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Nymphal and adult biology of the red-shouldered stink bug, *Thyanta perditor* (F.), on cultivated and on wild plants

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Abstract

Laboratory studies demonstrated that nymphs of *Thyanta perditor* (F.) completed their development on seed heads of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), and on immature and mature seeds of black jack (*Bidens pilosa* L.), whereas no nymphs passed to the third instar when fed with leaves of any plant evaluated. At adult emergency, fresh body weight of females and males was greater on seed heads of wheat and barley (range 59–77 mg) compared to adults reared on immature and mature seeds of black jack (36–51 mg). Survivorship of adults after 40 days post eclosion on wheat seed heads, on green bean (*Phaseolus vulgaris* L.) pods, and on maize (*Zea mays* L.) seeds was > 80%, and was lower on barley seed heads (50%) and on soybean (*Glycine max* Merr.) pods (<20%). For wild plants, on mature seeds and on black jack plant, the survivorship was higher than 50%. For reproduction, the most suitable diets were maize seeds, and wheat and barley seed heads (cultivated), and black jack (wild) plants. Twenty-eight days post eclosion, fresh body weight increased on all cultivated plants (26–62%), and less on the wild black jack plant (16%); on immature seeds and inflorescences of black jack, and on immature fruits of milkweed (*Euphorbia heterophylla* L.), adults lost weight (-10, -26, and -17%, respectively).

Keywords Heteroptera · Pentatomidae · Biology · Host plants

Introduction

Stink bugs (Pentatomidae) are mostly phytophagous and polyphagous on several cultivated plants of agricultural importance (Panizzi et al. 2000). The genus *Thyanta* encompass species commonly found on soybean (*Glycine max* Merr.), wheat (*Triticum aestivum* L.), and cotton (*Gossypium hirsutum* L.), among other crops. *Thyanta custator* (F.) and *T. custator acerra* McAtee (Reay-Jones 2010, 2014; Suh et al. 2013) are the most commonly referred species in the Nearctic (US), while in the Neotropics (Brazil), the red-shouldered stink bug, *Thyanta perditor* (F.), is the most common species, found mainly on wheat (Perez et al. 1980) and on soybean (Panizzi and Herzog 1984) crop plants.

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Several stink bug species pests in the Neotropics, such as *Dichelops melacanthus* (Dallas) (Chocorosqui and Panizzi 2008), *Nezara viridula* (L.) (Panizzi and Meneguim 1989), *Euschistus heros* (F.) (Costa et al. 1998; Malaguido and Panizzi 1999), and *Piezodorus guildinii* (Westwood) (Panizzi and Smith 1977; Panizzi et al. 2002), had their biology evaluated on different cultivated and on wild plants. However, *T. perditor*, although considered a potential pest, has been little investigated and information on its nymphal and adult biology on cultivated and on wild plants is scarce. The few studies found in the literature report its damage to wheat plants (Ferreira and Silveira 1991), and notes on its biology on black jack (*Bidens pilosa* L.), soybean (Panizzi and Herzog 1984), and wheat (Perez et al. 1980).

In this study, we report results obtained in laboratory bioassays, in which we evaluated the performance of nymph and adult *T. perditor* feeding on cultivated and on wild plants. The cultivated plants chosen were soybean, maize (*Zea mays* L.) wheat, barley (*Hordeum vulgare* L.), and green bean (*Phaseolus vulgaris* L.), and the wild plants chosen were the weeds black jack, milkweed (*Euphorbia heterophylla* L.), signal grass (*Brachiaria plantaginea* Link), and flax-leaf fleabane (*Conyza bonariensis* L.). This plant selection was

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done based on the presence of *T. perditor* on these plants or on its potential to colonize them. Therefore, we tested the hypothesis that these cultivated and wild plants may or may not support the nymphal and adult biology of *T. perditor*.

