Arthropod-Plant Interactions (2017) 11:89–97
DOI 10.1007/s11829-016-9469-1

ORIGINAL PAPER

Influence of fall armyworm previous experience with soybean genotypes on larval feeding behavior

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Received: 6 February 2016 / Accepted: 14 October 2016 / Published online: 29 November 2016

Abstract Previous experience on host plants can modify insect feeding behavior. Because insect habituation and induction of preference to host plants are variable across species of plants and insects, it is necessary to investigate each insect-plant interaction to determine whether this phenomenon occurs or not in the system. In this study we investigated the potential occurrence of habituation and induction of preference in fall armyworm (FAW) *Spodoptera frugiperda* to soybean genotypes. Neonate FAW larvae reared on artificial diet were divided into four treatment groups and fed for one generation with either the resistant soybeans PI 227687 or IAC 100 or the susceptible soybeans BRS Valiosa RR or IGRA RA 626 RR. Biological parameters of FAW were recorded. Eggs obtained from FAW of each genotype group were separated, and the newly hatched larvae were fed on the same genotypes experienced by their parents for additional 8 days. FAW larval preference and leaf area consumed were evaluated in choice feeding assays with the four soybean genotypes within a 24-h period. Genotypes PI 227687 and IAC 100 negatively affected FAW development, demonstrating they are FAW-resistant. FAW larvae exposed to both resistant genotypes consumed more foliage of genotype IGRA RA 626 RR in the choice assays, whereas larvae reared on both susceptible genotypes did not show any preference. From our preliminary study, FAW does not show habituation and induction of preference toward the experienced soybean genotypes. The importance of our findings to host plant resistance and insect-plant biology fields is discussed.

Keywords Learning · Habituation · Induction of preference · Sensitization · *Spodoptera frugiperda*

Handling Editor: Michael Smith.

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Introduction

Previous experience of insects on host plants can modify their subsequent feeding (Karowe 1989) and oviposition (Jaenike 1988) behavior through learning. Non-associative learning processes include habituation, sensitization, and induction of preference. Habituation is one of the most common and simplest types of learning and is characterized by the insect's gradual waning in responsiveness to a stimulus through experience, that is, after repetitive or constant contact of the insect to the stimulus. Sensitization is the opposite and occurs when repetitive contact with the stimulus leads to an increased response (Mathews and Mathews 2010). Induction of preference occurs when an insect alters its regular feeding behavior and tends to prefer feeding on a particular host plant experienced before; this type of non-associative learning is thought to involve different mechanisms of learning (Bernays and Chapman 1994; Schoonhoven et al. 2005). On the other hand, the influence of larval experience on adult feeding/oviposition behavior is known as pre-imaginal conditioning (Thorpe and Jones 1937). In recent years, the effects of insect learning have been extensively investigated and reported to occur in many herbivorous insect species (Bernays and Chapman 1994; Liu and Liu 2006; Moreau et al. 2008; Janz et al. 2008).

Insect learning has been confirmed in both larval and adult stages of holometabolous species (Bernays 1995). Lepidopterans, dipterans, and coleopterans often exhibit habituation and induction of preference to feeding deterrents in the larval stage (Jermy 1987). Adults usually show shifts in oviposition preferences induced by previous experience (Li and Liu 2004). Depending on the species of insect and host plant, insect learning can last from minutes, hours, and days (Bernays and Chapman 1994) to many generations (Wilson and Starks 1981).

Plant genotypes expressing moderate-to-high insect-resistant traits can be used as an additional tactic for insect pest control. Host plant resistance (HPR) is an advantageous control method for both farmers and the environment. Because insect experience is one of the biotic factors that may influence the expression of plant resistance (Smith 2005), the occurrence of learning in test insect individuals should be given special attention in HPR studies. This topic deserves attention prior to genotype-screening assays as the occurrence of insect habituation and induction of preference to genotypes and species of plants could substantially alter the outcome of the feeding assays. Because insect learning is variable across species, it is necessary to investigate each interaction between herbivores and plants to elucidate whether insect learning occurs or not in a given plant-insect system. In cases with no previous knowledge of the occurrence of any type of learning in a test insect species, it is common to exclude this effect from the experiment; this is done by standardizing the insect diet before the assay is set up, that is, excluding the host plant on which the test insect was previously fed to avoid misleading results (Smith 2005; Boiça Júnior et al. 2013).

