View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by CONICET Digital

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Subscriber access provided by VICTORIA UNIVERSITY

Article

Effect of select volatiles on two stored pests: the fungus Fusarium verticillioides and the maize weevil Sithophilus zeamais.

María Paula Zunino, Jimena María Herrera, Romina Paola Pizzolitto,
Hector Rubinstein, Julio Alberto Zygadlo, and Dambolena José

J. Agric. Food Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jafc.5b02315 • Publication Date (Web): 10 Aug 2015

Downloaded from http://pubs.acs.org on August 18, 2015

Just Accepted

“Just Accepted” manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides “Just Accepted” as a free service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. “Just Accepted” manuscripts appear in full in PDF format accompanied by an HTML abstract. “Just Accepted” manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are accessible to all readers and citable by the Digital Object Identifier (DOI®). “Just Accepted” is an optional service offered to authors. Therefore, the “Just Accepted” Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the “Just Accepted” Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these “Just Accepted” manuscripts.

ACS Publications

Journal of Agricultural and Food Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Published by American Chemical Society. Copyright © American Chemical Society. However, no copyright claim is made to original U.S. Government works, or works produced by employees of any Commonwealth realm Crown government in the course of their duties.

Effect Of Selected Volatiles On Two Stored Pests: The Fungus *Fusarium verticillioides* And The Maize Weevil *Sithophilus zeamais.*

María P. Zunino[1,2], Jimena M. Herrera[1,2], Romina P. Pizzolitto[1,2], Héctor R. Rubinstein[3], Julio A. Zygadlo*[1,2], José S.Dambolena[1,2].

[1] Instituto Multidisciplinario de Biología Vegetal (IMBiV-CONICET), Universidad Nacional de Córdoba - ICTA, Avenida Vélez Sarsfield 1611, X5016GCA, Córdoba, Argentina.

[2] Instituto de Ciencia y Tecnología de los Alimentos (ICTA), FCEFyN –UNC, Avenida Vélez Sarsfield 1611, X5016GCA, Córdoba, Argentina.

[3] CIBICI (CONICET), Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, X5016GCA, Córdoba, Argentina.

* Corresponding author. Instituto Multidisciplinario de Biología Vegetal (IMBiV-CONICET), Universidad Nacional de Córdoba - ICTA, Avenida Vélez Sarsfield 1611, X5016GCA, Córdoba, Argentina. Phone/fax: +54 0351 4334141.e-mail: jzygadlo@efn.uncor.edu (Julio A. Zygadlo).

ABSTRACT

New agronomic practices and technology enabled Argentina a larger production of cereal grains, reaching a harvest yielded of 26.5 million metric tons of maize, of which, about 40% was exported. However, much of the maize production is lost annually by the attack of fungi and insects (2.6 million tons). In the study, the antifungal effect of selected volatiles on *Fusarium verticillioides,* its mycotoxin production, and repellent and insecticidal activities against weevill *S. zeamais,* insect vector of *F. verticillioides,* were evaluated. Compounds tested were (2E)-2-hexenal, (2E)-2-nonenal, (2E,6Z)-2,6-nonadienal, 1-pentanol, 1-hexanol, 1-butanol, 3-methyl-1-butanol, pentanal, 2-decanone and 3-decanone, which occur in the blend of volatile compounds emitted by various cereal grains. The most active antifungal were the aldehydes (2E)-2-nonenal, (2E)-2-hexenal and (2E,6Z)-2,6-nonadienal [Minimum Inhibitory Concentration (MIC) values of < 0.03mM, 0.06mM and 0.06mM, respectively]. The fumonisin B_1 (FB_1) occurrence also was prevented because these compounds completely inhibited the fungal growth. The best insecticidal fumigant activities against maize weevil were shown by 2-decanone and 3-decanone [Lethal Concentration (LC_{50}) $\leq$ 54.6 µl/L (<0.28 mM)]. Although, all tested compounds showed repellent activity against *S. zeamais* at a concentration of 4 µl/L, the (2E,6Z)-2,6-nonadienal was the most active repellent compound. These results demonstrate the potential of (2E,6Z)-2,6-nonadienal to be used as a natural alternative to synthetic pesticides on *F. verticillioides* and *S. zeamais*.