Materials and methods

Stink bug colony and plants

Adults and nymphs of *T. perditor* were field-collected on black jack plants at the Embrapa National Wheat Research Center Field Experimental Station located in Passo Fundo, RS, Brazil (28°15′ S, 52°24′ W). Bugs were taken to the Laboratory of Entomology, where a colony was established. They were placed into plastic cages ($25 \times 20 \times 20$ cm) lined with filter paper, and kept in a walk-in chamber at 25 ± 1 °C temperature, $65 \pm 10\%$ relative humidity, and photoperiod of 14L:10D hours.

As food source, black jack plants carrying all reproductive structures (inflorescence, immature and mature seeds) were provided, which were replaced twice a week. Deposited egg masses were collected daily from the rearing cages and placed inside Petri dishes (9.0×1.5 cm) containing a fresh green bean pod (*Phaseolus vulgaris* L.) to obtain nymphs. Nymphs were used for the nymphal biology tests. Nymphs were also allocated in plastic cages ($25 \times 20 \times 20$ cm) and fed with a mixture of green bean pods, raw shelled peanuts (*Arachis hypogaea* L.), and branches of black jack containing inflorescence, immature and mature seeds, to obtain adults for the adult biology tests.

Plants of wheat cv. BRS Parrudo, barley cv. BRS Cauê, and soybean cv. BRS 284 and of black jack, milkweed, signal grass, and flax-leaf fleabane were obtained in the greenhouse. Seeds were placed in pots (5 L) containing a mixture of prepared soil every 2 weeks to grow the plants. These plants were cultivated during June to November 2016. Fresh green bean pod and maize (immature seed head) of unknown cultivars were obtained in local grocery stores.

Nymphal biology on cultivated and on wild plants

On the first day of the second instar (first instar stays grouped and do not feed), nymphs were carefully collected from the colony using a fine paint brush and placed into Petri dishes $(9.0 \times 1.5 \text{ cm})$ lined with filter paper. A wet cotton placed on a plastic lid (2 cm diameter) was used to provide water. The following plants were used as food source (10 nymphs/food) and replaced twice per week: cultivated—wheat, barley, soybean, green bean, and maize; wild—black jack, milkweed, signal grass, and flax-leaf fleabane.

During the vegetative plant stage, we evaluated from cultivated plants, leaves of maize, soybean, barley, and wheat, and from wild plants, leaves of black jack, signal grass, milkweed, and flax-leaf fleabane. During the reproductive plant stage, we evaluated from cultivated plants, immature seed heads of wheat and barley, immature pods of soybean and green bean, and immature seeds of maize. From the wild plants, we tested inflorescences, and immature and mature seeds of black jack, immature fruits of milkweed, immature seeds of signal grass, and mature seeds of flax-leaf fleabane.

The Petri dishes were placed randomly in an environmental chamber maintained at 25 ± 1 °C and $65 \pm 5\%$ RH and photoperiod of 14L:10D. Daily observations were made on nymph survivorship and development. From these observations, we calculated nymphal developmental time (for each instar and from second instar to adult) and percent mortality during each instar and from second instar to adult. At adult emergence, fresh body weight was measured using an electronic balance (Sartorius model BP210S).

Adult biology on cultivated and on wild plants

The adult performance was evaluated on five cultivated plants (wheat, barley, soybean, green bean, and maize) and on two wild plants (black jack and milkweed). The following treatments were used: cultivated plants—immature seed heads of wheat and barley, immature pods of soybean and green bean, and immature seeds of maize; wild plants plants of black jack (carrying vegetative and reproductive structures), inflorescences, immature seeds, and mature seeds of black jack, and immature fruits of milkweed.

On the day of emergence, adults obtained from the laboratory colony were separated, weighed, and placed in pairs (female + male) in plastic boxes $(11 \times 11 \times 3.5 \text{ cm})$ lined with filter paper and added a food source (n = 10 pairs per food treatment); for the treatment with black jack plant, a plastic cage $(25 \times 20 \times 20 \text{ cm})$ was used. Wet cotton placed on a plastic lid (2 cm diameter) was used to provide water. The boxes were placed randomly in an environmental chamber maintained at 25 ± 1 °C and $65 \pm 5\%$ RH and photoperiod of 14L:10D.