Most studies on insect learning have been performed with parasitoid species, but less information is found in the literature on the learning of insect pests to plant genotypes differing in insect-resistance levels. Among the available studies, adult *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) preferred feeding on plants of *Solanum tuberosum* or *S. dulcamara* on which their larvae were previously fed (Rossetto 1973). Rossetto (1972) concluded that the weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) did not show pre-imaginal conditioning when reared on either sorghum or corn. Female *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae) preferred ovipositing on foliage of the same grape cultivar experienced by the larvae (Moreau et al. 2008). More recently, Santa-Cecília et al. (2013) reported that, although there is no pre-imaginal conditioning in *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae), individuals of this species showed initial preference to the host for which they had prior experience. Given the variable results on insect learning across species, the performance of more studies to investigate the influence of insect learning on feeding preference among plant genotypes will significantly contribute to the HPR and insect-plant biology research fields.

In this study, we investigated the potential occurrence of habituation and induction of preference in a highly polyphagous insect species (Capinera 2002), the fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). The fall armyworm (FAW) is widespread throughout the American continent (Sparks 1979) and is one of the insect species most benefiting by successional cultivation of soybeans, cotton, and maize in Brazil mainly due to its generalist feeding habit (Santos et al. 2009). Thus, FAW holds great potential to become an important pest of soybeans in the future as the exposure of this crop to FAW populations has increased in recent years in the country (Sá et al. 2009; Bueno et al. 2011). In addition to its economic importance, FAW is a good model insect for studies on insect learning as polyphagous caterpillars are more prone to habituate to plant deterrents than oligophagous caterpillars (Bernays and Chapman 1994).

In this study, we examined the hypothesis that FAW habituates and has its feeding preference induced toward the soybean host used as larval food. Thus, we aimed at investigating the influence of resistant and susceptible soybean genotypes on FAW larval habituation and induction of preference.

Materials and methods

Experimental conditions, testing insects, and genotypes of soybeans

The experiment was conducted in the laboratory and greenhouse of the School of Agriculture and Veterinary Sciences, São Paulo State University, in Jaboticabal, state of São Paulo, Brazil. Laboratory assays were performed under environmentally controlled conditions (26 ± 2 °C temperature, 60 ± 10% temperature, and 12L: 12D h photoperiod).

Soybean genotypes were selected for this study based on differences in the levels of resistance and susceptibility against another armyworm species, *Spodoptera eridania* (Cramer) (Souza et al. 2012, 2014). Genotypes PI 227687 and IAC 100 were chosen as the resistant genotypes, while BRS Valiosa RR or IGRA RA 626 RR were chosen as the susceptible genotypes. Seeds of the soybean genotypes were sown in 5-l pots filled with soil (dystrophic red latosol) (Centurion et al. 1995), sand, and bovine manure at a 3:1:1 ratio and were kept in the greenhouse until use in the assays. Because the soybean reproductive stage is critical for fall armyworm infestations (Panizzi et al. 2012), soybean plants were used at the R3–R4 growth stage (Fehr and Cavines 1977) in all assays. Moreover, the use of R3–R4 soybeans is more adequate for differentiating the expression of resistance to FAW than V4–V5 soybeans (Boiça Júnior et al. 2015).

FAW larvae used in the experiment were obtained from a colony maintained for ~2 years in the laboratory (~20 generations). During this period, wild FAW individuals collected in maize fields of Jaboticabal county, SP, Brazil, were introduced into the laboratory colony to maintain genetic variability. The larvae were reared on an artificial diet based on wheat germ, soybean bran, casein, and brewer's yeast (Greene et al. 1976), and the adults were fed a 10% honey solution. Because we used soybean genotypes not previously tested for resistance against FAW, we recorded biological parameters of FAW developed on leaves of the soybean genotypes for one generation before evaluating the insect habituation and induction of preference.

Evaluation of FAW development on resistant and susceptible soybean genotypes

To evaluate FAW development on soybeans we randomly assigned 60 neonate larvae from the laboratory colony to each soybean genotype, totaling 240 larvae in the experiment. Each larva represented one replicate and the experimental unit. We recorded the following biological parameters of FAW: duration and survival of larva, pupa, and larva to adult, weights of larva and pupa, and fecundity of unfed and fed adults.

Neonate FAW larvae were carefully transferred into 9-cm-diameter petri dishes lined with filter paper moistened with distilled water using a fine paintbrush. Each petri dish received one larva, and the petri dishes were distributed in a completely randomized design. The FAW larvae were fed with soybean leaves collected from the mid part of plants at R3–R4 reproductive stages (Fehr and Cavines 1977). Twelve-day-old larvae were weighed on an analytical scale (Ohaus Corp., Barueri, state of São Paulo, Brazil) and thereafter returned to the petri dishes. Twenty-four hours after pupation, the pupae were weighed and kept in the petri dishes until emergence of adults.

