

Competition between the phytophagous stink bugs *Euschistus heros* and *Piezodorus guildinii* in soybeans

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Abstract

BACKGROUND: The abundance and contribution of the neotropical brown stink bug, *Euschistus heros* (F.), and the redbanded stink bug, *Piezodorus guildinii* (West.), to the composition of insect pests of soybean, *Glycine max* (L.), fields have changed both spatially and temporally in neotropical soybean production areas. Therefore, we assessed the competitiveness of each species in direct competition experiments following an additive series. We performed mixed (adult) insect infestations in soybean plants and evaluated the fitness of each species and the soybean yield.

RESULTS: While the competitive ability of *E. heros* was significantly compromised by increments in conspecifics and heterospecifics (i.e. *P. guildinii*), the competitive ability of *P. guildinii* was compromised by the presence of heterospecifics (i.e. *E. heros*). The reproductive output of *P. guildinii* remained unaffected by increments in *E. heros* or of *P. guildinii*. Intriguingly, despite the fact that *P. guildinii* apparently lost the competition with *E. heros*, almost no pod production was observed in any plant colonised by the former.

CONCLUSIONS: The higher abundance of *E. heros* in neotropical soybean fields seems to result from higher competitive ability than its heterospecific competitor *P. guildinii*, which may prevent the higher losses caused by *P. guildinii*.

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Keywords: phytophagous pentatomids; population growth; interspecific competition; intraspecific competition

1 INTRODUCTION

Intra- and interspecific competition dynamics are major ecological processes that shape the patterns of insect abundance, distribution and diversity.^{1–3} However, despite the strong evidence that herbivore competition is ubiquitous, the vast majority of investigations focus on leaf-chewing or grain-feeding insects,^{4–9} leaving the competitive interactions between vascular-feeding – and especially, seed-sucking insects (e.g. pentatomids) – as a largely unexplored subject.

Several species of these phytophagous pentatomids overlap in both niche and range.^{10–14} In commercial fields of soybeans, *Glycine max* (L.), the competition among herbivorous pentatomids will not only unbalance the stink bug assemblages but also determine the integrated pest management actions adopted to reduce economic losses by these key pest species. For instance, the annual impact of the stink bug complex on Brazilian soybean yield and quality is a combination of economic losses from reduced seed quality, direct yield losses and chemical control costs.^{15,16} In neotropical savannah-like areas, which are prevalent in mid-South America, the current pentatomid complex attacking soybean encompasses three main species: the neotropical brown stink bug *Euschistus heros* (F.), the southern green stink bug *Nezara viridula* (L.) and the redbanded stink bug *Piezodorus guildinii* (West.).^{10,12,17} However, the abundance and contribution of each of these stink bug species to the soybean yield and quality

have changed spatially and temporally in these neotropical soybean production areas.

While *E. heros* is now more widespread and occurs in greater numbers than *P. guildinii* and *N. viridula*, it rarely occurred in the region until the 1970s.^{12,18,19} Although *P. guildinii* currently exhibits lower abundance and distribution, it usually causes more seed injury per insect than *E. heros* and *N. viridula*,²⁰ and has been responsible for the green bean syndrome observed in Brazilian soybeans.²¹ Interestingly, *P. guildinii* was of secondary importance in the US soybean producing areas for many years, but it has recently become a major pest of soybean in both Louisiana and Texas.^{22–25}

The number of recorded host plants used by *E. heros* in neotropical regions is lower than the number recorded for *P. guildinii* or *N. viridula*,^{11,12} which mainly restricts the populations of the former to soybean fields. Other factors such as the widespread adoption of no-tillage cultivation systems, the introduction of multiple cropping and higher insecticide tolerance have certainly contributed

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to the expansion of *E. heros* in Brazilian territory and into adjacent countries such as Argentina.^{11,12,15,26,27} In the present study, we investigated the role of competition as a contributing factor for the increased incidence and importance of *E. heros* in neotropical soybean fields over time. We expected that the current prevalence of *E. heros* over other stink bug species would be due to its higher competitiveness.

