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A test of economic thresholds for soybeans exposed to stink bugs and defoliation

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A B S T R A C T

Economic thresholds (ET) for gross tissue removal and piercing-sucking damage by stink bugs are well-established for soybean (*Glycine max*). However, little is known about the interaction effects of these injuries. During the 2017/18 and 2018/19 crop seasons, field trials were carried out to assess the interaction of defoliation and stink bug (*Euschistus heros*) infestation and its impact on soybean yield with special respect to oil and protein content and quality. During the 2020/21 crop season, five of the treatments from previous crop season trials were chosen to be repeated. No interaction between defoliation and damage caused by stink bugs was found for any tested parameter. Cages infested with 2 stink bugs m^{-1} in the vegetative stage exhibited a reduction of yield compared with cages infested with 0 and 1 stink bug m^{-1}, but only during the 2018/19 crop season. Although small alterations in the tested parameters were observed under certain circumstances, overall, the currently recommended ETs for each type of injury proved sufficient. These ETs are: 30% defoliation at the vegetative soybean stage; 15% defoliation at the reproductive soybean stage; density of 2 stink bugs m^{-1} in soybean fields for grain production; 1 stink bug m^{-1} in soybean fields for seed production. Those ETs are still valid and can be used by soybean producers separately for defoliation and stink bug infestation.

1. Introduction

Injuries caused by arthropods on cropped plants are one of the major obstacles in sustainable food chain systems, impacting production, quality, availability and distribution. The global yield loss of soybean attributed to pests and disease is an estimated 21.4% (Savary et al., 2019). In Brazil, the annual loss is over 4.3 million tons, and almost 84 million tons of insecticides are used in pest management every year (Oliveira et al., 2014). Integrated pest management (IPM) seeks to maintain yields by conserving natural enemies while using insecticides only when necessary (Bottrell and Schoenly, 2018; Bueno et al., 2021; Dara, 2019). IPM is based on the premise that plants tolerate certain levels of injuries without yield reduction (Higley and Pedigo, 1996). Therefore, the use of any pest management measures, including chemical pesticides, is only appropriate when pest numbers reach Economic Thresholds (ETs) (Peterson and Higley, 2002). An ET is defined as the most appropriate time to start pest management in order to avoid economic yield loss (Bueno et al., 2013, 2021; Pedigo et al., 1986). Management at pest numbers below the recommended ETs can result in inconsistent economic returns (Henry et al., 2011; Higley and Pedigo, 1996), while leading to an increase of pest resistance (Sosa-Gómez and Silva, 2010) and a disruption of the environment (Bueno et al., 2021).

In Brazil, the most important soybean pests are the defoliating larvae of the lepidopteran families Noctuidae and Erebidae, as well as the piercing-sucking stink bug complex (Hemiptera: Pentatomidae) (Bueno et al., 2017). Stink bugs include at least 54 different species reported in soybean fields (Panizzi and Slansky, 1985) of which *Euschistus heros* (Fabricius, 1794) (Hemiptera: Pentatomidae) is the most abundant and economically important in South America (Panizzi and Correa-Ferreira, 1997).

Soybean ETs slightly vary worldwide. In Brazil, the ETs for soybean defoliation are 30% during the vegetative stage and 15% during the reproductive state (Hayashida et al., 2021), while in the USA they are 35% and 20%, respectively (Andrews et al., 2009). There are also differences in the recommended ETs for stink bug infestation. In Brazil, the threshold is two pentatomids per meter on soybean fields used for grain production, and one pentatomid per meter on soybean fields used for seed production (Bueno et al., 2015). In Mississippi, USA, the ET is 3.3

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stink bugs per meter, regardless of grain or seed production (Catchot, 2008).

Despite well-established ETs for both defoliation and stink bug management in soybean, there is a general lack of information on the interaction of multiple injuries (Hutchins et al., 1988). Although under field conditions, soybean plants are frequently attacked simultaneously by defoliators and stink bugs (Bueno et al., 2021), little is known about the impact of the interaction between the two pest groups on soybean yield and possible implications for ETs. An ET refinement, or even the development of a multiple-species ET, may be necessary if the interaction between defoliation and stink bug infestation on soybean plants causes higher losses in quantity or quality than either pest alone. Therefore, this study evaluated the interaction between different levels of artificial defoliation and stink bug densities on soybean yield and seed quality, and whether independent ETs for each pest are sufficient to avoid economic loss.

2. Material and methods

Three independent field trials were carried out during the 2017/18, 2018/19, and 2020/21 crop seasons (Table 1) to test different scenarios of defoliation and stink bug infestation occurring simultaneously. Trials were located at the Embrapa Soja Experimental Station (Warta District, Londrina County, Paraná, Brazil; 23°11′ S, 51° 11′ W). During the 2017/18 and 2018/19 crop seasons, trials were carried out in a 3 × 3 full factorial randomized block design with 9 treatments (3 defoliation levels X 3 stink bug infestation levels) and 4 replicates each (Table 1). Each replicate was formed by a 1m-long soybean row of 12 plants inside a cage. The cage was 1 m^3 (1m × 1m x 1m) in size and consisted of iron bars covered with a fine nylon garden netting barrier made of high-density polyethylene (mesh size 0.7 mm). Each cage had a door fitted with a Velcro strip, to allow for evaluation and maintenance during trials (Gomes et al., 2020).

In trial 1, both defoliation and stink bug infestation were imposed during the soybean vegetative stage. In trial 2, defoliation was imposed during the vegetative stage while stink bug infestation was imposed during the reproductive stage. In trial 3, both defoliation and stink bug infestation were imposed during the reproductive stage (Table 1). Each trial tested a different combination of defoliation and stink bug infestation rates frequently occurring in soybean fields.

Defoliation and stink bug infestation started at the vegetative soybean stage V1 (Fehr et al., 1971) (causing vegetative injuries) and finished with harvest (causing reproductive injuries), a period ranging from 112 to 117 days in different crop seasons (Table 1). The tested artificial defoliation levels at the vegetative soybean stage were 0% (control), 16.7% (representing 50% of the current ET) and 33.3% (current ET). At the reproductive soybean stage, levels were 0% (control), 8.3% (representing 50% of the current ET) and 16.7% (current ET). Stink bug infestation was examined at both the vegetative and reproductive soybean stage with 0, 1 and 2 adults of *E. heros* per meter (1m-long soybean row with 12 plants), which is the current ET for the R3 to R6 reproductive development stages of soybean (Bueno et al., 2015) (Table 1).

In the 2020/21 crop season, in contrast to the other crop seasons (2017/18 and 2018/19) trials were not carried out in a 3 × 3 full factorial randomized block design. Instead, trial sizes were reduced and only the combinations of defoliation and stink bug injuries with the most interesting results (five treatments) recorded in the earlier crop seasons were selected. The 2020/21 trials were carried out in a complete randomized block design with 5 treatments (Table 1) and 4 replicates of the same size as described for the previous crop seasons.

The soybean cultivar BRS 1010 IPRO was used, an early-maturing cultivar (maturity group 6.1) with an indeterminate growth habit and high yield potential. In each replicate, 20 seeds per meter were sown. One week after emergence, plants were thinned out to leave a standardized 12 plants per meter.

Table 1
Treatments of three independent field trials evaluating different economic thresholds for IPM decisions in soybeans under the perspective of stress interaction between defoliation and stink bugs (SB). Description of the 3 × 3 factorial randomized block design (3 defoliation levels X 3 stink bug infestation levels) used during the 2017/2018; 2018/2019 crop seasons and selected treatments during the 2020/2021 crop season.