KEYWORDS: *Fusarium verticillioides* - Fumonisin B_1 - Volatile organic compounds – Kernels - *Sitophilus zeamais.*

INTRODUCTION

New agronomic practices and technology enabled Argentina a larger production of cereal grains, reaching a harvest yielded of 26.5 million metric tons of maize, of which, about 40% was exported.[1] However, much of the maize production is lost annually by the attack of fungi and insects (2.6 million tons).[2-4] *Fusarium verticillioides* (Sacc.) Niremberg (e.g. *F. moniliforme* Sheldon) is one of the most frequent fungal pathogens associated with maize worldwide. In addition, some isolates of this species are able to produce the mycotoxin fumonisin B_1 (FB_1) on the maize in the field and/or during the storage, that represents a considerable problem due to their immunotoxic, neurotoxic, hepatotoxic, nephrotoxic, and carcinogenic effects on animals.[5,6] The contamination of maize by *F. verticillioides* and fumonisin can occur in pre and post-harvest stages.[7, 8] However, fumonisin is mostly produced during grain storage, when the temperature, humidity and the presence of such as *S. zeamais* enable production of secondary metabolites by the fungus.

As a primary pest of stored maize, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) contributes to the dispersal of fungal spores [9-11] and through feeding damage provides entry points for fungal infections.[12]

Synthetic pesticides are used to preserve maize grains from deterioration by stored pests. However, the development of resistant populations of fungi [13] and insects,[14-16] problems in the human health and other negative effects on the environment [12] have generated considerable interest in the preservation of grains by the use of natural compounds.[17] In recent years, semiochemicals have been of increasing interest in the search for natural control of stored grain pests.[18,19] Many volatile organic compounds (VOCs) emanating from kernels and seeds (e.g. maize, soybeans, barley, wheat) [19-23] are lipoxygenase (LOX)-derived products, affect both fungal growth [24] and the behavior of fungi-vectoring insects.[25] The antifungal effects of VOCs against *Aspergillus carbonarius*, *Fusarium proliferatum* and *Aspergillus flavus*, and the antimycotoxin activity against *Aspergillus* spp. have been previously reported.[26, 27] Nevertheless, to our knowledge, only the VOC (2E)-2-hexenal has been tested for its effect on *F. verticillioides* growth and FB_1 biosynthesis.[28] On the other hand, the insecticidal activity of the VOC components, alkyl ketones [29] and C6- and C9-aldehydes [30] against *Tribolium castaneum, Rhyzopertha dominica, Sitophilus granarius, Sitophilus oryzae* and *Cryptolestes ferrugineus* have been reported. However, no insecticidal studies against the main pest of stored maize, *S. zeamais*,[14] have yet been performed. The aim of this investigation was to determine the antifungal effect of ten recognized VOCs from cereal kernels on *F. verticillioides*, its mycotoxin production, and the insecticidal effects against its insect vector *S. zeamais*.

MATERIALS AND METHODS

Chemicals

The chemicals (2E)-2-hexenal (w256005, purity >95%), (2E)-2-nonenal (255653, 97%), (2E,6Z)-2,6-nonadienal (w337706, >96%), 1-pentanol (76929, >99%), 1-hexanol (471402, >99%), 1-butanol (281549, >99%), 3-methyl-1-butanol (309435, >99%), pentanal (w309818, >97%), 2-decanone (w510637, >98%), 3-decanone (268194, 98%) and propionic acid (101362192, 99.5%) were purchased from Sigma-Aldrich (Buenos Aires, Argentina). DDVP (dicholrvos, positive control, technical grade, >98 % purity) was purchased from Chemotécnica S.A (Buenos Aires, Argentina).

Fungal strain

An isolate of *Fusarium verticillioides* (Sacc) Niremberg (= *F. moniliforme* Sheldon teleomorph *G. fujikuroi* (Sawada) Ito in Ito & Kimura [31] strain 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. This fumonisin-producing strain was isolated from maize in California.[32]

Inoculum preparation

F. verticillioides M3125 was grown in Czapek-dox agar Petri plates for 7 days at 28 ºC in the dark, to allow profuse sporulation. Then, sterile distilled water was added to each plate and a conidia suspension was obtained by scraping the colony surface with a sterile Drigalsky spatula, which was then filtered through a cheesecloth. The conidial concentration (1×10^{6} conidia/mL) was standardized using a haemocytometer.

