Crop Protection 155 (2022) 105936

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Simulated soybean pod and flower injuries and economic thresholds for *Spodoptera eridania* (Lepidoptera: Noctuidae) management decisions

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A R T I C L E I N F O

Keywords:
Injury-damage relation
Cry1Ac
Pest tolerance
Economic injury level
Florivory
Armyworm

A B S T R A C T

Although defoliation economic thresholds (ETs) in soybean are well-established, there are concerns about *Spodoptera* spp. and *Helicoverpa* spp. damaging soybean pods and flowers. Moreover, *S. eridania* is the most common species of its genus feeding on soybean in Brazil. On this account, we quantified the feeding of *S. eridania* larvae on leaves, pods and flowers of BRS388RR and BRS1001IPRO cultivars in the laboratory. In the field, trials were conducted testing artificial injuries for 3 consecutive crop seasons. Results indicated that third-instar *S. eridania* can grow when feeding on pods (from plants at R3 stage). However, the larval stage is lengthened (27.4 days) compared with larvae feeding on leaves (19.9 days). Tests with older pods (stages R5.1 and R5.5) resulted in 100% mortality. For neonates, 100% mortality was observed for both pod and pod + flower feeding. Also, soybean tolerated all tested injuries in the field, including 25% perforated pods with 16.7% defoliation or 100% flower removal, without yield reduction. Overall, *S. eridania* is a leaf feeder. Older larvae (from 3rd instar) can feed on pods at an early plant reproductive stage (R3 stage). However, soybean tolerance to pod and flower injuries is high, even when occurring in combination with defoliation. Therefore, an ET of 25% of injured pods or a general defoliation ET of 15% at the reproductive stage is safe. Flower removal at R2 reproductive stage by *S. eridania* should not trigger management because no reduction in yield was observed even when 100% of flowers were removed.

1. Introduction

Soybean [*Glycine max* (L.) Merrill] is one of the most economically important crops worldwide, representing approximately half of the total production of vegetable oils and proteins (Oerke, 2006). Unfortunately, soybean yield can be drastically reduced by pests. Among defoliators, larvae in the genus *Spodoptera* (Guenée, 1852), especially *Spodoptera eridania* (Stoll, 1782) (Lepidoptera: Noctuidae) are important because of their high abundance in soybean fields and their injury potential on both leaves and plant reproductive structures (Bueno et al., 2011; Santos et al., 2010). *Spodoptera eridania* and *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) have been recently reported as invasive species in Africa (Goergen et al., 2016; Tepa-Yotto et al., 2021), and *S. frugiperda* has also appeared in India (Ganiger et al., 2018) and Australia (EPPO, 2020) and was categorized as A1 (quarantine pest) in Europe (EPPO, 2019). Furthermore, *S. eridania* is one of the most abundant generalist pests in Central America, South America and the Caribbean, feeding on more than two hundred plant species (Pogue, 2002; Capinera, 2005; Tay and Gordon, 2019; Parra et al., 2021).

Since 2013, commercial *Bt* soybean traits expressing toxins of the bacteria *Bacillus thuringiensis* have been adopted, first in Brazil and now worldwide, with the exception of the United States (ISAAA, 2021). Despite the Cry1Ac toxin being highly active against most Lepidoptera, *Spodoptera* spp. and *Helicoverpa zea* (Boddie, 1850) (Lepidoptera: Noctuidae) are naturally tolerant to it (Bernardi et al., 2014). Because of the high adoption of *Bt* soybean [more than 30 million hectares in Brazil alone in the 2020/21 crop season (Spark, 2021)] and the consequent reduction of insecticide sprays against lepidopteran larvae, *Spodoptera*

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https://doi.org/10.1016/j.cropro.2022.105936
Received 29 November 2021; Received in revised form 27 January 2022; Accepted 30 January 2022
Available online 1 February 2022

spp. occurrence in soybean fields has become more common (Bueno et al., 2018). Consequently, growers are increasingly concerned not only about *Helicoverpa* spp. but also about *Spodoptera* spp. damaging soybean pods and flowers (Bueno et al., 2018; Conte et al., 2019). It is well documented that *H. zea* feed on all soybean host plant tissue types (Reisig et al., 2017), but reports on *Spodoptera* spp. capacity of feeding on pods or flowers are less frequent and based only upon growers' perception (Bueno et al., 2018; Conte et al., 2019). Also, there is only a small number of studies on soybean tolerance to pod and flower injuries (Reisig et al., 2017). The majority of studies in the literature on the impact of pod loss on soybean yield has been conducted using later maturing determinate cultivars (McAlister and Krober, 1958; Hicks and Pendleton, 1969; Kincade et al., 1971; Smith and Bass, 1972; McPherson and Moss, 1989). Only a few studies used early maturing indeterminate cultivars (Adams et al., 2015), which are the most common cultivars in Brazil (Bueno et al., 2021) and the USA (Heatherly, 1999).