Trial	2017/2018 and 2018/2019 crop seasons				2020/2021 crop season
	Soybean vegetative stage		Soybean reproductive stage		Selected treatment (soybean development stage)
	Defoliation (%)	Stink bug. m^{-1}	Defoliation (%)	Stink bug. m^{-1}	
1	0; 16.7 and 33.3	0; 1 and 2 adults	0	0	**1)** 0 (control); **2)** 16.7% defoliation (vegetative) + 1 SB (vegetative); **3)** 16.7% defoliation (vegetative) + 2 SB (vegetative); **4)** 33.3% defoliation (vegetative) + 1 SB (vegetative); **5)** 33.3% defoliation (vegetative) + 2 SB (vegetative).
2	0; 16.7 and 33.3	0	0	0; 1 and 2 adults	**1)** 0 (control); **2)** 16.7% defoliation (vegetative) + 1 SB (reproductive); **3)** 16.7% defoliation (vegetative) + 2 SB (reproductive); **4)** 33.3% defoliation (vegetative) + 1 SB (reproductive); **5)** 33.3% defoliation (vegetative) + 2 SB (reproductive).
3	0	0	0; 8.3 and 16.7	0; 1 and 2 adults	**1)** 0 (control); **2)** 8.3% defoliation (reproductive) + 1 SB (reproductive); **3)** 8.3% defoliation (reproductive) + 2 SB (reproductive); **4)** 16.7% defoliation (reproductive) + 1 SB (reproductive); **5)** 16.7% defoliation (reproductive) + 2 SB (reproductive).

Stink bugs were counted and defoliation was imposed on the new leaves twice a week. Cages were reinfested whenever a lower number (due to insect escape or death) than specified for each treatment was noted. Additionally, artificial defoliation was imposed on the newly grown leaves after each evaluation, according to the determined treatment. Where zero stink bugs were required for the treatment, we removed any observed insects from the cages. Furthermore, to ensure that no other insects were affecting the experiment, thiamethoxam + lambda-cyhalothrin 26.5 + 35.25 g.a.i. ha^{-1} (Engeo Pleno® 250 mL ha^{-1}) was sprayed on a regular basis (every 21 days) using a CO_2 pressurized back sprayer (Herbicat®, Catanduva, SP, Brazil) set to a spray volume of 150 L ha^{-1}. Herbicides and fungicides were applied when necessary (two herbicide applications between the third and sixth week after the emergence of plants, and three fungicide applications at the reproductive phase, 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 for all treatments, including the control treatments where plants were not injured.

2.1. Defoliation and stink bug injuries

Artificial defoliation was carried out twice a week by manually removing the number of leaves according to each treatment with scissors, following the method of Gazzoni and Moscardi (1998). This procedure was performed on all plants in each replicate (Table 1).

In the trials conducted in 2017/18, adult stink bugs used to infest replicates were from laboratory colonies, reared according to Silva et al. (2008). The stink bugs were originally collected from soybean fields in the Embrapa Soybean Experimental Farm, Londrina, State of Paraná, Brazil (23° 11′ 11.7″ S and 51° 10′ 46.1″ W). The population had been kept in the laboratory for approximately seven years, during which new field insects had been introduced each year to maintain colony quality. Two-day old adults were used for the infestation trials to replace missing stink bugs whenever necessary to keep a constant level of insects according to each treatment. During the second and third crop seasons (2018/19 and 2020/21), wild adults of *E. heros* were collected from soybean plants in the surrounding area of the experiment (23° 11′ 11.7″ S and 51° 10′ 46.1″ W) and used in the trials for infestation.

2.2. Yield parameters

All plants 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 the productivity for 13% seed moisture was calculated. In addition to yield, we recorded the weight of 1000 grains (g), oil and protein contents (%), the number of pods containing 0, 1, 2, 3 and 4 grains, and the total number of pods per replicate.

The protein and oil contents of the soybean samples were determined by Near Infrared Reflectance spectroscopy using the Thermo Scientific™ Antaris™ II FT-NIR analyzer (Thermo Fisher Scientific Waltham, MA, USA), reading three different curves for each sample. The results presented here are the mean of the three readings, expressed as percentage on a dry basis (Mertz-Henning et al., 2017).

In addition to these parameters, soybean grain quality was evaluated. For this purpose, 30 g of soybean yield were sampled and classified following the national standard quality legislation (Brasil, 2007). Additionally, a tetrazolium test was carried out in order to further inspect the seeds for stink bug damage. A scale of 6–8 (%) indicates the percentage of seeds with sufficient damage to make them unviable (percentage of dead soybean). In addition, the viability and vigor of soybean grains after stink bug damage of different intensities was evaluated. The tetrazolium test was performed using two subsamples of 50 grains per sample, which were humidified in paper with distilled water for 16 h at 25 °C. Subsequently, the seeds were submerged in a solution of 0.075% of 2,3,5-triphenyl tetrazolium chloride and were placed in an oven at 40 °C for 2 h and 30 min in the dark. Afterwards, the seeds were washed and individually inspected by cutting them longitudinally through the center of the embryonic axis, following the methodology described by França-Neto and Krzyzanowski (2018).

2.3. Statistical analysis

Data from all tests were analyzed with R software, using the interface Rstudio and the packages "dplyr" and "ExpDes.pt" (Ferreira et al., 2014).

Since trials were replicated in the crop seasons of 2017/18 and 2018/19, the possibility of a combined analysis of these data sets was checked (Moore and Dixon, 2015). However, the interaction of year, defoliation and stink bug infestation was not significant ($p < 0.05$) and the result of the higher residual sum of square divided by the lower residual sum of square was lower than seven, indicating that the years should not be combined in the analyses (Ferreira, 2018). Data were subjected to tests for normality (Shapiro and Wilk, 1965) and homoscedasticity (Burr and Foster, 1972). Where necessary, data was transformed into Box-Cox (for weight of 1000 grains in trial 3 from crop season 2017/18; immature grains (g) in trial 1 and fermented grains (g) in trial 3 from crop season 2017/18) or sin(x) (for grains damaged by insects in trial 2 from crop season 2018/19) prior to the analysis of variance (ANOVA). Since there was no interaction between stink bug infestation and defoliation in the factorial analyses ($p_{SBxDefol} > 0.05$), the main effects were tested separately for these parameters. Means were compared by a Tukey test where the F statistic showed significant values ($\alpha \leq 0.05$).

3. Results

There was no interaction observed between the levels of artificial defoliation and stink bug infestation for any of the evaluated parameters in any trial (Tables 2–5). There was no reduction in yield for any of the levels of stink bug infestation and defoliation (%) during the 2017/18 crop season. In the 2018/19 crop season, yield was reduced (only at trial 1) where 2 stink bugs m^{-1} were imposed during the whole vegetative stage (Table 2). During the 2020/21 crop season, when the five selected treatments from previous crop seasons were repeated, no yield reduction was observed for any of the combinations (trials 1, 2 and 3) of defoliation and stink bug infestation (Table 6).

In both the 2017/18 and 2018/19 crop seasons, defoliation (%) reduced the weight of 1000 grains (g) compared with the control (0% defoliation) only when imposed during the entire soybean reproductive development stage at the current ET (16.7% defoliation) but without yield reduction (kg ha^{-1}) (trial 3) (Table 2). In contrast, no reduction in the weight of 1000 grains (g) for any of the combinations of stink bug infestation and defoliation (trials 1, 2 and 3) were recorded when the five selected treatments were repeated in the 2020/21 crop season (Table 6).

The number of grains in each soybean pod from different treatments was evaluated in an attempt to understand any possible yield difference. Although this parameter varied between different levels of stink bug infestation, it was not impacted by defoliation (Fig. 1). Neither was there any interaction between stink bug infestation and defoliation intensity (Fig. 1).

Overall, stink bug infestation had low impact on protein content and no impact on oil content of the harvested soybean. In 2017/18 and 2018/19, only during the latest crop seasons, protein levels were higher than the control when 2 stink bugs m^{-1} were imposed during the entire reproductive stage (trial 2) (Table 3). Likewise, no impact of defoliation on both oil and protein content was observed for the 2017/18 and 2018/19 crop seasons (Table 3). In the 2020/21 crop season, protein content also increased when stink bugs were imposed during the entire reproductive stage (trial 2) but this result was not confirmed in trial 3, which was exposed to the same stink bug density during the entire reproductive stage (Table 7).

