Australian Journal of
Crop Science
AJCS
AJCS 17(12):935-943 (2023)
ISSN:1835-2707
doi: 10.21475/ajcs.23.17.12.p4004

Evaluation of damage caused by stink bug *Diceraeus melacanthus* (Dallas) and *Euschistus heros* (F.) (Heteroptera: Pentatomidae) to the maize seedlings

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Abstract

Understanding of different damage potentials between insect species feeding on maize [*Zea mays* (L.)] is critical for elaborating control decision tools. There is a lack of information on the damage that the stink bug *Diceraeus melacanthus* (Dallas) and *Euschistus heros* (F.) may cause to maize seedlings, especially under high densities. Therefore, we compared the damage of these species to maize in a greenhouse using four infestation levels (zero, two, four, and eight adults per plant), in a randomized complete block design with six replications. Five weekly evaluations were performed after infestation, and plants were harvested at physiological maturity. The results indicated that *D. melacanthus* caused more damage than *E. heros* in all infestation levels. Two adults of *D. melacanthus* per plant for 21 days resulted in the death of 96% of the plants. *Euschistus heros* caused four-times less damage to early-stage maize plants than *D. melacanthus*. Based on the regression analysis, the infestation of one *E. heros* adult per plant reduced the number of kernels per row and weight of harvested grain by 11%.

Keywords: *Zea mays*, stink bug, Hemiptera, damage score, second season maize.
Abbreviations: V_E–V_6 seedling emergence to sixth leaf collar; V_1 first leaf collar.

Introduction

Maize, *Zea mays* (L.) (Poales: Poaceae) is the most important cultivated cereal crop in the world, with production above 1.080 billion tons produced in 2020 (CONAB, 2021). In Brazil, the maize production system includes two seasons: the first is planted during September-December and harvested in February-May. In contrast, the second is planted in February-March and harvested in June-July (Chiesa et al., 2016). The use of no-tillage cultivation of maize after soybean harvest (maize second season), environmental conditions, crop rotation, and the massive adoption of genetically modified plants provide survival conditions and food supply for many polyphagous pests such as stink bugs (Engel et al., 2020; Panizzi et al., 2022; Jacobi et al., 2022). Among the many species of stink bugs that can feed on maize, *Diceraeus melacanthus* (Dallas) and *Euschistus heros* (F.) (Heteroptera: Pentatomidae) are the most important and abundant in this production system (Smaniotto and Panizzi, 2015). Populations of both species are increased at the end of the soybean cycle and remains in the area after soybean harvest and attack again when new maize seedlings germinated (Silva et al., 2013). Stink-bug adults and nymphs usually feed on the stems of maize seedlings, and their damage is highest during early vegetative stages (Roza-Gomes et al., 2011; Silva et al., 2019; Fernandes et al., 2020; Bryant et al., 2021; Jacobi et al., 2022). Symptoms of stink-bug damage on maize seedlings include leaf discoloration and twisting, dead heart, tillering, and plant death, depending on the intensity of the damage and species (Roza-Gomes et al., 2011; Crosariol Netto et al., 2015; Gomes et al., 2020; Bryant et al., 2021; Jacobi et al., 2022). The time it takes for the damage symptoms to appear and feeding behavior differences among stink-bug species are some of the issues faced during field scouting and field management practices improvement (Depieri and Panizzi, 2011). The simultaneous occurrence of *D. melacanthus* and *E. heros* feeding on maize seedlings is frequent, and the understanding of their potential for damage is critical for control decision-making, which should be based on the number of stink bugs per plant (Silva et al., 2021). Previous studies have focused on evaluating the damage of different stink-bug species on maize (Copatti and Oliveira, 2011; Torres et al., 2013; Vasconcelos et al., 2014; Gomes et al., 2020). However, there is a lack of information about the potential for damage by *E. heros* to maize seedlings (Gomes et al., 2020), especially when a maize seedling is attacked by three or more adults and compared with the damage caused by *D. melacanthus*. Therefore, this study compared the damage caused by these two stink bug species at different infestation levels.

