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Impact of soybean injury by *Euschistus heros* (Fabricius, 1794) (Hemiptera: Pentatomidae) on feeding preference and survival of *Lasioderma serricorne* (Fabricius, 1792) (Coleoptera: Anobiidae), and on soybean quality maintenance under storage

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ABSTRACT

Soybean cultivars tolerant to *Euschistus heros* injury - known as 'Block® Technology' cultivars - were recently released by Embrapa Soja to be cropped in Brazil. Thus, this study aimed to determine feeding preferences and survival rates of *Lasioderma serricorne* in stored soybean of different cultivars ('Block® Technology' and susceptible cultivars), as well as to analyze the grain quality of soybean cultivars after being exposed to two different *E. heros* outbreak levels (4-5 adults/m and ≤2 adults/m). Two 'Block® Technology' soybean cultivars (BRS 543 RR and BRS 1003 IPRO) and four susceptible soybean cultivars (BRS 1010 IPRO, BRS 1061 IPRO, NA 5909 RG and NS 5959 IPRO) were studied. The higher level of *E. heros* abundance (4-5 adults/m) reduced the nutritional quality of soybean for *L. serricorne*. It preferred and more severely attacked stored soybean which had been exposed to a lower *E. heros* level of abundance (≤2 adults/m) in the field. Also, the higher abundance of *E. heros* in the field triggered higher percentages of fermented grains, which increased with storage time. In conclusion, the studied soybean cultivars tolerant to *E. heros* in the field were not tolerant to *L. serricorne* injury under storage conditions. Moreover, despite higher tolerance of 'Block® Technology' cultivars to *E. heros*, our data show that long-lasting outbreaks of 4–5 adult stink bugs/m (R4 and R5 stages) should be avoided in the field since they triggered a higher percentage of fermented grains during storage, mainly in the first 3 months of storage.

1. Introduction

Among the soybean pests occurring in South America, the piercing-sucking stink bug complex (Hemiptera: Pentatomidae) stands out as the most important group (Bueno et al. 2015, 2021). Stink bugs reported in soybean fields include at least 54 different species (Panizzi and Slansky Junior, 1985) of which *Euschistus heros* (Fabricius, 1794) (Hemiptera: Pentatomidae) is the most abundant and economically important in South America, mainly in the central region of Brazil, at latitudes between 0° and 23° (Panizzi and Corrêa-Ferreira, 1997; Bueno et al., 2021). Those pests feed directly on soybean pods from plants in the field, seriously affecting the yield and negatively impacting the physiological and sanitary quality of harvested grains and seeds (Corrêa-Ferreira and Azevedo, 2002). Moreover, field outbreaks of different stink bug species can trigger an increase of fermentation and acidity rates in stored soybean, in addition to causing necrosis of cotyledons and embryonic axes (Panizzi and Slansky Junior, 1985). Despite the impact of insects in the field on the product's quality during storage, any relationship between the intensity of injury caused by *E. heros* in the field and the occurrence and development of pest species during storage is still virtually unknown.

After harvest, soybean grains and seeds are stored for different periods of time until reaching their final destination. During storage, soybean is also vulnerable to pests (Lorini, 2012; Silva et al., 2018). The cigarette beetle, *Lasioderma serricorne* (Fabricius, 1792) (Coleoptera: Anobiidae), is considered one of the economically most serious insect

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pests during storage (Hori et al., 2011; Naveena et al., 2019). It is found in different regions, feeding on different stored products (Verma, 2012; Zanuncio et al., 2014; Silva et al., 2018; Naveena et al., 2019; Azmiera et al., 2020; Vishali et al., 2022). Recently, the species has become greatly important to Brazilian producers because of its significant abundance in stored soybean (Lorini, 2012; Silva et al., 2018). It is a major threat during soybean storage since it is reducing the weight, germination rates and commercial quality of grains (Silva et al., 2018).

Adults of *L. serricorne* do not feed on soybean seeds but dig holes to create suitable oviposition sites (Boateng et al., 2017). After the incubation period, emerged larvae are extremely active and feed heavily on grains and seeds (Gunasekaran, 2001), causing severe damage (Lorini et al., 2015; Silva et al., 2018). These injuries reduce the nutritional value of soybean and facilitate contamination by pathogens, especially fungi of the genera *Aspergillus*, *Penicillium*, and *Fusarium*, which impact quality and vigor of stored soybean grains and seeds (Panizzi and Slansky Junior, 1985; Corrêa-Ferreira and Sosa-Gómez, 2017; Silva et al., 2018). However, *L. serricorne* feeding preference and survival rates and, consequently, its potential to damage stored grains, vary between different food sources (Jacob, 1993; Saeed et al., 2004; Lorini, 2012; Naveena et al., 2019; El-Fouly et al., 2021). Thus, understanding the feeding habits and survival of *L. serricorne* on stored soybeans is of great theoretical and practical interest in order to define the best management strategies (Lorini, 2012; Silva et al., 2018). Therefore, this study was carried out to determine feeding preference and survival rates of *L. serricorne* in stored soybean of different cultivars (both 'Block® Technology' and susceptible cultivars). In addition, we aimed to determine the maintenance of grain quality of different soybean cultivars harvested after exposure to one of two different stink bug outbreak levels in the field (4-5 adult stink bugs/m and ≤2 adult stink bugs/m).

2. Materials and methods

The experiment was carried out with *E. heros* infestation under field conditions and repeated during two consecutive crop seasons (2019/20 and 2020/21) at the Embrapa Soybean Experimental Station (Warta District, Londrina County, Paraná, Brazil; 23°11′ S, 51° 11′ W, 630 m of altitude) in a 6 × 2 factorial randomized block design (6 soybean cultivars X 2 stink bug infestations) (Table 1) with 4 replicates. Each replicate measured 4 × 6 m, where voile screen-cages (3 m width x 4 m length and 2 m height) were set up to cover plants and keep stink bug infestation constant over time. After harvesting, the soybean was stored in an air-conditioned laboratory with temperature at 25 ± 3 °C, and relative humidity of 60 ± 20%. The tests with *L. serricorne* were performed in the same laboratory, simulating commonly applied soybean storage conditions (Coradi et al., 2020a).