2 MATERIALS AND METHODS

2.1 Insects

A colony of *E. heros* was established, starting from insects maintained at Embrapa Genetic Resources and Biotechnology (Brasília, DF, Brazil). The colony of *P. guildinii* was established from nymphs and adults collected in soybean fields near Embrapa Rice and Beans (Santo Antônio de Goiás, GO, Brazil – geographic coordinates 16° 29' 13.15" S, 49° 17' 54.55" W). The colonies were reared under controlled conditions (27 ± 2 °C, 60 ± 20% relative humidity, with an L:D photoperiod of 14:10 h). All the developmental stages of the stink bugs were reared following methods previously described elsewhere.^{28–30} Field-collected individuals from soybean farms in the regions of Tangará da Serra (State of Mato Grosso, Brazil) and from the experimental soybean fields at the Federal University of Viçosa (Viçosa, State of Minas Gerais, Brazil) and at Embrapa Rice and Beans (Santo Antônio de Goiás, GO, Brazil) were routinely introduced into the laboratory colonies to increase the genetic variability of insects used in the experiments.

2.2 Cultivation conditions and experimental plots

The indeterminate soybean variety NA 8015 RR (Nidera Sementes, São Paulo, Brazil), maturity group 8.0, was sowed under no-tillage at the experimental farm of Embrapa Rice and Beans (16° 29' 30.8" S, 49° 17' 41.7" W) on 12 December 2014. Agronomic practices used were those recommended for soybean production in Brazilian savannah ('Cerrado'). Seeds were inoculated with *Bradyrhizobium elkanii* (6 mL kg⁻¹ seeds) (Nitragin®; Novozymes, São Paulo, SP, Brazil) and sowed at a rate of 16 seeds m⁻¹ with a 0.45 m row spacing (~355 000 plants ha⁻¹).

When soybeans reached the R3 stage, field cages (1.0 × 1.0 × 1.0 m) were placed over one row of soybean plants. Plants inside the cages were manually weeded, leaving only four soybean plants per cage. To prevent any damage caused by other insects and disease, 4 days before the stink bug infestation the plants inside the cages were sprayed with beta-cyfluthrin (125 g AI L⁻¹; Bayer CropScience Ltda, São Paulo, Brazil) at a rate of 0.25 g AI ha⁻¹ and a fungicide mixture of azoxystrobin and cyproconazole at rates of 60 and 24 g AI L⁻¹ respectively (azoxystrobin 200 g AI L⁻¹, cyproconazole 80 g AI L⁻¹; Syngenta Proteção de Cultivos Ltda., São Paulo, Brazil).

2.3 Competition experiments

The assays were implemented following an additive series expanded to a bivariate factorial design replicated in four soybean fields, each one containing each treatment. The experiment was carried out in a single season because the varying densities of both insect species encompass the species composition and outcome to be expected in different years. Prior to infestation, the plants were inspected to ascertain the absence of insects. Sixty-seven days after planting, sexually mature adults (16–18 days after emergence) of *E. heros* and *P. guildinii* were confined inside cages in a sex ratio of 1:1 (male:female). Although insect mortality was

never higher than five insects per experimental unit, 3 days after the infestation the cages were checked and dead insects were replaced to maintain the initial population. The control treatment consisted of cages without insects.

Competition experiments were designed for *E. heros* and its heterospecific competitor *P. guildinii* using an additive series, as suggested by Snaydon.³¹ Mixed infestations were established on four soybean plants; the initial number of insects of one species was fixed at 10, whereas the other species had an increasing number of insects varying from 0 to 10. Therefore, each species with a variable number of insects started the competition at initial proportions of 0, 0.17, 0.29, 0.38 and 0.50 against the second species, respectively, which exhibited a fixed total number of insects. The insects remained under competition with evaluations at 15, 30 and 45 days after initial infestation (DAI). At each evaluation, we recorded the total number of insects (nymphs and adults) of each species per experimental unit (i.e. a cage with four plants). The instantaneous rate of population increase (r_i) for each species in each experimental unit was calculated at 45 DAI using the formula $r_i = [\ln(N_f/N_i)]/\Delta T$, where N_f and N_i are the final and initial number of live insects, respectively, and ΔT is the duration of the experiment in days.^{32,33} At maturity, all four plants from each cage were harvested, and yield parameters such as number of empty pods (no observable seeds), 100 seeds weight, total seed yield and number of seeds per pod were assessed.