Results

Plant damage score

Species and insect density per plant influenced the damage. At 14 DAI (second evaluation), 88% of the plants infested with two *D. melacanthus* (Fig. 1a) presented scores above five, which represents leaf development problems and meristem damage. The other 12% of the plants died (score = 8). All plants subjected to four-insect density were severely damaged, resulting in 62% of dead plants (Fig. 1b). Eight *D. melacanthus* per plant resulted in 88% of dead plants after 14 d (Fig. 1c), and all the remaining plants (12%) showed symptoms of dead heart (score = 7). At the end of the infestation (21 DAI), all plants infested with two *D. melacanthus* (Fig. 1a) had the apical meristem affected (score = 5), and 92% and 100% of the plants were dead when infested with four and eight adults, respectively (Fig. 1b, c). Plants infested with two *E. heros* were mostly (88%) damaged (score < 2) after 14 days of infestation (Fig. 1a). The same was true for 79% of the plants under the density of four adults (Fig. 1b), and 22% of the plants infested with eight adults (Fig. 1c). At the end of infestation (21 DAI), 88% of the plants infested with two *E. heros* remained with scores equal to or lower than two (Fig. 1a), while 58% and 30% of the plants had a density of four and eight insects, respectively (Fig. 1b, c). *Euschistus heros* damage for 21 days was not enough to cause plant death at any insect density.

At all infestation rates, *D. melacanthus* caused higher damage than *E. heros* in maize plants (Fig. 2). No differences were observed between *D. melacanthus*-infested rates, and in the two evaluations following insect removal (7 and 14 DAIR) plant damage triggered by *D. melacanthus* continued to increase in all insect densities ($P > 0.05$). Treatments infested with *E. heros* were not influenced by the number of adults per plant (Fig. 2). The damage scores in maize plants were similar in different densities of *E. heros*, differing from non-infested plants. However, two weeks after insect removal (14 DAIR), 83% and 75% of the plants showed a reduction in symptoms of damage caused by *E. heros* in all adult densities per plant (Fig. 3).

Plant height

Maize plant height was affected by stink-bug species and population per plant. At all infestation rates, plants infested with *D. melacanthus* showed reduced size when compared with those infested with *E. heros* (Fig. 4). This parameter was affected by *E. heros* densities. However, the density of two and four adults of *E. heros* per plant was not sufficient to reduce plant height compared to non-infested plants. In contrast, *E. heros*-infested plants with eight adults were smaller than the control plants. After the infestation period and insect removal, *E. heros*-infested plants continued to grow. At seven and 14 DAIR, plants were taller in all treatments (i.e., insect densities) (Fig. 5). *Diceraeus melacanthus* also affected plant height significantly. All infestation-rate plots differed from the non-infested plots, and no differences were observed regardless of the infestation. No plant height difference was observed, either 7 or 14 DAIR for this specie ($P > 0.05$).

Stem diameter

There was a significant effect of the interaction between stink-bug species and the infestation rate. Plants subjected to *D. melacanthus* damage presented thinner stems than plants infested with *E. heros*, regardless of the number of insects (Fig. 6). The stem diameter of *E. heros*-infested plants did not differ from non-infested plants, regardless of the infestation rate. In contrast, the stem diameter of plants infested with two, four, and eight adults of *D. melacanthus* differed from the control, and no significant difference was verified among *D. melacanthus*-infested plants at any infestation rate.

In all densities, the stem diameter of *E. heros*-infested plants was larger after both 7 and 14 DAIR (Fig. 7); while plants infested with *D. melacanthus* had no difference in stem diameter after the infested period ($P > 0.05$).

Leaf flag and main cob height

Because of the high damage severity in *D. melacanthus*-infested plants, it was not possible to evaluate leaf flag or cob height, as none of these treatments reached the reproductive stage. Different infestation rates of *E. heros* did not affect the total plant height ($P = 0.4470$, F = 0.91, df = 3) or main cob height ($P = 0.6616$, F = 0.54, df = 3).