Soybean quality measurements (Brazilian National Standard Quality Test according to Brasil, 2007 and tetrazolium test according toFrança-Neto and Krzyzanowski, 2018) varied related to stink bug and defoliation injury. However, the overall impact of stink bug infestation was low and defoliation apparently had no impact on the evaluated soybean-quality parameters (Tables 4, 5, 8 and 9).

Stink bug infestation of 2 insects m^{-1} increased the amount of fermented grains (g) and grains damaged by insects (g) (DBI) when imposed during the entire reproductive stage in trial 3, but only during crop season 2017/18. However, in trial 2, at the same stink bug infestation rate and soybean stage, no increase in fermented grains was observed either in 2017/18 or in 2018/19 (Table 4). An increase in fermented grains was observed again in the 2020/21 crop season at both stink bug infestation levels during the entire reproductive stage in trials 2 and 3, but no differences in the number of grains damaged by insects (g) (DBI) were recorded in those trials. Furthermore, no differences between treatments were observed for either fermented grains (g) or grains damaged by insects (g) (DBI) in trial 1, when stink bug infestation

Table 2
Yield (Kg.ha^{-1}) and weight of 1000 grains (WTG) at different levels of interaction between defoliation and stink bug infestation evaluated during the 2017/2018 and 2018/2019 crop seasons in three independent field trials (Trial 1: 0%; 16.7% (½ET); 33.3% (ET) defoliation and 0; 1; 2 stink bug adults/meter during the vegetative soybean development stage; Trial 2: 0%; 16.7% (½ET); 33.3% (ET) defoliation during the vegetative stage and 0; 1; 2 stink bug adults m^{-1} during the reproductive soybean development stage and Trial 3: 0%; 8.3% (½ET); 16.7% (ET) defoliation and 0; 1; 2 stink bug adults m^{-1} during the reproductive stage).

Injury		Trial 1		Trial 2		Trial 3	
		Yield (kg.ha^{-1})	WTG (g)	Yield (kg.ha^{-1})	WTG (g)	Yield (kg.ha^{-1})	WTG^1(g)^1
2017/2018 Crop Season							
Stink bug (adult.m^{-1})	0	4240.5 ± 216.2^{ns}	141.8 ± 2.2^{ns}	3679.1 ± 290.0^{ns}	131.0 ± 2.5^{ns}	3467.1 ± 206.4^{ns}	130.2 ± 1.9^{ns}
	1	3804.4 ± 275.4	137.6 ± 2.8	3812.5 ± 221.6	131.3 ± 2.1	3855.3 ± 199.1	135.4 ± 2.7
	2	4123.7 ± 199.2	139.7 ± 1.6	3547.0 ± 256.8	135.8 ± 3.9	3859.7 ± 231.7	135.1 ± 2.9
Defoliation (%)	0	4022.8 ± 302.^{ns}	138.4 ± 3.2^{ns}	3664.8 ± 265.2^{ns}	130.4 ± 2.3^{ns}	3892.4 ± 275.2^{ns}	138.8 ± 2.5 A
	½ ET	3918.6 ± 221.9	140.4 ± 1.6	4030.1 ± 189.9	133.5 ± 1.3	3768.0 ± 134.6	133.7 ± 2.2 AB
	ET	4227.2 ± 163.1	140.4 ± 1.8	3343.7 ± 273.0	134.3 ± 4.4	3521.6 ± 211.8	128.3 ± 2.2 B
Statistics	P_{SB}; P_{Defol}; $P_{SBxDefol}$	0.45; 0.68; 0.79	0.50; 0.80; 0.95	0.77; 0.19; 0.87	0.49; 0.66; 0.66	0.36; 0.48; 0.85	0.26; 0.02; 0.68
	F_{SB}; F_{Defol}; $F_{SBxDefol}$	0.82; 0.40; 0.42	0.72; 0.22; 0.18	0.27; 1.80; 0.31	0.73; 0.43; 0.61	1.07; 0.75; 0.33	1.45; 4.73; 0.58
2018/2019 Crop Season							
Stink bug (adult.m^{-1})	0	4415.7 ± 192.4 a	155.0 ± 2.3^{ns}	2958.2 ± 170.2^{ns}	151.6 ± 2.6^{ns}	3246.4 ± 210.1^{ns}	150.4 ± 3.5 a
	1	4358.3 ± 179.1 a	151.6 ± 3.1	3087.2 ± 171.6	151.2 ± 2.6	2827.5 ± 188.0	141.3 ± 2.4 b
	2	3751.8 ± 255.7 b	143.8 ± 5.0	2886.6 ± 163.5	151.8 ± 2.2	3066.6 ± 157.6	147.8 ± 2.5 ab
Defoliation (%)	0	4519.9 ± 213.3^{ns}	150.5 ± 3.2^{ns}	3073.6 ± 139.1^{ns}	155.7 ± 2.2^{ns}	3139.0 ± 198.7^{ns}	149.7 ± 3.2 A
	½ ET	4079.9 ± 253.5	148.5 ± 5.2	3026.2 ± 177.2	149.2 ± 1.9	3037.2 ± 176.7	149.1 ± 2.9 AB
	ET	3926.0 ± 179.0	151.41 ± 2.8	2832.3 ± 182.5	149.7 ± 2.8	2964.3 ± 200.8	140.6 ± 2.2 B
Statistics	P_{SB}; P_{Defol}; $P_{SBxDefol}$	0.04; 0.09; 0.27	0.07; 0.92; 0.50	0.66; 0.52; 0.35	0.98; 0.080.79	0.20; 0.75; 0.05	0.05; 0.03; 0.12
	F_{SB}; F_{Defol}; $F_{SBxDefol}$	3.82; 2.68; 1.38	2.95; 0.08; 0.86	0.42; 0.67; 1.16	0.02; 2.87; 0.43	1.70; 0.30; 2.74	3.53; 4.20; 2.03

Means (±SE) followed by the same letter in a column (low-case letter for stink bugs and upper-case letter for defoliation) did not differ statistically from each other according to the Tukey test ($p > 0.05$) in the same crop season. ^{ns}ANOVA non-significant. ^{1}Original means followed by statistics performed on data transformed by Box-Cox.

Table 3
Oil and Protein content (%) (Means ± SE) at different levels of interaction between defoliation and stink bug infestation evaluated during the 2017/2018 and 2018/2019 crop seasons in three independent field trials (Trial 1: 0%; 16.7% (½ET); 33.3% (ET) defoliation and 0; 1; 2 stink bug adults m^{-1} during the vegetative soybean development stage; Trial 2: 0%; 16.7% (½ET); 33.3% (ET) defoliation the during vegetative stage and 0; 1; 2 stink bug adults m^{-1} during the reproductive soybean development stage and Trial 3: 0%; 8.3% (½ET); 16.7% (ET) defoliation and 0; 1; 2 stink bug adults m^{-1} during the reproductive soybean development stage).

