46}$ = 0.55; P = 0.58).

3.2. Experiment 2

3.2.1. Flowering

In experiment 2, *H. armigera* herbivory at flowering caused yield loss but simulated herbivory did not ($F_{2,56}$ = 8.44; P < 0.001; Fig. 3). Herbivory by *H. armigera* decreased the number of pods per plant ($F_{2,56}$ = 10.72; P < 0.001; Fig. 4), but it did not affect the number of undamaged seeds per pod ($F_{2,56}$ = 1.743; P = 0.18) or the mean seed weight ($F_{2,56}$ = 1.88; P = 0.16). Damaged seeds were detected in both herbivory treatments (4/20 replicates in the *H. armigera* treatment and 2/20 in the simulated treatment), but not in the control (Table A1). There was no statistically significant impact of herbivory treatment on the count of damaged seeds (χ^2 = 4.5484; df = 2; P = 0.10) nor the weight of damaged seeds (χ^2 = 4.3547; df = 2; P = 0.11).

Herbivory at flowering decreased the number of pods per raceme ($F_{2,56}$ = 5.52; P = 0.0065; Table 4), with plants subject to *H. armigera* herbivory having the fewest. Herbivory also influenced the number of pod-bearing racemes per plant ($F_{2,56}$ = 3.51; P = 0.037), but Fisher's-LSD test did not detect a significant difference among treatment means, indicating the significant result may be a type 1 error. The intra-plant yield distribution (i.e. mainstem vs branches) was not influenced by herbivory treatments at flowering ($F_{2,56}$ = 0.538; P = 0.59), nor was harvest index ($F_{2,56}$ = 0.335; P = 0.717), plant height ($F_{2,56}$ = 0.119; P = 0.888), or number of branches ($F_{2,56}$ = 2.84; P = 0.07). Herbivory did however influence total plant biomass ($F_{2,56}$ = 12.42; P < 0.001), with *H. armigera* damaged plants having the least.

3.2.2. Podding

Herbivory at podding influenced seed yield ($F_{2,50}$ = 4.65; P = 0.014; Fig. 3), with *H. armigera* herbivory resulting in the lowest yield. Herbivory did not influence number of pods per plant ($F_{2,50}$ = 2.11; P = 0.13; Fig. 4), seeds per pod ($F_{2,50}$ = 3.11; P = 0.053), or mean seed weight ($F_{2,50}$ = 0.21; P = 0.81). At podding, damaged seed count (χ^2 = 19.67; df = 2; P < 0.001) and damaged seed weight (χ^2 = 18.397; df = 2; P < 0.001) were both influenced by injury treatment (Table A1). At harvest, damaged seeds were detected in the *H. armigera* herbivory (11/15 replicates) and simulated herbivory (9/19 replicates) treatments.

Herbivory at podding did not influence the number of pods per raceme ($F_{2,50}$ = 0.05; P = 0.86) nor the number of racemes per plant ($F_{2,50}$ = 0.873; P = 0.42). Herbivory did not affect the proportion of yield allocated to the mainstem ($F_{2,50}$ = 2.992; P = 0.059) but *H. armigera* herbivory did decrease harvest index ($F_{2,50}$ = 4.23; P = 0.020) and total plant biomass ($F_{2,50}$ = 5.27; P = 0.00838). Herbivory treatments did not affect plant height ($F_{2,50}$ = 2.009, P = 0.145), but there was a difference among treatments in the number of side branches