Table 1
Stink bug infestation levels of the soybean cultivars evaluated in the experiment.

Cultivar	Infestation level	Maturity group	Feature	Growth habit
BRS 543 RR	≤2 stink bugs.m^{-1} 4-5 stink bugs.m^{-1}	6.0	Tolerant to stink bugs (block technology)	Indetermined
BRS 1003 IPRO	≤2 stink bugs.m^{-1} 4-5 stink bugs.m^{-1}	6.3	Tolerant to stink bugs (block technology)	Indetermined
NS 5959 IPRO	≤2 stink bugs.m^{-1} 4-5 stink bugs.m^{-1}	5.9	Susceptible to stink bugs	Indetermined
BRS 1010 IPRO	≤2 stink bugs.m^{-1} 4-5 stink bugs.m^{-1}	6.1	Susceptible to stink bugs	Indetermined
NA 5909 RG	≤2 stink bugs.m^{-1} 4-5 stink bugs.m^{-1}	6.7	Susceptible to stink bugs	Indetermined
BRS 1061 IPRO	≤2 stink bugs.m^{-1} 4-5 stink bugs.m^{-1}	6.1	Susceptible to stink bugs	Indetermined

2.1. Origin of insects used in the experiments

Adults of wild stink bugs were collected in the field from soybean plants in the surrounding area of the experiment (23° 11′ 11.7″ S and 51° 10′ 46.1″ W, 630 m altitude). For this, a ground cloth (1.0 m × 1.2 m) was positioned parallel to soybean rows to cover the ground under each of two adjacent soybean rows. Plants were shaken and all adult insects that had dropped on the ground cloth were manually collected, put into a cage and taken to the laboratory. In the laboratory, insects were visually identified as described by Panizzi et al. (2012). Identification was confirmed by a dichotomous key (Rolston, 1974) for 5% of the insects visually identified as *E. heros*. Considering the significant population genetic structure of *E. heros* between northern and southern Brazilian regions (Zucchi et al., 2019) and that an occurrence of different species of the genus *Euschistus* in the same Brazilian soybean fields has not been reported (Hickmann et al., 2021), the adopted procedures were precise enough to ensure correct identification of the stink bug species. Thus, the insects collected in the field were used to infest the experimental units (protected in cages) with 4–5 stink bugs/m (Table 1).

Plants treated with ≤2 stink bugs/m were not covered by cages and the natural field population of the stink bug was evaluated weekly and controlled with insecticides if necessary. The registered insecticide used was thiamethoxam + lambda-cyhalothrin 26.5 + 35.25 g.a.i. ha^{-1} (Engeo Pleno® 250 mL ha^{-1}). It was sprayed with a CO_2 pressurized backpack sprayer (Herbicat Ltda, Catanduva, São Paulo, Brazil) adjusted to spray a volume of 150 L ha^{-1} using a hollow cone, model TXVK-8 tip. Spraying was carried out under appropriate environmental conditions (winds below 6 km h^{-1}, relative humidity above 50%, and a maximum temperature of 25 °C) to keep the *E. heros* population at ≤ 2 bugs per meter.

Lasioderma serricorne used in the experiment during soybean storage were obtained from a laboratory colony kept at the Post-harvest Seeds and Grains laboratory of the Technological Nucleus Dr. Nilton Pereira da Costa, Embrapa Soybean, where the insects had been maintained since 2010. Thus, the insects used in the experiments were approximately in the 50th generation, reared under controlled conditions (temperature of 25 ± 2 °C and relative humidity of 50 ± 10%). In the laboratory, the insects were maintained on a diet described by Ferri et al. (2012), based on 200 g cornmeal, wheat germ and yeast crushed in a proportion of 5:2:1, with the addition of 100 g whole wheat grains. The food was placed in a glass jar of 500 ml volume. Afterwards, 100 newly emerged, non-sexed adult individuals of *L. serricorne* were released inside the jar, for population multiplication. To cover the jars, the plastic screw caps were fitted with a mesh screen, internally coated with filter paper. The mass rearing jars were kept in the laboratory in an aired place until the emergence of new adults to be used in the experiments or for maintenance of insect cultures.

2.2. Conduction of the soybean in the field

The soybean cultivars (Table 1) were sown in parallel plots (3 ha each plot) using 15 seeds per linear meter and 0.45 m row spacing, on December 1, 2019 and December 16, in the first and second year of the experiment, respectively. To prevent injury by other insect pests present in the field, *Bt* insecticide (*Bacillus thuringiensis* 13.44 g.a.i. ha^{-1}; Dipel® 400 mL ha^{-1}) was sprayed three times during each crop season, using a CO_2 pressurized back sprayer (Herbicat®) set for a spray volume of 150 liters ha^{-1} before starting stink bug infestation, at R4 soybean developmental stage (Fehr et al., 1971). *Bacillus thuringiensis* is an extensively used insecticide against lepidopterous pests with minimal effects on *E. heros* (Hartman et al., 2016). There was no significant occurrence of pest species other than lepidopterous larvae and *E. heros* in the field, thus guaranteeing an isolated effect of *E. heros* in the experiment.

An herbicide (glyphosate 1440 g.a.i. ha^{-1}; Roundup 3L ha^{-1}) and fungicides (azoxystrobin + cyproconazol 93.33 g.a.i. ha^{-1}; Priori Xtra® 300 mL ha^{-1}) were also applied when necessary. The herbicide was sprayed twice between the third and sixth week after the emergence of plants. The fungicides were sprayed three times in the reproductive stage, starting between R1 and R2, followed by additional sprayings at 20 to 30-day intervals. *Bacillus thuringiensis*, herbicide and fungicide sprayings were performed evenly over the total area of the experiment on all cultivars. This ensured that any potential negative effects of those products on *E. heros* were the same in each treatment, mitigating any bias on the results of the experiment.

Evaluated cultivars were infested with one of two levels of stink bug abundance at the early R4 reproductive stage (Fehr et al., 1971) (Table 1). Infestation was controlled from R4 until the completion of R5 (Fehr et al., 1971), which simulates the most common *E. heros* occurrence in soybean fields in Brazil during the most sensitive soybean development stages to pest injury (Oliveira et al., 2022). Stink bug infestation levels were controlled as previously described in section "2.1. Origin of insects used in the experiments".

