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Effect of fungal volatile organic compounds on a fungus and an insect that damage stored maize

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ABSTRACT

The silo is an environment where a large number of biological interactions take place such as: insect-microorganism-grain interactions, which can generate great economic losses due to the deterioration in quality of the grain and the presence of mycotoxins. In recent years, particular interest has been focused on the search for environmentally friendly insecticides that will provide pest control in stored grains. The volatile organic compounds (VOCs), of a fungal origin, were evaluated for the control of maize grain pests: the insect *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), the fungus *Fusarium verticillioides* and its mycotoxin, fumonisin B_1 (FB_1). The most active fumigant compound tested was 1-octen-3-ol (LD_{50} = 27.7 μL/L air), followed by 3-octanol and 3-octanone (LD_{50} = 43.2 and 219.7 μL/L air, respectively). The fungal VOCs also showed repellent activity against *S. zeamais*, with antifugal activity against *F. verticillioides* growth being inhibited at concentrations greater than 0.53 mM, while its mycotoxin production capacity was inhibited depending on the compound concentration. At the repellent concentration, the fungal VOCs showed low phytotoxicity activities. The results presented in this paper demonstrate the potential of fungal VOCs as biopesticides, because they may control granivorous insects, fungal growth and FB_1 production, which consequently is of economic importance and might improve food safety of stored grains.

1. Introduction

The environment of a grain silo includes numerous interactions, such as grain-fungal, grain-insect and insect-fungal (Cox and Collins, 2001; Cox, 2004), which can generate great economic losses due to deterioration in quality of the grain and the presence of mycotoxins. Among the main pests found in stored maize grains in Argentina are the insect *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and the filamentous fungus *Fusarium verticillioides* (Sacc.) Nirenberg (=*Fusarium moniliforme* Sheldon teleomorph *G. Fujikuroi* (Sawada) Ito in Ito & Kimura) (Bartosik, 2014; Chulze et al., 2000).

F. verticillioides is the main cause of maize ear rot in Argentina (Chulze et al., 2000) and is the major producer of the mycotoxin called fumonisin. This mycotoxin represents a major problem due to its toxicological implications in humans and farm animals (Theumer et al., 2010 and references therein). The fumonisins are subdivided into the groups FA_1, FA_2, FB_1, FB_2 and FB_3 (Abodo-Becognee et al., 1998; Zhang et al., 2013), with those of group B being the ones of most importance owing to their toxicity and the frequency that they appear in nature, especially fumonisin B_1 (FB_1) (Rheeder et al., 2002). Maize infection by *F. verticillioides* occurs mainly via grain stigmata or grain wounds, which at all stages of its development causes maize ear rot in the pre- and post-harvest stages (Munkvold and Desjardins, 1997; Martinez et al., 2010). However, the highest production of fumonisin takes place during grain storage, when the temperature, humidity and the presence of insect vectors such as *S. zeamais* favor fungal growth and secondary metabolite production (Chulze, 2010).

Sitophilus zeamais is an insect that causes extensive damage among stored grains, particularly maize (Trematerra et al., 2013). Although the coats of the grains form a defensive barrier against microbial action, the damaging action of *S. zeamais* on the maize grain coats can lead to fungal infection and subsequent mycotoxin production. The systematic and intensive use of synthetic

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insecticides, particularly when used recommended treatment concentrations, has caused the development of a resistent insect population (Boyer et al., 2012; references therein; Corrêa et al., 2011; Guerra Pimentel et al., 2008). Therefore, in recent years, increasing interest has been focused on the search for new insecticides and/or environmentally friendly strategies that can provide effective pest control in the stored grains (Abebe et al., 2009; Hardin et al., 2010). This has been the starting point of many studies that have used natural products such as essential oils as potential pesticides (Zunino et al., 2012).

It has been proposed that the biological interactions generated during the storage of grains, and among grains, insects and fungi, are mediated by volatile organic compounds (VOCs) (Germinara et al., 2008; Trematerra et al., 2013 and references therein). These studies have revealed the importance for an organism to be able to recognize the chemical signals from the environment that surrounds it, because an incorrect identification could result in poor nutrition, in its intoxication, or it being the target of a predator. Certain VOCs (oxylipins) are originated mainly from the oxidation of unsaturated fatty acids by the lipoxygenase (LOX) enzyme. Related to this, the plant LOX was reported to oxidize linoleic acid to yield 13-hydroperoxide or 9-hydroperoxide and their related hexanal and (3Z)-nonenal, compounds, respectively (Combet et al., 2006). Mushrooms and fungi can also generate VOCs by fungal LOX activity (10-LOX), which catalyzes the stereospecific oxidation of linoleic fatty acid to (8E, 12Z, 10S)-hydroperoxy-8,12-octadecadienoic acid and is finally decomposed to (8E)-10-oxo-8-decenoic acid and 1-octen-3-ol, and their derivatives, 3-octanol, 1-octen-3-one and 3-octanone (Buśko et al., 2010; Combet et al., 2006; Husson et al., 2001).

