Diceraeus melacanthus (Dallas) (Hemiptera: Pentatomidae) development, preference for feeding and oviposition related to different food sources

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ABSTRACT

The dispersion of *Diceraeus melacanthus* (Dallas) from soybean (first crop season) to maize (second crop season) is facilitated by the presence of weeds (e.g. *Commelina benghalensis*) and soybean grains on the ground. Understanding insect development and behavior on different food sources is important to develop pest management strategies. Thus, three independent experiments were conducted to study *D. melacanthus* nymph development, and adult preference for feeding and oviposition in different food source scenarios in the field. The first two trials studied development and food preference of *D. melacanthus* related to different food sources (*C. benghalensis* branches, moistened soybean grain + soybean seedlings, maize seedlings + *C. benghalensis* branches, moistened soybean grains + maize seedlings, moistened soybean grains + *C. benghalensis* branches, maize seedlings, as well as a standard diet). The third trial evaluated *D. melacanthus* oviposition preferences between the aforementioned plants. Overall, moistened soybean grains resulted in better nutritional quality, thus being crucial for *D. melacanthus* development, triggering faster nymph development as well as better overall adult fitness. The combination of soybean grains and maize seedlings or *C. benghalensis* branches showed great potential to benefit *D. melacanthus*, since these complementary food sources improved stink bug fitness, increasing mainly adult longevity, fecundity and egg viability. Therefore, it can be concluded that in order to reduce *D. melacanthus* outbreaks in second season maize, it is important to reduce soybean harvest loss (reducing, therefore, moistened soybean grain on the ground) and eliminate stink bug associated plants like *C. benghalensis*.

Introduction

Agriculture has been intensified worldwide. In some areas, mainly in the neotropics, climatic conditions allow the cultivation of two or even three crops per year. This intensive land use causes a "green bridge" that provides plant availability as food for pests throughout the year. This permanent abundance of food triggers pest outbreaks and consequently crop damage (Bueno et al., 2021), mainly caused by polyphagous pest species (Pedigo, 2002). This was recently observed for the green-belly stink bug, *Diceraeus melacanthus* (Dallas) (Hemiptera: Pentatomidae) in the soybean *Glycine max* (L.) Merrill production system cultivated as first crop (summer) followed by maize, *Zea mays* L. as second crop (autumn/winter) (Bueno et al., 2015; Corrêa-Ferreira and Sosa-Gómez, 2017; Barão et al., 2020). Consequently, this pest has increased in importance in soybean fields, mainly occurring after the end of the first crop season, seriously impacting maize cropped during the

second season (Bueno et al., 2015; Duarte et al., 2015). Although usually being found in several legumes, including soybeans, since the 1970s (Panizzi et al., 1977), *D. melacanthus* has been increasingly reported to attack maize seedlings as well (Ávila and Panizzi, 1995; Panizzi and Chocorosqui, 2000). Not only is the occurrence of *D. melacanthus* favored by the presence of cultivated plants throughout the year (green bridge) (Bueno et al., 2015; Chiesa et al., 2016; Corrêa-Ferreira and Sosa-Gómez, 2017), but also by several non-cultivated plants present in the production system, such as the weed *Commelina benghalensis* (Chocorosqui and Panizzi, 2008; Silva et al., 2013). Moreover, according to Lemos et al. (2012) the presence of *C. benghalensis* in maize fields poses a significant competition to cultivated plants, and can be considered one of the most important weeds of maize in some important Brazilian agricultural sites (Rocha et al., 2000). Those non-cultivated plants can be alternative sources for stink bug feeding, shelter and oviposition, therefore increasing pest outbreaks and crop damage (Panizzi, 2000; Panizzi and Lucini, 2022).

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In soybean, nymphs and adults of *D. melacanthus* pierce and suck nutrients directly from pods, negatively impacting crop yields by harming the physiological and sanitary quality of the seeds (Corrêa-Ferreira and Azevedo, 2002). In contrast, when feeding on maize these insects suck their nutrients from plant seedlings, causing injuries that can be lethal, depending on attack intensity and crop development stage (Panizzi and Chocorosqui, 2000). Despite the increasing damage caused by *D. melacanthus* in soybean and maize within the last years, few biological and behavioral aspects related to different food sources have been recorded to date. In this context, it is important to highlight that the change in feeding behavior observed for this stink bug species, shifting from reproductive structures of soybean during the summer to vegetative tissues of other plant species such as maize in the autumn/winter, can be attributed to the low availability of preferred hosts (Manfredi-Coimbra et al., 2005).

The food quality found by *D. melacanthus* throughout the year plays an important role because its foraging strategies aim to optimize the composition and correct balance of nutrients. Therefore, understanding the response of *D. melacanthus* to different food sources is of great theoretical and practical interest for the development of durable pest management strategies (Després et al., 2007; Kianpour et al., 2014). The control of polyphagous and mobile pests requires management systems that focus not only on a single-season crop in an individual field or farm but also on growing systems in extensive areas of agricultural landscapes (Abel et al., 2007; Wu, 2007; Herde, 2009).