Maize harvest

No cobs were produced in plants under any infestation rate of *D. melacanthus*. Data analysis showed no effect of *E. heros* infestation rate on the main cob length ($P = 0.1972$, F = 1.76, df = 3) or the number of kernel rows per cob ($P = 0.8641$, F = 0.24, df = 3). In contrast, the interaction of *E. heros* infestation level, number of kernels per row ($P \leq 0.05$, $R^2 = 0.97$, Fig. 8a), and harvested grain weight ($P \leq 0.05$, $R^2 = 0.95$, Fig. 8b) was adjusted to a quadratic curve. According to the proposed model, the presence of one *E. heros* per plant reduced 11% of the number of kernels per row and harvested grain weight; two *E. heros*, 20%; four *E. heros*, 32%; and six *E. heros*, 37% reduction in relation to non-infested plants (Fig. 8a, b). The weight of 1,000 kernels was not analyzed because of the low number of kernels produced in plants under high infestation levels.

Discussion

The damages under greenhouse conditions demonstrated that maize plants are susceptible to stink-bug damage immediately after emergence, as reported by other authors (Rodrigues, 2011; Silva et al., 2019). For both species, the damage increased proportionally to the number of insects, which was not surprising for *D. melacanthus* based on published results (Roza-Gomes et al., 2011; Torres et al., 2013; Bridi et al., 2016; Gomes et al., 2020). However, between two and eight *E. Heros* per plant, there were no differences in damage to maize seedlings, confirming previous studies when plants were submitted to lower insect densities (Torres et al., 2013; Gomes et al., 2020).

Damage in plants infested with *E. heros* reflected in plant height and stem diameter reduction, confirming the potential damage of this species to maize. Due to the high damage caused by *D. melacanthus* to the plant, it was impossible to set apart the differences between infestation levels, as indicated by Rodrigues (2011) and Bridi et al. (2016). The results were flat in all our treatments. Severe symptoms and high mortality of plants infested with *D. melacanthus* were expected, whereas the economic thresholds established by other colleagues are lower than those we tested, varying from 0.03 stink bugs per plant during V_E–V_6 vegetative stage (Silva et al., 2021), 0.08 at V_E (Rodrigues, 2011), 0.18 at V_1 (Duarte et al., 2015), to 0.5 per plant at V_E–V_7 (Gomes et al., 2020). *Euschistus heros* infestations were not associated with plant death, confirming the results of Copatti and Oliveira (2011) studying plants under the density of three adults and similar environmental conditions. However, they did not report tillering because insect feeding had not been sufficiently severe. This reinforces that severe symptoms are only observed in plants under much higher densities of *E. heros*, as reported for other stink bug species (Townsend and Sedlacek, 1986; Sedlacek and Townsend, 1988).

Differences in plant mortality and damage resulted in a lower recovery capacity of plants infested with *D. melacanthus* than those with *E. heros.* Domiciano et al. (2004) analyzed the damages caused by *Diceraeus* sp. in maize and wheat and concluded that plants with low and medium damage (scores < 4) presented higher recovery compared with severely damaged plants (scores > 5), in accordance with our results. Failure to recover from damage in *D. melacanthus*-infested plants even after insect removal may be related to the metabolic cost involved in chemical defenses induced after stink bug attack or due to inter-plant competition for resources (Maddonni and Otegui, 2004; Jacobi et al., 2021), especially under greenhouse condition where the solar incidence is limited. Another hypothesis is that severely damaged plants have impaired development, tending to be dominated by larger plants (Maddonni and Otegui, 2004). In contrast, we observed a damage reduction in the plants two weeks after *E. heros* removal. These results show that in vegetative-phase maize can recover from damage caused by less than eight *E. heros* per plant. Similar results were found by Copatti and Oliveira, (2011) and Vasconcelos et al. (2014), who reported lower adult densities per plant. One explanation is that lighter damages, such as the presence of yellow spots, could be related to the higher period of probing than that of feeding, as Depieri and Panizzi (2011) concluded that the damage of *E. heros* in soybean seeds is proportional to the feeding period.