Five FAW couples that had emerged on the same day from each soybean treatment were separated and housed in PVC cages (10 cm diameter × 21 cm height) for evaluation of adult fecundity. The adults were fed a 10% honey solution imbibed in a cotton pad inside a small plastic coffee cup. Mortality of FAW adults was checked daily to compute data for longevity of fed adults. The remaining adults emerging from each soybean treatment were kept in the petri dishes from which they had emerged and were not offered any kind of food; mortality was recorded daily, and data were computed for longevity of unfed adults.

Evaluation of FAW's previous experience with soybean genotypes on larval feeding behavior

Aiming to evaluate the occurrence of habituation in FAW, we separated the eggs laid by FAW females reared in each soybean treatment from the previous biological assay. The egg masses were kept in 9-cm-diameter petri dishes lined with moistened filter paper until hatching. Next, neonate larvae were individually transferred to petri dishes lined with moistened filter paper using a fine paintbrush. The larvae were offered and developed on leaves collected from the mid part of R3–R4-stage soybeans of which their parents had experience. The larvae were fed on each soybean treatment for 8 days, after which the occurrence of habituation was evaluated through a choice feeding assay.

Eight-day-old larvae from each treatment group were separated and carefully transferred into petri dishes (14 cm diameter) containing four soybean leaf discs (2.5 cm diameter) using a fine paintbrush. The leaf discs were punched out from leaves collected from the mid part of R3–R4-stage soybean genotypes. Four 8-day-old larvae were released per petri dish using a paintbrush, and each petri dish with four leaf discs and four FAW larvae represented one experimental unit. The release of one larva per leaf disc in the choice assays proved to be adequate for evaluating FAW feeding preference on soybeans (Boiça Júnior et al. 2015). Treatments were replicated five times and were arranged in a completely randomized design. Larval choice was recorded 1, 6, 12, and 24 h after the release of the larvae in the petri dishes. At the end of the experiment (after 24 h), leaf area consumed was measured using an LI-COR 3100A leaf area meter (LI-COR, Lincoln, NE, USA).

Statistical analysis

Data of biological parameters and feeding preference of FAW were checked for normality of residuals and homogeneity of variances by the Kolmogorov-Smirnov and Levene tests, respectively. When data of the biological parameters did not fit into a curve of normal distribution and/or were heteroscedastic, they were analyzed by the Kruskal-Wallis non-parametric test; normally distributed and homoscedastic data were analyzed by one-way analysis of variance (ANOVA). Treatment means were separated by Tukey post hoc test ($\alpha = 0.05$) when ANOVA was significant. For FAW feeding preference data, leaf area consumed was normally distributed and showed homogeneity of variances, and therefore analysis was by one-way ANOVA. When significant, mean separation of treatments was calculated by Fisher's LSD test ($\alpha = 0.05$). Statistical analysis was performed in the SAS version 9.0 software (SAS Institute 2002).

Results

Evaluation of FAW development on resistant and susceptible soybean genotypes

We observed significant differences ($H_{3,166} = 116.55$; $P < 0.0001$) of soybean genotypes in the duration of FAW larval stage (Table 1). Larvae fed genotype IGRA RA 626 RR took 11.8–25.8% less time to complete the larval stage than larvae fed the other genotypes. Genotype BRS Valiosa RR showed intermediate duration of larval stage, whereas genotypes IAC 100 and PI 227687 negatively affected FAW larval growth, extending the stage to 24.8 and 25.1 days, respectively. Larvae of *S. frugiperda* fed leaves of PI 227687 genotype had significantly affected survival ($H_{3,236} = 38.16$; $P < 0.0001$), with a 60% decrease from the initial population of larvae (Table 1). The other soybean genotypes did not differ from each other, and the rates of larval survival ranged from 75 to 97.7%.

For the pupal stage, there were no differences in either the duration of the period ($H_{3,143} = 5.37$; $P = 0.1465$) or the survival ($H_{3,166} = 8.74$; $P = 0.3300$) of FAW across the soybean genotypes (Table 1). Mean duration and survival of the pupal stage among genotypes were 11.5 days and 87.3%, respectively. These results possibly indicate that there is little influence of FAW larval feeding between resistant and susceptible genotypes on the time the pupae spend until adulthood and on the survival.