Samples of *E. heros* were taken on a weekly basis from early R4 until the completion of R5 soybean developmental stage in order to keep stink bug infestation as constant as possible in accordance to each pre-established treatment (Table 1). Sampling was performed using a ground cloth (1.0 m × 1.2 m) positioned parallel to soybean rows to cover the ground under each of two adjacent rows (Bueno et al., 2021). In each treatment, three random samples were taken from 1 m sections of rows, counting individuals longer than 0.5 cm (corresponding to adults and nymphs from 3rd to 5th instars). Whenever *E. heros* infestation was below 4 stink bugs/m inside the cages, wild stink bugs were collected in nearby soybean fields and released inside the cages to keep infestation at the pre-established level of 4–5 insects/m. Stink bug infestation ≤2 insects/m was kept at natural conditions, without chemical control (but would have been used if necessary) since the natural annual population never grew above this level, in neither season. At the end of R5 and beginning of R6 soybean development stage (Fehr et al., 1971), field cages were removed, and the insecticide thiamethoxam + lambda-cyhalothrin 26.5 + 35.25 g.a.i. ha^{-1} (Engeo Pleno® 250 mL ha^{-1}) was sprayed over all cultivars to control stink bugs until harvesting. Soybean was harvested in each replicate when grains were fully mature (R8 phenological stage) (Fehr et al., 1971). Each sample was threshed separately and stored to carry out evaluations of *L. serricorne* damage as well as grain quality at different points in time during storage.

2.3. Feeding preference and survival of *Lasioderma serricorne*

Immediately after harvesting, soybean grains were moisture standardized through shade drying, ensuring a moisture ≤14%, which is common for soybean storage in Brazil. This was required because the loss of viability and vigor of stored soybean under high RH conditions is a well-known phenomenon (Shelar et al., 2008). Moisture was checked using a humidity measurer model G650i (Gehaka®, São Paulo, SP, Brazil). Subsequently, the samples were homogenized in a grain homogenizer (Boerner®, Chicago, USA), divided into two equal parts and quartered in a multichannel cereal divider. After the homogenization procedure, samples were packed in paper boxes (1 kg) and stored until performing the feeding preference and survival tests, as well as the commercial evaluation and classification of soybeans, which were conducted as described in the following section (2.4).

The development and preference tests of *L. serricorne* were carried out in a wooden apparatus named 'preference arena' introduced by Lorini et al. (2018). In brief, the preference arenas measure 50 cm × 50 cm x 10 cm, and are divided into 16 compartments, with a maximum capacity of 500 g grains each, and fitted with a lid of transparent glass (Fig. 1). Experimental design was a 6 × 2 factorial randomized block (6 soybean cultivars X 2 stink bug infestations) (Table 1) with 4 replicates. Each preference arena was considered one replicate. Feeding preference and survival of *L. serricorne* between different soybean cultivars were examined 8 months after harvesting and storage in the 2019/20 crop season and repeated immediately after harvesting in the 2020/21 crop season.

For the evaluation of *L. serricorne* preference between soybean cultivars, 100 g of soybean were placed in each section of the 'preference arena' and 400 adults of *L. serricorne* (1–7 days old) were released in the center of the arena (Fig. 1). Sixty days after *L. serricorne* infestation (60 DAI), the soybeans of each section were removed and the number of insects (dead *L. serricorne*) determined. After this evaluation, the soybean content of each section of the 'preference arena' was placed in a 500 ml glass jar covered with filter paper. The number of *L. serricorne* alive (second generation) was determined after 90 DAI, and *L. serricorne* consumption of each soybean was evaluated. The content of each jar was sieved to remove the insects to be counted. The food consumption by *L. serricorne* was measured as described by Lorini et al. (2012) and Lorini et al. (2018) and briefly summarized in the following. The fine residues of the food (flour) of each glass jar were separated by the aid of a 20-micron-mesh sieve (Retsch®, Haan, Germany), weighted and considered directly related to the *L. serricorne* consumption of each treatment.

Data analysis was performed to confirm the assumptions of normality of residues (Shapiro and Wilk, 1965) and homogeneity of variance of treatments (Burr and Foster, 1972) and thus the reliability of the analysis of variance (ANOVA). The results of both seasons (2019/20 and 2020/21) were subjected to ANOVA through joint analysis of the harvestings and to the F significance test ($p \leq 0.05\%$). The means were compared by Tukey's test ($p \leq 0.05\%$) using the statistical software SASM-Agri.

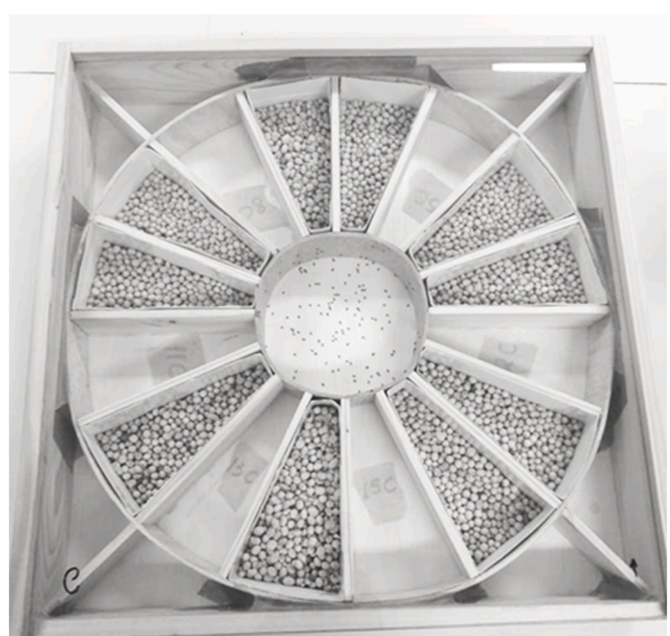

Fig. 1. Preference arena used in the *Lasioderma serricorne* trials.