Based on the results of the stink bug infestation on vegetative maize plants, we observed significant differences in damage potential between *D. melacanthus* and *E. heros*, reinforcing the results under low insect densities (Torres et al., 2013; Gomes et al., 2020). However, it was expected that high density levels of *E. heros* per plant would result in damages comparable to that caused by low *D. melacanthus* density. Copatti and Oliveira (2011) also observed differences between these two stinkbug species and reported that it took an average of three *E. heros* to cause the same damage as that of one *D. melacanthus.* Nevertheless, in our study, *E. heros* density four times higher than that of *D. melacanthus* was insufficient to result in similar damage.

There is still no suitable explanation for the difference between species discussed previously. However, some hypotheses exist. Rostrum length cannot be considered because it is similar between species (Depieri and Panizzi, 2010). As suggested by Liu and Bonning (2019), the pentatomid stink bugs utilize a similar suite of proteases and nucleases to digest plant material. The difference in other salivary components may elicit a distinct maize seedling self-protection response, which may result in volatile organic compounds emission that induces stink bug feeding avoidance (Roza-Gomes et al., 2011; Giacometti et al., 2020; Jacobi et al., 2021). Panizzi and Silva (2009) related stink bug damage to its stylet penetration frequency. However, (Depieri and Panizzi, 2011) verified that the damage caused by *D. melacanthus* in soybean seeds was not proportional to the feeding period, as noted for *E. heros*.

Another hypothesis is the feeding preferences of both species. *Diceraeus melacanthus* prefers feeding in seedlings near the soil surface, mainly in the downward position (Panizzi and Lucini, 2019), whereas *E. heros* prefer feeding in the whorl and expanded leaves (Author personal observation). Stink bugs can use different strategies according to the feeding site, which could help explain the damage severity differences (Lucini and Panizzi, 2017). Even though this was not evaluated, the feeding site preference among species requires more investigation to refine potential management strategies.

Regarding the final plant height and cob height, no differences were observed in maize plants infested with *E. heros*, suggesting a full recovery of plants under all insect infestation levels even after 21 days of feeding. In comparison, no viable cobs were produced in plants infested with *D. melacanthus,* regardless of infestation levels. Gomes et al. (2020) found differences in cob height in plants infested with 0.7 *D. melacanthus*, approximately one-third of the lowest population in our study. The effect of initial damage caused by *D. melacanthus* on maize yield has been widely studied under lower densities (Duarte et al., 2015; Bridi et al., 2016; Gomes et al., 2020; Silva et al., 2019, 2021), and no additional results were found in higher populations due to damage severity. Conversely, in the tested populations (above one adult per plant), *E. heros* influenced the number of kernels per row and harvested grain weight. This was observed because plants define their productive potential in stages V_1–V_6 (Fancelli and Dourado Neto, 1997), which matches the infestation period, and any disturbance can result in yield losses, especially because new maize leaves cannot compensate for the damaged ones. According to Gomes et al. (2020), *E. heros* has a low potential to damage maize plants, as no impact was observed in yield in densities as small as one stink bug per plant. However, in our results, similar infestation rates of *E. heros* reduced the number of kernels per cob row and harvested grain weight. Therefore, management recommendations for growers should be considered when the population is higher than one stink bug per plant. However, further research is needed to refine these results, considering control costs and production value under field conditions. In summary, our results regarding the maize vegetative and reproductive phases show that the damage caused by *D. melacanthus* and *E. heros* in early-stage maize under population densities up to 8 adults per plant are not equivalent under greenhouse conditions.

Fig 1. Damage score frequency of maize plants submitted to two (a), four (b), and eight (c) adults of *Diceraeus melacanthus* and of *Euschistus heros* per plant, at 7, 14, 21, 28, and 35 days after infestation.