The availability of weeds, volunteer soybean plants (plants emerging resulting from seeds dropped during harvest) or nearby crops as food sources and crop rotation sequences play an important role in population dynamics and outbreaks of polyphagous herbivores. Therefore, the identification of insect preferences, biology, and feeding behavior is crucial to find economically and ecologically sustainable solutions to the problems caused by these herbivores (Behmer, 2009). For that reason, studies that aim to evaluate biological traits, including food preference and reproduction of the green-belly stink bug in different host plants (Panizzi and Lucini, 2022), as found in two-season cropping systems, are crucial to obtain optimally designed pest management strategies. In this context, the objective of this work was to evaluate *D. melacanthus* development and preferences for feeding and oviposition, related to different food sources.

Materials and Methods

Three different trials were carried out to study *D. melacanthus* development and preference for feeding and oviposition in different food source scenarios. The first and second trials were carried out inside biochemical oxygen demand climate chambers (BODs) (ELETROLab®, model EL 212, São Paulo, SP, Brazil) set at 80 ± 10% relative humidity, temperature of 25 ± 2°C, 14L:10D photoperiod in a completely randomized design. The third trial was carried out under field conditions without environmental control in a completely randomized block design.

Insect rearing

Stink bugs used in trials 1 and 2 (laboratory trials) originated from a colony of *D. melacanthus* kept under controlled conditions (25 ± 2°C, 70 ± 10% relative humidity and 14L:10D photoperiod) according to Panizzi (2000) and Silva et al. (2008), as briefly described in the following. Insects were collected from maize fields in Londrina, PR, Brazil (23°11′11.7″S e 51°10′46.1″ W), in 2016/17 and reared in the laboratory for fifteen generations. *Diceraeus melacanthus* adults were maintained in plastic boxes (20 x 20 x 24 cm high) lined with filter paper and fabric (raw

cotton) on the sides to serve as oviposition substrate. A standard diet was offered, composed of bean pods (*Phaseolus vulgaris* L.), soybean seeds (*Glycine max* L.), raw shelled peanuts (*Arachis hypogaea* L.), sunflower seeds (*Helianthus annuus* L.), and privet fruits (*Ligustrum lucidum* Ainton). Cages were cleaned and food was replaced every two days. Furthermore, egg masses were removed on a daily basis and placed in plastic boxes (11 x 11 x 3.5 cm) lined with filter paper containing one bean pod as food source for the nymphs. Upon reaching the 4th instar, nymphs were transferred to plastic boxes until they reached adulthood, containing the same food and following the procedure described above. Insects from this colony were used for trials or colony maintenance.

Tested food sources

Plants used in the experiments were maize (cv. BM810), soybean (cv. BRS 388 RR), and the weed *C. benghalensis,* all having been cultivated in greenhouses. Plant tissues (trials 1 and 2) were removed as required for each treatment and taken to BODs (ELETROLab®, model EL 212, São Paulo, SP, Brazil) set at 80 ± 10% relative humidity, temperature of 25 ± 2°C, 14L:10D photoperiod, being offered to the insects. For the experiment on *D. melacanthus* development (trial 1), soybean and maize were sown three times a week (4 L pots) and plants of 10 cm height were used in the trials. *Commelina benghalensis* was sown in 2 L pots and the apical parts of the plants (10 cm) were used.

The moistened soybean grains were kept on mesh screen to avoid direct water contact inside plastic boxes (11 x 11 x 3.5 cm) containing 100 mL of water, for 24 hours to induce turgidity, thereby simulating the conditions of soybean grains found on the ground in maize fields during the second crop. Subsequently, the moistened soybean grains were removed from that container and two grains were used for each replicate.

For the feeding preference experiment (trial 2), maize, soybean, and *C. benghalensis* were sown in pots (4 L) inside the greenhouse with daily automatic irrigation. Soybean and maize seedlings of 10 cm height were used in the experiments. Apical portions of branches of 10 cm tall *C. benghalensis* plants were used in order to provide an equivalent amount of food irrespective of which plant was used in a trial. The moistened soybean grains were used following the same methodology described above.

For the oviposition preference experiment (trial 3), plants were sown in circles on the experimental field at Embrapa Soja inside screened cages (6 m x 4 m). Each circle was composed of maize (maize plants in the reproductive stage R2), soybean (soybean plants in the reproductive stage R4) (Fehr et al., 1971), soybean seedlings, maize seedlings, and *C. benghalensis*. Each cage contained five circles of 1 m in diameter (Fig. 1). Maize and soybean plants to be used at the reproductive stage were sown first, while those to be used as seedlings were sown seven days before starting the experiment. *Commelina benghalensis* was transplanted from pots to circles at the flowering stage, seven days before the experiment.