Injury		Trial 1		Trial 2		Trial 3	
		Oil (%)	Protein (%)	Oil (%)	Protein (%)	Oil (%)	Protein (%)
2017/2018 Crop Season							
Stink bug (adult.m^{-1})	0	23.6 ± 0.2^{ns}	33.8 ± 0.3^{ns}	22.7 ± 0.2^{ns}	34.9 ± 0.2^{ns}	23.0 ± 0.2^{ns}	35.0 ± 0.3^{ns}
	1	23.7 ± 0.2	34.2 ± 0.3	22.5 ± 0.3	35.1 ± 0.3	22.8 ± 0.2	35.1 ± 0.3
	2	23.1 ± 0.2	34.6 ± 0.2	23.1 ± 0.2	35.3 ± 0.2	23.1 ± 0.2	34.9 ± 0.2
Defoliation (%)	0	23.4 ± 0.2^{ns}	34.1 ± 0.3^{ns}	22.7 ± 0.2^{ns}	35.3 ± 0.2^{ns}	23.0 ± 0.2^{ns}	35.0 ± 0.3^{ns}
	½ ET	23.4 ± 0.2	34.3 ± 0.3	22.9 ± 0.3	34.9 ± 0.3	22.9 ± 0.2	35.2 ± 0.3
	ET	23.6 ± 0.2	34.1 ± 0.3	22.7 ± 0.2	35.1 ± 0.2	23.0 ± 0.2	34.8 ± 0.2
Statistics	P_{SB}; P_{Defol}; $P_{SBxDefol}$	0.11; 0.60; 0.22	0.21; 0.84; 0.53	0.24; 0.89; 0.59	0.56; 0.42; 0.45	0.32; 0.81; 0.36	0.87; 0.47; 0.36
	F_{SB}; F_{Defol}; $F_{SBxDefol}$	2.44; 0.52; 1.53	1.65; 0.17; 0.81	1.51; 0.12; 0.71	0.60; 0.89; 0.96	1.18; 0.21; 1.15	0.14; 0.77; 1.14
2018/2019 Crop Season							
Stink bug (adult.m^{-1})	0	22.1 ± 0.3^{ns}	35.5 ± 0.4^{ns}	21.2 ± 0.2^{ns}	37.5 ± 0.4 b	21.1 ± 0.4^{ns}	37.1 ± 0.5^{ns}
	1	22.1 ± 0.3	35.8 ± 0.3	20.9 ± 0.4	37.9 ± 0.5 ab	20.8 ± 0.3	37.6 ± 0.4
	2	21.7 ± 0.6	36.3 ± 0.7	20.2 ± 0.3	38.8 ± 0.3 a	20.4 ± 0.3	38.2 ± 0.4
Defoliation (%)	0	22.3 ± 0.4^{ns}	35.2 ± 0.4^{ns}	20.8 ± 0.3^{ns}	38.0 ± 0.4^{ns}	20.8 ± 0.2^{ns}	37.9 ± 0.3^{ns}
	½ ET	21.5 ± 0.5	36.6 ± 0.7	20.7 ± 0.3	38.3 ± 0.4	21.1 ± 0.2	37.4 ± 0.4
	ET	22.0 ± 0.3	35.8 ± 0.3	20.9 ± 0.4	37.9 ± 0.5	20.5 ± 0.4	37.7 ± 0.6
Statistics	P_{SB}; P_{Defol}; $P_{SBxDefol}$	0.44; 0.40; 0.13	0.75; 0.48; 0.11	0.06; 0.84; 3.23	0.04; 0.81; 3.44	0.34; 0.49; 0.79	0.29; 0.79; 1.30
	F_{SB}; F_{Defol}; $F_{SBxDefol}$	0.85; 0.94; 1.97	0.29; 0.76; 2.10	3.23; 0.17; 0.76	3.44; 0.22; 1.74	1.14; 0.73; 0.42	1.30; 0.24; 0.43

Means (±SE) followed by the same letter in a column (low-case letter for stink bugs) did not differ statistically from each other according to the Tukey test ($p > 0.05$) in the same crop season. ^{ns}ANOVA non-significant.

and defoliation was imposed during the soybean vegetative stage (Table 8).

Stink bug infestation alone led to an increase of the number of immature grains (g) compared with the control in trial 2 in the 2017/18 crop season, when 2 stink bugs m^{-1} were imposed during the entire reproductive stage (Table 4). However, no differences due to stink bug infestation were observed in any other trial or crop season (Tables 4 and 8).

Stink bug infestation also had a higher impact on the percentage of dead embryos (%), vigor (%) and viability (%) of harvested grains compared with the control, but only when insects were present during the reproductive stage. Results greatly varied between crop seasons (Tables 5 and 9). The percentage of dead embryos (%) increased when 2 insects m^{-1} were imposed during the soybean reproductive stage at trial 3 in crop season 2017/18. In the following season (2018/19), both 1 and 2 stink bugs m^{-1} increased the dead embryo rate (%) compared with control. However, in trial 2, under the same stink bug infestation rate and soybean stage, there was no increase in either the 2017/18 or the 2018/19 crop season (Table 5). It is important to mention that during the 2020/21 crop season, no differences in the percentage of dead

Table 4
Main attributes of National Standard Quality Test (IN11; Brasil, 2007) of 30 g samples from different levels of interaction between defoliation and stink bug infestation evaluated during the 2017/2018 and 2018/2019 crop seasons in three independent field trials (Trial 1: 0%; 16.7% (½ET); 33.3% (ET) defoliation and 0; 1; 2 stink bug adults m^{-1} during the vegetative soybean development stage; Trial 2: 0%; 16.7% (½ET); 33.3% (ET) defoliation the during vegetative stage and 0; 1; 2 stink bug adults m^{-1} during the reproductive soybean development stage and Trial 3: 0%; 8.3% (½ET); 16.7% (ET) defoliation and 0; 1; 2 stink bug adults m^{-1} during the reproductive soybean development stage).