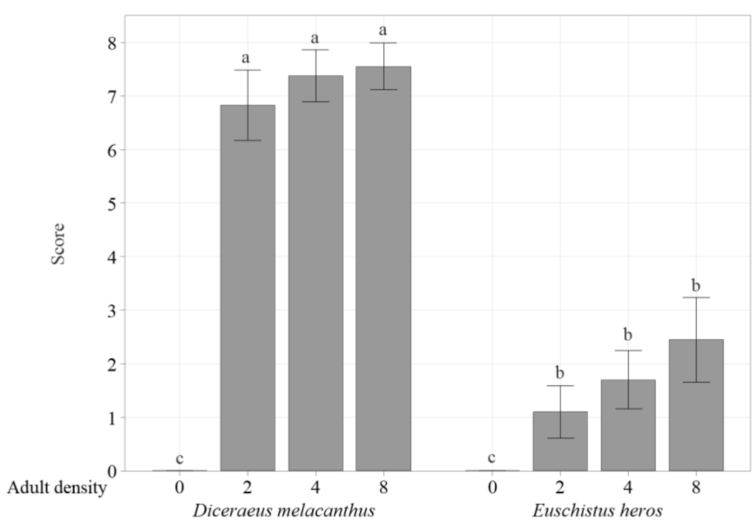

Fig 2. Mean damage score of maize plants infested with *Diceraeus melacanthus* or *Euschistus heros* at the densities of two, four, and eight adults per plant across five assessment dates (7, 14, 21, 28 and 35 DAI). Data were fitted to a linear mixed model and estimated marginal means were calculated and compared using Tukey's HSD test. The treatments followed by the same letter are not significantly different (α = 0.05). Raw values of the five assessments are presented in the figure.

Table 1. Description of the damage score applied to maize plants resulting from stink bug feeding, adapted from Silva et al. (2019).

Score	Damage description
0	No damage
1	Less than 3 small yellow spots on the leaves (<1 cm)
2	4 to 7 yellow spots, 1 to 3 brown spots on the leaves (<1 cm), plants with normal growth, and normal apical meristem
3	Less than 3 large brown spots (>2 cm)
4	4 to 7 brown spots on the leaves, plant growth reduction, normal apical meristem
5	More than 8 brown spots, apical meristem affected, reduced growth, and twisted whorl
6	Plant with reduced growth and dead heart
7	Dead heart and tillering
8	Dead plant

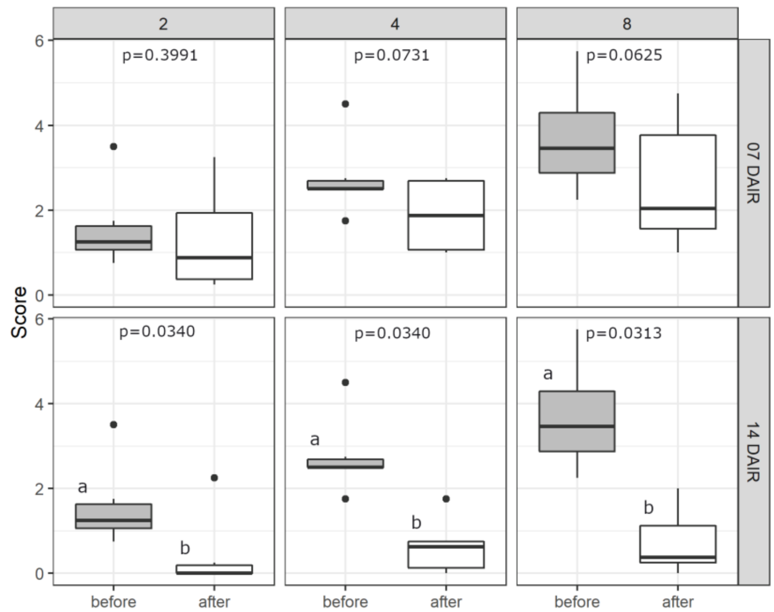

Fig 3. Damage score comparison of maize plants infested with two, four, and eight *Euschistus heros* adults before insect removal (i.e., 21 days after infestation) against 7 and 14 days after insect removal (DAIR). A separate Wilcoxon signed-rank test was performed for each comparison. Treatments followed by different letters are statistically different (α = 0.05).