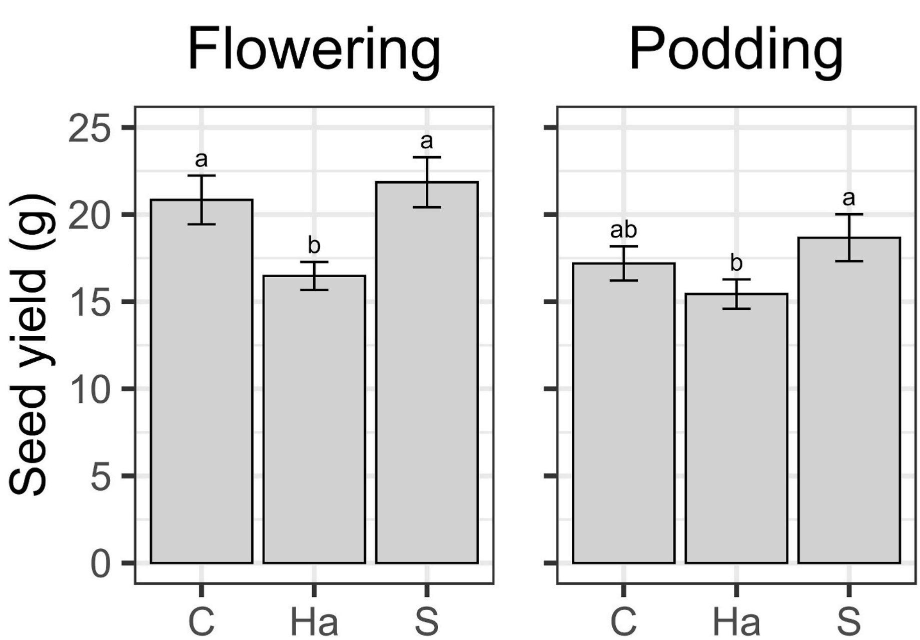

Fig. 3. Seed yield per plant when injured in the flowering and podding crop stages in Experiment 2. Bars represent means and error bars are standard errors. Different letters indicate significant differences (P < 0.05) between means (among injury treatments, within a crop stage) according to Fisher's protected LSD test. C = control (no herbivory), Ha = *H. armigera* larva (actual herbivory), and S = simulated herbivory.