Injury		Trial 1			Trial 2			Trial 3		
		Fermented (g)	Immature (g)[1]	DBI (g)[2]	Fermented (g)	Immature (g)	DBI (g)[2,3]	Fermented (g)[1]	Immature (g)	DBI (g)[2]
2017/2018 Crop Season										
Stink bug (adult.m^{-1})	0	0.0 ± 0.0 ^{ns}	0.1 ± 0.1 ^{ns}	0.6 ± 0.2 ^{ns}	0.1 ± 0.1 ^{ns}	0.1 ± 0.1 ^{ns}	0.9 ± 0.3 b	0.3 ± 0.2 b	0.14 ± 0.06	1.4 ± 0.5b
	1	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.00 ± 0.00	0.0 ± 0.0	2.5 ± 0.8 ab	0.1 ± 0.1 ab	0.08 ± 0.04	3.0 ± 0.7 ab
	2	0.1 ± 0.1	0.0 ± 0.0	0.6 ± 0.2	0.4 ± 0.3	0.1 ± 0.0	3.0 ± 0.5 a	0.4 ± 0.2 a	0.03 ± 0.01	4.1 ± 0.5 a
Defoliation (%)	0	0.1 ± 0.1 ^{ns}	0.0 ± 0.0 ^{ns}	0.7 ± 0.2 ^{ns}	0.1 ± 0.1 ^{ns}	0.1 ± 0.1 ^{ns}	2.1 ± 0.6^{ns}	0.3 ± 0.2 ^{ns}	0.11 ± 0.04 a	3.0 ± 0.7 ^{ns}
	½ ET	0.2 ± 0.1	0.1 ± 0.1	0.5 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	1.9 ± 0.4	0.1 ± 0.1	0.09 ± 0.05 ab	2.7 ± 0.5
	ET	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.3 ± 0.3	0.0 ± 0.0	2.5 ± 0.7	0.4 ± 0.2	0.05 ± 0.04 b	2.8 ± 0.8
Statistics	P_{SB}; P_{Defol}; $P_{SBxDefol}$	0.37; 0.89; 0.82	0.38; 0.85; 0.45	0.94; 029; 0.14	0.24; 0.68; 0.57	0.57; 0.46; 0.52	0.03; 0.75; 0.95	0.03; 0.18; 0.60	0.57; 0.01; 0.94	0.01; 0.95; 0.19
	F_{SB}; F_{Defol}; $F_{SBxDefol}$	1.04; 0.12; 0.38	1.01; 0.16; 0.96	0.06; 1.29; 1.95	1.53; 0.39; 0.75	0.57; 0.80; 0.82	4.11; 0.30; 0.18	3.96; 1.86; 0.71	0.57; 5.22; 0.20	5.58; 0.05; 1.67
2018/2019 Crop Season[1]										
Stink bug (adult.m^{-1})	0	16.7 ± 3.8 ^{ns}	0.4 ± 0.2 ^{ns}	3.0 ± 0.6 ^{ns}	23.7 ± 3.8 ^{ns}	3.4 ± 1.0 b	3.1 ± 0.6 ^{ns}	20.2 ± 3.7 ^{ns}	2.06 ± 0.82 ^{ns}	2.8 ± 0.6 ^{ns}
	1	22.1 ± 5.4	0.5 ± 0.2	2.1 ± 0.5	26.7 ± 4.4	2.4 ± 0.6 b	2.7 ± 0.45	25.4 ± 3.8	2.53 ± 0.94	3.9 ± 0.5
	2	23.3 ± 7.8	1.5 ± 1.0	1.5 ± 0.3	31.7 ± 3.4	7.8 ± 1.7 a	3.9 ± 0.5	29.3 ± 3.5	2.29 ± 0.57	4.3 ± 0.5
Defoliation (%)	0	18.0 ± 5.6 ^{ns}	0.4 ± 0.2 ^{ns}	2.7 ± 0.4 ^{ns}	24.2 ± 3.1 B	5.5 ± 1.8 ^{ns}	2.6 ± 0.5 ^{ns}	22.9 ± 3.4 ^{ns}	2.2 ± 0.5 ^{ns}	3.8 ± 0.5 ^{ns}
	½ ET	25.4 ± 7.3	1.5 ± 1.0	2.2 ± 0.6	33.0 ± 3.9 A	3.2 ± 1.0	3.4 ± 0.6	22.5 ± 2.9	1.6 ± 0.5	3.4 ± 0.6
	ET	18.7 ± 4.2	0.5 ± 0.2	1.8 ± 0.5	25.7 ± 4.4 AB	4.9 ± 1.0	3.7 ± 0.6	29.5 ± 4.6	3.2 ± 1.1	3.9 ± 0.5
Statistics	P_{SB}; P_{Defol}; $P_{SBxDefol}$	0.71; 0.63; 0.72	0.20; 0.11; 0.17	0.08; 0.33; 0.20	0.07; 0.04; 0.05	0.01; 0.38; 0.24	0.27; 0.34; 0.71	0.22; 0.33; 0.48	0.93; 0.40; 0.90	0.14; 0.74; 0.95
	F_{SB}; F_{Defol}; $F_{SBxDefol}$	0.34; 0.47; 0.52	1.72; 2.43; 1.75	2.89; 1.15; 1.63	3.04; 3.63; 2.78	5.99; 1.01; 1.49	1.40; 1.13; 0.54	1.60; 1.18; 0.90	0.08; 0.94; 0.27	2.17; 0.30; 0.17

Means (±SE) followed by the same letter in a column (low-case letter for stink bugs and upper-case letter for defoliation) did not differ statistically from each other according to the Tukey test ($p > 0.05$) in the same crop season. ^{ns}ANOVA non-significant. [1]Data transformed to Box-Cox. [2] DBI = Damaged by insects. [3]Data transformed to sin(x).

embryos (%) were recorded between the tested treatments (Table 9).

Vigor (%) was reduced by exposure to 2 stink bugs m^{-1} during the reproductive stage (trials 2 and 3) but only in the crop season of 2018/19 (Table 5). Viability was reduced by 2 stink bugs m^{-1} during the reproductive stage (trial 3) but only in the crop season of 2018/19. However, in trial 2, under the same stink bug infestation density and soybean stage, this reduction in vigor was not recorded for the 2017/18 crop season (Table 5).

4. Discussion

Soybean-IPM is based on the premise that soybean plants can tolerate a certain level of injury without relevant yield loss (Bueno et al., 2013, 2021; Higley and Pedigo, 1996). This was taken into consideration while establishing the ETs for both defoliation (Batistela et al., 2012; Hayashida et al., 2021) and stink bug feeding (Bueno et al., 2015). The adoption of ETs has contributed to a decrease in insecticide use (Bueno et al., 2021). However, ETs were developed for one type of injury each and growers are concerned when multiple pest guilds are present simultaneously in numbers below the individual ETs. Without relevant tests, our understanding of the effectiveness of the traditional ET approach for IPM is limited (Hutchins et al., 1988).

Most tests to establish ET thresholds have focused on species of the same injury guild, producing data on common injuries such as stand reduction, leaf-mass consumption, assimilate removal, water-balance disruption, fruit destruction, or architecture modification (Pedigo et al., 1986). To our knowledge, this is the first study evaluating a possible interaction between defoliation (gross tissue removal) and piercing-sucking injury (triggered by Hemiptera feeding) in soybean, with plants in the vegetative and reproductive stages of development.

Since there was no interaction between defoliation and stink bug injuries for any of the trials and evaluated parameters, the results herein reported indicate that ETs for defoliation and stink bug infestation can be used independently for soybean IPM decisions. The recommended ETs for lepidopteran larvae (30% defoliation in the vegetative stage or 15% defoliation in the reproductive state) (Batistela et al., 2012; Hayashida et al., 2021) should be used regardless of levels of stink bug infestation in the field. Likewise, the ET of 2 stink bugs m^{-1} for soybean grain production and 1 stink bug m^{-1} for crop seed production (Bueno et al., 2015) should be used for *E. heros* and under the defoliation level equal to or lower than 30% which was the limit tested in this study.

Trials were carried out during three consecutive crop seasons (2017/18, 2018/19, and 2019/20) with consistent results although small variations were observed. Simmons and Yeargan (1990) also reported a lack of interaction between defoliation and stink bug feeding in soybean, but for different pest species. They found no significant interaction between artificial defoliation and the green stink bug, *Acrosternum hilare* (Say) feeding on soybean for yield, number of seeds, or seed size.

Table 5
Results of Tetrazolium test (França-Neto and Krzyzanowski 2018)): Dead embryo (Stink bug scale 6–8) (%), vigor (%) and viability (%) at different levels of interaction between defoliation and stink bug infestation evaluated during 2017/2018 and 2018/2019 crop seasons in three independent field trials (Trial 1: 0%; 16.7% (½ET); 33.3% (ET) defoliation and 0; 1; 2 stink bug adults m^{-1} during the vegetative soybean development stage; Trial 2: 0%; 16.7% (½ET); 33.3% (ET) defoliation during the vegetative stage and 0; 1; 2 stink bug adults m^{-1} during the reproductive soybean development stage and Trial 3: 0%; 8.3% (½ET); 16.7% (ET) defoliation and 0; 1; 2 stink bug adults m^{-1} during the reproductive soybean development stage).