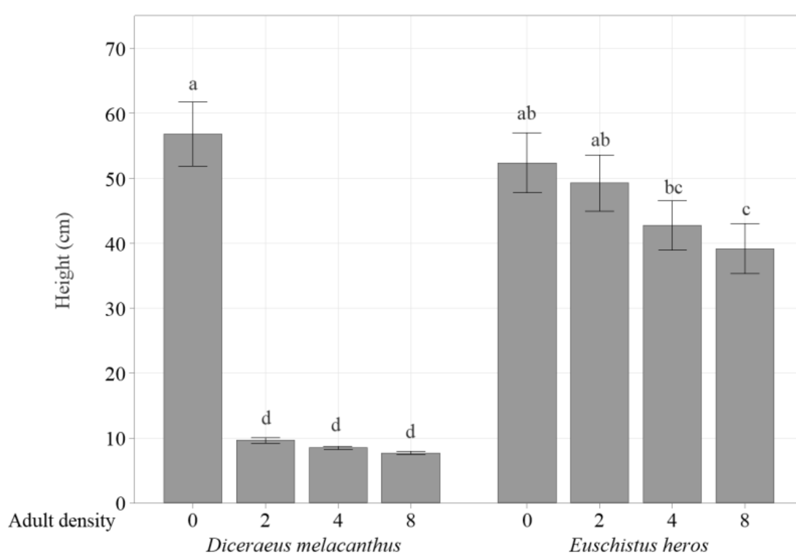

Fig 4. Mean height (cm) of maize plants infested with *Diceraeus melacanthus* or *Euschistus heros* at the densities of two, four, and eight adults per plant across five assessment dates (7, 14, 21, 28 and 35 DAI). Data were fitted to a linear mixed model and estimated marginal means were calculated and compared using Tukey's HSD test. The treatments followed by the same letter are not significantly different (α = 0.05). Raw values of the five assessments are presented in the figure.

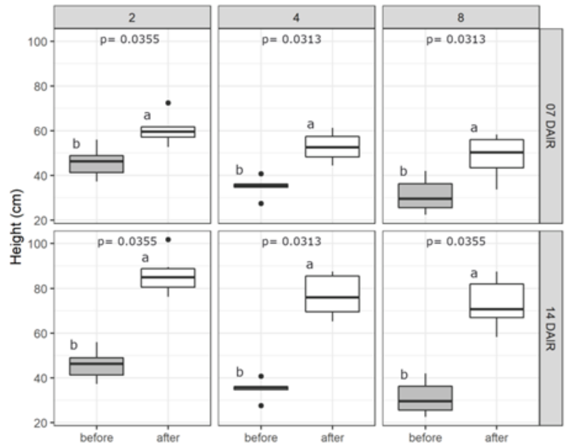

Fig 5. Height (cm) comparison of maize plants infested with two, four, and eight *Euschistus heros* adults before insect removal (i.e., 21 days after infestation) against 7 and 14 days after insect removal (DAIR). A separate Wilcoxon signed-rank test was performed for each comparison. Treatments followed by different letters are statistically different (α = 0.05).

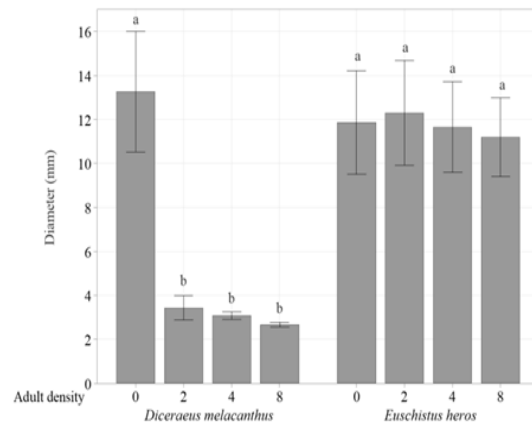

Fig 6. Mean stem diameter (mm) of maize plants infested with *Diceraeus melacanthus* or *Euschistus heros* at the densities of two, four, and eight adults per plant across five assessment dates (7, 14, 21, 28 and 35 DAI). Data were fitted to a linear mixed model and estimated marginal means were calculated and compared using Tukey's HSD test. The treatments followed by the same letter are not significantly different (α = 0.05). Raw values of the five assessments are presented in the figure.