2.4 Statistical analysis

The number of live insects per experimental unit was subjected to repeated-measures (multivariate) analysis of variance because determination of insect numbers per plant was carried out on the same plots at each evaluation date,^{34,35} thereby avoiding the problem of pseudoreplication in time.^{35–37} This analysis was carried out using the PROC MANOVA procedure with the PROFILE statement, as suggested by von Ende.³⁸ When necessary, *post hoc* Tukey's HSD tests ($\alpha = 0.05$) were performed to compare treatment means. The instantaneous rate of increase in each species at 45 DAI was subjected to analyses of covariance with the species presence as the independent variable and the proportional increase in density as a covariate (PROC GLM procedure). Complementary regression analyses were performed when necessary (PROC REG procedure). The assumptions of normality and homogeneity of variance were checked, and no data transformation was necessary (UNIVARIATE procedure). Comparisons of soybean yields among the treatments were made by the PROC ANOVA procedure, and *post hoc* Tukey's HSD tests ($\alpha = 0.05$) were performed to compare treatment means. All statistical procedures above were performed using SAS/STAT software for Windows (SAS Institute, Cary, NC).³⁹

3 RESULTS

3.1 Population increase through time

Repeated-measures ANOVA for the number of live *E. heros* indicated significant effects of the interaction of the presence and increasing proportion of heterospecifics ($P < 0.05$) (Table 1). Significant effects were also found for evaluation day (time) and for the interactions between time and the initial proportion of the species that had its initial proportion changed ($P < 0.05$) (Table 1). As shown in Fig. 1A, 15 days after the infestation, the number of live *E. heros* was unaffected by increments in *P. guildinii*, but in the other two assessments (i.e. 30 and 45 DAI) the quantities of live

Table 1. Repeated-measures ANOVA for the total number of live insects from competition experiments between *E. heros* and *P. guildinii*^a

	<i>E. heros</i>				<i>P. guildinii</i>		
Sources of variation	df	<i>F</i>		<i>P</i>	<i>F</i>	<i>P</i>	
Between samples							
Species (S)	1	29.61		<0.0001*	0.18	0.67	
Initial proportion (P)	4	0.79		0.54	1.15	0.35	
S × P	4	4.71		0.0046*	0.73	0.57	
Error	30	–		–	–	–	
Sources of variation	df _{den} /df _{num}	Wilks' lambda	<i>F</i>	<i>P</i>	Wilks' lambda	<i>F</i>	<i>P</i>
Within samples							
Time (T)	2/29	0.6104	9.26	0.0008*	0.6000	9.67	0.0006*
T × S	2/29	0.8484	2.59	0.09	0.7624	4.52	0.0196*
T × P	8/58	0.3489	5.02	<0.0001*	0.7839	0.94	0.49
T × S × P	8/58	0.6243	1.93	0.07	0.8221	0.75	0.65
a* Significant at <i>P</i> < 0.05.							

^aSignificant at $P < 0.05$.