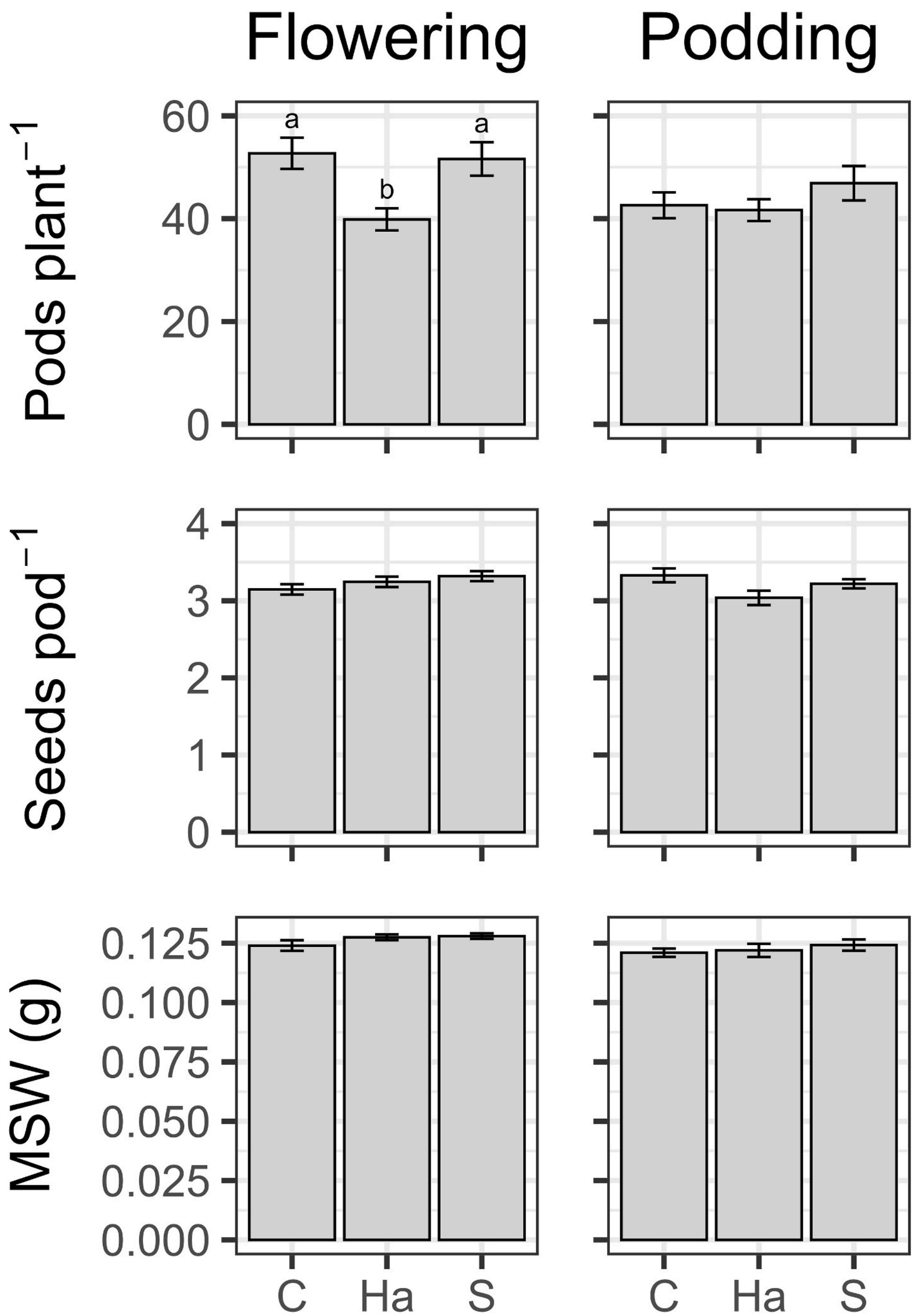

Fig. 4. Yield components (pods per plant, seeds per pod, and mean seed weight (MSW)) for plants injured in the flowering and podding crop stages in Experiment 2. Bars represent means and error bars are standard errors. Different letters indicate significant differences (P < 0.05) between means (among injury treatments, within a crop stage) according to Fisher's protected LSD test. C = control (no herbivory), Ha = *H. armigera* larva (actual herbivory), and S = simulated herbivory.

Table 4
Mean (±SE) yield and growth characteristics taken from plant samples at harvest maturity in Experiment 2. Seed yield and its components are displayed in Figs. 3 and 4. Different letters indicate significant differences (P < 0.05) among means (among injury treatments, within a crop stage) according to Fisher's protected LSD test.

Plant characteristic	Flowering			Podding		
	Control	*Helicoverpa armigera*	Simulated herbivory	Control	*Helicoverpa armigera*	Simulated herbivory
Pods per raceme	3.48 ± 0.12a	2.97 ± 0.09b	3.30 ± 0.12 ab	3.16 ± 0.15	3.23 ± 0.16	3.26 ± 0.14
Pod-bearing racemes per plant	15.4 ± 0.9	13.4 ± 0.5	15.75 ± 0.896	13.55 ± 0.57	13.2 ± 0.78	14.37 ± 0.84
Yield allocated to mainstem (%)	48.4 ± 2.4	47.2 ± 2.8	44.8 ± 2.4	50.65 ± 2.8	53.87 ± 3.39	45.2 ± 2.5
Harvest index	0.437 ± 0.006	0.431 ± 0.005	0.4345 ± 0.005	0.439 ± 0.005a	0.415 ± 0.008b	0.427 ± 0.004 ab
Total plant biomass (g)	47.195 ± 2.6a	38.30 ± 1.74b	50.21 ± 3.098a	39.16 ± 2.13 ab	37.57 ± 2.07b	43.77 ± 2.98a
Plant height (cm)	101.4 ± 1.75	101.2 ± 1.4	100.3 ± 1.91	97.45 ± 1.56	102.00 ± 2.04	100.37 ± 1.30
Branch count	5.3 ± 0.34	4.35 ± 0.31	5.65 ± 0.52	5.15 ± 0.34 ab	4.13 ± 0.27b	5.42 ± 0.33a

on plants ($F_{2,50} = 4.485$; $P = 0.016$).

3.3. Larval performance

In Experiment 1, larval RGR was higher in the flowering treatment than the podding treatment ($F_{1,28} = 20.73$; $P < 0.001$; Fig. 5), and all larvae in both treatments reached 6th instar. In Experiment 2 there was no difference in larval RGR between crop stages ($\chi^2 = 0.071111$; df = 1; P = 0.79; Fig. 5). All larvae at the flowering stage reached 6th instar, but at the podding stage only 73% of larvae reached 6th instar, the remainder only developed to the fifth instar.

4. Discussion

In this study we examined the effect of simulated and actual *H. armigera* herbivory on pigeonpea plants at different crop stages (flowering and podding). Across two experiments we obtained different results. In the first experiment, herbivory treatments decreased plant yield at podding, but plants tolerated both herbivory treatments at flowering. In the second experiment, although *H. armigera* herbivory caused yield loss at flowering, the simulated herbivory treatment did not. Then at podding *H. armigera* herbivory decreased yield compared to the simulated herbivory treatment but not compared to the control.

Pigeonpea plants typically tolerate actual and simulated herbivory occurring at flowering, but this is not always the case given the differences across our experiments and treatments. It has been long demonstrated that pigeonpea can tolerate substantial physical removal of flowers (Table 1), but our study documents this in response to actual *H. armigera* feeding. Our results indicate that compensation does not always occur in response to floral feeding. In Experiment 2 flowering plants did not compensate for *H. armigera* feeding although they did compensate in response to the simulated herbivory treatment. The reason for the lack of compensation in flowering plants in the second experiment is unclear, but it may be related to the prevailing abiotic crop growing environment. Alternatively, previous aphid feeding may have influenced the pigeonpea tolerance response by either depleting resources available for compensation and/or by inducing defence responses in the affected plants that negatively impacted subsequent *H. armigera* feeding (Quijano-Medina et al., 2023) such that the damage was insufficient to induce a compensation response. Interactions amongst feeding guilds and their effects on plant response are likely to further complicate the development of economic thresholds for pigeonpea.

