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Insect-corn kernel interaction: Chemical signaling of the grain and host recognition by *Sitophilus zeamais*

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ABSTRACT

In living organisms, the cuticle has structural functions and is involved through chemical signaling in biological interactions such as plant-insect and provides protection against biotic and abiotic factors, thereby avoiding desiccation or the attack of predators. The objective of this study was to investigate the participation of the epicuticle in the maize kernel-*Sitophilus zeamais* interaction. The GC-MS analysis of the epicuticle extract demonstrated the presence of aliphatic hydrocarbons, alcohols, ethers, fatty acids, sterols and their derivatives. The results of bioassays show that the epicuticle of maize has a primordial role in its interaction with *S. zeamais*, and participates in the recognition and attraction to the food source, as well as regulating its reproduction. In addition the compounds present in the epicuticle extract may act as signal molecules and development regulators. This study reveals the effect of the maize kernel cuticle on *Sitophilus* behavior and contributes to the understanding of the interaction.

1. Introduction

In natural and agronomic ecosystems, the interactions between plants and phytophagous insects are initially mediated by chemical signals such as volatiles from plants (Germinara et al., 2008). The silo is a new environment where a large number of biological interactions are produced and these cause significant economic losses (Cox, 2004; Cox and Collins, 2002).In this new environment, the insect *Sitophilus zeamais* (Motschulsky) is considered the principal and the most dangerous plague in tropical and subtropical climates of stored maize kernels (Tefera et al., 2011). This insect affects the harvest by lowering the quality of kernels and germination, also due to an increase in fungal infections by transporting spores and facilitating the penetration of hyphae through the damage done in the grain (Nesci et al., 2011; Yuya et al., 2009). Although numerous investigations have studied damage to stored grains caused by insects in different biological systems, the signal that initiates the infection process is still unknown.

The first barrier of contact between organisms and the environment is the cuticle (Welti and Wang, 2004; Zunino and Zygadlo, 2005). In plants, this provides protection against the biotic and abiotic factors, and is involved in the plant-insect interactions affecting the behavior of predators and/or parasitoids (Bargel et al., 2006; Lemieux, 1996; Yeats and Rose, 2013). In general, the cuticle is composed of a complex mixture of long-chain non-polar compounds such as hydrocarbons, wax esters, aldehydes, ketones, long chain alcohols, fatty acids, terpenoids and sterols (Lemieux, 1996; Lucini et al., 2006). These compounds can act as precursors of hormones and pheromones, regulate development processes and/or modulate interactions between organisms (Kosma et al., 2010; Lemieux, 1996; Lucini et al., 2006). Although numerous studies have reported on the relationship between plant foliar waxes and insects (Braccini et al., 2015; Kosma et al., 2010; Li and Ishikawa, 2006; van Loon et al., 1992), little is known about the role of the kernel cuticle in grain-insect interactions. Several authors have observed that the cuticular waxes of the wheat grain play an

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important role in oviposition and alimentation of *S. granarius* (Nawrot et al., 2010; Niewiada et al., 2005). However, there are few investigations about the effect of the cuticle on the maize kernel-*S. zeamais* interaction. García-Lara et al. (2003) observed that the hardness of the kernels is negatively correlated with the susceptibility index and damage caused by *S. zeamais.* Moreover, the polyphenolic compounds of the cuticle of maize kernel were related with resistance to attack by *S. zeamais* (García-Lara et al., 2003; Panagabko et al., 2000; Sen et al., 1994). These results suggest a participation of the cuticle kernel in the interaction with the insect. Based on this, the aim of the present work was to investigate the participation of the epicuticle of the maize kernel as a chemical signal in the interaction with *S. zeamais*, which provided findings towards a better management of this pest in the silo, translating this into a lesser economic loss for farmers.

2. Methods and materials

2.1. Kernels and insects

Maize kernels were obtained from Manfredi Experimental Station (INTA, Córdoba, Argentina) and kept in closed containers at −20 °C and 70 ± 5% relative humidity (r.h.). The varieties used were CV: ACA468MGRR2 Nº station: 229 (ACA) and Illinois CV: 1767 MG Rep 2. Nº station: 222 (ILLI), of which were harvested in 2014. These maize varieties show resistance (ACA) and susceptibility (ILLINOIS) to fungal infection, allowing us to suppose that this could affect the insect's response. *Sitophilus zeamais* adults, without differentiation of age or sex (except in the progeny assay), were reared on maize kernels and maintained under laboratory conditions (28 ± 2 °C and 70 ± 5% r.h.) until being used in the bioassays.