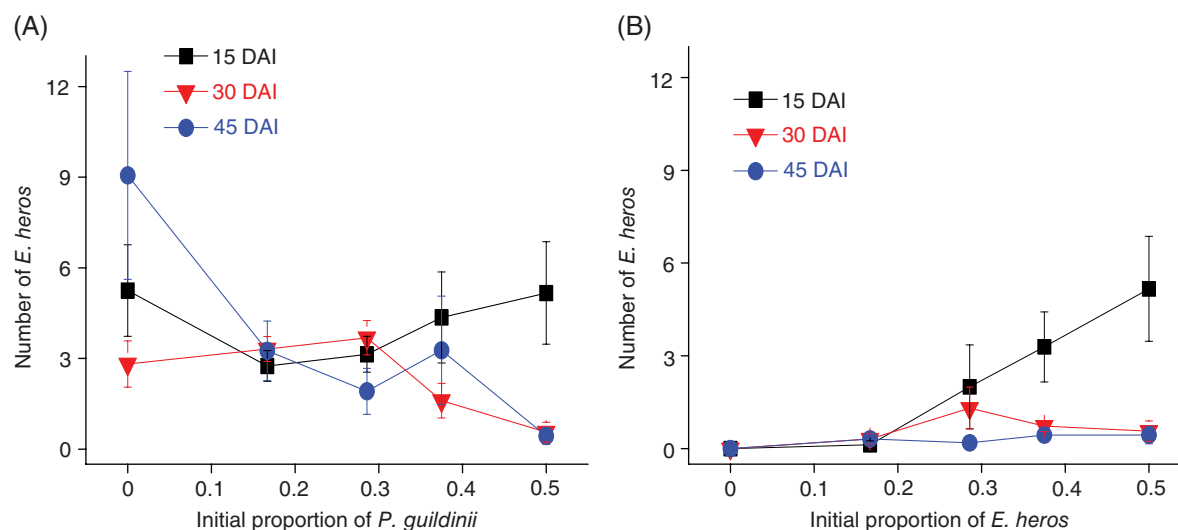


Figure 1. Total number of living *E. heros* obtained through 45 days of competition between *E. heros* and *P. guildinii*. (A) Although not affected at the beginning of the competition (i.e. 15 DAI), *E. heros* abundance was significantly reduced in the other two assessments (i.e. 30 and 45 DAI) in the experimental units that had higher initial proportions of *P. guildinii*. (B) Increasing the initial proportion of *E. heros* caused significant increments in the number of *E. heros* at the beginning of the competition period (i.e. 15 days after infestation) but not in the other evaluations. The box plots indicate the range of data (lower and upper quartiles and extreme values) and the median values.

E. heros were significantly reduced when the competition began with higher proportions of *P. guildinii*. Increments in the initial proportion of *E. heros* caused significant increments in the number of live *E. heros* at the beginning of the competition period (i.e. 15 days after infestation) but not in the other evaluations (Fig. 1B).

Repeated-measures ANOVA for the number of live *P. guildinii*, however, found no significant effects from the species that had their initial proportion changed or the initial proportion of each species or from their interactions ($P > 0.05$) (Table 1). The abundance of *P. guildinii* was significantly affected by time and its interaction with the species that had their initial proportion changed ($P < 0.05$) (Table 1). For the abundance of *P. guildinii*, while the number of live *P. guildinii* was significantly reduced over time when *E. heros* was the species with a variable number of insects (Fig. 2A), variations in the initial proportion of *P. guildinii* did not affect conspecific abundance over the study time (Fig. 2B).

3.2 Competition and the instantaneous rate of population increase (r_t)

The models of the analyses of covariance for the total number of live insects of each species after 45 days of competition were significant (Table 2). However, while the growth rate of *E. heros* was significantly affected by increasing the level of competition (i.e. increasing the initial number of insects) with their conspecifics or with *P. guildinii* (Fig. 3A), the growth rate of *P. guildinii* was unaffected by increasing the initial proportion of conspecifics or of *E. heros* (Fig. 3B).

3.3 Yield parameters

The production of empty pods was significantly ($F_{9,39} = 65.9$, $P < 0.001$) increased by the presence of the stink bugs (Fig. 4). For the treatment where *E. heros* was the only stink bug present, the percentage of empty pods was significantly lower than in all the treatments containing *P. guildinii*, but it was higher than in