Results of duration and survival of the FAW larva-to-adult period were similar to results found for the larval parameters (Table 1). Genotype IGRA RA 626 RR showed the shortest larva-to-adult period, differing significantly ($H_{3,143} = 113.35$; $P < 0.0001$) from the other genotypes; BRS Valiosa RR was intermediate, and genotypes IAC 100 and PI 227687 caused a 6-day elongation in FAW larva-to-adult duration relative to the period spent on IGRA RA 626 RR. Survival of FAW larva to adult was significantly lower ($H_{3,143} = 50.86$; $P < 0.0001$) in the PI 227687 genotype, with rates of survival 1.2–1.6 times lower than rates recorded in the other genotypes, which did not differ from each other.

Weights of larvae ($H_{3,221} = 128.71$; $P < 0.0001$) and pupae ($H_{3,163} = 54.39$; $P < 0.0001$) of FAW were significantly affected by the soybean genotypes (Fig. 1). Larvae fed leaves of IGRA RA 626 RR obtained the highest weights of larvae, differing from the other genotypes. Feeding on foliage of genotype BRS Valiosa RR yielded larvae with intermediate larval weights, whereas IAC 100 and PI 227687 yielded the lowest larval weights; larvae of FAW fed the latter two genotypes exhibited weights of larvae 65 and 76% lower than weights of larvae fed genotype IGRA RA 626 RR, respectively. The lowest weights of larvae of FAW fed genotypes IAC 100 and PI 227687 resulted in pupae with the lowest weights, differing significantly from weights of pupae reared on IGRA RA 626 RR and BRS Valiosa RR.

Longevity of *S. frugiperda* (Fig. 2) differed significantly between genotypes for unfed adults ($H_{3,101} = 26.83$; $P < 0.0001$) but not when adults were fed a honey solution ($F_{3,36} = 0.75$; $P = 0.5318$). FAW unfed adults lived 1.5–1.6 times longer when developed on IGRA RA 626 RR than on the resistant genotypes IAC 100 and PI 227687. Longevity of FAW unfed adults in genotype BRS Valiosa RR was not significantly different from that in IGRA RA 626 RR and IAC 100. Longevity of fed adults was greater than twofold longer than longevity of unfed adults.

Taking all results together from the biological parameters recorded from FAW, the soybean genotypes herein evaluated are classified according to the levels of resistance to FAW as follows: PI 227687, highly resistant (HR); IAC 100, moderately resistant (MR); BRS Valiosa RR, susceptible (S); IGRA RA 626 RR, highly susceptible (HS).

Evaluation of FAW's previous experience with soybean genotypes on larval feeding behavior

In choice feeding assays with FAW larvae that had previously fed on one of the four soybean genotypes, larval

Table 1 Means (±SE) of duration and survival of larva, pupa, and larva-to-adult periods of *Spodoptera frugiperda* developed on soybean genotypes

Genotypes	Larva[a]		Pupa		Larva to adult[a]	
	Period (day)	Survival (%)	Period (day)	Survival (%)	Period (day)	Survival (%)
PI 227687 (HR)[b]	25.1 ± 0.16a	41.7 ± 6.42b	11.1 ± 0.24	80.0 ± 8.16	36.2 ± 0.48a	60.8 ± 5.45b
IAC 100 (MR)	24.8 ± 0.45a	75.0 ± 5.64a	11.5 ± 0.12	82.2 ± 5.76	36.3 ± 0.45a	78.6 ± 4.06a
BRS Valiosa RR (S)	21.1 ± 0.48b	97.7 ± 3.60a	11.5 ± 0.12	98.2 ± 1.82	32.6 ± 0.16b	97.9 ± 2.07a
IGRA RA 626 RR (HS)	18.6 ± 0.24c	75.0 ± 5.64a	11.7 ± 0.14	88.9 ± 4.74	30.4 ± 0.24c	82.0 ± 3.85a

[a] Means followed by different letters within columns are significantly different by Kruskal-Wallis test ($P < 0.05$)

[b] *HR* highly resistant, *MR* moderately resistant, *S* susceptible, *HS* highly susceptible

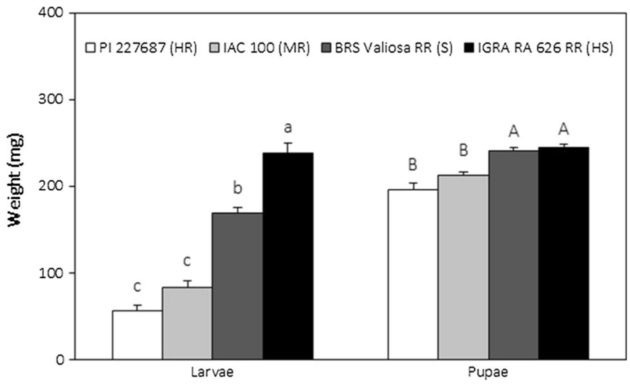

Fig. 1 Fresh weight (mg) of larvae and pupae of *Spodoptera frugiperda* developed on soybean genotypes. *Bars with different letters* for the same biological parameter are significantly different by Kruskal-Wallis test ($P < 0.05$). *HR* highly resistant, *MR* moderately resistant, *S* susceptible, *HS* highly susceptible