2.2. Kernel epicuticle extraction and GC-MS analyses

The epicuticle components of the two varieties of kernels were removed using a methodology of Russin et al. (1997), with some modifications. Briefly, the extraction was carried out using chloroform (3:2 kernels/ml of chloroform) for 30 s, for washing only the most superficial components of the grain (epicuticle), then the solvent was evaporated and the extract was resuspended, and its weight was quantified.

The GC-MS analysis of the epicuticle extract composition was performed on a Clarus SQ 8T GC/Mass Spectrometer (Perkin Elmer). The methodology used was that reported by Nawrot et al. (2010) with some modifications of equipment. A Perkin Elmer Elite 5MS column (30 m × 0.25 mm x 0.25 μm of thick) was used. The GC oven temperature was programmed from 40 °C to 320 °C at a rate of 4 °C/min, followed by a 20 min isothermal run. Helium was used as the carrier, and the injector temperature was 300 °C in splitless mode. The mass spectrometer was fitted with an electron ionization source operated at 70 eV, the source temperature was 230 °C, and the interface temperature was 280 °C with a solvent delay of 6 min. Mass spectra were recorded from m/z 45–400 amu in the full scan mode. Identification of the compounds was carried out using the NIST2005 library.

2.3. Thickness of the epicuticle

To compare the epicuticle thicknesses, digital photographs of the kernels of both varieties, with or without the epicuticle, were taken using a magnifying glass Olympus SZX16 coupled to an Olympus camera DP71. To carry this out, the methodology of Jacobsen et al. (1971) with some modifications, was used. The kernels of both varieties, with or without epicuticle, were boiled for 15 min in distilled water and placed in a solution of hydrochloric acid (20%) for 36 h. Then, the kernels were washed with distilled water and cut transversely with a knife. To visualize the epicuticle, the sections were stained with Sudan IV, washed and then observed. Four replicates of each treatment were used.

2.4. Repellent/attraction activity bioassay

To determine the effect of the epicuticle components in the kernels, the behavior of the *S. zeamais* was compared for kernels with (control) or without the epicuticle (treatment), using a two-choice olfactometer bioassay according to Herrera et al. (2015). Briefly, two flasks (250 ml) were connected by a glass tube (30 cm × 1 cm diameter) with a small hole (1 cm × 1 cm) in the middle (15 cm from the two flasks), with entry points between the flasks and the tube being sealed with rubber plugs, which were covered with parafilm to prevent gas leakage. Before connecting the flasks and the tube, 14 maize kernels were placed in the flasks (flask A was control: kernels with epicuticle; and flask B was the treatment: kernels without epicuticle). Then for each experimental set, groups of twenty insects, deprived of food for at least 24 h, were released in the hole of the glass tube and tested for 2 h in a climatic chamber. The experiments being carried out between 10:00 and 16:00 h and the response index (RI) was calculated (see below). The position of the flasks was changed at each replication. To discount the effect of the solvent on the response of the insect, a solvent control was performed using14 kernels with epicuticle in one flask vs 28 kernels (14 with and 14 without epicuticle) in other flask, under the same conditions using both maize varieties. After this, the same two-choice olfactometer was used to evaluate preference for increasing concentrations of epicuticle extract compared with the control.

The experiments were performed seven times for each assay, with each group of insects only being used once. For each trial, the RI was calculated from equation 1:

$$RI = \left[\frac{(T - C)}{Tot}\right] \times 100$$

where, T was number of insects responding to the treatment; C was number of insects responding to the control and Tot was total number of insects responding to the bioassay. In this case, insects in the flask with kernels with an epicuticle or the extract were considered treatment values and insects in the flask with kernels without an epicuticle or solvent were considered control values. Insects that did not show any response in the experiment were not taken into account (Phillips et al., 1993). Positive values of RI indicated attraction to the treatment, while negative ones revealed repellence. For the statistical analysis, the *paired-sample t-test* was used for the choice of insects and *ANOVA* for the comparison of the response indexes of both varieties.

2.5. Susceptibility of kernels to insect attack. Determination of kernel damage and loss of weight

This experiment was carried out for 20 days under laboratory conditions in 250 mL-flasks simulating store conditions. In each flask, 14 kernels (treatment or control), previously weighed, were introduced and 20 insects were released. After 20 days the kernels were weighed and the percentage of damage was determined by counting the number of perforated grains. For the statistical analysis the one-way *ANOVA* was used comparing the results obtained, for each determined variable, between both treatments (with and without epicuticle). The experiment was performed seven times.