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Volatile organic compounds from the interaction between *Fusarium verticillioides* and maize kernels as a natural repellents of *Sitophilus zeamais*

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ABSTRACT

Maize kernels are exposed to *Sitophilus zeamais* attack and *Fusarium verticillioides* infestation during storage, which can result in product deterioration and economic losses. The objective of this study was to evaluate the involvement of the Volatile Organic Compounds (VOCs) emitted by the fungi-corn system in grain-insect interactions. Volatiles emitted by healthy maize kernels were different from those emitted by fungal infected kernels, with the latter being enriched by alcohols, ketones and sesquiterpenes, which were considered early indicators of fungal contamination. The results demonstrated that the kernels exposed to the fungal VOCs and their pure compounds (1-octen-3-ol and 3-octanol) were less attractive and less damaged by *S. zeamais* than controls. In addition to compound adsorption, other processes may have caused the protective effect of exposed kernels against insect damage. This is the first contribution of the role of the fungal volatiles on the behavior of *S. zeamais*, and could provide an important contribution to the conservation of stored grains and pest management and an early indicator of fungal contamination.

1. Introduction

Grains are stored in bulk before their commercialization or consumption. In this environment biological interactions occur, such as grain-microorganism, grain-insect, insect-microorganism and/or grain-grain (Cox, 2004), which often produce economic losses. Among the main pests that affect stored maize grains are fungi and insects.

The fungal pathogens *Fusarium verticillioides* (Sacc.) Nirenberg (=*F. moniliforme* Sheldon teleomorph *G. Fujikuroi* (Sawada) Ito in Ito & Kimura) are the main cause of ear rot of corn in Argentina (Chulze, 2010) and one of the major producers of fumonisins (FBs) which can cause health problems to humans and farm animals (Theumer et al., 2010). The principal FBs production can takes place during grain storage, when temperature and humidity conditions allow the synthesis of these fungal secondary metabolites. *Sitophilus zeamais* is a primary pest that affects stored grain and, the damage produced by this represents a gateway to fungal infection acting as a vector of the fungal spores (Ferreira-Castro et al., 2012).

During maize storage, the competition for substrate between fungi and insects may occur and both pests must develop diverse strategies to compete and persist in sulk stored grain. The volatile organic compounds (VOCs) emitted by living organisms are known to play a critical role in tritrophic interactions, acting as a signal to unstressed plants to adjust their defensive systems (Wenda-Piesik et al., 2010). For instance, oxylipins, synthetized by lipoxygenases (LOXs), and the sesquiterpenes have function being related to the defense against pests and pathogens (Engelberth, 2011; Ghirardo et al., 2012). Although the involvement of VOCs in insect-plant-microorganism interactions has been well-described (Wenke

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et al., 2010), the role of fungal VOCs in grain-insect-pathogen interactions is poorly understood. Our previous studies have demonstrated the antifungal and antimycotoxicogenic activities of some fungal VOCs (1-octen-3-ol, 3-octanol and 3-octanone) (Herrera et al., 2015) on *F. verticillioides,* and also their insecticide effects on *S. zeamais.* These results have suggested that *Fusarium* can release these fungal VOCs to prevail over competitors present in the storage environment. Thus, the objective of this study was to evaluate the involvement of VOCs emitted by a fungi-maize grain system on the behavior of *Sitophilus zeamais,* and their role in grain-insect interactions under storage conditions.

2. Materials and methods

2.1. Kernels, insects, fungal strain and inoculum preparation

Maize kernels were obtained from Experimental Station Manfredi (INTA, Córdoba, Argentina) and kept in closed containers at -4 °C and 70± 5% relative humidity (r.h.). *Sitophilus zeamais* adults, without differentiation of age or sex, were used on maize kernels in the bioassays, which were maintained under laboratory conditions (28 ± 2 °C and 70± 5% r.h.). The *Fusarium verticillioides* strain M3125, provided by Dr. Robert Proctor, United States Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research, Peoria, IL, USA (Leslie and Summerel, 2006) was employed in all assays. Inoculum was obtained by growing it on Czapek-dox agar Petri plates for 7 days at 28 °C in the dark to allow profuse sporulation.