2.4. Soybean commercial evaluation and classification

In addition to the development and host preference test of *L. serricorne*, the commercial classification of soybeans at different storage times was conducted. For the 2019/20 crop season, soybeans were classified after 10 months of storage and for the 2020/21 season immediately after harvesting, and repeated after three and six months of storage. For each storage period, soybean grains were classified according to the normative ruling (Normative Instruction n. 11) of May 15, 2007, of the Brazilian Ministry of Agriculture, Livestock, and Supply (Coradi et al., 2020b; Vinhote et al., 2021). Twenty grams of soybean were inspected in detail as described in the following:

a) Impurity: all materials retained by the sieves other than soybean, including pods not threshed, with a tolerance of 1% (the soybean husk retained by sieves is not considered impurity);
b) Foreign matter: all materials that pass through sieves with the following characteristics: sheet thickness 0.8 mm, number of holes 400/100 cm^2, hole diameter 3.0 mm with a tolerance of 1%;
c) Moldy: all beans or pieces of beans with fungi (mold or mildew) visible to the naked eye, with a tolerance of 6%;
d) Fermented: all beans or pieces of beans that, due to the fermentation process, have undergone a visible change in the color of the cotyledon other than the one defined for sour beans;
e) Germinated (or sprouted): all beans or pieces of beans that have a visible radicle;
f) Immature: oblong-shaped beans that are intensely green, not having reached their full physiological development and which may be wrinkled;
g) Damaged by stink bugs: beans or pieces of beans that have altered and deformed spots in the pulp, are perforated or were attacked by stink bugs, in any of their developmental phases;
h) Total damage: the sum of all the above with a tolerance of 8%.
i) Greenish: the beans or pieces of beans with complete physiological development with a greenish cotyledon, with a tolerance of 8%;
h) Shriveled: beans with irregular shapes which are wrinkled, atrophied and devoid of internal mass.
j) Broken: all pieces of beans, including cotyledons, that are retained in a sieve with round holes of 3.0 mm in diameter;
l) Moisture: the percentage of water found in the product sample free of foreign matter and impurities, determined by an official method or by an apparatus that gives an equivalent result, with a tolerance of 14%.
m) Burned: all carbonized beans or pieces of beans;
n) Sour: all beans or pieces of beans that are visibly fermented in their entirety and with marked dark brown color, affecting the cotyledon;

3. Results

3.1. Feeding preference and survival of Lasioderma serricorne

There was a significant interaction between soybean cultivars and stink bug infestation levels in the joint analysis of the 2019/20 and 2020/21 crop seasons for all evaluated biological parameters of *L. serricorne* (Tables 2–4). Sixty days after *L. serricorne* infestation (60 DAI) in the 'preference arena', higher numbers of *L. serricorne* adults were recorded for soybeans of the cultivar BRS 543 RR compared with NS 5959 IPRO from fields under ≤2 *E. heros* adults/m infestation. The other studied soybean cultivars did not differ neither from BRS 543 RR nor from NS 5959 IPRO. In contrast, no differences in *L. serricorne* numbers were recorded between cultivars from fields with 4–5 *E. heros* adults/m infestation (Table 2). However, when comparing *L. serricorne* preference within soybean cultivars from fields with different levels of *E. heros* infestation, there was a overall higher number of *L. serricorne* adults on grains from fields with ≤2 *E. heros* adults/m than on grains from fields with 4–5 *E. heros* adults/m. This relationship between *L. serricorne* preference and previous *E. heros* infestation in the field was not recorded for the other studied cultivars (Table 2).

The number of *L. serricorne* recorded in each cultivar at 90 DAI (a point in time that corresponds to the second generation of these insects) was significantly higher for cultivars from fields with lower *E. heros* infestation (≤2 adults/m), except for the cultivar BRS 1061 IPRO. For this cultivar, abundance of *L. serricorne* was similar regardless of previous *E. heros* infestation in the field (Table 3). Concerning the number of *L. serricorne* on different cultivars, results differed between *E. heros* infestation levels. The lowest numbers of *L. serricorne* were found for

Table 2
Numbers of *Lasioderma serricorne* 60 days after infestation (DAI) in different stored soybean cultivars previously infested by *Euschistus heros* in the field.

Cultivar	Number of *L. serricorne*					
	≤2 stink bugs/meter in the field			4-5 stink bugs/meter in the field		
BRS 543 RR	13.0 ± 1.2	a	A	6.6 ± 0.8	a	B
BRS 1003 IPRO	8.8 ± 1.0	ab	A	9.9 ± 1.1	a	A
NS 5959 IPRO	6.6 ± 0.8	b	A	7.1 ± 0.9	a	A
BRS 1010 IPRO	10.9 ± 1.4	ab	A	8.8 ± 1.2	a	A
NA 5909 RG	10.7 ± 1.1	ab	A	6.0 ± 0.8	a	B
BRS 1061 IPRO	7.1 ± 1.7	ab	A	5.3 ± 1.4	a	A
Statistics				$F_{cultivar} = 2.93$ $F_{stink\ bug} = 9.49$ $F_{cultivar\ x\ stink\ bug} = 4.26$ $F_{crop\ season} = 114.8$ $F_{block} = 22.97$ $P_{cultivar} = 0.0193$ $P_{stink\ bug} = 0.0030$ $P_{cultivar\ x\ stink\ bug} = 0.0021$ $P_{crop\ season} < 0.0001$ $P_{block} < 0.0001$ $DF_{residue} = 23$		

Joint analysis of the 2019/20 and 2020/21 crop seasons. Means ± SEM followed by the same lower case letter in columns (between cultivars) and the same upper case letter in rows (between stink bug populations) did not statistically differ (Tukey's Test, $p > 0.05$). Data analyzed assuming the Poisson distribution.

Table 3
Numbers of *Lasioderma serricorne* 90 days after infestation (DAI) in different stored soybean cultivars previously infested by *Euschistus heros* in the field.