Development of D. melacanthus *feeding on different sources (trial 1: no-choice test)*

The first trial evaluated the development of *D. melacanthus* when feeding on different food sources. It was carried out in a completely randomized design with seven treatments (different food sources) and four replicates. Studied food sources were: 1) *C. benghalensis* branches, 2) moistened soybean grains + soybean seedlings, 3) maize seedlings + *C. benghalensis* branches, 4) moistened soybean grains + maize seedlings, 5) moistened soybean grains + *C. benghalensis* branches, 6)
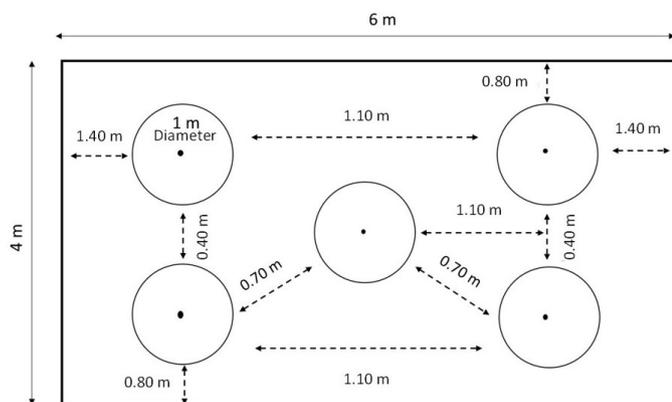
Figure 1 Scheme (not drawn to scale) of the cages used for the oviposition preference experiment (trial 3), depicting the circles inside each cage in which different plants were randomly offered to the stink bugs.

Figure 2 Arena (not drawn to scale) used for the feeding preference experiment (trial 2) of *Diceraeus melacanthus* between different food sources.

maize seedlings, and 7) a standard diet described by Silva et al. (2008) containing bean pods (*Phaseolus vulgaris* L.), soybean grains (*Glycine max* L.), peanut (*Arachis hypogaea* L.), sunflower seeds (*Helianthus annuus* L.) and privet fruits (*Ligustrum lucidum* Ainton).

Each replicate was composed of 12 first instar stink bugs in plastic boxes (11 x 11 x 3.5 cm), totaling 48 stink bugs per treatment (4 replicates of 12 insects each). First instar nymphs were maintained inside plastic boxes (11 x 11 x 3.5 cm) lined with moistened filter paper, and provided with the respective food sources, together with plastic Eppendorf® microtubes equipped with water-soaked cotton balls. Food was replaced and evaluations were made daily.

When the nymphs reached adulthood, weight (mg) and pronotum width (mm) of the insects were evaluated after 24 hours of adult emergence. The insects were weighed using a precision analytical scale (Shimadzu, model Ay220), and the pronotum width was measured using the Image J – Version 1.47 software on photographs taken with a stereoscopic microscopic of 40x magnification, using the software Leica Application Suite. The nutrient index was calculated as follows (Funayama, 2004): live weight of insects (mg)/pronotum width (mm)^3; in order to better understand the impact of different food sources (Funayama, 2004; Scaccini et al., 2020).

In addition, adults were separated by sex and fifteen couples per treatment were used (divided in 3 replicates of 5 couples each) and examined until their death. The couples were maintained inside plastic boxes (11 x 11 x 3.5 cm), and fed according to each treatment. Cotton balls, which are their preferred substrate, were used for oviposition. Egg masses were removed every second day and placed in Petri dishes (60 mm x 10 mm diameter) for egg counting and to check viability. Duration of the nymphal period (days), adult longevity (days), weight of males and females (mg), pronotum width (mm), female fecundity (total number of eggs produced by each female during its lifespan), and egg viability (%) (number of eggs from which nymphs hatched/ total number of eggs of each female x 100) were evaluated.

Feeding preference of D. melacanthus *adults between different food sources (trial 2)*

The second trial was carried out in the laboratory under the same controlled conditions as trial 1. The objective was to study feeding preferences of *D. melacanthus* adults between different food sources common for stink bugs early in the second crop season, after soybean harvest. The experiment was performed in a completely randomized
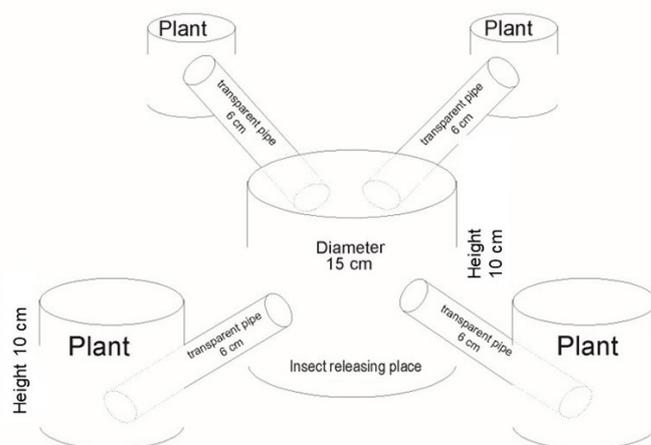
design and comprised six choice tests carried out inside arenas. In each test, two food sources were placed opposite of each other (Fig. 2): 1) soybean seedlings (SS) versus *C. benghalensis* branches (CbB), 2) soybean seedlings (SS) versus moistened soybean grains (MSG), 3) maize seedlings (MS) versus *C. benghalensis* branches (CbB), 4) maize seedlings (MS) versus moistened soybean grains (MSG), 5) maize seedlings (MS) versus soybean seedlings (SS), 6) *C. benghalensis* branches (CbB) versus moistened soybean grains (MSG). Each comparison was replicated 15 times. Each arena was composed of one plastic pot (10 cm height x 15 cm diameter) in the center and four plastic pots (8 cm height x 10 cm diameter), placed equidistantly and connected by a transparent pipe (6 cm length x 25.4 mm internal diameter) to the center pot where the insect was released (Fig. 2).