Furthermore, despite *Spodoptera* spp. having been extensively evaluated in the laboratory (Bortoli et al., 2012; Montezano et al., 2013, 2014, 2015, 2019; Silva et al., 2017), information on their biological and nutritional parameters feeding on soybean flowers and pods is scarce. In addition, to our knowledge, soybean tolerance to injury on both pods and flowers has been rarely investigated. However, such information is crucial for recommendations for integrated pest management (IPM) decisions (Higley eand Pedigo, 1996). Soybean-IPM is based on the premise that not all insect species demand management action, and that some levels of infestation and injury are tolerated by soybean plants, resulting in no economic yield loss (Bueno et al., 2013, 2021). Therefore, IPM programs are based on the concepts of economic injury level (EIL) as the lowest pest density that causes economic damage, and economic threshold (ET) as the appropriate time when control should begin to prevent pest population density from causing injury that reaches the EIL (Stern et al., 1959; Pedigo et al., 1986).

The recommended ETs for lepidopteran larvae which feed exclusively on soybean leaves is well-established despite slight differences around the world. In Brazil, management measures are initiated either when 20 large (≥1.5 cm) larvae are counted per sample-cloth (1-m-soybean line), or when 30% defoliation in the vegetative stage or 15% defoliation in the reproductive state is observed (Batistela et al., 2012; Hayashida et al., 2021). In the USA, a typical ET is reached at a defoliation level of 35% in the vegetative stage or 20% in the reproductive stage (Andrews et al., 2009). The capacity of *Spodoptera* spp. to injure soybean pods and flowers was never evaluated for the establishment of these ETs. Therefore, it is of theoretical and practical interest to study *Spodoptera* spp. damage potential on soybean flowers and pods as well as to determine plant tolerance to these kind of injuries in conjunction with damage to leaves in order to establish an appropriate ET for Soybean-IPM decisions. To this end, not only did this study seek to determine the impact of pod and flower injuries on soybean yield but also *S. eridania* capacity of triggering those injuries. This species was chosen because it is the most common species of the *Spodoptera* spp. and *Helicoverpa* spp. complex found on soybean in Brazil. Also, the current ET for *H. zea* takes into account its larvae capacity of damaging pods and flowers (Herbert et al., 2003), which is not the case for *Spodoptera* spp., to our knowledge.

2. Material and methods

Five trials were carried out between 2016 and 2019. Two of these trials were carried out under controlled conditions [25 ± 2 °C, relative humidity (RH) of 70 ± 10% and photoperiod of 14/10h (L/D)], evaluating *S. eridania* feeding on leaves, pods and flowers of soybean cultivars BRS 388 RR and BRS 1001 IPRO at the R3, R5.1 and R5.5 stages (Fehr et al., 1971) and quantifying the impact on biological traits of the larvae. Three plant reproductive growth stages [young (R3), middle (R5.1) and mature development (R5.5)] were used in order to represent pod tissues better (R3) or less (R5.1 and R5.5) suitable for insect feeding (Edwards and Singh, 2006), providing a better understanding of the insect injury capacity. Also, three additional experiments were carried out under field conditions during three consecutive soybean seasons (2016/2017, 2017/2018 and 2018/2019) at Embrapa, in the municipality of Londrina (S 23° 11′ 11.7"; WO 51° 10′ 46.1″) in the northern state of Paraná (PR), Brazil, studying soybean tolerance to simulated plant injury, as briefly described in the following.

2.1. *Spodoptera eridania* laboratory feeding studies

One experiment was carried out in 2016 using the BRS 388 RR cultivar (maturity group 6.4 and indeterminate growth habit; Roundup Ready cultivar) and a second experiment was carried out in 2017 using BRS 1001 IPRO cultivar (maturity group 6.2 and indeterminate growth habit; *Bt* soybean cultivar). *Spodoptera eridania* larvae used in both experiments were obtained from insect colonies kept at Embrapa Soybean where insects were field collected in soybean and kept for one year in the colony according to previously described methodology (Silva et al., 2017).

In the first experiment (2016) *S. eridania* egg masses were individually arranged in Gerbox cases (crystal polystyrene boxes 11 × 11 × 4 cm) lined with filter paper moistened with distilled water. After hatching, all larvae received the same diet (pods + leaves from soybean cultivar BRS 388 RR at R3 developmental stage). Food was replaced daily. After 4 days of hatching, only larvae recently molted to the 3rd instar were selected and individualized in 50 ml plastic cups (Copaza®, Içara, SC, Brazil), covered with absorbent paper (Scott®, São Paulo, SP, Brazil) and cardboard lids. This procedure assured that first and second instars lasted 4 days altogether. They were then fed daily according to each treatment. Treatments were: 1) Leaves from plants at R3 development stage; 2) Leaves + pods from plants at R3 development stage; 3) Pods from plants at R3 development stage; 4) Leaves from plants at R5.1 development stage; 5) Leaves + pods from plants at R5.1 development stage; 6) Pods from plants at R5.1 development stage; 7) Leaves from plants at R5.5 development stage; 8) Leaves + pods from plants at R5.5 development stage; 9) Pods from plants at R5.5 development stage. Each treatment had 3 replicates. Each replicate contained 15 individual larvae. Replicate results were the average value of the 15 individualized larvae. The evaluated parameters were the time required for larvae to pupate [larval development time (days)] and mortality (%).