At the end of 40 days of adult life, survivorship, fecundity (i.e., number of eggs and egg masses), and egg fertility (i.e., egg hatchability) were recorded. The parameters calculated were percent of ovipositing females, number of egg masses and eggs per female deposited, and percent of egg hatched. In addition, adults (females and males) had their fresh body weight, 28 days post eclosion, measured which was used to calculate percent of weight gain.

Statistical analysis

Data were previously analyzed using the Bartlett test to check for homogeneity of variances (P < 0.05), and then transformed to log(x) when necessary to attend the pre-requisites of analysis of variance (ANOVA). Nymphal and adult biology data were analyzed using ANOVA, and means were compared using the Tukey test (P < 0.05), except for comparison of the female's body weight at the first day of the adult life, where means between barley (immature seeds head) and black jack (mature seeds) were compared using Student's *t* test. All statistical analyses were carried out using R program version 3.3.1 (R Development Core Team 2016).

Results

Nymphal biology on cultivated and on wild plants

When fed during the vegetative stage of cultivated and wild plants, *T. perditor* nymphs were unable to complete their development (no nymphs reached the third instar). Nymphs

survived on average from 4.7 to 5.1 days in the presence of leaves of cultivated plants (wheat = 5.1 ± 0.7 days, maize = 4.8 ± 0.4 days, barley = 4.8 ± 0.5 days, and soybean = 4.7 ± 0.5 days), and from 2.9 to 5.4 days on wild plants (signal grass = 5.4 ± 0.5 days, milkweed = 4.9 ± 0.5 days, flax-leaf fleabane = 4.5 ± 0.4 days, and black jack = 2.9 ± 0.3 days). On water only, nymphs survived for 4.5 ± 0.3 days.

When fed during the reproductive stage of cultivated plants, *T. perditor* nymphs were able to complete their development when fed on immature seed heads of barley (60%) and wheat (30%), but not on immature seeds of maize or on immature pods of soybean and of green bean (Table 1). On wild plants, *T. perditor* nymphs completed their development only on immature (40%) and on mature (70%) seeds of black jack. On mature seeds of flax-leaf fleabane, on inflorescences of black jack, on immature seeds of signal

Table 1 Mean (\pm SE) developmental time and survivorship of *Thyanta perditor* nymphs feeding on reproductive structures of cultivated and wild plants in laboratory conditions

Food source [initial # of nymphs] ^a	Instar duration (days)	Total devel-	Survi-			
	Second	Third	Fourth	Fifth	opmental time (days)	vorship (%)
Cultivated						
Barley (immature seed heads) [10]	$10.3 \pm 0.7a$ [6] (40%) ^b	5.5±0.3a [6] (0%)	7.2±0.5b [6] (0%)	10.7±0.4ab [6] (0%)	33.7±0.9a	60
Wheat (immature seed heads) [10]	10.0±0.9a [5] (50%)	5.5±0.5a [4] (10%)	7.0±0.0b [3] (10%)	9.7±0.3b [3] (0%)	$31.0 \pm 1.0a$	30
Maize (immature seeds) [10]	- [0] (100%)	-	-	-	-	0
Green bean (imma- ture pods) [10]	- [0] (100%)	-	-	-	-	0
Soybean (immature pods) [10]	- [0] (100%)	-	-	-	-	0
Wild						
Black jack (imma- ture seeds) [10]	5.3±0.2b [10] (0%)	4.7±0.3a [10] (0%)	7.1±0.6b [10] (0%)	14.0±0.8a [4] (60%)	$31.0 \pm 1.7a$	40
Black jack (mature seeds) [10]	4.2±0.1b [10] (0%)	6.1±0.8a [9] (10%)	11.4±1.1a [9] (0%)	11.7±0.8ab [7] (20%)	$34.3 \pm 1.9a$	70
Flax-leaf fleabane (mature seeds) [10]	4.5±0.5b [2] (80%)	9.0±0.0 ^c [1] (10%)	-	-	_	0
Black jack (inflo- rescences) [10]	$5.0 \pm 0.0^{\circ}$ [1] (90%)	-	-	-	-	0
Signal grass (immature seeds) [10]	- [0] (100%)	-	-	-	_	0
Milkweed (imma- ture fruits) [10]	- [0] (100%)	-	-	-	-	0