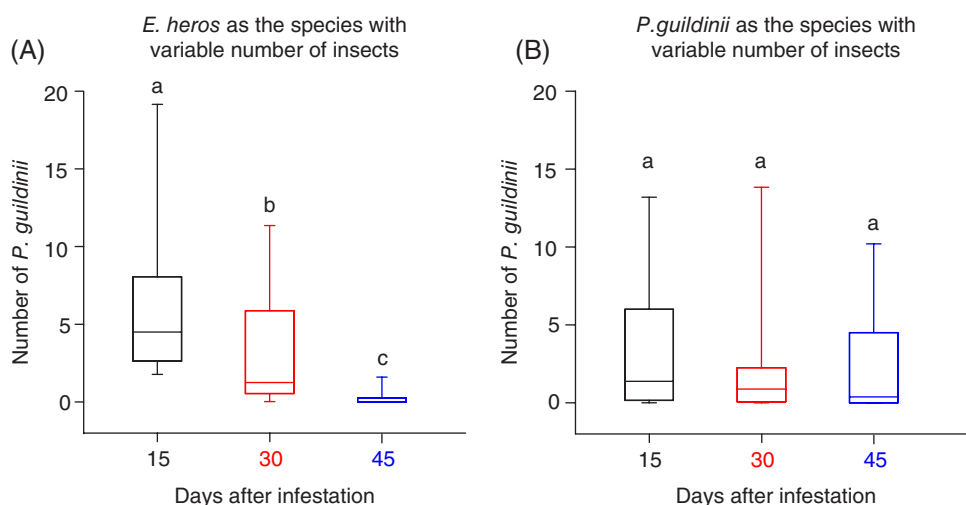


Figure 2. Total number of living *P. guildinii* obtained through 45 days of competition between *E. heros* and *P. guildinii*. The abundance of *P. guildinii* was significantly reduced over time when *E. heros* (A) was the species with a variable number of insects, but not when *P. guildinii* (B) was the species with a variable number of insects at the beginning of the competition period. The box plots indicate the range of data (lower and upper quartiles and extreme values) and the median values.

Table 2. Analyses of covariance for the total number of live insects after 45 days (one generation) of competition between *E. heros* and *P. guildinii*^a

Sources of variation	df	<i>E. heros</i>		<i>P. guildinii</i>	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Model	3	7.00	0.0008*	9.80	< 0.0001*
Error	36	—	—	—	—
Species with increasing density	1	1.87	0.18	17.17	0.0002*
Initial proportion	1	17.62	0.0002*	0.28	0.60
Interaction	1	0.23	0.63	2.56	0.12

^a* Significant at $P < 0.05$.

the treatment without stink bug infestation (Fig. 4A). The presence of stink bugs also significantly ($F_{9,39} = 19.6$, $P < 0.001$) reduced the production of seeds (Fig. 4B). While *E. heros* alone significantly reduced the seed production, the production of marketable seeds was completely absent (except in one experimental unit) in all the treatments containing *P. guildinii* (Fig. 4B). For the treatments that produced marketable seeds (i.e. treatments with no stink bug infestations and those infested only by *E. heros*), we observed that the presence of stink bugs did not affect the 100 seeds weight ($F_{1,7} = 0.75$, $P = 0.49$) but significantly reduced ($F_{1,7} = 6.29$, $P = 0.046$) the number of seeds per pod (no stink bug infestation: 2.5 ± 0.19 seeds pod⁻¹; infested only by *E. heros*: 1.9 ± 0.15 seeds pod⁻¹).

4 DISCUSSION

Here, we assessed for the first time the competitiveness of two co-occurring stink bug pests (*P. guildinii* and *E. heros*) of neotropical soybean fields cultivated under Brazilian savannah conditions. The study was performed in four soybean fields, allowing spatial extrapolation, and in a single crop season. The varying densities simultaneously used of both species allow for their likely field variation in different seasons and years without the need to

replicate such complex design through time, unless the underlying mechanisms determining the competition outcome are the target of attention, which was not the study objective.

The coexistence of such phytophagous stink bugs in neotropical soybean fields has been reported since the 1970s.^{10,19} However, while *E. heros* was of rare occurrence in this region at that time,¹⁸ it overtook the economic importance of *P. guildinii* and of the southern green stink bug *N. viridula*, which previously had higher abundance and wider territorial distribution,^{12,15,40–42} becoming the most abundant stink bug pest of soybeans in Brazil.^{12,15}

Although the mechanisms determining the competitive outcome among phytophagous stink bug species in soybean fields have not been investigated, we had the expectation that *E. heros* would present higher competitiveness in neotropical soybean fields than their heterospecific stink bug competitors. Such suspicion derived from the fact that the number of recorded host plants used by *E. heros* in the region is smaller than that recorded for *P. guildinii* and *N. viridula*.^{11,12,17} Our findings confirmed such expectations and demonstrated that the actual prevalence of *E. heros* over other stink bug species is at least partially due to its higher competitiveness.