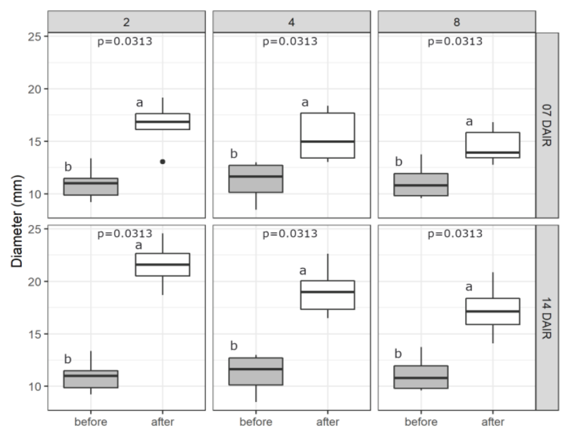

Fig 7. Stem diameter (mm) comparison of maize plants infested with two, four, and eight *Euschistus heros* adults before insect removal (i.e., 21 days after infestation) against 7 and 14 days after insect removal (DAIR). A separate Wilcoxon signed-rank test was performed for each comparison.Treatments followed by different letters are statistically different (α = 0.05).

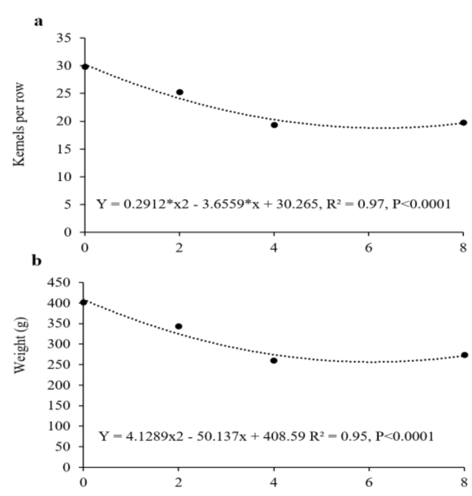

Fig 8. Number of kernels per row (a) and harvested weight (b) of maize plants submitted to different *Euschistus heros* infestation levels per plant for 21 days.

Although *D. melacanthus* is considered the primary stink bug pest in maize in Brazil, the simultaneous occurrence with *E. heros* in the field and the damage differences between these species should be accounted during stink bug sampling and for elaborating control decision tools. Both species have the potential to damage plants and reduce maize yield. Damage ratings for control decision making in vegetative maize are shown to be inappropriate for stink bugs. This reinforces the attention needed to determine species composition in fields, mainly because monitoring and sampling stink bugs in maize are made by visual inspection and counting. Moreover, differences in insect behavior or feeding-position preference might bring extra barriers to correctly identifying or precisely quantifying different stink-bug species.

Materials and methods

Trial conditions and experimental design

The study was conducted in a greenhouse (25 ± 5°C, RH: 80 ± 10%) in Toledo, Paraná, Brazil (53°45'32.62'' S, 24°40'20.06'' W) from February to April 2018. Treatments consist of two stink-bug species (*D. melacanthus* and *E. heros*) and four infestation levels per plant (zero, two, four, and eight adults). There were eight treatments arranged in a randomized complete block design with six replications, totaling 48 experimental plots (cages with 0.6 m × 0.6 m; 4 plants/cage). The maize hybrid 30F53VYH (Leptra®) was treated with the fungicide Derosal Plus® (carbendazim, 150 g L^{-1}; thiram, 350 g L^{-1}). Plants were grown in conventionally prepared soil and fertilized with 300 kg ha^{-1} of 06–24–12 (NPK). Seeds were manually planted using one seed per planting hole (0.4 m × 0.4 m per plant). After emergence, seedlings were enclosed by a 0.6 m × 0.6 m × 2.0 m (l × w × h) *voile* cage. The cages were fixed using suspended chains attached to the top of the greenhouse.