Means (\pm SE) followed by the same letter in the column (cultivated and wild plants together) are not significantly different (Tukey's test, P < 0.05)

^aInitial number of nymphs

^b% Mortality of each instar

^cData were not considered for statistical analyses because only one individual survived

grass, and on immature fruits of milkweed, nymphs did not complete development (Table 1).

In general, the higher mortality was observed during the second instar, except on immature seeds of black jack where the fifth instar had highest mortality (Table 1). Even though some differences were observed during each instar, the total nymphal developmental time was not significantly different among those fed on immature seed heads of barley and wheat for cultivated plants, and on immature and mature seeds of black jack for wild plants (ranged on average from 31 to 34.3 days) (Table 1). On immature seeds of maize, immature pods of soybean and green bean, immature seeds of signal grass, and immature fruits of milkweed, no nymphs pass to the 3rd instar.

At the first day of the adult life, fresh body weight of females and males showed statistical differences when fed on cultivated and on wild plants on which nymphs completed development. Both females and males presented a significantly higher fresh body weight when fed on immature seed heads of the cultivated plants (barley and wheat; range 59–77 mg) compared to wild plants (immature and mature seeds of black jack; range 35.5–51 mg). On immature seeds of black jack, males presented the lowest body weight, whereas on mature seeds of this weed plant they remained in an intermediate position (Fig. 1).

Adult biology on cultivated and on wild plants

Survivorship of females and males *T. perditor* up to 40 days of life was variable according to the food source utilized.

ab

Black jack

(mature seeds)

□Males

Females

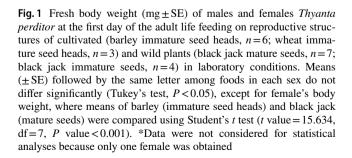
b

Black jack

(immature

seeds)

Wild



Wheat (seed

heads)

100

80

60

40

20

0

Barley (seed

heads)

Cultivated

Fresh body weight (mg)

On the cultivated plants, on wheat seed heads, green bean pods, and maize seeds, both females and males presented over 80% of survivorship after 40 days (Fig. 2a–c); however, the survivorship of females and males decreased over time on barley seed heads (50%) and mostly on soybean pods (20 and 10%, respectively) (Fig. 2d, e).

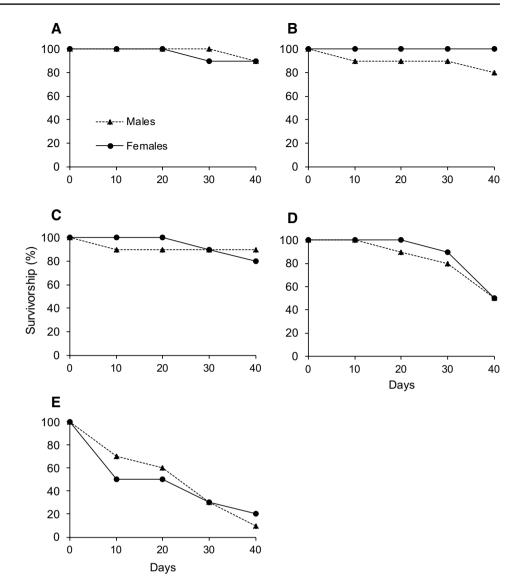
On the other hand, on all wild plants, the survivorship of both genders decreased over time. Passed 40 days, females and males fed on black jack plants (Fig. 3a) and males fed on mature seeds of black jack (Fig. 3b) showed survivorship higher or equal to 50%. On immature seeds and on inflorescences of black jack, and immature fruits of milkweed, survivorship decreased more sharply, in which more than 50% of males and females were dead after 20 days, and none reached day 40 (Fig. 3c–e).