In 2017, the trial was carried out again with the same procedures. However, this time larvae were individualized at hatching and tested treatments were: 1) Leaves from plants at R3 development stage; 2) Leaves + pods from plants at R3 development stage; 3) Pods from plants at R3 development stage; 4) Leaves + flowers from plants at R3 development stage; 5) Leaves + pods + flowers from plants at R3 development stage; 6) Pods + flowers from plants at R3 development stage; 7) Artificial diet (Greene et al., 1976). Each treatment had 3 replicates. Each replicate was comprised of 20 individual larvae. Replicate results were the average value of the 20 individualized larvae. The total larval development time (days) and mortality (%) were evaluated.

In order to have enough soybean plant tissues in different reproductive stages for feeding the larvae, plants were cultivated in a greenhouse. Periodically, soybean was sowed (5L plastic pots, with sterilized soil and 5 seeds.pot^{-1}) and each plant structure was collected at each reproductive stage (R3, R5.1 and R5.5) (Fehr et al., 1971). On a daily basis, plant tissues were removed from each plant. Leaf positioning varied from the first to the third completely expanded leaf, depending on leaf availability. Pods and flowers were collected from the upper half of the plant canopy. After the collection of plant tissues (leaves, pods and flowers), they were cleaned by immersion in sodium hypochlorite (4%) and rinsed in distilled water for three to 5 s. Water was removed with paper towels before offering the plant tissues to the experimental insects. The artificial diet (8 g per replicate) was replaced daily to avoid dehydration, and larval survival was recorded daily.

2.2. Artificial injury on soybean tissues in field studies

Besides analyzing *S. eridania* feeding capacity on pods and flowers, it was also important to quantify plant tolerance to pod and flower injury. Therefore, field trials were sowed on October 17, 2016 (2016–2017 crop season) with soybean cultivar BRS 388 RR, on October 24, 2017 (2017–2018 crop season) with soybean cultivar BRS 388 RR, and on October 22, 2018 (2018–2019 crop season) with soybean cultivar BRS 1001 IPRO. A row spacing of 0.45 m was used for both cultivars, and 15.1 and 14.5 seeds per linear meter were used for BRS 388RR and BRS 1001 IPRO, respectively.

During the 2016–2017 crop season, the study was carried out in a randomized block experimental design, testing six pod injury levels (0%, 5%, 10%, 15%, 20%, and 25%), with five replicates (4 rows of 6 m each). At the R4 development stage (Fehr et al., 1971) the total number of pods was recorded for the two central rows of each replicate, and then, the number of pods to be injured was calculated according to each treatment. Pods to be injured were randomly selected with equal distribution of injuries on top, middle and bottom pods of the plants. Injury was imposed at the most apical grain of the selected pods by completely perforating the pod and grain using a 4 mm hand drill (Bosch®, Belo Horizonte, MG, Brazil). When Lepidoptera larvae feed on smaller immature soybean pods, complete abscission of the damaged pod is commonly observed, which is not the case when they feed on fully elongated pods (Adams et al., 2015). Thus, injuring plants at R4 reproductive growth stage allows us to completely damage a single seed, since it can be identified in the pods, besides observing pod abortion due to injury. In most of the previously published studies (McAlister and Krober, 1958; Kincade et al., 1971; Smith and Bass, 1972; McPherson and Moss, 1989; Adams et al., 2015) whole pods were removed while this study aimed to understand plant capacity to tolerate pod injury at early reproductive stages.

During the 2017–2018 crop season, the artificial injury study was carried out using a 3x4 factorial in randomized block experimental design with three defoliation levels (0%, 8.3%, and 16.7%) and four pod injury levels (0%, 15%, 20% and 25%) with four replicates (4 rows x 4 m each). Considering that defoliation ET for soybean at reproductive stage is 15% in Brazil (Batistela et al., 2012) and that 25% pod injury was the highest injury level tested during 2016–2017 crop season; using both values as factors in a factorial analysis, we expected to have a good understanding of pod injury and defoliation interaction in the field. As previously described, pods were damaged at the R4 development stage (Fehr et al., 1971). At the same time, artificial defoliation was carried out by manually removing 0, 1/2 or 1/4 of the central leaflet of all leaves of the plant using scissors (Gazzoni and Moscardi, 1998), corresponding to each defoliation level. This procedure was performed on all leaves of all plants of the central rows of each replicate (Batistela et al., 2012). Defoliation was carried out on the new leaves at weekly intervals until reaching R7 plant reproductive stage (Fehr et al., 1971), since an indeterminate cultivar was used (Batistela et al., 2012). Pod injury was performed only once during R4 plant reproductive stage. The single-day pod injury was adopted in order to facilitate the injury procedure since we failed to mark pods and recognize the new pods each week.