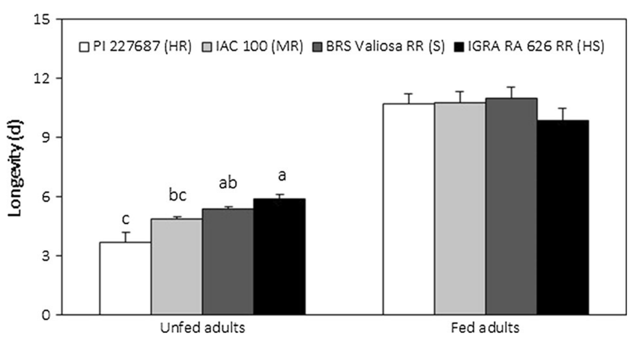

Fig. 2 Longevity (*d*) of unfed and fed adults of *Spodoptera frugiperda* developed on soybean genotypes. *Bars with different letters* for the same biological parameter are significantly different by Tukey test ($P < 0.05$). *HR* highly resistant, *MR* moderately resistant, *S* susceptible, *HS* highly susceptible

preference followed a similar trend regardless of the genotype experienced by FAW; the larvae preferred the susceptible genotypes over the resistant genotypes in most time points assessed (Fig. 3). FAW larval choice was highest 12 h after the release of the larvae in the petri dishes, except in the choice assay with IGRA RA 626 RR-experienced larvae, being the highest larval choice observed at 1 and 6 h.

Leaf area consumed by FAW larvae differed between soybean genotypes depending on whether the larvae were exposed to resistant or susceptible genotypes prior to choice assays (Fig. 4). When FAW larvae were developed on genotype IAC 100 prior to feeding assays, the larvae preferred genotype IGRA RA 626 RR ($F_{3,16} = 11.11$; $P = 0.0012$) when given a choice among the four genotypes of soybeans; leaf area consumed on IGRA RA 626 RR was on average 3.5-fold greater than leaf area consumed on the other genotypes. A similar trend occurred when FAW was previously developed on the other resistant genotype, PI 227687; larvae experienced in this genotype significantly preferred ($F_{3,16} = 4.72$; $P = 0.0048$) genotype IGRA RA 626 RR, followed by BRS Valiosa RR in the choice feeding assay. On the other hand, when FAW experienced the susceptible genotypes BRS Valiosa RR ($F_{3,16} = 1.36$; $P = 0.3964$) and IGRA RA 626 RR ($F_{3,16} = 1.02$; $P = 0.3983$), larval preference was not significantly different when given a choice among the four soybean genotypes.

Discussion

Evidence indicates that previous experience of insects on host plants can change feeding behavior in some species and that habituation and induction of preference may be responsible for this shift. Habituation is characterized by the gradual decline in insect responsiveness to a stimulus after repetitive contact to plant stimulus, enabling the insect to eat a previously unacceptable host plant. Induction of preference is a switch in insect regular feeding behavior in which the insect tends to prefer feeding on a particular previously experienced host plant (Bernays and Chapman 1994; Schoonhoven et al. 2005). Here we examined FAW feeding preference in choice feeding assays using groups of larvae that had experienced one of four soybean genotypes to demonstrate the potential occurrence of habituation and induction of preference. Results of this study showed that FAW larvae did not prefer feeding on the experienced genotype, and we suggest that habituation and induction of preference do not occur in FAW, at least when the larvae are reared on soybeans for a short time period. The importance of these findings for the host plant resistance and insect-plant biology research fields is discussed below.

When FAW was developed on foliage of the four soybean genotypes, PI 227687 and IAC 100 negatively affected the development, yielding increased FAW mortality rates, lengthening of duration of the larval stage and complete development, and reduced weights of the larvae and pupae. Both soybean genotypes were selected for this experiment as resistant standards based on their moderate-to-high levels of resistance expressed to another *Spodoptera* species (Souza et al. 2012, 2014), and from the results of our biological assay they proved to be FAW-resistant as well. Several studies have demonstrated the negative effects of the genotypes PI 227687 (Hatchett et al. 1976; Smith and Gilman 1981; Yanes and Boethel 1983; Hoffmann-Campo et al. 1994; Souza et al. 2012, 2014; Costa et al. 2014a, b) and IAC 100 (Lambert and Kilen 1984; Machado et al. 1999; Oliveira et al. 1993; Castiglioni and Vendramim 1996; Lourenção et al. 2000; Souza et al. 2012, 2014) on other insect species, indicating these