However, other factors such as favoured biology (i.e. higher number of generations per year), the widespread adoption of no-tillage cultivation and the introduction of multiple cropping have certainly contributed to the expansion of *E. heros* in Brazilian territory and into Argentina.^{12,15,27} The climate conditions in the Brazilian savannah (mid-west Brazil), where soybean production has increased considerably since the 1970s, may also represent a major factor for *E. heros* expansion in the neotropical region.^{11,15,43} Furthermore, despite the limited amount of information regarding the differential susceptibility to insecticides among these stink bug species in Brazil,^{44–46} *Euschistus* stink bugs seem to be more tolerant to insecticides than *P. guildinii* or *N. viridula*,^{47,48} which might also have contributed to the prevalence of *E. heros*.

Interestingly, other investigations in southern Brazil^{49,50} and recent surveys of stink bug species in some US states (e.g. Louisiana and Texas) have reported *P. guildinii* as the major pest of soybean fields,^{22–25} prevailing inclusive over of *Euschistus* stink bugs. Such shifts in the composition and relative abundance of the stink

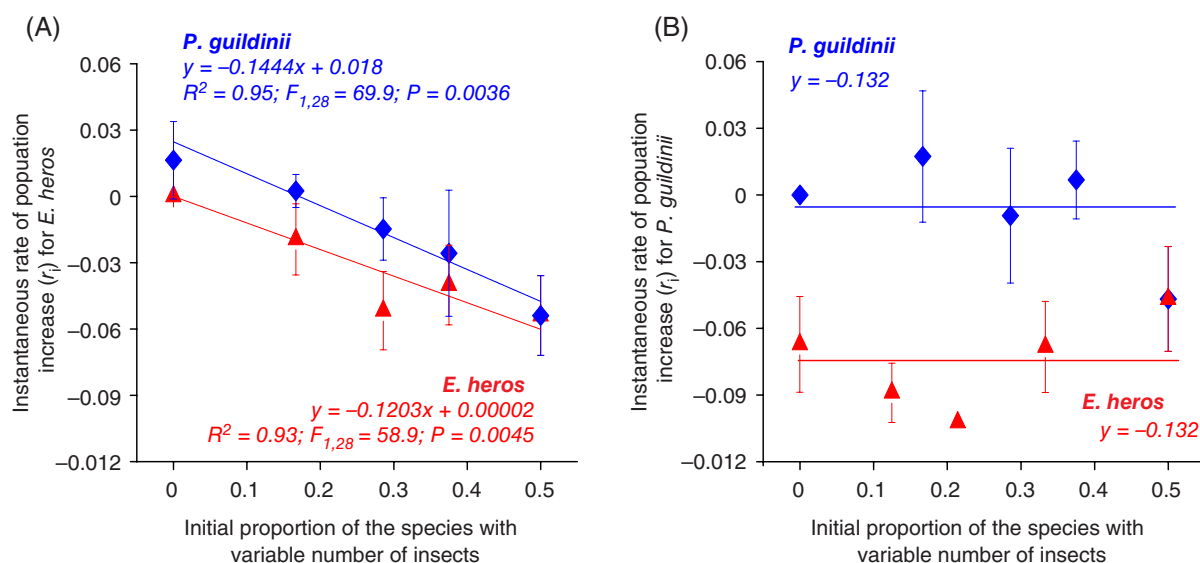


Figure 3. Instantaneous rate of population increase (r_i) of *E. heros* (A) and *P. guildinii* (B) obtained through 45 days of competition between *E. heros* and *P. guildinii*. The curves refer to the species with a fixed number of insects and the species with a variable number of insects, as indicated on each curve. The symbols represent the mean of four replicates and the vertical bars represent the standard error.