Maize and soybean seedlings of 10 cm height and the most recent *C. benghalensis* branches (10 cm from the apical part) were used in the experiment. The food combinations were placed inside plastic pots (8 cm height x 10 cm diameter) on opposite sides of the arena. Four *D. melacanthus* adults that were starved for 24 hours were released inside the center pots. Feeding preference was evaluated 24 and 48 hours later, by counting the number of stink bugs on each plant or insects on the petri dish showing on no apparent food choice. The experiment was performed in an environmentally controlled room with 25 ± 2°C temperature, 80 ± 10% relative humidity, and 14L:10D photoperiod.

Oviposition preference of D. melacanthus *adults between different plants (trial 3)*

The third trial was carried out under field conditions, without environmental control with temperatures varying from 22ºC to 34ºC and relative humidity from 60% to 80%, at Embrapa, in the municipality of Londrina (S 23°11'11.7"; WO 51°10'46.1") in the northern state of Paraná (PR), Brazil. The experiment was carried out to evaluate the oviposition preference of *D. melacanthus* adults between: soybean plants in the reproductive stage R4 (SP-R4) (Fehr et al., 1971), maize plants in the reproductive stage R2 (MP-R2), soybean seedlings (SS), maize seedlings (MS), and *C. benghalensis* plants (CbP) beginning to bloom. The plants were covered with a cage (6 m x 4 m), in a completely randomized block design with five replicates.

The plants were grown in circles directly in the soil (each circle was composed of one plant of each treatment), each cage contained five circles (Fig. 1). Adults (14-d old) of *D. melacanthus* from the insect rearing were

used. Twenty couples were released in each circle, totalling 100 couples per cage. Evaluations were made 72 hours after adult release. The variables evaluated were: number of eggs and number of egg masses per plant species.

Statistical analyses

Results were submitted to exploratory analysis to verify the assumptions of normality of residuals (Shapiro and Wilk, 1965), homogeneity of treatment variance, and additivity of the model (Burr and Foster, 1972) to allow for ANOVA. The significance of differences was identified using the Tukey test at 5% probability (SAS Institute, 2009).

Results

Development of D. melacanthus *feeding on different sources (trial 1: no-choice test)*

The developmental time (days) of nymphs differed between the evaluated food sources (Table 1). No insect survived on *C. benghalensis* branches alone as food source. Shorter nymph developmental time was recorded for insects feeding on diets containing moistened soybean grains or on the standard diet (control). However, moistened soybean grains + soybean seedlings resulted in the shortest adult longevity. Maximum adult longevity was recorded for both the standard diet and the combination of moistened soybean grains + maize seedlings,

despite these treatments being statistically similar to that of combined maize seedlings, maize seedlings + *C. benghalensis* branches, and of moistened soybean grains + *C. benghalensis* branches (Table 1).

Regarding adult weight, results were quite similar for females and males. The highest male and female weight was recorded for insects fed with the standard diet (control) (Table 2). Female weight in this treatment was statistically similar to that in moistened soybean grains + soybean seedlings, moistened soybean grains + maize seedlings, moistened soybean grains + *C. benghalensis* branches, and maize seedlings for male weight, and statistically similar to moistened soybean grains + soybean seedlings, moistened soybean grains + maize seedlings, and moistened soybean grains + *C. benghalensis* branches (Table 2).

There was no difference in pronotum size (mm) between *D. melacanthus* adults reared on different food sources (Table 2). Food sources containing moistened soybean grains and maize seedlings were always among the ones triggering higher values for the number of eggs per female and for egg viability (percentage of eggs with emergence of nymphs) (Table 2). There were no larger source-dependent differences in size or weight of insects, resulting in a similar nutrient index for all studied food sources.

Feeding preference of D. melacanthus *adults between different food sources (trial 2)*

Feeding preference of *D. melacanthus* adults varied significantly between different food sources (Fig. 3). There was a higher number

Table 1
Development time of nymphs and adult longevity of *Diceraeus melacanthus* (trial 1) fed with different food sources in the laboratory under controlled conditions (T: 25 ± 2 °C, RH 80 ± 10% and photoperiod of 14/10 h L/ D).