During the 2018–2019 crop season, the study was carried out in a randomized block experimental design, testing four flower removal levels (0%, 25%, 50%, and 100%), with five replicates (4 rows of 4 m each). At the R2 development stage (Fehr et al., 1971) the total number of flowers was recorded for the two central rows of each replicate, and then the number of flowers to be removed was calculated according to each treatment. Afterwards, the required number of flowers according to each treatment was manually removed using tweezers. Flower injury was randomly distributed on the plants, in equal parts on top, middle and bottom plant canopies. The R2 plant reproductive stage was used because it has the highest number of flowers (full bloom) (Fehr et al., 1971). We failed to mark plant growth in order to recognize new flowers each week, therefore injuring was performed only on one single day. Despite not exactly simulating insect feeding behavior, the study is an important contribution to understand soybean plant tolerance to this type of stress.

To prevent interference from insect defoliators, insecticides were applied weekly on the plots, using a CO_2 pressurized back sprayer (Herbicat®, Catanduva, SP, Brazil) set for a spray volume of 150 liters ha^{-1}. Additionally, herbicides and fungicides were applied when necessary [two herbicide applications between the third and sixth week after emergence of plants, and three fungicide applications at the reproductive plant stage, starting between R1 and R2 (Fehr et al., 1971), followed by additional applications at 20 to 30-day intervals]. These applications were performed equally over the total area of all treatments, including the control area where plants were not injured.

At harvest, the two central rows of each plot were manually harvested and threshed for evaluation. The weight and moisture content of each sample was recorded (moisture meter G800, Gehaka Agri, São Paulo-SP, Brazil) and productivity for 13% seed moisture was estimated. In addition to yield, the weight of 1000 grains was also recorded.

2.3. Statistical analysis

Results were submitted to exploratory analysis to verify the assumptions of normality of residuals (Shapiro and Wilk, 1965), homogeneity of treatment variance, and additivity of the model (Burr and Foster, 1972) to allow for ANOVA. When significant differences were detected, they were identified using the Tukey test at 5% probability (SAS, 2009). Pod abortion (%) was calculated as following: Pod abortion (%) = 100 –(100 x number of injured pods at harvest/number of injured pods at R4). Since pod identification with pen ink did not last until harvest, pod abortion in the control treatment was not measured.

3. Results

3.1. Spodoptera eridania laboratory feeding studies

In the first laboratory experiment, the third instar of *S. eridania* was able to feed and develop on soybean pods (from plants at R3 stage). However, the larval stage was lengthened by 37.7% (27.4 days) compared with larvae feeding on leaves (19.9 days) (Table 1). 100% mortality of larvae was observed when tested with pods from older plants (R5.1 and R5.5 stage). When larvae that fed on leaves were compared with those that fed on leaves + pods, the larval stage span were similar for all tested soybean plant stages. However, the time (days) to complete the larval stage were longer when leaves or leaves + pods were from older plant development stages compared to younger plant development stages (Table 1).

In the second experiment, in which neonates were individualized in each treatment, 100% mortality was recorded when both pods and pods + flowers were tested. When pods were offered with leaves, larval mortality was lower and similar to the artificial diet treatment (control) (Table 1).

3.2. Soybean artificial injury on plant tissues in field studies

Overall, soybean plants tolerated all tested types and intensities of injuries without yield reduction (Tables 2–4). Plant tolerance included the highest tested pod injury of 25%, during the 2016/2017 crop season (Table 2). Pod injury in combination with defoliation during crop season 2017/2018 (Table 3) and flower removal during crop season 2018/2019 (Table 4) were also tolerated without reduction of yield or weight of 1000 seeds. Tested injury intensity reached 25% of perforated pods in combination with 16.7% of defoliation at R4 plant development stage or even 100% of flower removal during R2 plant development stage, with injured plants having the same yield as uninjured plants (Tables 3 and 4).

The number of injured pods per 5.4 m^2 during crop season 2016/

Table 1
Spodoptera eridania biological characteristics (temperature of 25 ± 2 °C; relative humidity of 70 ± 10% and 14/10 h Light/Dark photoperiod).