Insect source and infestation

Adult stink bugs were collected in Toledo's surrounding field areas and taken to rearing *voile* cages (0.4 m × 0.4 m × 0.6 m) inside the greenhouse (25 ± 5°C, RH: 70 ± 10%). The food source was composed by a mixture of pods of green bean (*Phaseolus vulgaris* L.), sunflower (*Helianthus annuus* L.), raw shelled peanut seeds (*Arachis hypogaea* L), and maize seedlings (7–10-days old). The eggs were collected daily and placed in identical cages with the same food source to obtain adults (first-generation) for the artificial infestation (5–15-days old). Random insects (not sexed) were counted in the right amount to infest one cage and starved for 24 hours (in the presence of water only) before infestation. Stink bugs were maintained in contact with the plants for 21 days, starting at the V_1 growth stage (Ritchie and Hanway, 1982). Every two days, dead insects were replaced. After the infestation period, insects and cages were removed, and plants were sprayed with Engeo Pleno® (thiamethoxam, 141 g L^{-1}; lambda-cyhalothrin, 106 g L^{-1}) to avoid damage by resulting nymphs.

Evaluations

Assessments were performed weekly during five weeks [7, 14, 21, 28, and 35 days after infestation (DAI)]. The evaluated parameters were: a) stem diameter (mm), b) plant height (cm), and c) damage score. The stink bug damage to maize plants was evaluated using a visual damage score adapted from Silva et al. (2019), as shown in Table 1. Two weeks after flowering, ear and plant height were measured. We considered the height from the ground to the node attachment of the highest developed ear shank and the height from the ground to the base of the flag leaf, respectively. Ears were manually harvested after physiological maturity, and the following parameters were evaluated: main cob length, number of kernel rows per cob, number of kernels per row, and weight of 1,000 kernels. Weight and moisture were recorded and corrected for production per plot (adjusted to 13% moisture).

Statistical analysis

Generalized linear mixed-effects models were constructed for infestation levels and stink-bug species using the 'lme4' package in R (Bates et al., 2015; R Core Team, 2018). Plant height, stem diameter, and damage score were fitted to Gaussian models with assessment dates (DAI) as random effects. Gaussian and Gamma models were constructed with raw and log-transformed data. The quality of model fit was assessed using 'performance' and the 'DHARMa' packages (Lüdecke et al., 2021; Hartig, 2022). Models were checked for singularity, heteroscedasticity, normality of residuals, and outliers. The 'Emmeans' package was used to assess differences among estimated marginal means (Length et al., 2022). Model selection was AIC-based and resulted in models with log-transformed data with Gaussian distribution and identity link function for all three dependent variables. Plant damage scores were also presented as cumulative occurrence frequency for each stink bug species. Wilcoxon signed-rank test was used to compare the damage score, plant height, and stem diameter of maize plants before and after insect removal. For each variable, two paired tests were performed to compare the assessment on the day of insect removal with the assessment either 7 or 14 days after insect removal (DAIR). These analyses were carried out using the R package 'stats'. Production values were regressed (P < 0.05) against insect densities, testing linear, quadratic, and cubic models. The selected model presented the lowest significant P and the highest determination coefficient (R^2).

Conclusion

Diceraeus melacanthus caused more damage to maize seedlings than *E. heros* in all infestation levels. On the other hand, *E. heros* caused four times less damage to early-stage maize plants than *D. melacanthus*, but the infestation of even one *E. heros* adult per plant reduced the number of kernels per row and harvested grain weight by 11%. This information is critical for developing effective control decision tools and measures against these stink bug species in maize crops.

Conflict of interests

The authors have declared that no competing interests exist. Corteva agriscience™ provided support in the form of salaries for authors Ademar N. Istchuk, Josemar Foresti, Paulo R. da Silva, and Renata R. Pereira, but did not have any additional role in the conduction of trials, data collection and analysis.

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