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Behavioral responses of *Sitophilus zeamais* Motschulsky adults to conditioned grain kernels

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ABSTRACT

Laboratory experiments were conducted in order to assess the intra- and inter-specific interactions in cereal kernels and behavioral responses of maize weevil (*Sitophilus zeamais*) adults. For that purpose, conditioning of kernels was carried out in plastic boxes containing two caps; one with 2 g of maize, wheat or barley of different "kernel status": mechanically damaged, insect damaged or intact kernels, and the other with 2 g of whole maize kernels to be contaminated by the volatile semiochemicals released from the cap with the different "kernel status" grains. Depending on the test, kernel conditioning was carried out for various intervals, i.e., 1, 7 and 14 days. In the first experiment, three traps (three-choice tests) containing the semiochemically conditioned pairs maize–maize, maize–wheat or maize–barley kernels, with mechanically damaged, insect-damaged and intact kernels, were used. In the second experiment, two traps (two-choice tests) containing the conditioned pairs of maize–maize or maize–wheat or maize–barley kernels, with mechanically damaged, insect-damaged and intact kernels, were used. In the first experiment, when maize kernels were conditioned with maize kernels, significantly more *S. zeamais* adults were found in traps containing kernels conditioned by insect damaged kernels compared to traps with kernels conditioned by mechanically damaged or intact kernels at 1 and 14 days of conditioning intervals. When maize kernels were conditioned with wheat kernels, significantly more *S. zeamais* adults were found in traps containing kernels conditioned by insect damaged kernels compared to traps with kernels conditioned by mechanically damaged or intact kernels only at the 14-day conditioning interval. When maize kernels were conditioned with barley kernels, significantly more *S. zeamais* adults were found in traps containing kernels conditioned by insect damaged kernels compared to traps with kernels conditioned by mechanically damaged kernels again only at the 14-day conditioning interval.

In the second experiment, when maize kernels were conditioned with maize kernels, significantly more *S. zeamais* adults were found in traps containing kernels conditioned by insect damaged kernels compared to traps with kernels conditioned by mechanically damaged kernels at all conditioning intervals, but results for maize kernels conditioned with wheat kernels were less clear. When maize kernels were conditioned with barley kernels, significantly more *S. zeamais* adults were found in traps containing kernels conditioned by mechanically damaged kernels compared to traps with kernels conditioned by insect damaged kernels at all conditioning intervals.

1. Introduction

Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae) is a major pest of stored grains, particularly maize in tropical and subtropical regions (Aitken, 1975; Longstaff, 1981; Throne, 1994; Likhayo and Hodges, 2000). Adults of *S. zeamais* attack sound kernels while their larvae develop inside kernels (Storey, 1987). Infestations in the field, with adults penetrating the husk, continuing during storage and causing severe losses (Caswell, 1962; Haines, 1991). The damaged kernels have low nutritional value, low rate of germination, low commercial value and decreased weight, and increased susceptibility to fungal infestation (Dharmaputra et al., 1994; Yuya et al., 2009). According to Faroni (1992) the *S. zeamais* larva consumes approximately two thirds of the endosperm. This

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species, along with the related *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), responds to the aggregation pheromone known as "Sitophilure" (4S, 5R)-5-hydroxy-4methyl-3-heptanone (Schmuff et al., 1984; Phillips et al., 1985; Walgenbach and Burkholder, 1986). However, species of the genus *Sitophilus* respond also to specific phagostimulants, which are often recovered from kernels that were previously contaminated or conditioned (Trematerra et al., 1999, 2000; Niewiada et al., 2005; Athanassiou et al., 2006). Trematerra et al. (1999) noted that specific parts of wheat kernels are much more attractive than others for *S. oryzae* adults. Moreover, *Sitophilus granarius* (L.) feeds on the embryo, but it oviposits away from the embryo to protect the immatures from injury by other adults (Gołębiowska et al., 1978; Niewiada et al., 2005).

While there is clear evidence that some volatiles can be attractive or repulsive for *Sitophilus* adults (Niewiada et al., 2005; Wakefield et al., 2005; Germinara et al., 2007, 2008), the effect of cross-contamination of grain kernels requires additional attention. Trematerra et al. (2007) found that *S. zeamais* was more attracted to kernels that were previously exposed to insect-damaged kernels in comparison to intact or mechanically damaged kernels. In the same study, the authors noted that intact kernels were more attractive than mechanically damaged maize kernels. Yet, there is still inadequate information on the attractiveness of maize kernels that were conditioned by other grain commodities such as wheat and barley. Moreover, the duration of this interaction among kernels, i.e. the interval that intact kernels remain with damaged kernels, may play a critical role in weevil host finding. In our experiment we examined the intra- and inter-specific interactions in cereal kernels and behavioral responses of *S. zeamais* adults.