Injury		Trial 1			Trial 2			Trial 3		
		Dead embryo (%)	Vigor (%)	Viability (%)	Dead embryo (%)	Vigor (%)	Viability (%)	Dead embryo (%)	Vigor (%)	Viability (%)
2017/2018 Crop Season										
Stink bug (adult. m^{-1})	0	0.5 ± 0.3 ^{ns}	93.2 ± 1. ^{ns}	96.9 ± 0.5 ^{ns}	0.9 ± 0.4 ^{ns}	92.7 ± 1.0 ^{ns}	95.4 ± 0.7 ^{ns}	1.2 ± 0.4 b	90.9 ± 1.6 ^{ns}	95.3 ± 0.5 ^{ns}
	1	0.2 ± 0.1	93.6 ± 0.8	96.5 ± 0.5	2.8 ± 0.9	89.2 ± 1.7	95.3 ± 0.8	2.3 ± 0.5 ab	90.1 ± 1.2	95.1 ± 0.7
	2	0.4 ± 0.2	94.6 ± 1.1	97.2 ± 0.7	2.8 ± 0.5	88.4 ± 1.4	94.4 ± 0.6	3.1 ± 0.4 a	88.6 ± 1.0	95.2 ± 0.5
Defoliation (%)	0	0.7 ± 0.3 ^{ns}	93.3 ± 1.1 ^{ns}	96.8 ± 0.5 ^{ns}	1.9 ± 0.6 ^{ns}	89.3 ± 1.6 ^{ns}	94.8 ± 0.9 ^{ns}	2.7 ± 0.5 ^{ns}	89.5 ± 1.0 ^{ns}	94.3 ± 0.5 ^{ns}
	½ ET	0.3 ± 0.1	92.9 ± 1.1	96.7 ± 0.5	2.3 ± 0.7	90.7 ± 1.5	95.1 ± 0.7	1.7 ± 0.5	90.3 ± 1.3	95.9 ± 0.7
	ET	0.2 ± 0.1	94.8 ± 0.8	97.1 ± 0.7	2.3 ± 0.7	90.3 ± 1.6	95.3 ± 0.7	2.3 ± 0.46	89.8 ± 1.2	95.3 ± 0.2
Statistics	P_{SB}; P_{Defol}; $P_{SBxDefol}$	0.42; 0.14; 0.13	0.75; 0.41; 0.32	0.71; 0.88; 0.81	0.12; 0.93; 0.96	0.12; 0.81; 0.59	0.59; 0.86; 0.94	0.02; 0.28; 0.32	0.36; 0.87; 0.80	0.98; 0.17; 1.00
	F_{SB}; F_{Defol}; $F_{SBxDefol}$	0.91; 2.16; 1.95	0.30; 0.92; 1.24	0.35; 0.13; 0.40	2.29; 0.08; 0.16	2.36; 0.21; 0.71	0.53; 0.15; 0.20	4.90; 1.33; 1.25	1.06; 0.14; 0.41	0.02; 1.89; 0.01
2018/2019 Crop Season										
Stink bug (adult. m^{-1})	0	5.2 ± 1.7^{ns}	74.4 ± 4.4^{ns}	81.3 ± 3.6^{ns}	9.5 ± 2.2^{ns}	49.1 ± 7.6 a	58.9 ± 7.0 a	5.8 ± 1.4 b	53.3 ± 5.9 a	64.0 ± 5.5 a
	1	6.1 ± 2.5	71.8 ± 5.7	80.0 ± 5.1	7.9 ± 1.6	48.6 ± 4.6 a	59.9 ± 4.2 a	10.3 ± 2.2 a	39.2 ± 5.3 ab	49.1 ± 5.5 ab
	2	6.8 ± 3.0	69.8 ± 8.8	77.1 ± 8.4	10.3 ± 1.5	26.9 ± 4.5 b	37.5 ± 4.1 b	10.2 ± 1.2 a	28.2 ± 3.7 b	40.5 ± 3.7 b
Defoliation (%)	0	5.3 ± 2.5 ^{ns}	78.1 ± 6.1 ^{ns}	84.9 ± 5.2 ^{ns}	6.6 ± 0.7 ^{ns}	42.2 ± 6.8 ^{ns}	52.1 ± 6.3 ^{ns}	9.7 ± 2.02^{ns}	37.8 ± 4.2 ^{ns}	48.2 ± 4.4^{ns}
	½ ET	8.2 ± 3.2	63.1 ± 8.0	71.4 ± 7.8	12.1 ± 2.1	37.3 ± 6.5	49.6 ± 5.9	6.8 ± 1.2	47.5 ± 5.3	58.6 ± 5.2
	ET	4.6 ± 1.3	74.9 ± 4.4	82.0 ± 3.6	9.0 ± 1.9	45.2 ± 6.1	54.7 ± 6.1	9.8 ± 1.8	35.3 ± 7.2	46.8 ± 6.7
Statistics	P_{SB}; P_{Defol}; $P_{SBxDefol}$	0.91; 0.59; 0.43	0.87; 0.21; 0.24	0.87; 0.23; 0.16	0.55; 0.58; 0.61	0.01; 0.58; 0.05	0.01; 0.78; 0.08	0.04; 0.38; 080	0.01; 0.25; 0.72	0.01; 0.23; 0.75
	F_{SB}; F_{Defol}; $F_{SBxDefol}$	0.10; 0.53; 1.00	0.14; 1.65; 1.48	0.14; 1.59; 1.79	0.61; 0.56; 0.68	5.63; 0.56; 2.75	6.30; 0.25; 2.42	3.83; 1.01; 0.41	5.65; 1.48; 0.52	5.31; 1.55; 0.48

Means (±SE) followed by the same letter in a column did not differ statistically from each other according to theTukey test (p > 0.05) in the same crop season. ^{ns}ANOVA non-significant.

Table 6
Yield ($Kg.ha^{-1}$) and weight of 1000 grains (WTG) (Means ± SE) at selected treatments of different levels of interaction between defoliation and stink bug infestation evaluated during the 2020/2021 crop season trials (Trials 1 and 2: 16.7% = ½ET and 33.3% = ET for defoliation (%) during vegetative stage; Trial 3: 8.3% = ½ET and 16.7% = ET for defoliation% during reproductive stage).

Treatment		Trial 1 (defoliation and stink bug injuries during the vegetative soybean development stage)		Trial 2 (defoliation and stink bug injuries during the vegetative and reproductive soybean development stages, respectively)		Trial 3 (defoliation and stink bug injuries during the reproductive soybean development stage)	
		Yield ($kg.ha^{-1}$)	WTG	Yield ($kg.ha^{-1}$)	WTG	Yield ($kg.ha^{-1}$)	WTG
Control (0 injury)		4035.4 ± 106.9^{ns}	132.8 ± 1.7^{ns}	3296.8 ± 376.1^{ns}	133.1 ± 4.8^{ns}	2267.9 ± 141.6^{ns}	121.6 ± 3.2^{ns}
½ Defoliation ET + 1 stink bug		3595.4 ± 369.9	137.0 ± 3.0	3120.5 ± 585.5	125.1 ± 3.3	2135.5 ± 64.5	118.3 ± 6.0
½ Defoliation ET + 2 stink bugs		3273.2 ± 195.4	134.8 ± 2.3	2820.3 ± 123.2	125.3 ± 1.6	2512.8 ± 324.1	131.6 ± 0.7
Defoliation ET + 1 stink bug		3500.7 ± 353.8	137.0 ± 2.0	2249.7 ± 262.2	119.4 ± 4.4	2461.9 ± 216.3	126.1 ± 2.6
Defoliation ET + 2 stink bugs		3769.5 ± 197.0	129.0 ± 1.4	2524.9 ± 281.6	124.7 ± 4.8	2261.6 ± 217.3	117.3 ± 7.0
Statistics	$P_{treatment}$	0.46	0.12	0.07	0.04	0.71	0.27
	P_{block}	0.96	0.54	0.01	0.00	0.44	0.83
	$F_{treatment}$	0.96	2.29	2.94	3.53	0.54	1.47
	F_{block}	0.09	0.76	6.45	7.62	0.98	0.29
	$DF_{residue}$	12	12	12	12	12	12

^{ns}ANOVA non-significant ($p > 0.05$).