Cultivar	Number of L. *serricorne*					
	≤2 stink bugs/meter in the field			4-5 stink bugs/meter in the field		
BRS 543 RR	7.0 ± 0.9	a	A	0.3 ± 0.2	c	B
BRS 1003 IPRO	5.9 ± 0.8	a	A	2.5 ± 0.5	ab	B
NS 5959 IPRO	6.7 ± 0.9	a	A	0.9 ± 0.3	abc	B
BRS 1010 IPRO	8.1 ± 1.5	a	A	2.0 ± 0.7	abc	B
NA 5909 RG	1.9 ± 0.5	b	A	0.7 ± 0.3	bc	B
BRS 1061 IPRO	3.1 ± 0.8	ab	A	3.1 ± 0.8	a	A
Statistics				$F_{cultivar} = 5.64$ $F_{stink\ bug} = 64.27$ $F_{cultivar\ x\ stink\ bug} = 5.32$ $F_{crop\ season} = 2.12$ $F_{block} = 13.94$ $P_{cultivar} = 0.0002$ $P_{stink\ bug} < 0.0001$ $P_{cultivar\ x\ stink\ bug} = 0.0004$ $P_{crop\ season} = 0.1506$ $P_{block} < 0.0001$ $DF_{residue} = 23$		

Joint analysis of the 2019/20 and 2020/21 crop seasons.Means ± SEM followed by the same lower case letter in columns (between cultivars) and the same upper case letter in rows (between stink bug populations) did not statistically differ (Tukey's Test, $p > 0.05$). Data analyzed assuming the Poisson distribution.

Table 4
Consumption by *Lasioderma serricorne* (mg) of grains of stored soybean cultivars harvested after different infestation levels of *Euschistus heros* in the field.

Cultivar	*L. serricorne* consumption (mg)					
	≤2 stink bugs/meter in the field			4-5 stink bugs/meter in the field		
BRS 543 RR	158.0 ± 42.2	a	A	13.6 ± 3.5	b	B
BRS 1003 IPRO	81.7 ± 22.0	a	A	27.9 ± 7.3	ab	B
NS 5959 IPRO	115.4 ± 30.1	a	A	13.2 ± 3.4	b	B
BRS 1010 IPRO	238.7 ± 91.9	a	A	27.1 ± 10.4	ab	B
NA 5909 RG	70.4 ± 18.8	a	A	11.4 ± 2.9	b	B
BRS 1061 IPRO	97.5 ± 37.6	a	A	91.3 ± 35.6	a	A
Statistics				$F_{cultivar} = 3.95$ $F_{stink\ bug} = 86.26$ $F_{cultivar\ x\ stink\ bug} = 3.62$ $F_{crop\ season} = 20.88$ $F_{block} = 11.40$ $P_{cultivar} = 0.0035$ $P_{stink\ bug} < 0.0001$ $P_{cultivar\ x\ stink\ bug} = 0.0061$ $P_{crop\ season} < 0.0001$ $P_{block} < 0.0001$ $DF_{residue} = 23$		

Joint analysis of the 2019/20 and 2020/21 crop seasons. Means ± SEM followed by the same lower case letter in the column (between cultivars) and the same upper case letter in rows (between stink bug populations) did not statistically differ (Tukey's Test, $p > 0.05$). Data analyzed assuming the gamma distribution.

cultivars NA 5909 RG and BRS 543 RR from *E. heros* infestation levels of ≤2 adults/m and 4-5 adults/m, respectively (Table 3).

Food consumption (mg) by *L. serricorne* (Table 4) after 90 DAI showed the same trend as their abundance (Table 3). Consumption (mg) was significantly higher for cultivars from fields with lower *E. heros* infestation (≤2 adults/m), except for the cultivar BRS 1061 IPRO (Table 4). There were no differences between cultivars from fields with an *E. heros* infestation level of ≤2 adults/m. In contrast, higher *L. serricorne* consumption was recorded for BRS 1061 IPRO and lower consumption for both BRS 543 RR, NS 5959 IPRO, and NA 5909 RG from fields previously infested with 4–5 adults of *E. heros*/m, while onsumption of cultivars BRS 1003 IPRO and BRS 1010 IPRO did not differ from these (Table 4).

3.2. Soybean commercial evaluation and classification

There were significant differences in percentage of damage caused by *E. heros* recorded for the samples of soybean grains harvested in the 2019/20 and 2020/21 crop seasons (Tables 5–8). The classification characteristics of foreign matter, moldy, greenish, and moisture were always below 1%, 6%, 8%, and 14%, respectively. These are equivalent to the maximum limits established by IN11 for soybean commercialization (Vinhote et al., 2021) (Tables 5–8). However, it is important to point out that these values were higher for soybean with an *E. heros* infestation of 4–5 adults/m for almost all tested soybean cultivars (Tables 5–8). Values were lower shortly after harvest (Table 6) but increased during storage, mainly in the first three months (Tables 7 and 8).

No burned or sour beans were recorded in any of the treatments. Values for germinated and shriveled beans were zero in 2019/20 (Table 5) as well as after harvest in 2020/21 (Table 6). After three and six months of storage in 2020/21 values for shriveled beans were still zero while low numbers of germinated beans were recorded for BRS 543 RR at both *E. heros* infestation levels (Tables 7 and 8). Values for immature beans were also low at all evaluated time points and both levels of *E. heros* infestation in both seasons (Tables 5–8).

During the 2019/20 season, impurity values were always below the IN11 established limit of 1% for soybeans at the *E. heros* infestation level of ≤2 adults/m. On the other hand, they were always above this limit for soybeans at the *E. heros* infestation level of 4–5 adults/m (Table 5). Similarly, during the 2020/21 crop season, impurity values for soybean at the higher *E. heros* infestation level were higher than for soybeans with lower *E. heros* infestation with the exception of BRS 1003 IPRO (Table 6). Impurity did not change over time, remaining the same at three (Table 7) and six (Table 8) months of soybean storage.

Total damage was usually higher for soybean from fields with higher *E. heros* infestation (Tables 5–8) although during the 2019/20 crop season it was only higher than the limit of 8% for BRS 543 RR and NA 5909 RG (Table 5). Similarly, during the 2020/21 season, total damage at harvest was higher than 8% for both NA 5909 RG and BRS 1061 IPRO from fields with 4–5 *E. heros*/m (Table 6). After three months of storage, the values for total damage were higher than 8% for all soybean cultivars from fields with 4–5 *E. heros*/m (Table 7). After six months of storage the value for total damage for BRS 1061 IPRO from fields with ≤2 *E. heros*/m was also higher than 8% (Table 8).