2. Materials and methods

2.1. Insects and kernels

Adults of *S. zeamais*, of mixed sex and age, were taken from cultures kept under laboratory conditions on maize kernels at 28 ± 1 °C and 70 ± 5% relative humidity (r.h.) and continuous darkness.

Conditioning of kernels was carried out in plastic boxes (9 cm × 12.5 cm × 4.5 cm) containing two plastic caps (3 cm in diameter, 1.5 cm in height); a cap with 2 g of maize, wheat or barley with different "kernel status": mechanically damaged, where the kernels were broken by hammer, or insect damaged, where culture kernels were left for 30 days with adults of *S. zeamais*, or intact kernels, and another cap with 2 g of whole maize kernels to be contaminated by the volatile semiochemicals released from the different "kernel status" caps. Depending on the test, kernel conditioning was carried out for various intervals, i.e., 1, 7 and 14 days. All conditioning tests were conducted in controlled rooms set at 27 ± 1 °C with 70 ± 5% r.h. and continuous darkness.

The tests were carried out in a cylindrical arena of plexi-glass (45 cm in diameter, 30 cm in height) (Trematerra et al., 2007). Two or three modified Flit-Track M^2 trap-devices (Trécé Inc, Adair, OK, USA) were placed in the arena. In each trial, 50 adult beetles of mixed sex and age were released at the center of the arena. The number of trapped insects was checked 15 h after their introduction in the arena. Teflon paint was used to prevent maize weevils from escaping from the traps or the arena.

2.2. Experiment 1

Three traps (three-choice tests) loaded with kernels of different "status" were used. Nine replicates were performed for each case study, using 450 insects for each case. The conditioning intervals were 1, 7, and 14 days.

Case study 1. Three traps containing maize kernels conditioned either by mechanically damaged, insect damaged or intact maize kernels were compared.

Case study 2. Three traps containing maize kernels conditioned either by mechanically damaged, insect damaged or intact wheat kernels were compared.

Case study 3. Three traps containing maize kernels conditioned either by mechanically damaged, insect damaged or intact barley kernels were compared.

2.3. Experiment 2

The conditions were as above, but in this case, two traps (two-choice tests) were placed in the arena. Six replicates were performed for the case studies, using 300 insects for each case. The conditioning intervals were 1, 7, and 14 days.

Case study 4. Two traps containing maize conditioned either by mechanically damaged or insect damaged maize kernels, by insect damaged or intact maize kernels, or by mechanically damaged or intact maize kernels, were compared.

Case study 5. Two traps containing maize conditioned either by mechanically damaged or insect damaged wheat kernels, by insect damaged or intact wheat kernels, or by mechanically damaged or intact wheat kernels, were compared.

Case study 6. Two traps containing maize conditioned either by mechanically damaged or insect damaged barley kernels, by insect damaged or intact barley kernels, or by mechanically damaged or intact barley kernels, were compared.

2.4. Data analysis

Before the analysis, counts were transformed as suggested by Trematerra et al. (2000). For experiment 1, data were submitted to a two-way ANOVA, with "kernel status" and conditioning interval as main effects and means were separated by using the Tukey–Kramer honestly significant difference (HSD) test at $P < 0.05$. In the case of experiment 2, for each bioassay series, the data were analyzed separately by using the two-tailed t-test at $P < 0.05$ (Snedecor and Cochran, 1980). All analyzes were done using the SPSS software version 18 (SPSS Inc., 2010).

3. Results

3.1. Experiment 1

In the first, three-choice experiment, kernel status was significant for all three grain combinations; $F = 24.3$, $P < 0.01$, df = 2, 80; $F = 9.9$; $P < 0.01$; df = 2, 80, and $F = 4.2$, $P = 0.02$, df = 2, 80, for maize/maize, maize/wheat, and maize/barley, respectively. No significant differences were noted among conditioning periods; $F = 0.3$, $P = 0.31$, df = 2, 80; $F = 0.6$, $P = 0.55$, df = 2, 80, and $F = 0.0$, $P = 0.97$, df = 2, 80 or for interactions $F = 0.8$, $P = 0.52$, df = 4, 80; $F = 1.3$, $P = 0.28$, df = 4, 80, and $F = 0.6$, $P = 0.66$, df = 4, 80 for maize/maize, maize/wheat, and maize/barley, respectively.

When maize kernels were conditioned with maize kernels (case study 1) for 1 or 14 days, significantly more *S. zeamais* adults were found in traps containing kernels conditioned by insect damaged kernels compared to traps with kernels conditioned by mechanically damaged or intact kernels (Table 1). However, when maize kernels were conditioned with wheat or barley kernels (case studies 2, 3), significantly more *S. zeamais* adults were found in traps containing kernels conditioned by insect damaged kernels when