There is considerable evidence in the scientific literature that soybean yield and seed quality are more susceptible to injuries during the reproductive stage than in the vegetative stage (Batistela et al., 2012; Mertz-Henning et al., 2017). Intriguingly, the results for trial 1 indicate yield loss when two stink bugs m^{-1} were present during the vegetative stage although only in one of the tested crop seasons (2018/19, trial 1). It is important to point out that stink bug infestation was imposed during the entire vegetative stage, from V1 to the early soybean reproductive stage R1 (Fehr et al., 1971). This is unusual and not likely to happen in a commercial field (Oliveira et al., 2022). Despite such high insect pressure, other important parameters such as the weight of 1000 grains, total number of pods, oil and protein content, and quality parameters were

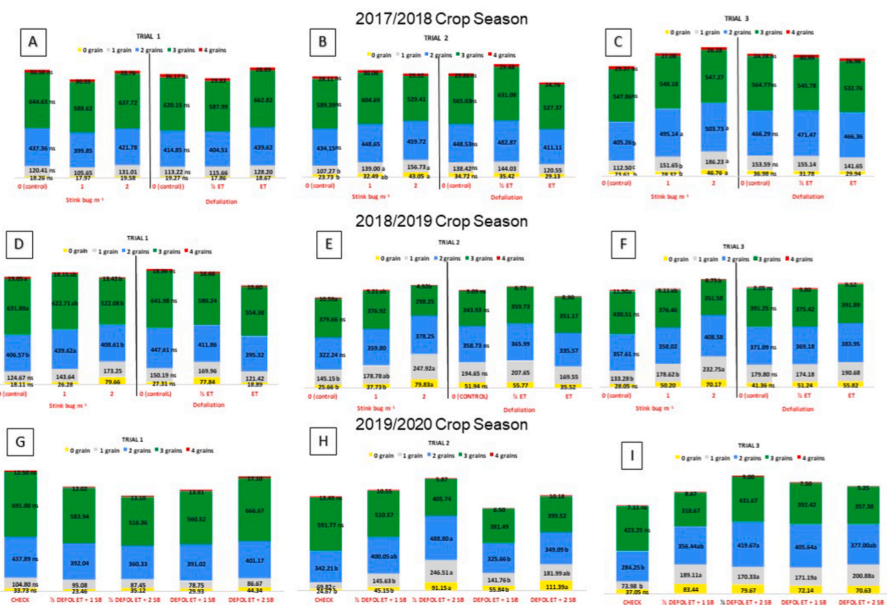

Fig. 1. Number of pods containing 0, 1, 2, 3 and 4 grains in different scenarios of defoliation and stink bug interaction evaluated during the 2017/2018 and 2018/2019 crop seasons in three independent field trials (A–F); and during the 2020/2021 crop season with selected treatments (G–I). Trials 1 and 2: 16.7% = ½ET and 33.3% = ET for defoliation (%) during vegetative stage; Trial 3: 8.3% = ½ET and 16.7% = ET for defoliation% during reproductive stage. Means followed by the same lowercase letter for stink bug, and uppercase letter for defoliation did not significantly differ ($p \geq 0.05$). ns = non-significant.

Table 7
Oil and protein content (%) at selected treatments of different levels of interaction between defoliation and stink bug infestation evaluated during the 2020/2021 crop season trials (Trials 1 and 2: 16.7% = ½ET and 33.3% = ET for defoliation (%) during the vegetative stage; Trial 3: 8.3% = ½ET and 16.7% = ET for defoliation% during the reproductive stage).

Treatment		Trial 1 (defoliation and stink bug injuries during the vegetative soybean development stage)		Trial 2 (defoliation and stink bug injuries during the vegetative and the reproductive soybean development stages, respectively)		Trial 3 (defoliation and stink bug injuries during the reproductive soybean development stage)	
		Oil (%)	Protein (%)	Oil (%)	Protein (%)	Oil (%)	Protein (%)
Control (0 injury)		23.0 ± 0.9^{ns}	35.2 ± 1.3^{ns}	23.7 ± 0.3 a	33.8 ± 0.1 b	23.9 ± 0.2^{ns}	32.6 ± 0.5^{ns}
½ Defoliation ET + 1 stink bug		22.5 ± 1.0	35.7 ± 1.2	22.2 ± 0.2 ab	35.00 ± 0.6 b	22.3 ± 0.6	35.5 ± 0.9
½ Defoliation ET + 2 stink bugs		23.1 ± 0.3	35.00 ± 0.2	21.0 ± 0.5 b	38.0 ± 0.3 a	23.7 ± 0.2	34.0 ± 0.3
Defoliation ET + 1 stink bug		23.1 ± 0.5	35.1 ± 1.0	22.8 ± 0.6 a	35.2 ± 0.7 ab	21.9 ± 0.9	36.5 ± 1.6
Defoliation ET + 2 stink bugs		23.8 ± 1.2	34.8 ± 1.0	22.0 ± 1.0 ab	36.5 ± 1.3 ab	22.1 ± 0.4	35.7 ± 0.7
Statistics	$P_{treatment}$	0.87	0.95	0.01	0.00	0.06	0.07
	P_{block}	0.58	0.06	0.01	0.06	0.43	0.42
	$F_{treatment}$	0.30	0.16	6.35	6.64	3.07	2.83
	F_{block}	0.68	3.25	6.74	3.28	0.99	1.01
	$DF_{residue}$	12	12	12	12	12	12

Means (±SE) followed by the same letter in a column did not differ statistically from each other according to theTukey test ($p > 0.05$). ^{ns}ANOVA non-significant.

not impacted by stink bug infestation during the vegetative stage. The Neotropical Brown Stink Bug *E. heros* is able to feed during the soybean's vegetative stage and can trigger the plant's defense response (Timbó et al., 2014). This might be a possible explanation for the observed impact on pod composition and yield, which occurred only in one of the crop seasons and was not observed in the other two crop seasons.

Brazil has adopted the same ET for different species of stink bug pests in soybean. However, the damage potential of some pentatomid species, such as *Piezodorus guildinii* (Westwood) (Hemiptera: Pentatomidae) has been reported to be higher than other stink bug complexes, possibly because of the length of the mouthparts and its unique salivary compounds (Depieri and Panizzi, 2011; Sosa-Gómez et al., 2020). In addition, *P. guildinii* is reported to cause foliar retention (Husch et al., 2014), which can also impact harvest results. Such differences might be considered in future research in order to develop a multiple guild ET. Despite being the most common species, *E. heros* is considered less harmful than other stink bug species, with densities up to 12 adults m^{-1} for 21 days at the R6 stage not reducing crop yield (Scopel et al., 2016).

Our findings indicate that defoliation, when kept at the ET (16.7% defoliation) throughout all of the reproductive stages (from R1 to R8), can lower the weight of 1000 grains but with no impact on yield. Previous studies have reported that defoliation and the consequent reduction of the leaf area index are responsible for decreasing the weight of 1000 grains (Glier et al., 2015; Hayashida et al., 2021) and are directly correlated with yield loss (Dalchiavon and De Passos E Carvalho, 2012). In contrast, the present study found a decrease in weight of 1000 grains but no overall yield loss.

Plants adopt different strategies to avoid reduction in fitness. One of these strategies is the reallocation of primary metabolites (Zhou et al., 2015). It is possible that when our tested plants were experiencing defoliation and stink bug injury, they reallocated photo assimilates from the developing pods and grains to new ones. Our tested cultivar has an indeterminate growth habit and this might explain why the observed decrease in the weight of 1000 grains had no impact on overall yield.

The soybean grains produced in Brazil have higher oil and protein content than those of other major global exporting countries (Thakur and Hurburgh, 2007). Thus, besides soybean yield quantity, soybean quality parameters, particularly oil and protein content, are important

Table 8
Main attributes of National Standard quality test (IN11; Brasil, 2007) of 30g samples at different levels of interaction between defoliation and stink bug infestation evaluated during the 2020/2021 crop season trials (Trials 1 and 2: 16.7% = ½ET and 33.3% = ET for defoliation (%) during the vegetative stage; Trial 3: 8.3% = ½ET and 16.7% = ET for defoliation% during the reproductive stage).