Table 5
Commercial classification (%) of soybean by Normative Instruction N°. 11 after 10 months of storage. Different soybean cultivars cropped after exposure to two levels of infestation by *Euschistus heros* in the field. 2019/20 crop season.

Cultivars	Stink bug. m^{-1}	Impurity	Foreign matter	Moldy	Fermented	Germinated	Immature	Damaged by stink bugs	Total Damage	Greenish	Shriveled	Broken	Moisture
BRS 543 RR	≤2	0.23	0.00	0.00	0.00	0.00	0.00	0.17	0.17	0.29	0.00	0.49	11.9
BRS 543 RR	4–5	1.37	0.01	0.00	7.07	0.00	1.37	11.45	19.89	0.00	0.00	7.66	12.0
BRS 1003 IPRO	≤2	0.22	0.01	0.00	0.00	0.00	0.00	0.69	0.69	0.00	0.00	0.00	11.9
BRS 1003 IPRO	4–5	3.06	0.00	0.93	1.03	0.00	1.33	2.62	5.91	2.16	0.00	1.03	11.6
NS 5959 IPRO	≤2	0.11	0.00	0.00	0.00	0.00	1.12	0.44	1.56	1.22	0.00	0.58	10.7
NS 5959 IPRO	4–5	4.82	0.01	0.00	0.00	0.00	0.00	3.94	3.94	6.34	0.00	0.39	11.2
BRS 1010 IPRO	≤2	0.16	0.00	0.00	1.30	0.00	0.34	0.81	2.25	0.00	0.00	0.29	13.1
BRS 1010 IPRO	4–5	3.44	0.01	0.00	2.35	0.00	0.00	1.86	4.21	0.93	0.63	0.24	12.4
NA 5909 RG	≤2	0.41	0.00	0.00	0.14	0.00	0.39	1.76	2.29	0.00	0.00	0.00	8.5
NA 5909 RG	4–5	1.86	0.03	0.00	2.69	0.00	0.81	8.45	11.95	3.42	0.00	5.15	8.4

Table 6
Commercial classification (%) of soybean by Normative Instruction N°. 11 immediately after harvest. Different soybean cultivars cropped after exposure to two levels of infestation by *Euschistus heros* in the field. 2020/21 crop season.

Cultivars	Stink bug.m^{-1}	Impurity	Foreign matter	Moldy	Germinated	Immature	Total Damage	Greenish	Shriveled	Broken	Moisture
BRS 543 RR	≤2	0.59	0.00	0.00	0.00	0.00	0.96	0.00	0.00	0.58	10.5
BRS 543 RR	4–5	4.17	0.01	0.00	0.00	1.09	5.86	2.08	0.00	1.38	13.4
BRS 1003 IPRO	≤2	1.76	0.00	0.00	0.00	0.34	4.13	0.00	0.00	0.63	13.6
BRS 1003 IPRO	4–5	1.64	0.01	0.00	0.00	1.06	6.92	0.00	0.00	2.76	11.2
NS 5959 IPRO	≤2	2.04	0.00	0.00	0.00	0.00	1.60	1.78	0.00	0.39	13.3
NS 5959 IPRO	4–5	0.49	0.01	0.00	0.00	1.29	6.38	0.00	0.00	2.49	11.4
NA 5909 RG	≤2	1.36	0.00	0.00	0.00	0.00	0.95	0.00	0.00	0.49	12.4
NA 5909 RG	4–5	2.11	0.01	0.00	0.00	0.00	14.29	0.00	0.00	0.39	13.3
BRS 1061 IPRO	≤2	0.33	0.00	0.00	0.00	0.00	2.10	0.00	0.00	0.62	10.5
BRS 1061IPRO	4–5	1.85	0.02	0.00	0.00	0.00	9.10	1.47	0.00	0.49	11.4

Table 7
Commercial classification (%) of soybean by Normative Instruction N°. 11 after three months of storage. Different soybean cultivars cropped after exposure to two levels of infestation by *Euschistus heros* in the field. 2020/21 crop season.

Cultivars	Stink bug.m^{-1}	Impurity	Foreign matter	Moldy	Germinated	Immature	Total Damage	Greenish	Shriveled	Broken	Moisture
BRS 543 RR	≤2	0.59	0.00	0.00	0.09	0.00	1.99	0.00	0.00	1.47	10.5
BRS 543 RR	4–5	4.17	0.01	0.00	0.29	0.83	12.12	0.00	0.00	0.00	13.4
BRS 1003 IPRO	≤2	1.76	0.00	0.00	0.00	0.74	5.66	0.00	0.00	0.00	13.6
BRS 1003 IPRO	4–5	1.64	0.01	0.00	0.00	0.05	11.86	0.00	0.00	0.48	11.2
NS 5959 IPRO	≤2	2.04	0.00	0.00	0.00	0.00	2.60	0.00	0.00	0.38	13.3
NS 5959 IPRO	4–5	0.49	0.01	0.00	0.00	0.29	11.07	0.00	0.00	1.93	11.4
NA 5909 RG	≤2	1.36	0.00	0.00	0.00	0.15	3.18	0.00	0.00	0.00	12.4
NA 5909 RG	4–5	2.11	0.01	0.00	0.00	0.47	15.96	0.00	0.00	0.09	13.3
BRS 1061 IPRO	≤2	0.33	0.00	0.00	0.00	0.00	5.14	0.00	0.00	0.69	10.5
BRS 1061 IPRO	4–5	1.85	0.02	0.00	0.00	0.00	17.88	0.00	0.00	0.29	11.4

Table 8
Commercial classification (%) of soybean by Normative Instruction N°. 11 after six months of storage. Different soybean cultivars cropped after exposure to two levels of infestation by *Euschistus heros* in the field. 2020/21 crop season.