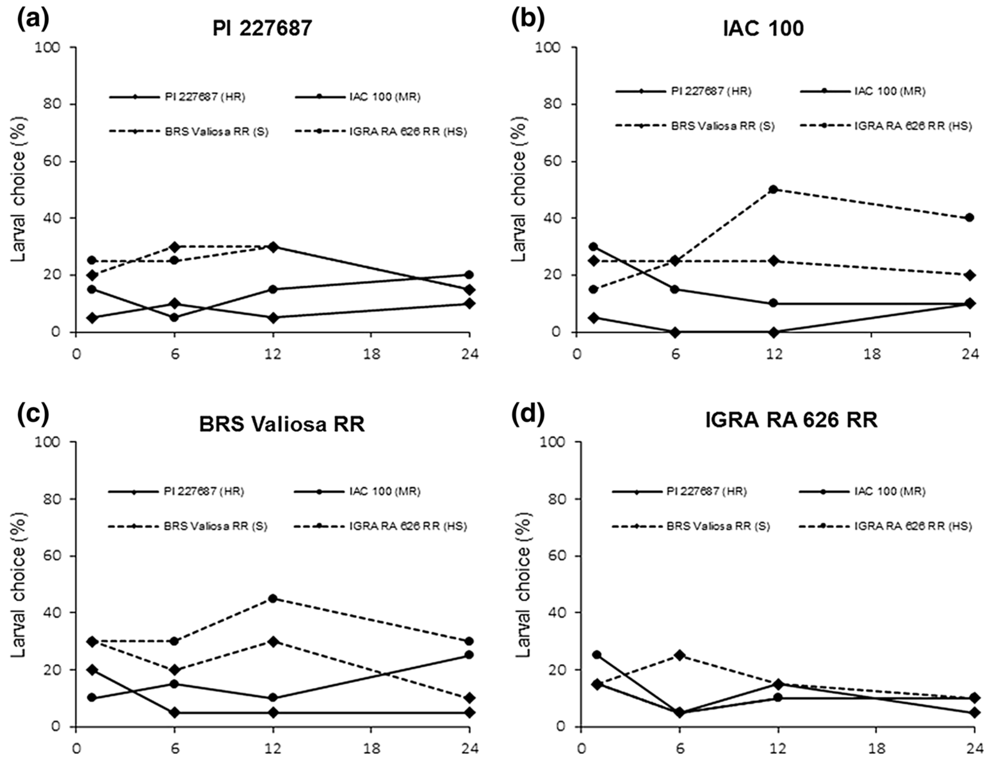

Fig. 3 Feeding preference (%) of *Spodoptera frugiperda* larvae after 1, 6, 12, and 24 h using larvae with experience of genotypes PI 227687 (**a**), IAC 100 (**b**), BRS Valiosa RR (**c**), and IGRA RA 626 RR (**d**) prior to choice assays. *HR* highly resistant, *MR* moderately resistant, *S* susceptible, *HS* highly susceptible