2.2. Maize inoculation

In order to determine the optimal experimental parameters for maize inoculations, preliminary evaluation were carried out using different days of exposure, grain weights and water contents, and varying the number and size of the mycelial discs. From these results, maize grains (25 g) were placed in a 250 ml Erlenmeyer flask and sterilized for 2 consecutive days in an autoclave for 15 min at 121 °C. Sterile distilled water (8 ml) was added to the autoclaved maize to obtain 35% humidity. Then, three 10-mm diameter mycelial discs of *F. verticillioides*, taken from the edge of the plate of a 7-day-old culture on Czapek-dox agar, were transferred aseptically to the conditioning corn. Flasks with inoculated maize were incubated for 7 days at 28 °C with manual periodic shaking to achieve a good homogenization, with flasks containing sterile corn without fungal inoculation being used as control. Four replications of each treatment were performed. These treatments were used as inductor of healthy grains.

2.3. Conditioning of kernels with VOCs from fungal infected kernels system

For the purpose of determining the capacity of the kernels to respond to VOCs emitted by *F. verticillioides* during infection, the conditioning of the kernels was carried out using a method described by Trematerra et al. (2013), with some modifications. Plastic containers (20 cm × 30 cm x 10 cm; 3 L) were divided by an interior wall into two compartments (A and B), connected by a free space between the dividing wall and the lid of the container. Inside each compartment, a glass plate (10 cm diameter x 3 cm high) was positioned. Then, on the plate in compartment A, 25 g of fungal infected kernels or a filter paper with 0.004 μl/L of 1-octen-3-ol (Sigma-Aldrich, ≥99%) and 3-octanol (Sigma-Aldrich, ≥98%), (minimum concentration of *S. zeamais* repellence previously determined) were placed, while on the plate from compartment B, 25 g of healthy whole maize kernels were placed to be exposed to the VOCs or pure compounds released from compartment A. For incubation, the plastic container was kept in a controlled room at 28± 2 °C with 70± 5% r.h. and continuous darkness for 6 days. As a control treatment, healthy whole maize kernels were deposited in both compartments (A and B) of another plastic container and maintained under the same conditions. The selection of the two fungal VOCs compounds was performed based on previous studies against *S. zeamais* in our laboratory (Herrera et al., 2015). In *in vitro* test, the 1-octen-3-ol and 3-octanol had greater insecticide activity and shown greater effect on seed germination considering that these compounds would affect the surrounding grains, even at very low concentration (Herrera et al., 2015). The 1-octen-3-ol was selected for the evaluation in this study because this is the first compound formed by fungal LOX activity (10-LOX) (Brash, 1999).

After the incubation period, the exposed maize kernels (compartment B) were used to study their effects on the dietary behavioral of *S. zeamais,* by testing the susceptibility of these kernels to insect attack and in the attraction-repellency tests (see sections 2.4 and 2.5). The kernels exposed to pure compounds were also used for volatile determination of these compounds (see section 2.6).

2.4. Repellent/attraction activity bioassay

To determine the effect of conditioned kernels on the behavior of *S.zeamais,* a Repellent/Attraction Activity bioassay was performed using a two-choice olfactometer, according to Herrera et al. (2015). This test allows the effect of conditioning related to the choice of *S. zeamais* between conditioned kernels and controls to be observed. Briefly, two flasks (250 ml) were connected by a glass tube of 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, maize samples were added to the flasks. Then, twenty insects, deprived of food for at least 24 h were placed in the hole of the glass tube, which were then released and tested for 2 h in a climatic chamber, with the experiments being carried out between 10:00 and 16:00 h and the response index (RI) calculated. The position of the flasks was changed at every replication. Insects were given a choice between the conditioned maize and control (treatments are listed in Table 2), and the experiments were performed five times for each assay, with each group of insect only being used once. For each trial, the RI was calculated by using the equation (1) RI = [(T-C)/Tot] x 100, where T is number of insects responding to the treatment, C is the number of insects responding to the control, and Tot is the total number of insects released. (Phillips et al., 1993). Insects that did not show any response in the experiment were not taken into account. Positive values of RI indicate attraction to the treatment, while negative ones indicate repellence.

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

To determine the susceptibility of conditioned kernels to insect attack, the grain damage and loss weight was measured. This experiment was carried out as above using a two-choice olfactometer bioassay (see section 2.4), but for this test the experiment was run for 20 days, after which, kernels of both flasks were weighed on an analytical scale and the number of damaged kernels and dead insects and the RI were determined.