For the reproductive performance of *T. perditor*, the percentage of females that oviposited varied from 30 to 100% among cultivated plants and from 0 to 100% among wild plants. On soybean pods, only 30% of females laid eggs; however, on the other cultivated plants (green bean pods, seed heads of wheat and barley, and maize seeds) the majority (>70%) of females oviposited (Table 2). On wild plants, all females laid eggs when fed on black jack plants; however, on mature and on immature seeds of black jack, only 40 and 10% of females laid eggs, respectively; on inflorescences of black jack and on immature fruits of milkweed, no females laid eggs (Table 2).

Regarding the fecundity of *T. perditor*, the number of egg masses/female was significantly higher when they were fed on maize seeds, and wheat and barley seed heads on cultivated plants; on the wild plants—black jack—fecundity was also significantly higher (over 2×more egg masses/female) compared to the remaining food sources (Table 2). As a result of this higher fecundity, the total number of eggs per female was also significantly higher (range 136–207 eggs) on these referred food sources; on the remaining food sources, the number of eggs/female was significantly lower (range 9–54 eggs) (Table 2).

Egg hatchability showed variability among the foods provided; on plants where females laid the highest number of eggs (maize seeds, wheat and barley seed heads, and black jack plants), it ranged from 56 to 68%. Percentage values of egg hatchability tended to be higher on green bean pods and on immature and mature seeds of black jack (>75%); however, few eggs were laid on these foods (Table 2).

Fresh body weight of adult *T. perditor* after 28 days presented significant differences among foods. On cultivated plants, the fresh body weight did not show significant differences among them (range 85–98 mg). Percentage gain weight was higher on maize seeds (62%), followed by wheat seed heads (43%), soybean pods (39%), green bean pods (34%), and barley seed heads (26%) (Fig. 4). On wild plants, fresh body weight was, in general, smaller, except for black Fig. 2 Survivorship (%) of adult females and males *Thyanta perditor* (n = 10) up to 40 days post eclosion feeding on cultivated plants in laboratory conditions. Wheat: immature seed heads (**a**), green bean: immature pods (**b**), maize: immature seeds (**c**), barley: immature seed heads (**d**), and soybean: immature pods (**e**)



jack plants (on average 86 mg), which did not differ significantly from the cultivated plants. Body weight decreased significantly on mature seeds of black jack (on average 77 mg), and achieved the lowest values on inflorescences and immature seeds of black jack, and on immature fruits of milkweed (< 58 mg). Percentage weight gain occurred only on mature seed of black jack (23%) and on black jack plants (16%), whereas on immature seeds and inflorescences of black jack and on immature fruits of milkweed, adults lost weight (-10, -26, and -17%, respectively) (Fig. 4).

Discussion

Thyanta perditor, as the majority of pentatomid pests of crops of economic importance (Panizzi 1997), feeds preferably on seeds, as reported by Ferreira and Silveira (1991) on wheat plants. In our study, this feeding preference for

reproductive structures was clearly demonstrated by the fact that *T. perditor* nymphs did not developed on vegetative structures (leaves) of both the cultivated and wild plants tested. In fact, nymphs survived for about 5 days only on these leaves before perishing, similar to what happened when they took water only. Even though *T. perditor* nymphs did not survive when fed only on vegetative structures (leaves), Tomacheski and Panizzi (2018) observed that *T. perditor* adults had preference for seedlings of soybean and maize compared to other cultivated plants, and for the weed plants, milkweed and black jack were preferred, when compared to both cultivated and wild plants.

