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Damage assessment of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in soybean reproductive stages

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ABSTRACT

Quantification of damage by *Helicoverpa armigera* on soybean is a key factor in understanding the potential of this pest to cause losses in Brazil soybean fields and determining the timing of its control. The objective of this study was to characterize and quantify the damage of *H. armigera* at two reproductive stages of soybean in Brazil. Four experiments were conducted at two soybean phenological stages, full bloom (R2) and early grain filling (R5.1) over two years. A range of *H. armigera* larval densities up to 8 larvae m^{-2} were infested on plants using third instar larvae for 15 days. Number of pods m^{-2}, seeds m^{-2}, seeds pod^{-1} and seed yield were assessed in the upper, middle and lower third of soybean plants. *H. armigera* caused more injuries in the middle part of the plant, followed by the upper and lower third. The rate of seed-yield loss at the R2 growth stage was 7.7 g per larva, significantly lower than the rate of loss at the R5.1 stage (10.6 g per larva). At both crop growth stages, there were losses in total pod numbers and seeds/pod with increasing larval density, but sometimes with an initial period of compensation. The response of average seed weight to increasing infestation varied with plant growth stage, decreasing at the R2 stage, but increasing or not changing at R5.1. The results demonstrate that relatively low infestations of *H. armigera* can cause significant reductions for soybean in Brazil, and it is necessary to intensify monitoring and use of efficient and alternative control methods for the use of insecticide. In conclusion, our study shows that growth stage R5.1 is more sensitive to damage by *H. armigera* than the R2 stage. Further studies should be carried out to quantify the damage of *H. armigera* in the vegetative stage of the soybean crop in Brazil and also on determinate cultivars.

1. Introduction

Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) is an important pest in Australia, Asia and Europe, where its causes severe damage to soybeans (Rogers and Brier, 2010a) and is currently one of most important pests of agriculture worldwide (Tay et al., 2013). In Brazil, the occurrence of *H. armigera* was confirmed in 2013 (Czepak et al., 2013) and its origin, as well as possible pathways of incursions, are still being studied (Arnemann et al., 2018). Since then, it has been reported in South (Arnemann et al., 2016), Central (Smith, 2014) and North America (USDA-APHIS, 2015) raising concerns about the proper management of this pest.

There are more than 180 plant species reported as hosts of *H. armigera* (Pogue, 2004; Tay et al., 2013). In Brazil, it has been found damaging cotton, maize, soybean, bean, tomato, sorghum, wheat, sunflower, fruit, vegetables, and some weeds (Avila et al., 2013; Arnemann et al., 2014; Fernandes et al., 2015). The damage caused by *H. armigera* to soybean during 2012/13 was estimated at $ 0.8 billion (Bueno and Sosa-Goméz, 2014), demonstrating the importance and the need for knowledge about damage caused by this pest species in Brazilian soybean fields.

Artificial defoliation in the vegetative stage and removal of pods in the reproductive stage have been used in attempts to understand the damage caused by *H. armigera* in soybean and to provide values for an economic threshold (ET) (Rowden, 1987; Timsina et al., 2007). However, these studies did not demonstrate the actual damage of *H. armigera* and did not define the yield loss per *H. armigera* larvae, a key parameter to determine ET (Pedigo and Rice, 2014). Potential damage from natural feeding may result in a variety of injury events, including: complete consumption of pods, consumption of developing pods and

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seeds, and destruction of the apical growing points, which reduce the plant's compensatory ability, are not captured by artificial depodding experiments. Rogers and Brier (2010a, b) published the only study that quantified the loss of soybean yield by actual *H. armigera* larvae, showing that damage depends on the maturation stage and the potential yield of soybean plants, climatic conditions and, especially, density by larvae. However, damage of *H. armigera* using soybean plants in the field has not been studied in the New World.

The ET quantifies the situation when the crop is at risk of yield losses and when insecticide sprays are necessary (Brier et al., 2010). At a practical level, this ET may vary from year to year, or even from one area to another within the same year, depending on environmental, economic and agricultural factors (Fathipour and Sedaratian, 2013). A precursor to establishing an ET for *H. armigera* on soybean under Brazilian growing conditions is the knowledge about the potential of *H. armigera* to cause seed yield loss in soybean fields using Brazilian-adapted varieties. Therefore, the objectives of this study were to characterize and quantify the damage of *H. armigera* on the reproductive stages of soybeans cultivated in Brazil.

2. Material and methods

2.1. Experiments

The experiments were conducted in Santa Maria - RS/Brazil (29° 43′ 40″S 53° 33′ 43″ W) over two summer cropping seasons (2013/14 and 2014/15), using the indeterminate cultivar BMX Potência RR, maturity group 6.7 (approximately 100 days for maturation). The sowing dates were 22/12/2013 and 24/11/2014, and seeds were treated with fungicide and insecticide (300 mL/100 kg seed of carboxin (200 g/L) + thiram (200 g/L) and 200 mL/100 kg seed of fipronil (250 g/L)), and fertilization based on soil analysis. The management of weeds, diseases and insects were made with applications of 2.5 L/ha glyphosate potassium (620 g/L), 300 mL/ha azoxystrobin (200 g/L) + cyproconazole (80 g/L) and 400 mL/ha of indoxacarb (150 g/L), respectively. The insecticide was sprayed up to 20 days before installation of the cages, to keep the area free of damage from other insects.