Food source		Nymphal duration (days)	Adult longevity (days)
Commelina benghalensis[1]		No survival	No survival
Moistened soybean grains + soybean seedlings		23.2 ± 0.3 b	53.2 ± 5.2 b
Maize seedlings + *C. benghalensis*		32.7 ± 0.5 a	71.4 ± 3.0 ab
Moistened soybean grains + maize seedlings		23.3 ± 0.4 b	79.5 ± 3.0 a
Moistened soybean grains + *C. benghalensis*		22.3 ± 0.3 b	70.6 ± 4.0 ab
Maize seedlings		33.9 ± 1.4 a	75.1 ± 7.3 ab
Standard diet[2]		23.1 ± 0.7 b	88.5 ± 4.8 a
Statistics	CV (%)	5.36	13.26
	P	< 0.0001	0.0026
	F	57.27	5.81
	DF$_{residue}$	18	17

Means ± SEM listed in columns and followed by the same letter are not significantly different according to the Tukey test (p ≥ 0.05). [1]Commelina benghalensis branches; [2]Standard diet described by Silva et al. (2008).

Table 2
Weight (mg) of males and females, pronotum width, fecundity, and egg viability of *Diceraeus melacanthus* (trial 1) fed with different food sources in the laboratory under controlled conditions (T: 25 ± 2 °C, RH 80 ± 10% and photoperiod of 14/10 h L/ D).

Food source		Weight (g)		Pronotum width (mm)	Fecundity (egg number/female)	Nutrient index[3]	Egg viability (%)
		Males	Females				
Commelina benghalensis[1]		No survival	No survival	No survival	No survival	No survival	No survival
Moistened soybean grains + soybean seedlings		0.0434 ± 0.0011 ab	0.0498 ± 0.0034 abc	6.97 ± 0.27[ns]	42.3 ± 31.0 c	0.1402 ± 0.0089[ns]	5.4 ± 2.3 c
Maize seedlings + *C. benghalensis*		0.0356 ± 0.0028 b	0.0448 ± 0.0017 bc	6.46 ± 0.05	52.9 ± 8.7 bc	0.1493 ± 0.0026	31.0 ± 4.3 bc
Moistened soybean grains + maize seedlings		0.0435 ± 0.0041 ab	0.0461 ± 0.0016 abc	6.61 ± 0.22	146.4 ± 24.2 b	0.1734 ± 0.0175	27.3 ± 24.8 bc
Moistened soybean grains + *C. benghalensis*		0.0460 ± 0.0004 a	0.0509 ± 0.0017 ab	6.82 ± 0.18	155.4 ± 17.8 b	0.1539 ± 0.0066	34.4 ± 16.4 bc
Maize seedlings		0.0373 ± 0.0015 ab	0.0397 ± 0.0034 c	6.31 ± 0.06	101.5 ± 19.2 bc	0.1565 ± 0.0034	81.6 ± 2.1 a
Standard diet[2]		0.0462 ± 0.0022 a	0.0559 ± 0.0004 a	6.54 ± 0.05	436.7 ± 7.3 a	0.1786 ± 0.0014	69.4 ± 8.9 b
Statistics	CV (%)	10.04	9.99	4.99	25.60	14.83	24.60
	P	0.0122	0.0053	0.1069	< 0.0001	0.2240	< 0.0001
	F	4.23	5.10	2.14	36.70	1.55	72.80
	DF$_{residue}$	16	17	18	11	18	9