Therefore, on vegetative stage of cultivated and on wild plants, *T. perditor* may exploit them as shelter and/or as plant juice source for rehydration, which is known to occur in other stink bug species, such as *Edessa meditabunda* (F.) (Lucini and Panizzi 2016), which feed preferably on plant stems (Silva et al. 2012), and *P. guildinii* (Westwood)

Fig. 3 Survivorship (%) of adult females and males Thyanta *perditor* (n = 10) up to 40 days post eclosion feeding on wild plants in laboratory conditions. Black jack: plants (a), black jack: mature seeds (b), black jack: immature seeds (c), black jack: inflorescences (d), and milkweed: immature fruits (e)

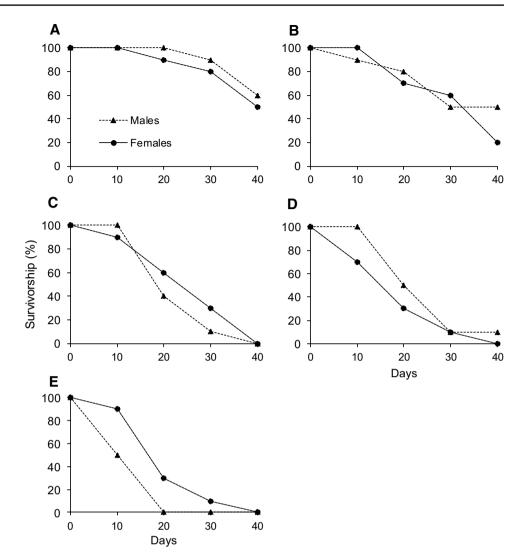


Table 2 Reproductive performance of females Thyanta perditor feeding on reproductive structures of cultivated and wild plants in laboratory conditions

Food source	Females laying	Number per female		Egg hatched (%)	
	eggs (%)	Egg masses	Eggs		
Cultivated					
Maize (immature seeds)	100 [10] ^a	$7.2 \pm 0.7a$	$206.9 \pm 35.3a$	67.7 ± 8.1a	
Wheat (immature seed heads)	100 [10]	5.3±0.6a	$201.1 \pm 23.4a$	63.5±10.9a	
Barley (immature seed heads)	80 [8]	4.0 ± 0.7 abc	$136.4 \pm 27.1a$	55.5 ± 12.5a	
Green bean (immature pods)	70 [7]	1.6 ± 0.3 cd	$30.3 \pm 5.3b$	76.2 ± 4.9a	
Soybean (immature pods)	30 [3]	2.0 ± 0.0 bcd	$54.3 \pm 10.6b$	46.4 ± 23.3a	
Wild					
Black jack (plants)	100 [10]	5.2 ± 0.8 ab	$200.2 \pm 36.6a$	66.5±6.1a	
Black jack (mature seeds)	40 [4]	$1.5 \pm 0.2d$	$21.0 \pm 5.6b$	79.6±2.5a	
Black jack (immature seeds)	10 [1]	2.0 ± 0.0^{b}	9.0 ± 0.0^{b}	$88.8\pm0.0^{\rm b}$	
Black jack (inflorescences)	0 [0]	_	_	_	
Milkweed (immature fruits)	0 [0]	-	_	_	

Means $(\pm SE)$ followed by the same letter in the column (cultivated and wild plants together) are not significantly different (Tukey's test, P < 0.05)

^aNumber of females that oviposited

^bData were not considered for statistical analyses because only one female survived

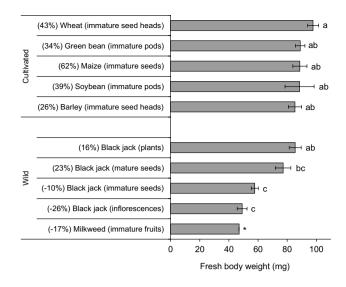


Fig. 4 Fresh body weight $(mg \pm SE)$ of (female + male) *Thyanta perditor* after 28 days of the adult life feeding on cultivated (wheat, n=19; green bean=19; maize=18; soybean=6; barley=17) and on wild (black jack plants, n=17; mature seeds=12; immature seeds=8; inflorescences=5; milkweed immature seeds=1) plants in laboratory conditions. Means (\pm SE) followed by the same letter are not significantly different (Tukey's test, P < 0.05). *Data were not considered for statistical analyses because only one individual survived. In parentheses, percentage of body weight gain in the period

(Lucini et al. 2016), which feed preferably on seeds (Panizzi and Smith 1977). Much probably, the nutrient concentration in leaves of cultivated and wild plants, therefore, is not enough to allow *T. perditor* nymphs to complete their development.