Larvae food source (plant stage)	Experiment 1 (BRS 388 RR)[1] (Individualized in each treatment from 3rd instar)		Experiment 2 (BRS 1001 IPRO) (Individualized in each treatment from neonates)	
	Larval duration (days)	Mortality (%)	Larval duration (days)	Mortality (%)
Leaves (R3)	19.9 ± 0.1 d	56.7 ± 13.1 b	19.69 ± 1.41^{ns}	43.3 ± 8.8 b
Leaves + Pods (R3)	19.9 ± 0.1 d	53.4 ± 12.8 b	20.02 ± 0.87	30.0 ± 2.9 bc
Pods (R3)	27.4 ± 0.2 a	48.5 ± 7.7 b	All dead	100.0 ± 0.0 a
Leaves + Flowers (R3)	Not tested	Not tested	20.05 ± 1.12	36.7 ± 8.8 b
Leaves + Pods + Flowers (R3)	Not tested	Not tested	20.41 ± 1.22	31.7 ± 7.2 bc
Pods + Flowers (R3)	Not tested	Not tested	All dead	100.0 ± 0.0 a
Leaves (R5.1)	22.0 ± 0.1 c	43.6 ± 14.2 b	Not tested	Not tested
Leaves + Pods (R5.1)	21.7 ± 0.4 c	47.9 ± 4.9 b	Not tested	Not tested
Pods (R5.1)	All dead	100.0 ± 0.0 a	Not tested	Not tested
Leaves (R5.5)	23.7 ± 0.2 b	21.1 ± 6.2 b	Not tested	Not tested
Leaves + Pods (R5.5)	24.5 ± 0.4 b	30.9 ± 1.4 b	Not tested	Not tested
Pods (R5.5)	All dead	100.0 ± 0.0 a	Not tested	Not tested
Artificial diet[2]	Not tested	Not tested	16.31 ± 1.31	5.0 ± 2.9 c
Statistics CV (%)	1.83	17.46	10.75	19.82
P	<0.01	<0.01	0.17	<0.01
F	126.81	19.02	1.99	41.39
$DF_{residual}$	14	18	10	14

Means (±SE) followed by the same letter in the column do not differ statistically from each other by Tukey test ($P > 0.05$). ^{ns}ANOVA non-significant. [1]Experiment carried out with all larvae fed on pods + leaves from soybean cultivar BRS 388 RR during both first and second instar when then diet was switched to different plant tissues. [2]Artificial diet previously described in the literature (Greene et al., 1976).

2017, when plants were at R4 development stage, was higher for the tested treatments of 15% injury (255.2 pods), 20% injury (326.2 pods) and 25% injury (388.6 pods) compared with 5% injury or 10% injury treatments (69.8 pods and 135.2 pods, respectively). However, some of those injured pods aborted. Pod abortion from R4 stage to harvest varied from 28.6% to 33.5% with no difference between the injury levels (Table 2).

In the following growing season (2017/2018) results were similar for injured pods as well as for plants with injured pods in conjunction with 16.7% leaf defoliation. There was no interaction between defoliation and pod injury regarding plant capacity to tolerate injuries. Moreover, even the 25% pod injury imposed at the R4 plant stage did not result in higher pod abortion (%). Pod abortion of injured pods ranged from 22.8 to 29.2% and did not vary among the pod injury intensities (Table 3).

When flowers were removed during 2018/2019 crop season, soybean plants also tolerated all tested levels of injuries (25, 50 and 100% of flower removal at R2 plant development stage) without reduction of yield or weight of 1000 seeds. Interestingly, even with 100% of flowers removed only once at the R2 stage, plants were able to produce new flowers and have the same yield with the highest number of pods per area (5114.2 pods/3.6 m^2) (Table 4).

4. Discussion

Soybean-IPM is based on the premise that soybean plants can tolerate a certain amount of injury without experiencing economically relevant yield loss (Higley and Pedigo, 1996; Batistela et al., 2012; Bueno et al., 2013, 2021). Despite the observed high tolerance to different stressors, the response of soybean plants to injury can vary among injury intensities as well as injuries to different plant structures (Higley and Pedigo, 1996). While soybean plant tolerance to defoliation has been extensively recorded in literature over the years (e.g., Begun and Eden, 1965; Turnipseed, 1972; Fehr et al., 1977; Hinson et al., 1978; Fehr et al., 1981; Pickle and Caviness, 1984; Hintz et al., 1991; Higley, 1992; Gazzoni and Moscardi, 1998; Haile et al., 1998; Board et al., 2010; Batistela et al., 2012; Hayashida et al., 2021), less attention has been given to soybean plant tolerance to both pod and flower injuries.

Lepidoptera larvae can cause defoliation, which is a minor injury and less economically important compared with the damage caused by larvae feeding on pods and flowers (Adams et al., 2015; Reisig et al., 2017). Therefore, not only is it of great theorical and practical interest to study plant tolerance to pod and flower injury but also the respective capacities of different insect species to injure those plant structures. While it is known that *H. zea* feed on all soybean plant tissue types (Reseig et al., 2017) including pods and flowers (Eckel et al. 1992a, 1992b), reports by farmers of *Spodoptera* spp. injuring both pods and flowers (Bueno et al., 2018; Conte et al., 2019) were not scientifically verified. In this study, soybean pods and flowers were found to be unsuitable as food for *S. eridania*, because larvae that fed exclusively on those plant parts had higher mortality and longer development times than those that fed on foliage. An extended duration of the larval period is a compensatory action for a larva to recover when feeding on a

Table 2
Soybean response to different levels of artificial pod injury performed during R4 reproductive stage, on the apical grain of randomly selected pods. Londrina, Paraná, Brazil (S 23° 11′ 11.7"; WO 51° 10′ 46.1″), 2016/2017 crop season.