Experimental units were cages (1 m × 1 m x 1 m), supported by a metal frame and covered with voile material. Inside of each cage, 18 soybean plants were distributed in two rows spaced at 0.5 m, with plants approximately equally spaced within rows. The experimental design was a randomized block design, with five treatments and four replications. Four experiments were carried out, two adding the insects at phenological stages R2 (full bloom) and two at R5.1 (grains perceptible to the touch, early grain filling) (Fehr and Caviness, 1977), with one of each growth stage in the cropping seasons 2013/14 and 2014/15.

The target treatments were densities of *H. armigera* larvae per cage (larvae/m^2), as follows: T1 - control (0 larvae/m^2); T2 - 2 larvae/m^2; T3 - 4 larvae/m^2; T4 - 6 larvae/m^2; T5 - 8 larvae/m^2. The *H. armigera* larvae used in the experiments were from the laboratory colony kept at the Integrated Pest Management Laboratory (LabMIP-UFSM). In 2013/14 the colony was started from caterpillars collected from soybean fields and the identification of the specimens was performed using the methodologies described by Hardwick (1965) and Pogue (2004). For the 2013/14 experiments, larvae were from the second and third generations. In 2014/15, the colony was retained and new larvae were collected and added into the colony to maintain high diversity and vigour.

Cages were installed when the soybean plants were in the desired stages and then infested with early third-instar larvae (approximately seven days after larval hatching). Before infestation, the larvae were fed with soybean leaves and pods for 24 h to become accustomed to the food. Early third-instar larvae were used because our experience, and that of Rogers and Brier (2010a, b), was that artificial infestations of third-instar larvae establish well on soybeans but that eggs, and first and second instar larvae establish poorly and unreliably. Additionally and as clarified previously, first and second instar larvae cause minimal, if any, seed loss. The cages were maintained at constant densities of larvae for a period of 15 days, by visually inspecting the caged plants every three days and when necessary, adding new larvae to maintain the treatment density. All 18 plants in each cage were carefully inspected, plant by plant, as well the entire soil area inside the cage. For the replacement and density correction within the cages (Table 1, Supplementary Table 1), the same population was kept in the laboratory to have the same age as the larvae used on the field experiments.

After the infestation period (15 days) the cages were removed and the total area was kept free of any pest until harvest, by weekly insecticide application. The 18 plants of each cage were harvested and the following soybean plants features were assessed: pods/m^2, seeds/m^2, seeds/pod and seed yield. The features were evaluated dividing the plant into upper, middle and lower third.

Table 1
Average number of larvae in each cage over the experimental period.

Treatment	Replication	Average Density			
		Growth stage R2		Growth stage R5.1	
		2013/14	2014/15	2013/14	2014/15
0	1	0	0	0	0
2	1	1.87	1.88	1.75	1.90
4	1	3.75	3.75	3.75	3.70
6	1	5.25	5.25	5.50	5.70
8	1	6.37	–	7.13	6.50
0	2	0	0	0	0
2	2	1.87	1.88	1.75	1.80
4	2	3.75	3.75	3.88	3.50
6	2	5.25	5.25	5.25	5.30
8	2	6.50	–	6.88	7.10
0	3	0	0	0	0
2	3	1.62	1.63	2.00	2.00
4	3	3.87	3.88	3.63	3.70
6	3	5.37	5.38	5.13	5.50
8	3	7.00	–	7.00	6.70
0	4	0	0	0	0
2	4	1.75	1.75	1.88	1.80
4	4	3.62	3.63	3.63	3.60
6	4	5.50	5.50	5.25	5.20
8	4	6.50	–	6.88	–

2.2. Statistical analysis

Statistical analyses were conducted in Genstat V18.2 (www.vsni.co.uk), and Sisvar (Ferreira, 2011) programs. The overall average larval density in a cage was calculated as the average of the series of average densities for that cage, based on the counts at the beginning and end of each three-day period. Preliminary assessment of the data pointed to two issues. Firstly, in both R2-stage experiments, the rate at which *H. armigera* larvae were missing - and so required replacement - was more than double for the 8 larvae/cage density than for the lower densities. *H. armigera* is highly cannibalistic at high densities (Zalucki et al., 1986); because this increased cannibalism would have affected both average density calculations and the yield/pest density relationships, the 8 larvae/cage treatment was removed from the analysis for the R2 growth stage experiments. Secondly, in the 2014/15 R5.1 experiment, Replication 4 of the 8 larvae/cage treatment had a higher undamaged yield potential than the other plots in this replication, as evidenced by the higher total pod and seed numbers in that plot compared to the other plots in this replication. Additionally, the Genstat model-checking routines flagged this plot as having large positive residuals in the pest-

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