The extensive biological activity against different organisms demonstrated by the fungal VOCs has been used to try to explain the biological interactions that occur inside the silo. It has been reported that 1-octen-3-ol compound inhibits the growth of *Botrytis cinerea* Pers.:Fr. [teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel], *Fusarium oxysporum* Schlechtend.:Fr. (Zhao et al., 2011) and *Aspergillus flavus Link* (Cleveland et al., 2009), as well as the conidia germination of *A. flavus* and *Penicillium* species (Chitarra et al., 2005; Cleveland et al., 2009). Although, the compounds 1-octen-3-ol and octanol have no apparent effect on mycotoxin synthesis (Cleveland et al., 2009), 1-octen-3-ol has shown insecticidal activity against *Tribolium castaneum* (Zhao et al., 2011), a pest in the food industry, and has also demonstrated attractant activity on several flies and beetles (Faldt et al., 1999). This broad spectrum of activities demonstrates the potential of these compounds for use in the preservation of foods, such as in stored grains in silos. In the present work, we have selected for our experiments the compounds 1-octen-3-ol, 3-octanol and 3-octanone, as they represent over 70% of the fungal VOCs produced by *Aspergillus, Penicillium, Fusarium* and *Alternaria* species (Combet et al., 2006; Jelén and Wasowicz, 1998).

One potential function of fungal VOCs in the interactions between fungus, insect and grain that occur in stored grains is discussed in this manuscript. Another objective of this investigation was to determine the insecticidal, repellent and acetylcholinesterase (AChE) activities of these VOCs against *S. zeamais,* as well as the inhibition of growth and fumonisin production by *F. verticillioides*, in order to establish a basis from new biopesticides.

2. Materials and methods

2.1. Organisms

Sitophilus zeamais were reared on sterilized whole maize grain in sealed containers. Insect rearing was carried out under controlled temperature and humidity (28 °C and 60–70%) and a light/dark regime of 12:12 (FAO, 1974). Adults of a strain of *S. zeamais* were obtained from Metán, Salta province, Argentina. The colony was maintained in our laboratory for one year without exposure to insecticides before testing. The unsexed adult weevils used in all the experiments were approximately 2 weeks old. All experiments were conducted under complete darkness in a controlled environment chamber (28 °C and 60–70% r.h.).

The fungal strain *F. verticillioides* M3125, provided by Dr. Robert Proctor, United States Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research, Peoria, IL, United States, was used for all experiments. It was isolated from maize in California and is a fumonisin-producing strain (Leslie et al., 1992).

As inoculum in the antifungal test, a conidia suspension (1×10^6 conidia/ml) was prepared adding sterile distilled water to a culture of *F. verticillioides* M 3125 grown in Czapek-dox agar Petri plates for 7 days at 28 °C in the dark.

2.2. Chemicals

The VOCs selected for use in the current work were: 1-octen-3-ol (98%) (Cat. N° 05284, Aldrich); 3-Octanone (98%) (Cat. N° 136913, Aldrich) and 3-octanol (98%) (Cat. N° W358118, Aldrich). The dichlorvos (DDVP, technical grade, >98% purity) was purchased from Chemotécnica S.A. (Buenos Aires, Argentina).

2.3. Fumigation toxicity assay

The insecticidal activity against *S. zeamais* was evaluated using fumigant toxicity assay described by Huang et al. (2000), with some modifications. Briefly, different amounts of pure VOCs at doses corresponding to 20–600 μL/L air were placed onto Whatman filter paper disks of 2 cm diameter. Only the lowest concentrations were diluted in n-hexane, and in these cases each filter paper disk was air dried for 2 min and placed on the underside of the screw cap of a glass vial (30 mL). To avoid direct contact of the weevils with VOCs, a nylon gauze piece was fitted 1 cm under the screw cap of each glass vial. Ten adult *S. zeamais* were placed into each vial, with the experiment being repeated five times/dose. Control treatments were performed under the same conditions with dichlorvos compound 0.06–20 μL/L air (positive control), or without pure compounds (negative control). Dichlorvos was used as a positive control due to their high vapor pressure and their known insecticide activity. Insect mortality was checked after 24 h, and the mortality percentages and LD_{50} values were calculated.

2.4. Anti-acetylcholinesterase test

Untreated *S. zeamais* adults (0.5 g) were separately homogenized in 5 ml of 0.1 M ice-cold phosphate buffer (pH 7.4) using a Teflon glass tissue homogenizer. The homogenates were centrifuged (5000 rpm for 20 min at 0 °C), and the supernatants used as the enzyme source for determination of AChE activity. Inhibition of AChE was determined by the colorimetric method of Ellman et al. (1961) using acetylthiocholine iodide (ATChI) at 0.25 mM (Sigma Aldrich Co., St. Louis, MO USA) as the substrate. Enzyme aliquots (100 μL) and 5,5-dithio-bis (2-nitrobenzoic) acid (DTNB) (100 μL of 0.01 M) were added to 0.1 M phosphate buffer (pH 7,4; 600 μL), and volatile compound test solutions (100 μL) prepared in absolute ethanol were added to this mixture. Control treatments were prepared by the addition of absolute ethanol (100 μL) instead of a volatile compound. These mixtures were incubated at 35 °C for 15 min, and the reactions were started by adding ATChI (100 μL). Absorbance was measured at 412 nm using a UV/VIS Spectrometer