Percentage of injured pods	R4		Harvest			
	Total number of pods/5.4 m^2	Number of injured pods/ 5.4 m^2	Total number of pods/5.4 m^2	Pod abortion (%)	Yield (Kg/ha)	Weight of 1000 seeds (g)
0	1605.6 ± 165.1^{ns}	0.0 ± 0.0 c[1]	7572.0 ± 383.2^{ns}	–[1]	4567.2 ± 384.5^{ns}	143.9 ± 3.6^{ns}
5	1394.4 ± 134.5	69.8 ± 6.7 b	7293.0 ± 208.1	28.6 ± 4.6^{ns}	4130.2 ± 419.2	139.2 ± 4.5
10	1351.2 ± 226.3	135.2 ± 22.6 b	8437.8 ± 833.2	33.5 ± 6.0	4888.3 ± 451.5	146.5 ± 3.5
15	1701.4 ± 206.9	255.2 ± 31.0 a	7588.0 ± 198.5	29.3 ± 4.8	4531.5 ± 481.3	143.6 ± 6.2
20	1631.2 ± 193.4	326.2 ± 38.7 a	7079.6 ± 421.4	31.9 ± 4.3	4062.0 ± 476.1	139.1 ± 5.9
25	1554.0 ± 171.0	388.6 ± 42.8 a	7361.4 ± 394.0	29.2 ± 3.3	4061.1 ± 470.1	138.9 ± 5.5
Statistics CV (%)	23.99	16.15	13.57	32.70	11.19	3.91
P	0.63	<0.0001	0.41	0.92	0.07	0.18
F	0.70	60.44	1.06	0.22	2.43	1.71
$DF_{residual}$	20	20	20	16	20	20

Means (±SE) followed by the same letter in the column do not differ statistically from each other by Tukey test ($P > 0.05$). Soybean cultivar BRS 388 RR (maturity group 6.4 and indeterminate growth habit; Roundup Ready cultivar). ^{ns}ANOVA non-significant ($P > 0.05$). [1]Non-evaluated parameter.

Table 3
Soybean response to different levels of artificial injuries on central leaflets and the apical grain of randomly selected pods, performed during R4 reproductive stage. Londrina, Paraná, Brazil (S 23° 11′ 11.7"; WO 51° 10′ 46.1″), 2017/2018 crop season.

Percentage of plant injury		R4		Harvest			
		Total number of pods/ 3.6 m^2	Number of injured pods/ 3.6 m^2	Total number of pods/ 3.6 m^2	Pod abortion (%)	Yield (Kg/ha)	Weight of 1000 seeds (g)
Injured pods	0	2749.9 ± 165.3^{ns}	0.0 ± 0.0 c[1]	5342.9 ± 109.8^{ns}	–[1]	4616.0 ± 104.3^{ns}	154.6 ± 2.2^{ns}
	15	3093.7 ± 127.8	464.1 ± 19.2 b	5389.8 ± 92.1	22.8 ± 3.2^{ns}	4495.8 ± 191.3	154.1 ± 2.1
	20	3154.9 ± 146.0	627.5 ± 28.8 a	5478.8 ± 90.0	29.2 ± 3.2	4444.0 ± 170.7	153.6 ± 2.4
	25	2776.8 ± 163.8	711.0 ± 39.8 a	5475.1 ± 100.6	28.3 ± 2.4	4402.2 ± 139.4	152.9 ± 1.5
Leaf loss	0	3021.0 ± 154.4^{ns}	451.4 ± 71.8^{ns}	5433.6 ± 93.4^{ns}	22.8 ± 2.9^{ns}	4608.7 ± 140.9^{ns}	154.7 ± 1.8^{ns}
	8.3	2900.6 ± 113.5	458.3 ± 77.8	5433.5 ± 88.91	32.2 ± 3.1	4543.8 ± 127.6	154.3 ± 1.9
	16.7	2909.9 ± 140.6	442.3 ± 73.7	5397.8 ± 72.7	25.1 ± 2.6	4316.0 ± 121.8	152.4 ± 1.6
Statistics	CV (%)	17.05	18.81	5.65	18.14	9.06	4.25
	$P_{\text{damaged pods}}$	0.12	<0.0001	0.64	0.17	0.61	0.93
	$P_{\text{defoliation}}$	0.75	0.8678	0.93	0.06	0.12	0.58
	$P_{\text{damaged pods *defoliation}}$	0.19	0.0834	0.34	0.19	0.17	0.69
	$F_{\text{damaged pods}}$	2.10	168.39	0.57	1.93	0.62	0.15
	$F_{\text{defoliation}}$	0.28	0.14	0.07	3.28	2.29	0.55
	$F_{\text{damaged pods *defoliation}}$	1.57	2.07	1.18	1.66	1.63	0.66

Means (±SE) followed by the same letter in the column (upper-case letter) do not differ statistically from each other by Tukey test ($p > 0.05$) in the leaf loss factor. Soybean cultivar BRS 388 RR (maturity group 6.4 and indeterminate growth habit; Roundup Ready cultivar). ^{ns}ANOVA non-significant ($P > 0.05$). [1]Non-evaluated parameter.