Means ± SEM listed in columns and followed by the same letter are not significantly different according to the Tukey test (p ≥ 0.05). [1]Commelina benghalensis branches; [2]Standard diet described by Silva et al. (2008). [3]Nutrient index: weight (mg)/pronotum width (mm)[3] (Funayama, 2004). [ns]ANOVA not significant.
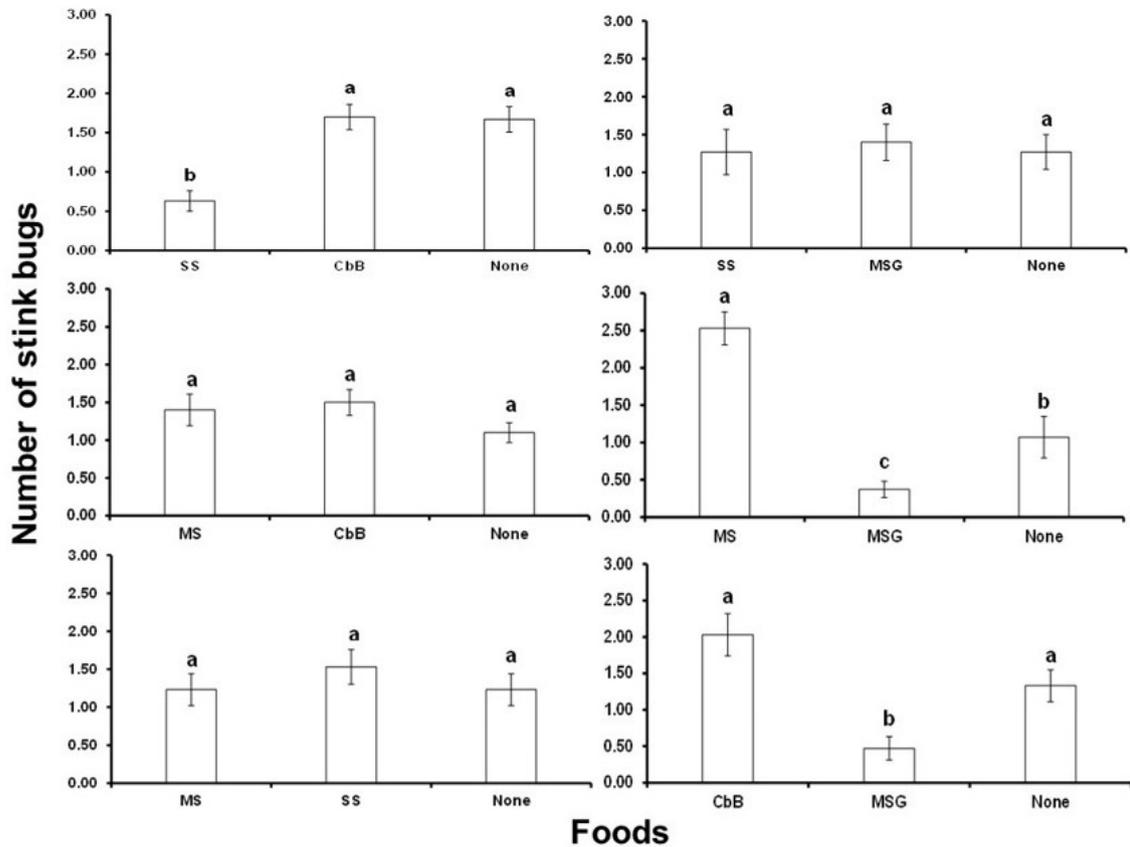
Figure 3 Number of stink bugs (means ± SE) per food source associated with the feeding preference (trial 2) of *Diceraeus melacanthus* adults. Bars represent the average of evaluations performed during 24 and 48 hours. Means followed by the same letter do not differ according to the Tukey test (p ≥ 0.05). Food: soybean seedlings (SS), *Commelina benghalensis* branches (CbB), maize seedlings (MS), moistened soybean grains (MSG), no food preference (NONE). Analysis performed on data transformed to $\sqrt{x+0.5}$. [ns]Anova not significant.

of *D. melacanthus* feeding on CbB compared with both SS and MSG. However, there was no difference in feeding preference of *D. melacanthus* between CbB and MS (Fig. 3). Neither was any preference recorded between SS and both moistened MSG and MS. In contrast, MS were more attractive to *D. melacanthus* adults than moistened soybean grains MSG (Fig. 3).

Oviposition preference of D. melacanthus *adults between different plants (trial 3)*

There was a significant preference of *D. melacanthus* for oviposition on *C. benghalensis* plants compared with the other treatments (Fig. 4). The number of egg masses was higher on CbP compared with SP-R4, SS, MP-R2, and MS (Fig. 4). Consequently, the highest number of eggs was also observed on *C. benghalensis* plants followed by soybean plants in the reproductive stage R4 (SP-R4), maize seedlings (MS), maize plants in the reproductive stage R2 (MP-R2) and soybean seedling (SS) (Fig. 4).

Discussion

Moistened soybean grains were found to be of important nutritional value and therefore crucial for *D. melacanthus* development, triggering faster nymph development as well as better overall adult fitness compared with the other studied food sources despite no significant differences between treatments in the nutrient index. The combination of moistened soybean grains + maize seedlings had a higher nutrient
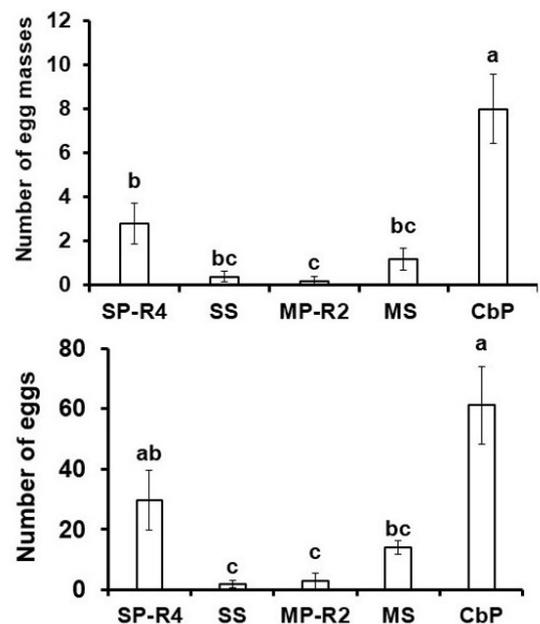