The fact that nymphs of *T. perditor* did survive on seed heads of barley and wheat in our laboratory tests explains in part the presence of adults on these plants in the field. However, their relatively low survivorship, as compared to other species of phytophagous pentatomids (see review in Panizzi 1997), may explain the fact that they are seldom observed on seed heads of cereal plants. Therefore, most adults observed on the seed heads might have developed as nymphs elsewhere. On the other cultivated Poaceae (immature seeds of maize) and Fabaceae (immature pods of soybean and green bean), no nymphs survived, demonstrating their unsuitability as food sources for *T. perditor* nymphs.

On wild plants, nymphs did survive and complete development on black jack (immature and mature seeds), confirming previous data on *T. perditor* nymphal biology on this food source (Panizzi and Herzog 1984). However, on our study, we add novel information on the relation of the bug with black jack, demonstrating that nymphs develop better on mature than on immature seeds, and that, although observed in the field, nymphs do not complete development when fed exclusively on black jack inflorescences. The other three remaining weed plants tested (flax-leaf fleabane mature seed, signal grass immature seed, and milkweed immature fruit) were unsuitable foods for nymphs.

The higher fresh body weight attained at adult emergency on seed heads of barley and wheat, and on mature seeds of black jack shows that, despite the high nymph mortality, the ones that are able to reach adulthood, originate bigger adults. The adult survivorship, reproduction, and weight gain data demonstrate that, in general, food sources suitable for nymphs were also suitable for adults. However, some drastic differences were observed. For example, all nymphs died when fed on immature seeds of maize; adult reproduction, however, was highest on this diet.

Maize seedlings may be attacked by stink bugs, such as the so-called in Brazil green-belly stink bugs, *D. melacanthus* (Dallas) (Chocorosqui and Panizzi 2008) and *D. furcatus* (F.) (Panizzi et al. 2015), and by the Neotropical brown stink bug, *E. heros* (F.) (Quintela et al. 2006). Moreover, Tomacheski and Panizzi (2018) reported the preference of *T. perditor* adults for maize seedlings compared to wheat and barley seedlings; however, it was considered an unexpected result, because *T. perditor* is not reported to occurs on maize, whereas on wheat it does (Smaniotto and Panizzi 2015). On maize seed heads, few reports are found in the literature on true bugs exploring this as a food source: the cosmopolitan southern green stink bug, *N. viridula* (L.) in Louisiana (USA) (Negron and Riley 1987), and the coreid, *Leptoglossus zonatus* (Dallas) in Brazil (Panizzi 1989).

Apparently, adult pentatomids and other true bugs are unable to identify maize seed heads as a rich and nutritious food source or are unable to reach the seeds covered by the maize seed head husk. Also, the fact that nymphs died when fed on exposed immature maize seeds while adults reached the highest reproduction suggest that the nymphal development and adult reproduction syndromes apparently require different nutrients and/or nutrients on different concentrations. Clearly, additional investigations should be conducted to confirm these data.

Interesting to note was the fact that on black jack immature seeds, both, nymphs and adults, did not perform well. The majority of nymphs did not complete development, adults (males and females) did not reach day 40 and few reproduced, and they lost weight after 28 days. On mature seeds, however, the opposite was observed, i.e., the majority of nymphs completed development, both males and females reached day 40, reproduction was high, and they gained weight after 28 days. In fact, in natural conditions, nymphs are exclusively observed feeding on mature seeds of black jack. Apparently immature seeds do not have all nutrients needed for nymph and adult to perform well or may present toxic allelochemicals that cause detrimental effects compared to mature seeds.

These laboratory studies coupled with field observations allow to conclude that the stink bug *T. perditor* may eventually reach pest status on the winter cereals of southern Brazil, such as wheat and barley. Among the wild plants, black jack is the most common host plant, while the other weed plants tested play a minor role on its biology.

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