Table 4
Soybean response to different levels of flower removal performed during R4 reproductive stage. Londrina, Paraná, Brazil (S 23° 11′ 11.7"; WO 51° 10′ 46.1″), 2018/2019 crop season.

Removed flowers (%)		R2		Harvest		
		Number of flowers/3.6 m^2	Number of removed flowers/3.6 m^2	Total number of pods/3.6 m^2	Yield (Kg/ha)	Weight of 1000 seeds (grams)
0		3128.4 ± 221.0^{ns}	0.0 ± 0.0 d[1]	4616.4 ± 162.9 b	2856.3 ± 171.8^{ns}	125.9 ± 1.4^{ns}
25		2948.8 ± 168.7	737.4 ± 42.0 c	5015.8 ± 182.1 ab	3084.2 ± 178.9	125.4 ± 2.6
50		2921.6 ± 152.2	1460.8 ± 76.0 b	4933.8 ± 84.2 ab	3068.5 ± 109.5	126.0 ± 1.3
100		3136.2 ± 324.0	3136.2 ± 324.0 a	5114.2 ± 103.8 a	2940.3 ± 162.5	124.8 ± 2.4
Statistics	CV (%)	14.53	11.81	4.52	9.17	2.32
	P	0.80	<0.0001	0.02	0.53	0.89
	F	0.34	202.10	4.69	0.78	0.21
	$DF_{residual}$	12	12	12	12	12

Means (±SE) followed by the same letter in the column do not differ statistically from each other by Tukey test ($P > 0.05$). Soybean cultivar BRS 1001 IPRO (maturity group 6.2 and indeterminate growth habit; *Bt* soybean cultivar). ^{ns}ANOVA non-significant ($P > 0.05$).

low-quality host and enhances the larva's ability to achieve greater weight before pupation (Behmer, 2009). However, this longer development time increases larvae mortality rates from biotic and abiotic factors, which is described in the literature as "The slow growth-high mortality hypothesis" (Chen and Chen, 2018).

Overall, *S. eridania* larvae develop faster feeding on young plant tissues and were only able to feed and develop on pods at the early reproductive stage (R3). This is probably because mature tissues are harder to consume and provide less nutrients (Coley et al., 2006). Lepidopteran performance during feeding has been reported to be affected by the physical characteristics of the plant and its tissue, such as hardness, size, shape and texture (Slansky Jr. and Rodriguez, 1987; Bruce et al., 2005; Perkins et al., 2010). Vegetal tissues greatly differ for plants between R3 and R5.5 stages. In addition, newly hatched larvae have rudimentary mandibles, which can limit their ability to feed on harder foods (Lincoln et al., 1993). Also, greater cuticular thickness and the presence of trichomes can negatively affect the feeding of small insects, mainly in the first instar, by reducing their movement capacity (Gaston and Reavey, 1991; Vendramim and Guzzo, 2009). This probably explains the higher mortality of neonates, when tested on pods, compared with third instar larvae, that survived feeding on pods at an early development stage.

It is important to consider that herbivores face many challenges with respect to diet selection as leaves have an array of chemical and physical defenses and are very low in protein compared with seeds or fruits (Mattson, 1980). Carbohydrate and protein content is important for the development of insects and can vary depending on the host tissue of the plant and its phenological stage. Younger leaves generally have higher protein concentrations (Mattson, 1980; Lincoln et al., 1993). Younger pods can offer higher concentrations of carbohydrates (Liu et al., 2004). This could explain field observations reported by growers that *Spodoptera* spp. can eventually cause injury to soybean pods (Bueno et al., 2018).

Despite our research findings indicating that *S. eridania* is not a common pod and flower feeder, plant tolerance results, especially regarding pod injury, can also be regarded as important, with necessary caution, for other pod feeders such as different species of the genus *Spodoptera* or other Lepidoptera species such as *H. zea*. Different pest species might cause injury to the same plant structure with different intensities. Despite those possible differences, this research illustrates an important preliminary step in determining EIL and ET for insect soybean pod and flower feeders.

Neither 25% injury to pods, nor 100% flower removal reduced the number of pods at harvest, and the resulting yields were nearly identical to control (without injury). Similar to our study, Smith and Bass (1972) did not report significant soybean yield reduction at up to 80% pod removal when injury was performed at R4 in the determinate soybean cultivar 'Bragg'. It is important to emphasize that in both studies, soybean was injured at early reproductive stages. Early soybean reproductive stages are not as susceptible to yield loss caused by pod injury compared with later soybean stages, especially at low to moderate injury levels. Compensation for fruit loss at later reproduction stages should be lower and require more time compared with fruit loss occurring during early reproductive stages (Thomas et al., 1974; Adams et al., 2015). While, admittedly, our single day pod injury did not perfectly simulate natural insect feeding, it has been used in the literature and proven to be helpful for a better understanding of plant tolerance to injury since it allows better control of injury intensity compared with natural insect infestation. Natural insect feeding would occur over a more progressive timing compared with one discrete pod injury, as performed in this study. However, this single-day injury study allows to determine plant tolerance or yield reduction due to the injury at a given development stage.