Figure 4 Number of egg masses and individual eggs found per type of host (plants) associated with the oviposition preference (trial 3) of *Diceraeus melacanthus*. Plants: Soybean plants in the reproductive stage R4 (SP-R4); soybean seedlings (SS); Maize plants in the reproductive stage R2 (MP-R2); maize seedlings (MS); *Commelina benghalensis* plants (CbP). Means followed by the same letter in each figure do not differ according to the Tukey test ($p ≥ 0.05$). Analysis performed on data transformed to $\sqrt{x+0.5}$.

index than maize seedlings alone and the treatment with a nutrient index similar to that of the standard diet (assumed to be of higher nutritional value). Adult size depends on the suitability of the nymphal diet (Shearer and Jones, 1996; Manners and Walter, 2009), and larger size can indicate higher fecundity and longevity (McLain et al., 1990; Arakawa et al., 2004). Not only the quantity but also the quality of food consumed by insects has strong effects on pest biology, physiology and behavior (Panizzi and Parra, 1991; Nation, 2002; Golizadeh et al., 2009; Cabezas et al., 2013). Thus, when insects feed on hosts of low nutritional value, they usually need compensatory strategies. These can, for example, include an extension of the feeding period (Behmer, 2009), as recorded here for nymphs feeding only on maize seedlings or on maize seedlings + *C. benghalensis* branches.

Overall, pentatomids are polyphagous pests (Panizzi and Lucini, 2022) which benefit from a mixture of plant species (Oda et al., 1981; Shearer and Jones, 1996). While some of the offered plant species provide nutrients that allow nymph development and/or adult survival and reproduction (acting as host plants), other species available in plant combinations may offer shelter and provide nutrients and water that is only sufficient for temporal sustainment (acting as associated plants) (Panizzi and Lucini, 2022). The most nutritious plants are usually preferred by the insects, but at low abundance pests are forced to explore the associated plants available (Bernays and Chapman, 1994). Thus, a higher number of associated plants with *D. melacanthus* seems to be essential for an increase of the pest population from the first (soybean) to the second crop season (maize) in Brazilian agriculture (Silva et al., 2013; Panizzi and Lucini, 2022).

Most of the weed species are reported in the literature as plants associated with stink bugs (Panizzi and Lucini, 2022), as observed for *C. benghalensis* in our study. However, cultivated plants can also be associated with stink bugs. For example, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) has been reported feeding on grapes although suffering 100% mortality of its nymphs (Pfeiffer et al., 2012; Smith et al., 2014). Acebes-Doria et al. (2016) and Stahl et al. (2021) reported no increase in nymph survival until the adult stage of *H. halys* with a mixed diet of grapes (probably acting as associated plant) and peaches (which seem to act as host plant) in comparison with peaches alone. This suggests that a greater diversity of associated plants (weeds or cultivated species) can play an important role in pest bioecology and status (Panizzi and Lucini, 2022), although they are not the single cause for pest outbreaks. Thus, maize seedlings or *C. benghalensis* acting as associated plants have great potential to favor *D. melacanthus* outbreaks when combined with moistened soybean grains remaining on the ground after harvest, since this complementary food sources improve *D. melacanthus* fitness, mainly increasing adult longevity, fecundity and egg viability. Stink bugs have been reported to use cultivated plants such as maize seedlings, or weeds such as *C. benghalensis* as a complementary diet and water source, as well as for shelter (Manfredi-Coimbra et al., 2005).

In this context, it is important to point out that *D. melacanthus* did not complete its life cycle feeding exclusively on *C. benghalensis,* even though this plant was significantly preferred by the insect in the feeding and oviposition preference experiments, confirming that weed serves as a complementary water source or diet. In general, *D. melacanthus* needs to feed on fruits or seeds to efficiently complete its development (Chocorosqui and Panizzi, 2008). Soybean grains are rich in proteins and carbohydrates of high nutritional value (Slansky and Panizzi, 1987; De Moraes et al., 2006), which is of crucial importance to the nutrition of pentatomids (Chocorosqui and Panizzi, 2008).

It was previously reported that *D. melacanthus* nymphs could not complete their cycle to the adult stage when mature soybean seeds are provided as food source (Chocorosqui and Panizzi, 2008). Mature

soybean seeds are harder to penetrate by stink bugs stylets because of their low water content (~13% moisture) (Panizzi and Rossini, 1987). However, in our study the soybean grains were moistened before being offered to *D. melacanthus* nymphs, which facilitated nymph feeding. This treatment therefore was a better representation of field conditions commonly observed, with grains on the ground that were dropped during soybean harvest.