Cultivars	Stink bug.m^{-1}	Impurity	Foreign matter	Moldy	Germinated	Immature	Total Damage	Greenish	Shriveled	Broken	Moisture
BRS 543 RR	≤2	0.59	0.00	0.00	0.09	0.00	1.76	0.00	0.00	0.07	10.5
BRS 543 RR	4–5	4.17	0.01	0.00	0.29	0.00	19.02	0.00	0.00	0.05	13.4
BRS 1003 IPRO	≤2	1.76	0.00	0.00	0.00	0.00	4.97	0.00	0.00	0.00	13.6
BRS 1003 IPRO	4–5	1.64	0.01	0.00	0.00	0.99	18.78	0.00	0.00	0.05	11.2
NS 5959 IPRO	≤2	2.04	0.00	0.00	0.00	0.00	3.95	0.00	0.00	0.00	13.3
NS 5959 IPRO	4–5	0.49	0.01	0.00	0.00	0.56	18.68	0.00	0.00	0.14	11.4
NA 5909 RG	≤2	1.36	0.00	0.00	0.00	0.00	3.02	0.00	0.00	0.00	12.4
NA 5909 RG	4–5	2.11	0.01	0.00	0.00	0.00	13.48	0.00	0.00	0.00	13.3
BRS 1061 IPRO	≤2	0.33	0.00	0.00	0.00	0.00	9.71	0.00	0.00	0.09	10.5
BRS 1061 IPRO	4–5	1.85	0.02	0.00	0.00	0.29	18.71	0.00	0.00	0.01	11.4

As expected, stink bug damage and consequently fermentation was almost always higher for the higher *E. heros* infestation during both 2019/20 (Table 5) and 2020/21 crop seasons (Fig. 2). During the 2020/21 season, an increase of *E. heros* damage and fermentation from harvest to three months of storage was observed, but the steepness of increase from three to six months of storage differed between the studied soybean cultivars (Fig. 2).

4. Discussion

The quality of consumed food affects the biological and physiological parameters of insects (Nation, 2002; Golizadeh et al., 2009). Thus, host preference and food consumption have direct influence on insect survival and several other biological features (Nation, 2002; Golizadeh et al., 2009). In this context, it is important to highlight that in our study all soybean cultivars from fields with lower *E. heros* infestation (≤2 adults/m) were more likely to be consumed by *L. serricorne* than the same cultivars from fields with higher *E. heros* infestation (4-5 adults/m) and therefore supported the development of a larger population of *L. serricorne* in the second generation (higher number of adult insects at 90 DAI). This was because the nutritional quality of soybean for *L. serricorne* was greatly reduced after being attacked by a higher number of *E. heros* in the field.

It is well-known that different physico-chemical properties of food interact with survival and reproduction rates as well as with the whole biology of *L. serricorne* (Edde, 2019). Therefore, the higher *E. heros* infestation in the field (4-5 adults/m) resulted in poorer quality of grains as also recorded in other tests of the Instruction Normative 11 (Vinhote et al., 2021). Furthermore, the longevity of adults of *L. serricorne* usually depends on the type and quantity of food consumed during the larval stage (Papadopoulou, 2006; Mahroof and Philips, 2008). The only exception was BRS 1061 IPRO, for which no consumption differences were recorded regardless of stink bug infestation.

In general, the food selection process of various insects is mediated by an interaction of the central nervous system with several sensory channels, providing different stimuli such as color, shape and texture. The appropriate combination of these factors drives the insect to choose one cultivar over another (Bruce et al., 2005). However, *L. serricorne* apparently did not show preferences between the studied soybean cultivars, according to the number of insects at 60 DAI in the 'preference arena'. In fact, host preference of *L. serricorne* is poorly understood as previously reported in the literature (Naveena et al., 2019). Overall, a