The number of pods produced by a soybean plant is often directly related to the number of flowers produced and the proportion of flowers that develop into pods. Therefore, yield is expected to be related to the number of flowers (Dominguez and Hume, 1978) or inversely related to the percentage of total flower and pod abortion (Brevedan et al., 1978; Heitholt et al., 1986). However, soybean plants normally abort 30%–85% of the flower buds they produce (Swen 1933; van Schaik and Probst 1958, Weibold et al., 1981). Not only is abortion common for soybean flowers but also for soybean pods (Adams et al., 2015; Reisig et al., 2017). Certainly, abortion of plant reproductive tissues is higher during times of plant stress, which might be caused by insects (Dybing et al., 1986; Egli 2005). However, soybean plants have capacity of compensating if pods or flowers are injured by pests. Therefore, the contribution of an individual flower or pod to the total yield is unknown and probably highly variable (Adams et al., 2015; Reisig et al., 2017). In addition, overcompensation might occur. Hicks and Pendleton (1969) observed that removing all flower buds triggered a significant increase in soybean yield compared with control due to overcompensation of the injury. This might make the relationship between yield and injury even more variable and challenging.

Overall, the lack of yield reduction recorded in this study indicates that 25% pod injury is below EIL for *Spodoptera eridania* management, at least at an early reproductive plant stage (R3-R4), and no ET should be established below this percentage. Moreover, flower removal at R2 reproductive stage by *S. eridania* should not trigger management because no reduction in yield was observed even with 100% of flower removal. It is important to mention that insect feeding is considered one of the causes of delayed maturity in soybeans, but more related to attacks of stink bugs (Harbach et al., 2016). On this context, no delayed maturity was observed in our trials despite the level of simulated injury on plants.

Also, the absence of interaction between defoliation and pod injury indicates that the general ET (Batistela et al., 2012; Hayashida et al., 2021) of 15% defoliation during the plant reproductive stage does not need to be adjusted for *Bt* soybean grown in South America. However, it is important to acknowledge that we did not find a yield response from up to 25% defoliation in the present study, suggesting that the 15%-defoliation ET is too conservative, especially considering that most of the soybean samplers commonly overestimate defoliation levels, which leads to unnecessary insecticide application (Wilhelm et al., 2000; Silva et al., 2019). Indetermined soybean cultivars have frequent production of new leaves, flowers and pods, which gives plants a compensatory capacity, even at high injury levels caused by defoliating insects (Hayashida et al., 2021).

Despite some required caution towards the results herein reported, due to the early soybean reproductive stage and the discrete pod and flower injuries used in our trials, it is important to mention that this is the first study to report the impact of injury on soybean plants by *Spodoptera* spp. by means of simulated injury to flowers and pods. Thus, the absence of yield reduction despite pod injury with and without defoliation suggests that the ET of 25% damaged pods is totally sufficient to prevent yield loss. Future studies should investigate differences in the performance between cultivars of determinant and indeterminate growth, to evaluate injury performed for a long period of time to better simulate insect feeding, and also test later soybean reproductive stages and different soybean cultivars. Soybean yields are directly related to the ability to intercept light by the canopy after defoliation, resulting in significant differences in the ET among cultivars (Haile et al., 1998; Stacke et al., 2018).

The results reported here are also important to mitigate the overuse of insecticides, especially for control of *Spodoptera* spp. in *Bt* soybean, which is one of the greatest challenges for a more sustainable soybean production in South America (Bueno et al., 2018, 2021). Currently, the application of insecticides, while disregarding the ET, is causing an environmental imbalance, allowing population increases of secondary pests (Bueno et al., 2013). This study clearly indicated no yield impact from simulated injury intensities to pods and flowers. Additionally, based on laboratory results, the capacity of *S. eridania* to injure soybean pods is expected to be restricted to early soybean reproductive stages (R3) and later larval instars (from 3rd instar).

5. Conclusions

Larval *Spodoptera eridania* had better development when fed on soybean leaves. Although larvae can feed on soybean pods, feeding is restricted to older larvae (from 3rd instar) and early plant reproductive stages (R3 stage). Soybean plant tolerance to pod and flower injuries is high, even in the combination of pod injury and defoliation. Therefore, an ET for *Spodoptera eridania* of 25% of injured pods as well as the general defoliation ET of 15% at the soybean reproductive stage are appropriate and no adjustment of either ET is required. Simulated flower removal by *Spodoptera eridania* feeding did not cause damage even when 100% of flowers were removed during a single event in the R2 plant development stage. This result may be limited to soybean cultivars with indeterminate growth habit, which are able to keep producing new flowers and pods to compensate for previous loss.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study was financed in part by National Council for Scientific and Technological Development (CNPq grant 11470 and 302645/2018–7) and by the CAPES Foundation (88887.374211/2019–00). This work was partially supported by Hatch Project accession no. 1019561 from the USDAUSDA National Institute of Food and Agriculture and FAPED (project 697).

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