Although maize and *C. benghalensis* are not the most suitable food for the development of the *D. melacanthus* nymphs, the weed is of great importance for triggering a significant competition between the weed and the cultivated maize plants (Rocha et al., 2000). Moreover, stink bug adults showed a feeding preference for these plants over moistened soybean grains. The food choice by the insect can vary according to different characteristics of the food, both physical or chemical, such as the nutritional composition and/or allelochemistry (Slansky and Panizzi, 1987), which could have influenced the study and needs further investigation in future work. Additionally, the attractiveness of certain plants to the insects can be related to volatiles being released in higher amounts (Karban et al., 2000), which also explains the choice of *D. melacanthus* for these plants.

Volatiles released by the plants are of importance for insects to locate the host plant, that is, they are essential for the insects to meet their nutritional demand and find an adequate place for reproduction and oviposition (Bruce et al., 2005). Therefore, the oviposition preference of *D. melacanthus* for *C. benghalensis* as opposed to the other cultivated plants present in the cage, can be attributed to higher water content and architecture of this plant species. Maize and soybean seedlings have a smaller leaf area, narrower leaves, and fewer ramifications compared with *C. benghalensis*, which explains the greater number of egg masses and consequently the higher number of eggs of *D. melacanthus* on this plant. These findings corroborate studies that have pointed out that plant architecture is a relevant factor, which directly influences insect abundance and distribution (Espírito Santo et al., 2007). Therefore, the preference to oviposit on *C. benghalensis* is probably related to the plant's architecture, which provides shelter for eggs and later for 1st and 2nd instar nymphs, which do not have much mobility and less feeding activity.

Overall, our results show that the combinations of different food sources studied, and which frequently occur in the soybean-maize two-season production system, favors *D. melacanthus* development. In this crop system, *D. melacanthus* strongly benefited from recent changes (last 30 years) in crop management practices, especially the replacement of the plowing system by the no-tillage cultivation (Panizzi and Lucini, 2022). Moreover, shelter offered by the no-tillage system between first and second crop seasons (Bueno et al., 2015; Corrêa-Ferreira and Sosa-Gómez, 2017), associated with the presence of weeds such as *C. benghalensis* and the presence of soybean grains remaining on the ground, improve conditions for *D. melacanthus* survival and outbreaks. Although exclusive feeding on branches and leaves of *C. benghalensis* (Chocorosqui and Panizzi, 2008) does not allow for survival and completion of the nymphal stage until adulthood, *D. melacanthus* uses *C. benghalensis* vegetative tissues to keep a sufficient level of body hydration and nutrients to increase survival in the period of absence of major sources of nutrients. This usually happens after soybean harvest, cultivated in the first crop season (summer) and the sowing of maize in the second crop season (autumn-winter) (Panizzi and Lucini, 2022). During this period, *D. melacanthus* can benefit from nutritive vegetative tissues of food sources using a cell rupture feeding strategy (Panizzi et al., 2021). Thus, the presence of weeds such as *C. benghalensis* as well as grains on the ground, which can even develop into soybean volunteer plants, can play an important role in *D. melacanthus* bioecology and pest status, particularly in the Neotropics, where the species is active

during the whole year (Panizzi, 1997; Panizzi and Lucini, 2022). This combination of associated plants offers food as well as shelter for *D. melacanthus,* which has triggered the species to be a key pest in maize (Silva et al., 2013) and also in soybean (Oliveira et al., 2022).

Therefore, our results illustrate the importance of avoiding both the presence of soybean grains/seeds on the ground as well as the presence of weeds such as *C. benghalensis* between first and second crop seasons in order to reduce green bridge effects.

Measures to prevent soybean harvest loss as well as efficient weed control strategies are essential to reduce *D. melacanthus* outbreaks in maize cropped in the second season. A management strategy that focuses not only on one crop season but also on the productive system, taking the whole agricultural landscape into consideration, is strongly required to avoid yield damages, especially for an efficient control of polyphagous and mobile pest species such as *D. melacanthus* (Abel et al., 2007; Wu, 2007; Herde, 2009). In addition, we assume that different microclimates between soybean harvest and maize sowing, which can be triggered by the different scenarios evaluated herein, may play an important role in *D. melacanthus* survival since the effect of temperature on insects is well-known, although it was not investigated in this study.

Therefore, it can be concluded that in order to reduce *D. melacanthus* outbreaks in maize, cropped in the second season, it is important to reduce soybean harvest loss and eliminate weeds that serve as water reservoirs and shelter for the stink bugs after soybean harvest and maize sowing. This recommendation is reinforced by our observation that moistened soybean grains in combination with *C. benghalensis* and maize seedlings triggered the highest *D. melacanthus* fitness among the different food sources studied here.

Acknowledgments

Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

APQ, AFB and ARP contributed to the study design. All authors wrote and reviewed the paper. APQ conducted sampling. AFB and ARP contributed to reagents/material/analysis tools. APQ, JR and DMS conducted the experiments. All authors have read and approved the manuscript.

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