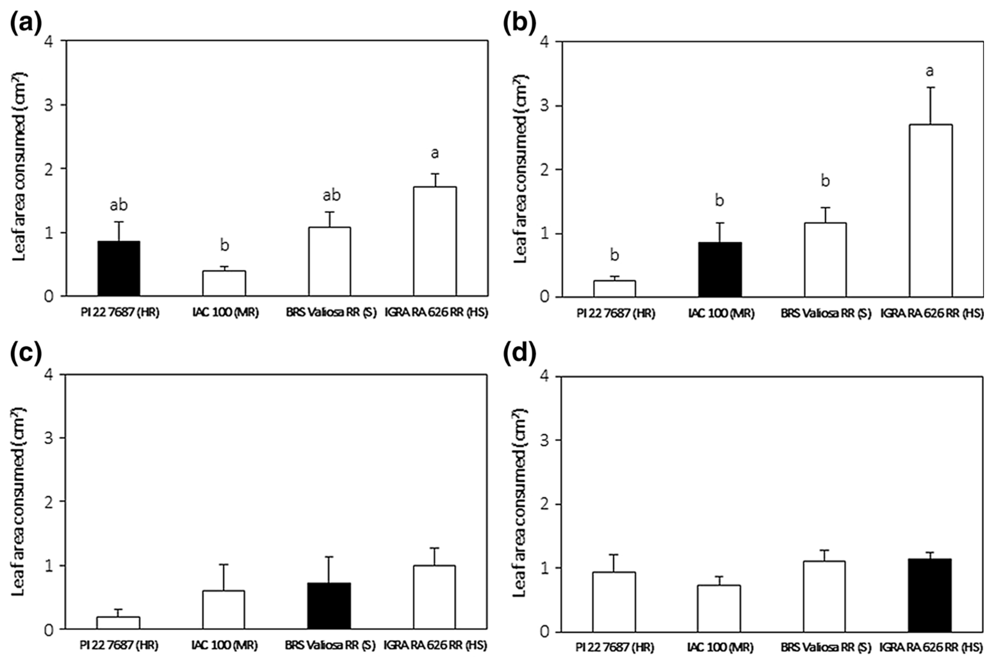

Fig. 4 Leaf area consumed by *Spodoptera frugiperda* larvae on leaf discs of soybean genotypes. *Dark-colored bars* represent the genotype experienced by *S. frugiperda* larvae prior to choice assays; PI 227687 (**a**), IAC 100 (**b**), BRS Valiosa RR (**c**), and IGRA RA 626 RR (**d**). *Bars with different letters* are significantly different by LSD test ($P < 0.05$). *HR* highly resistant, *MR* moderately resistant, *S* susceptible, *HS* highly susceptible

genotypes may bear genes conferring multiple insect resistance with potential use as donors in soybean breeding programs. To our knowledge, this study is the first to categorize soybean genotypes for levels of natural resistance against FAW, an insect pest with growing economic importance in many soybean-producing regions of Brazil (Bueno et al. 2011).

From the results of our bioassay, we suggest that genotypes PI 227687 and IAC 100 possess higher concentrations of defensive secondary compounds in their leaf tissues than in leaf tissues of genotypes IGRA RA 626 RR and BRS Valiosa RR. Some studies quantified insect-defensive compounds in genotypes PI 227687 and IAC 100, as in Piubelli et al. (2005). The authors correlated the expression of resistance in IAC 100 with higher concentrations of the flavonol rutin and of the isoflavonoid genistin, phenolic compounds serving a role in plant defense against many insects (Dixon and Steele 1999). Secondary compounds with antifeeding and antibiotic properties such as daidzein, coumestrol, sojagol, and glyceollins were extracted from PI 227687 leaf tissue by Sharma and Norris (1991). In another study, Hoffmann-Campo et al. (2001) determined the concentrations of the flavonols rutin and quercetin-3-*O*-glycosilgalactoside and of the isoflavonoid genistin in PI 227687. Therefore, the presence of higher concentrations of these and perhaps other phenolics and flavonoids in the PI 227687 and IAC 100 genotypes was likely responsible for the adverse effects on FAW development observed in our experiment.

We did not detect evidence for the occurrence of habituation and induction of preference in FAW to soybeans. This is clear when analyzing FAW larval choice over time and the leaf area consumed between genotypes in choice assays conducted with each group of genotype-experienced larvae. If habituation had occurred, we would have expected that FAW larvae that experienced either resistant soybean genotype (i.e., putatively possessing higher concentrations of deterrents) would be less deterred and would consume foliage of those genotypes to a greater extent than larvae that experienced the susceptible genotypes. Occurrence of induction of preference would be even clearer to distinguish as resistant genotype-experienced FAW would exhibit stronger arrestment toward resistant genotypes or would show increased leaf consumption on those genotypes compared to larvae that experienced the susceptible soybeans. However, in our study the opposite was found; when FAW larvae experienced either resistant genotype, greater leaf area consumed on IGRA RA 626 RR leaf discs was observed in choice assays. Moreover, in any soybean treatment group the percentage of FAW larval choice over time was never higher for the experienced genotype.

In addition to toxic metabolites, genotypes PI 227687 and IAC 100 possess many secondary compounds displaying antifeeding properties (Sharma and Norris 1991; Hoffmann-Campo et al. 2001; Piubelli et al. 2005). Lepidopterous larvae can exhibit habituation to feeding deterrents (Jermy 1987), which might ultimately lead to induced preference to substrates containing these compounds. According to our results, FAW larvae did not habituate to soybean deterrent compounds as larval feeding preference was not enhanced toward the resistant genotypes in the choice assays. We must stress the amount of time the larvae experienced the soybean genotypes prior to the feeding assays; the minimal duration of experience required to induce preference in larvae of *Pieris brassicae* (Linnaeus) (Lepidoptera: Pieridae) was 4 h (Ma 1972); within 24–48 h of experience in host plants preference was induced in larvae of *Manduca sexta* (Linnaeus) (Lepidoptera: Sphingidae) (Schoonhoven 1967). Although we exposed FAW larvae much longer than the period of time used in the aforementioned studies, further research is warranted to elucidate whether the time FAW experiences soybean genotypes influences its subsequent feeding preference.