At podding, most herbivory treatments caused yield loss and all herbivory treatments resulted in damaged (i.e. unmarketable) seeds. When herbivory occurs at this later crop stage, plants are unable to compensate in response as the plant's pod number has been set and seeds are filling with assimilates. Plants cannot set more pods, they cannot shift yield to branches, and they are limited in their ability to increase seed size. However, there is some potential for plants to re-flower, to set more pods, and this has been observed but only in response to severe damage (i.e. 100% of flowers and pods damaged; T.Volp, unpublished data). We initially expected that plants at podding may have been able to compensate for pest damage by increasing the mean seed weight of undamaged seeds, but our data did not support this.

To more effectively incorporate plant tolerance into pest management applications, we must gain a mechanistic understanding of how crop plants respond to pest herbivory. The general mechanisms by which plants tolerate herbivory are well known (Rosenthal and Kotanen, 1994; Strauss and Agrawal, 1999; Tiffin, 2000). In our study pigeonpea plants tolerated damage at flowering by compensatory growth (increasing seed size and branching), and redirection of assimilates (by redirecting yield to branches). Other tolerance mechanisms that are known from other systems include delaying development, increasing photosynthesis, and plant architecture pre-herbivory (Mitchell et al., 2016; Strauss and Agrawal, 1999; Tiffin, 2000). Our experiments were not designed to test for these mechanisms, although changes in plant photosynthesis in response to simulated flower and pod herbivory has been recorded for pigeonpea (Grover et al., 1985).

The key trait of importance for understanding tolerance in pigeonpea is the number of pods per plant, and, to a lesser extent, seed weight. Yield decreases caused by *H. armigera* herbivory at flowering are primarily a result of the pest decreasing the number of pods per plant. At podding, yield decreases are due to direct feeding damage to seeds resulting in damaged grain (i.e. decreasing undamaged seed weight). A plant's seed yield is a function of the three yield components displayed in

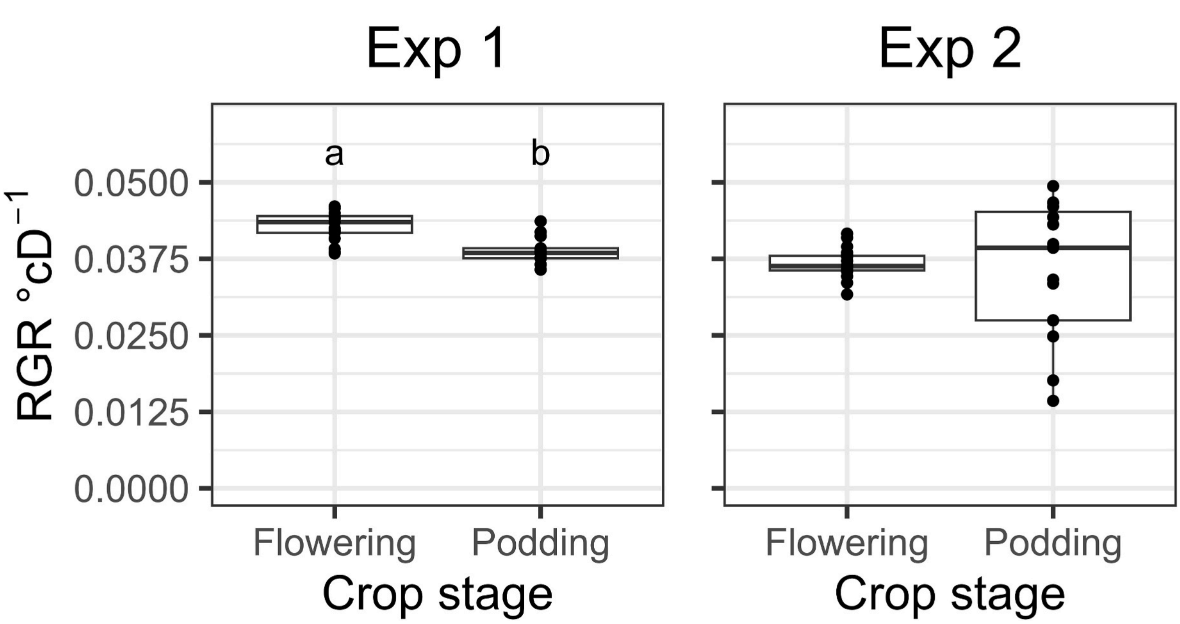

Fig. 5. Relative growth rates across both experiments for *H. armigera* larvae placed on pigeonpea plants at flowering and podding, consisting of the actual herbivory treatment. Black lines represent the mean, boxes are the interquartile ranges, and dots are the data from individual larvae. Different letters indicate significant differences (P < 0.05) between means (between crop stages, within an experiment) according to Fisher's protected LSD test. Exp 1 = experiment 1 and Exp 2 = experiment 2.