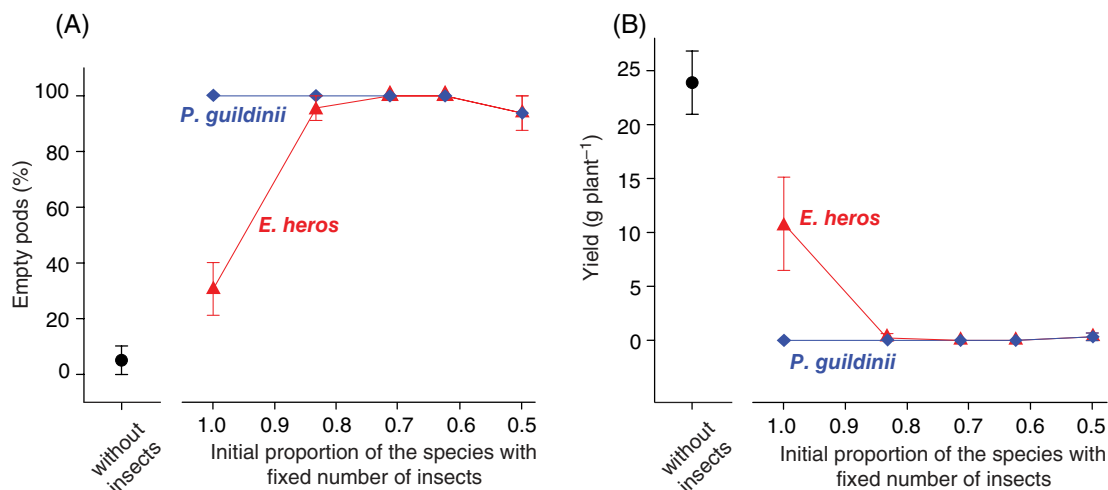


Figure 4. Evaluation of the number of empty pods (A) and productivity (B) of soybean plants in the presence and absence of stink bug infestations. The yield parameters of non-infested plants are indicated in black symbols. Linked symbols represent the yield of infested plants that had *E. heros* (red) or *P. guildinii* (blue) as the species with a fixed number of insects at the beginning of the competition period.

bug complex in soybean fields have raised concerns about the current economic thresholds used for these insects,^{26,41,51} pointing towards the adoption of multispecies action thresholds rather than single-species-based thresholds. In Brazil, the current economic threshold used for stink bugs is a multispecies threshold of two stink bugs bigger than 0.5 cm per m, which is regarded as a safe level regardless of the soybean cultivars.^{26,52}

Such multispecies economic thresholds have to be accurately determined, and there is a need to evaluate the damage potential of the different stink bug species. For instance, several investigations have reported that the damage caused by *P. guildinii* in Brazilian soybean fields is higher than that caused by other phytophagous stink bugs.^{20,21,53,54} The higher damage potential of *P. guildinii* has been attributed to their more deleterious salivary enzymes.⁵⁵

Although the insect colonies used in our experiment were from different sources, which might lead to potential bias due to inadvertent selection on the insect competitive abilities while

in the laboratory,⁵⁷ this is unlikely to have taken place because even the laboratory population used was subjected to periodic introduction of field insects from representative soybean fields, maintaining proper genetic diversity in the population. This investigation did not attempt to elucidate the mechanisms determining the outcome of competition between *E. heros* and *P. guildinii*, but it aimed to assess their competitiveness, coexistence and potential dominance. Further investigations aiming to evaluate the simultaneous contribution of multiple factors such as susceptibility to pesticides and to naturally occurring biological control agents (e.g. parasitoids of stink bug eggs) are needed. Additionally, our results indicate that, although *P. guildinii* is a weaker competitor than *E. heros*, it exhibits higher damage severity that completely suppresses marketable seed production whenever it is present. Thus, future research also needs to evaluate the relative impact of different stink bugs relative to damage potential, and to determine whether the current multispecies economic threshold

really reflects the needs of the neotropical soybean producing region.

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