An interesting fact occurred in the choice feeding assay using FAW larvae experienced in genotypes IGRA RA 626 RR and BRS Valiosa RR. Unlike the results found in the assays with resistant genotype-experienced larvae, in which they clearly preferred the highly susceptible IGRA RA 626 RR, larvae exposed to the susceptible genotypes did not exhibit any preference when given a choice. We suspect that susceptible genotype-experienced larvae, i.e., larvae reared on host plants with putatively reduced concentrations of feeding deterrents, had their feeding behavior somehow influenced when exposed to leaf discs of genotypes possessing higher concentrations of these compounds. This might be explained by the occurrence of the sensitization type of learning in those FAW larvae. However, for testing this hypothesis we would have to perform choice feeding assays with both experienced and naïve larvae.

Insects may be sensitized after repetitive contact with either intense deterrent or phagostimulant stimuli (Mathews and Mathews 2010). According to Bernays and Chapman (1994), insects' previous experience with deterrents can increase their responsiveness to the negative stimuli, consequently enhancing the efficiency of foraging for food because of a quicker decision not to eat the unsuitable food substrate. On the other hand, after experiencing positive stimuli insects may be more stimulated to search for other sources of high-quality nutrition (Bernays and Chapman 1994). In addition to suggesting that sensitization might have occurred in susceptible genotype-experienced FAW larvae, we do not rule out the possibility that larvae experienced with resistant soybeans were also sensitized. The occurrence of this type of learning might explain why resistant genotype-experienced larvae showed an increased response away from leaf discs of the resistant genotypes and toward the highly susceptible genotype IGRA RA 626 RR in choice assays. However, this is difficult to distinguish in choice feeding assays as naïve larvae would certainly prefer feeding on highly susceptible genotypes as well (Souza et al. 2012). Studies investigating sensitization at the physiological level may give insights into the mechanisms underlying the occurrence of this type of learning in FAW.

Most studies investigating insect learning have employed species of natural enemies, mainly parasitoids, but much less information is found on the learning of herbivores experiencing plant genotypes. In the few studies with herbivorous insects, previous insect experience on plant genotypes usually resulted in the insect conditioning to the host plant. Girousse et al. (1999) observed that when *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae) were fed on alfalfa plants, the aphids later caused different amounts of injury between resistant and susceptible cultivars of alfalfa. However, aphid prior feeding on faba beans did not result in differences of injury between resistant and susceptible cultivars of alfalfa. Similar trends occurred with the aphid species *Diuraphis noxia* (Kurdjumov) and *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae) between resistant and susceptible wheat genotypes (Schotzko and Smith 1991; Worrall and Scott 1991). Larvae of *Helicoverpa zea* (Boddie) showed different responses to maize silk extracts when the larvae experienced different hosts (Wiseman and Mcmillian 1980). *Leptinotarsa decemlineata* adults preferred feeding on plants of *S. tuberosum* or *S. dulcamara* as their larvae experienced the respective plant species prior to assays. Female *L. botrana* preferred ovipositing on the same grape cultivar used as larval food (Moreau et al. 2008). Conversely, Santa-Cecília et al. (2013) concluded that there is no pre-imaginal conditioning in *P. citri* when reared on coffee, citrus, or squash. These divergent results reinforce the hypothesis that insect learning is specific for determined species of insects and plants, and studies aiming at investigating the complex phenomenon of insect learning should address this topic for each insect-host plant interaction.

The results obtained herein will contribute to future studies on HPR using *S. frugiperda* on soybeans. The knowledge that habituation and induction of preference do not occur in FAW within a short period of larval experience can allow us to use this in screening trials of soybean genotypes to which FAW larvae were previously exposed. This includes both larvae kept in the laboratory and those collected from soybean-field plants. However, caution should be taken when using FAW larvae fed on the same host genotype for many generations as we do not yet know how learning affects FAW feeding behavior over time, and a future experiment is needed to elucidate this issue. We will conduct a follow-up study using the same soybean genotypes to evaluate the effects of FAW prior larval feeding on the adult oviposition behavior aiming to search for the occurrence of pre-imaginal conditioning in this herbivore. The answers of these questions will certainly aid in understanding the mechanisms underlying the adaptation processes in *S. frupigerpa* to its diverse host plants, in addition to helping in the design of more standardized and accurate genotype-screening assays.

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