Figs. 2 and 4: pods per plant, seeds per pod, and mean seed weight. In grain legume crops, seeds per pod and mean seed weight are traits under strong genetic control (Sadras, 2007) and our results demonstrate that there is only minor variation in these traits in response to herbivory treatments. Therefore, if grain legume crops are to tolerate herbivory they must mostly rely on the 'lever' of pods per plant, a highly plastic trait, which can shift with the tolerance mechanisms available to plants (compensatory growth and redirection of assimilates, in the present study).

It is important to note that across experiments we obtained differences in plant responses, as has been observed in other studies on pigeonpea. Chauhan et al. (1992) noted that pigeonpea compensated for simulated damage to pods in a low-yielding (dry season) experiment, but not in a high-yielding (wet season) experiment. This phenomenon was replicated in our study, whereby in Experiment 1 (lower yielding) both *H. armigera* and simulated herbivory at flowering did not cause yield loss. But in the higher yielding Experiment 2 (where control plants yielded almost double of those in Experiment 1) *H. armigera* feeding caused yield loss. Perhaps under high yield potential scenarios (e.g. wetter seasons) pigeonpea plants are more sink-limited than under drier conditions, when yield is constrained by the amount of photosynthates they can produce. Therefore, damage to sinks under sink-limited conditions would have greater impact on yield. Future work on plant response should place a greater emphasis on understanding the environmental controls on tolerance in flowering pigeonpea.

Our study demonstrates that under some circumstances simulated herbivory is a good mimic for *H. armigera* feeding, but in others it is not. Given that the outcomes of actual *H. armigera* feeding damage was only accurately replicated by simulated herbivory in one of our two experiments, our results illustrate the importance of multi-season experiments. Going forward we suggest that research on pigeonpea yield responses to pest damage still incorporates both techniques (i.e. simulated damage and real pest feeding) where possible but that if only one method is used then it should be carefully selected based on the aims of the specific study. There are some contexts where simulated herbivory is clearly more useful – e.g. screening a large number of pigeonpea cultivars for their tolerance capacity or to understand what phenotypic traits confer tolerance. However, for threshold development experiments, our results indicate that using *H. armigera* larvae is essential.

Results of our larval performance tests suggest that *H. armigera* larvae perform better on flowering plants than on podding plants. Although early instar *H. armigera* larvae tend to avoid pods and prefer to feed on flowers, we expected that larvae in our experiments would feed successfully on pods, as we used fourth instars, the stage by which larvae can feed on large pods 'cost-free' (Volp et al., 2024a). At flowering, larvae have access to a range of reproductive structures (bud initials, buds, flowers, small pods) compared to at podding (large pods only), so dietary mixing at flowering may have benefitted the performance of larvae at this phenological stage. In our study larvae were fed on artificial diet before being placed on plants, and this 'diet switch' may have rendered larvae less capable of pod feeding. Finally, at the podding stage tested in our experiments the pods were approaching maturity and as pods mature and harden, they likely become less palatable to larvae and this may have influenced the level of damage inflicted by larvae.

Substantial research is still required to properly understand and develop mechanistic models for how pigeonpea plants respond to *H. armigera* herbivory. This study shows that late podding pigeonpea cannot tolerate herbivory by *H. armigera* as the larvae directly damage pigeonpea seed. Tolerance of *H. armigera* herbivory at flowering represents a potential tactic to incorporate into pest management strategies, but considerable work is required to understand how tolerance is influenced by the crop's growing environment. Additionally, work is required to investigate how tolerance is influenced by pigeonpea genotype, any relevant crop management strategies, and how all these factors interact. The outcomes of this study are directly applicable to threshold development – as there are currently no empirical economic thresholds for *H. armigera* in pigeonpea. The experimental work required to develop pest thresholds for pigeonpea crops at the podding stage appears straightforward due to direct nature of yield loss from pest feeding. However, developing pest thresholds for flowering pigeonpea appears to represent a significant research undertaking.

CRediT authorship contribution statement

Trevor M. Volp: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Myron P. Zalucki:** Writing – review & editing, Methodology, Conceptualization. **Michael J. Furlong:** Writing – review & editing, Methodology, Conceptualization.

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Declaration of competing interest

The authors declare they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2026.107567.

Data availability

Data will be made available on request.

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