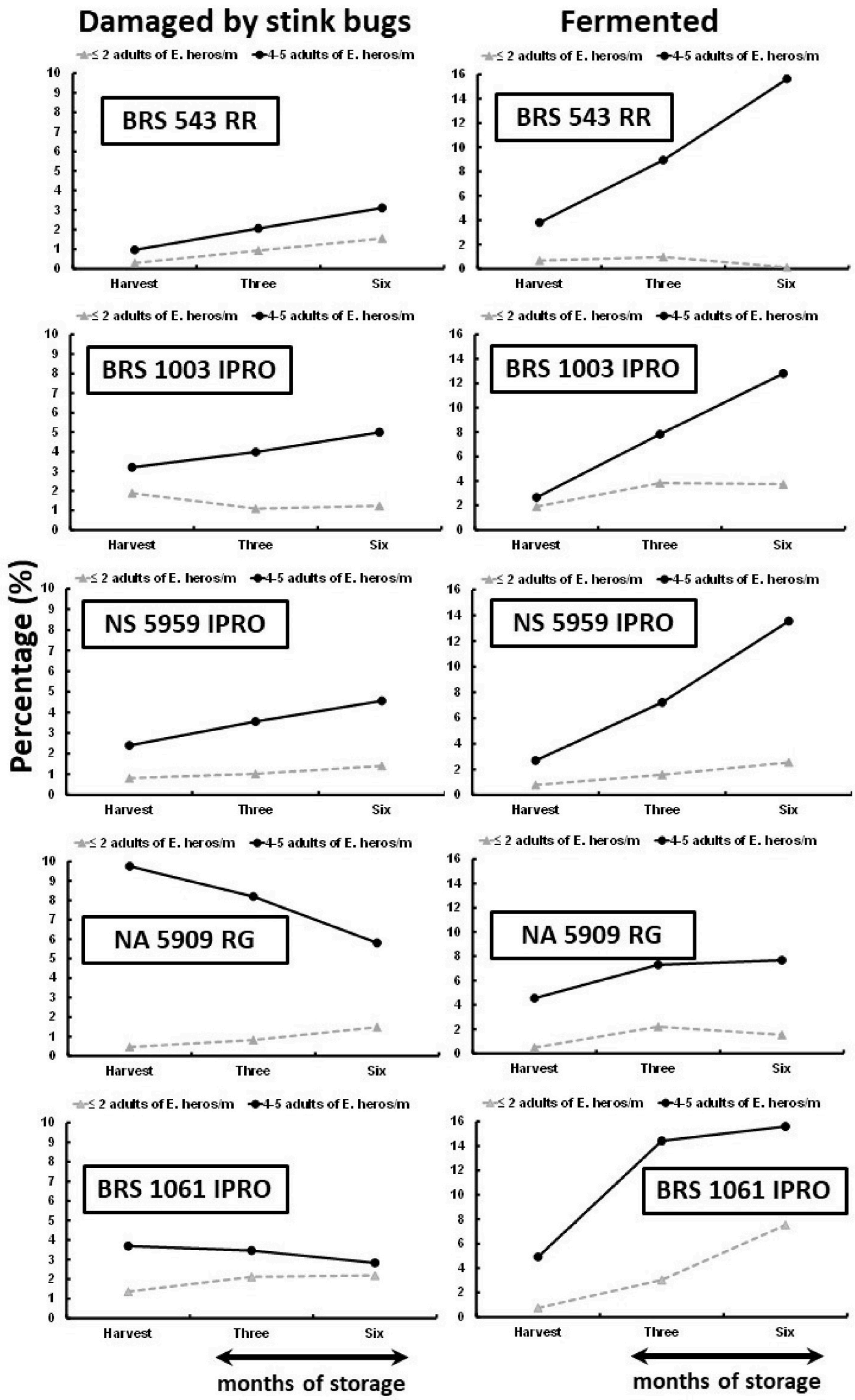

Fig. 2. Percentages (%) of damage by stink bugs and fermentation for the evaluated soybean cultivars at different storage time points according to Instructive Ruling number 11 (Vinhote et al., 2021).

carbohydrate diet is reported to be better for *L. serricorne* reproduction and development than oil crops and legumes (Babarinde et al., 2008). Thus, the differences recorded between the treatments in our study may partly be due to differences in the carbohydrate contents of different cultivars, which was not evaluated in our research and should be analyzed in future studies. To investigate nutritional factors impacting insect development is an important step when comparing different food sources and predicting which grain is more likely to be damaged by a pest (Scriber and Slansky Junior, 1981). This is crucial to design efficient management strategies in order to mitigate losses caused by *L. serricorne* during storage (Silva et al., 2018).

Previous studies have reported that field injuries in soybean pods trigger the plants to produce chemical substances for protection. Those are usually defense toxins that interfere with the development of pests, known as allelochemicals (Piubelli et al., 2005; Bortoli et al., 2012). Some other authors report deterrent and repellent properties of allelochemicals impacting the plant-pest relationship (Berenbaum and Neal, 1985; Ishaaya, 1986; Norris and Kogan, 2005). Thus, the higher *E. heros* infestation (4-5 adults/m) may have induced the presence of these allelochemicals in the tested soybean cultivars, leading to a lower nutritional quality of the soybean grains for *L. serricorne* consumption and development during storage. The presence of allelochemicals in different soybeans under higher and lower *E. heros* attack was not quantified during our study and should be further tested in future studies.

In addition, according to Chagas et al. (2019), *L. serricorne* has a preference for cultivars that allow its reproduction. The life cycle of this insect can vary between 30 and 90 days and produce between three and eleven generations per year (Lorini et al., 2015). In a study carried out by Lorini et al. (2018), *L. serricorne* population increased faster in stored soybeans with higher numbers of broken grains, which facilitates insect feeding and reproduction. However, when soybean grains are mechanically broken, the nutritional quality of the grains seems unaffected. In contrast, in our study the nutritional quality of grains was negatively impacted when soybeans were injured by stink bugs in the field, thus proving to be an even more important prerequisite for a stronger *L. serricorne* attack.

A preference for the diets available in the trials occurs only at the larval stages since adults of *L. serricorne* do not feed (Ferri et al., 2018). Lorini et al. (2012) found that the consumption of soybeans increased related to an increase in initial infestation, which supported the natural population growth of this species, suggesting a general adaptation of this pest to stored soybeans.

Not only did the *E. heros* infestation in the field impact soybean storage with regard to food consumption and development of *L. serricorne* but also to overall soybean quality over storage time. According to Bocatti et al. (2013), soybean quality during storage, defined by commercial standards, decreases with higher numbers of *E. heros* in the field. During storage, there is an increase in fermentation levels of stored grains directly related to *E. heros* infestation in the field. Soybean grains and seeds that suffered *E. heros* injury have higher rates of fermentation and increased acidity. Therefore, *E. heros* feeding reduces commercial soybean quality (Corrêa-Ferreira and Sosa-Gómez, 2017; Lorini et al., 2020) as well as the nutritional value of those grains for *L. serricorne* development.

It is noteworthy that the objective of the normative ruling number 11 (or Normative Instruction 11) is to establish the technical regulation for soybean, defining its official classification standards, with requirements for intrinsic and extrinsic identity and quality, sampling and marking or labeling for soybeans intended for export (Vinhote et al., 2021). In this ruling, the percentage of grains damaged by stink bugs must be divided by four for comparative purposes (Lorini, 2019). However, such damage is not necessarily discernible immediately after soybean harvest, but develops during storage and becomes more critical at the time of marketing (Corrêa-Ferreira and Sosa-Gómez, 2017). This explains the lower soybean quality recorded in our trial after longer periods of storage.

Therefore, despite *E. heros*-tolerant cultivars retaining their yield and seed quality at harvest under higher stink bug infestation in the field (Arias et al., 2018; Embrapa, 2019; Hoffmann-Campo et al., 2019), our results of an increased percentage of fermented grains during storage suggest that *E. heros* infestations of 4–5 adult stink bugs/m for long periods of time (from R4 until completion of R5 stages) should be avoided. This increase in fermentation levels and grain acidity during storage should be evaluated in detail in future research. Moreover, a greater tolerance of cultivars with ‘block technology’ to an *E. heros* infestation does not ensure a greater tolerance to a *L. serricorne* attack during storage. However, a higher abundance of *E. heros* in the field (4–5 stink bugs/m) reduces soybean nutritional quality for *L. serricorne,* which preferred and more severely attacked soybean under lower *E. heros* numbers in the field ($\leq$2 adults/m).

Authors’ statement

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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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