Treatments		Trial 1 (defoliation and stink bug injuries during the vegetative soybean development stage)			Trial 2 (defoliation and stink bug injuries during the vegetative and reproductive soybean development stages, respectively)			Trial 3 (defoliation and stink bug injuries during the reproductive soybean development stage)		
		Fermented (g)	Immature (g)	DBI (g)[1]	Fermented (g)	Immature (g)	DBI (g)[1]	Fermented (g)	Immature (g)	DBI (g)[1]
Control (0 injury)		9.5 ± 4.3 ^{ns}	0.4 ± 0.2 ab	7.2 ± 2.3 ^{ns}	6.4 ± 0.4 b	1.2 ± 0.4 ^{ns}	6.2 ± 0.8 ^{ns}	4.1 ± 1.1 b	0.76 ± 0.50 ^{ns}	5.1 ± 0.6 ^{ns}
½ Defoliation ET + 1 stink bug		12.5 ± 6.7	0.0 ± 0.0 b	8.6 ± 1.7	10.9 ± 2.1 a	1.4 ± 0.5	10.7 ± 1.5	20.8 ± 4.0 a	0.23 ± 0.06	7.7 ± 0.5
½ Defoliation ET + 2 stink bugs		4.8 ± 2.0	0.1 ± 0.1 ab	6.3 ± 1.5	25.1 ± 2.8 a	2.3 ± 0.2	10.1 ± 1.1	18.2 ± 7.5 a	0.58 ± 0.32	8.2 ± 2.5
Defoliation ET + 1 stink bug		7.9 ± 2.4	0.7 ± 0.2 a	8.7 ± 2.1	20.2 ± 1.5 a	2.2 ± 0.6	10.1 ± 2.1	19.1 ± 4.9 a	1.64 ± 0.57	10.6 ± 1.2
Defoliation ET + 2 stink bugs		6.8 ± 2.9	0.2 ± 0.1 ab	7.3 ± 2.9	24.0 ± 3.7 a	3.3 ± 1.9	9.9 ± 0.8	21.4 ± 4.5 a	3.29 ± 1.63	10.2 ± 2.0
Statistics	$P_{treatment}$	0.71	0.03	0.94	0.00	0.47	0.22	0.03	0.16	0.21
	P_{block}	0.33	0.23	0.85	0.05	0.23	0.65	0.20	0.70	0.77
	$F_{treatment}$	0.55	3.81	0.18	17.95	0.96	1.70	2.56	2.02	1.72
	F_{block}	1.26	1.66	0.27	3.50	1.66	0.56	1.79	0.49	0.38
	$DF_{residue}$	12	12	12	12	12	12	12	12	12

Means (±SE) followed by the same letter in a column did not differ statistically from each other according to the Tukey test ($p > 0.05$). ^{ns}ANOVA non-significant. [1]DBI = Damaged by insects.

Table 9
Results of Tetrazolium test (França-Neto and Krzyzanowski 2018): Dead embryo (Stink bug scale 6–8) (%), vigor (%) and viability (%) at different levels of interaction between defoliation and stink bug infestation evaluated during the 2020/2021 crop season trials (Trials 1 and 2: 16.7% = ½ET and 33.3% = ET for defoliation (%) during the vegetative stage; Trial 3: 8.3% = ½ET and 16.7% = ET for defoliation% during the reproductive stage).

Treatments		Trial 1			Trial 2			Trial 3		
		Dead embryo (%)	Vigor (%)	Viability (%)	Dead embryo (%)	Vigor (%)	Viability (%)	Dead embryo (%)	Vigor (%)	Viability (%)
Control (0 injury)		9.5 ± 2.9 ^{ns}	65.5 ± 11.5^{ns}	78.3 ± 7.4^{ns}	9.3 ± 0.9^{ns}	66.3 ± 2.1 a	77.7 ± 2.7 a	4.5 ± 1.9 ^{ns}	74.5 ± 4.0 ^{ns}	83.3 ± 3.7 ^{ns}
½ Defoliation ET + 1 stink bug		11.0 ± 3.8	69.3 ± 8.0	80.8 ± 4.7	16.3 ± 5.7	40.3 ± 6.7 ab	53.8 ± 6.8 ab	18.0 ± 4.5	41.0 ± 5.5	59.3 ± 5.5
½ Defoliation ET + 2 stink bugs		6.3 ± 3.3	75.8 ± 8.2	86.3 ± 5.1	22.0 ± 4.4	26.7 ± 6.3 b	46.0 ± 6.6 b	12.0 ± 4.1	48.5 ± 11.8	62.3 ± 11.3
Defoliation ET + 1 stink bug		11.0 ± 3.7	54.5 ± 12.5	71.5 ± 8.8	19.0 ± 2.4	33.8 ± 7.9 b	49.3 ± 6.2 b	19.0 ± 5.9	43.8 ± 9.6	59.0 ± 9.3
Defoliation ET + 2 stink bugs		10.8 ± 3.8	61.5 ± 12.7	74.0 ± 10.0	20.0 ± 0.4	31.7 ± 8.4 b	52.0 ± 8.5 ab	15.8 ± 4.0	47.0 ± 11.9	61.5 ± 11.7
Statistics	$P_{treatment}$	0.88	0.76	0.74	0.09	0.01	0.02	0.24	0.16	0.29
	P_{block}	0.78	0.84	0.92	0.13	0.28	0.26	0.89	0.44	0.25
	$F_{treatment}$	0.29	0.47	0.49	2.60	5.99	4.25	1.61	2.03	1.42
	F_{block}	0.36	0.28	0.16	2.34	1.42	1.54	0.21	0.98	1.56
	$DF_{residue}$	12	12	12	12	12	12	12	12	12

Means (±SE) followed by the same letter in a column did not differ statistically from each other according to the Tukey test ($p > 0.05$). ^{ns}ANOVA non-significant.

for industry purposes. Soybean oil is the most utilized domestic oil in Brazil, comprising about 90% of the total oil and vegetable fat used (Henning et al., 2018). Further, its protein supply accounts for nearly 60% of the world's vegetable protein (Liu, 1997).

In the last crop season (2020/21, trial 2), the protein content was observed to increase with the number of stink bugs. However, the oil content decreased. The inverse correlation between oil and protein content is well-documented in the literature, although its causes are debatable (Carrão-Panizzi et al., 2021; Mertz-Henning et al., 2017; Mourtzinis et al., 2017; Wijewardana et al., 2019). Changes in oil and protein were observed only in one trial. Moreover, the values for all trials were very similar to the national average (22.42% oil and 36.69% protein) (Henning et al., 2018; Hirakuri et al., 2018), which indicates that no important impact can be attributed to those plant injuries.

Despite the differences in soybean quality between treatments regarding the impact of stink bugs on the percentage of dead embryos (%), almost all values are within the limit determined by national legislation (Brasil, 2007). The percentage of dead embryos (%) is a parameter that assesses seed quality. The limit is 6% and the values observed in our trials are below that limit.

In the second and third crop season, the values for fermented soybean in all treatments exceed national limits. This increase in fermentation was potentially caused by the cages used in the experiments since cage effects have been previously reported (Simmons and Yeargan, 1990). Further studies are needed to evaluate the impact of cages for early-maturing, indeterminate growth, high yield cultivars, and their interaction with defoliation and stink bug feeding.

As stink bug density increased, the observed decrease in vigor and viability in some of the trials could be explained by proteases trigged by insect feeding. This may also contribute to the observed reductions in respiration and seed germination. When interpreting these results, it is necessary to consider that the plants were kept under injury during almost their entire reproductive stage. Infestation time also plays an important role in the intensity of seed damage (Scopel et al., 2016). However, adopting a lower ET does not increase yield and seed quality, nor does it provide any economic advantages (Bueno et al., 2015).

In conclusion, this study shows that injuries caused by defoliation and stink bug infestation under the ET do not have an impact on yield and its components, oil and protein content, or seed quality. An impact on these parameters was observed for a single injury under certain circumstances only in one trial in one season and therefore, the currently recommended ETs (30% defoliation at vegetative soybean stage, 15% defoliation at reproductive soybean stage and 2 or 1 stink bugs^{-1} for grain or seed production) can still be considered safe and can be used by soybean producers individually for defoliation and stink bugs.

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.

Data availability

Data will be made available on request.

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