Insects

Sitophilus zeamais (Motschulsky) were reared on sterilized whole maize grain in sealed containers. Insects were reared under controlled temperature and humidity (28 ºC and 60% - 70%) and a light/dark regime of 12:12.[33] 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. The male and female weevils used in all the experiments being approximately 2 weeks old. All experiments were conducted in complete darkness in a climatic chamber (28 °C and 60 -70% RH).

Effect of volatile organic compounds on fungal growth and fumonisin production

The antifungal activity of the VOCs was tested determining the radial growth of the fungal colony following a methodology proposed by Neri *et al.*[34] Briefly, a paper filter was placed on the inside cover of the maize meal extract agar (3%) Petri dish. The VOCs were added separately to 90-mm paper filter as pure liquid compounds, and the concentrations (0.03; 0.06; 0.13; 0.27; 0.53; 1.06; 2.12 and 4.24 mM) were expressed as 10^{-3} mol on filter paper per dish volume. A paper filter without VOCs was used as control. Then, 10 µL of a conidial suspension (1×10^{6} conidia/mL) of *F. verticillioides* M3125 was added aseptically to the centre of the Petri dishes. The maize meal extract Agar (3%) Petri dishes were then covered, wrapped in parafilm and incubated in the dark at 28°C. The colony diameter of *F. verticillioides* was measured after 7 days of incubation, and the colony area calculated using the formula for the area of a circle ($\pi * r^2$). Minimum inhibitory concentration (MIC) was defined as the lowest concentration of the VOCs at which no fungal growth was observed. To study the effects of the VOCs on FB_1 production, the inoculated plates were incubated in the dark at 28°C for 28 days. After this incubation, the parafilm and filter papers were removed and agar in the experimental plates was dried for 96 h at 60°C in a forced-air oven before being ground to a fine dry powder. Finally, 5 mL of water was added to the dried agar from each disk, and FB_1 was extracted by shaking the dried dishes with water for 120 min on an orbital shaker, with the mixture then being centrifuged at 5000 rpm for 15 min. The experiments were repeated two times in triplicate.[35]

Fumonisin B_1 quantitation

The quantitation of the samples was performed following a methodology proposed by Shephard *et al.*[36] Briefly, samples (1000 µL) from the FB_1 extracts were diluted with acetonitrile: water (1:1), and then an aliquot (50 µL) was derivatized prior to injection;

during 3.5 min with 200 μL of a solution, which was prepared by adding 5 ml of 0.1 M sodium tetraborate and 50 μL of 2-mercaptoethanol to 1 mL of methanol containing 40 mg of o-phthaldialdehyde. Derivatized samples were analyzed using Perkin Elmer HPLC equipped with a fluorescence detector, with the wavelengths used for excitation and emission being 335 nm and 440 nm, respectively, and with an analytical reverse phase C18 column (150 mm × 4.6 mm internal diameter and 5 μm particle size) connected to a precolumn C_{18} (20 mm × 4.6 mm and 5 μm particle size). For the mobile phase, methanol and NaH_2PO_4 0.1 M (75:25) were used, with the pH being set at 3.35 ± 0.2 with orthophosphoric acid and a flow rate of 1.5 mL/min. The quantitation of FB_1 was carried out by comparing the peak areas obtained from samples with those corresponding to the analytical standards of FB_1 (PROMEC, Program on mycotoxins and experimental carcinogenesis, Tygerberg, Republic of South Africa).

Insecticidal assay

Insecticidal effect on *S. zeamais* was tested using fumigant toxicity assay described by Huang *et al*.,[37] with some modifications. Briefly, different amounts of pure VOCs at concentrations 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 30s and placed on the underside of the screw cap of a glass vial (30 mL). Ten adult *S. zeamais* were placed into each vial, a nylon gauze piece was fitted 1cm under the screw cap of each glass vial, to avoid direct contact of the weevils with VOCs. The experiment was performed five replicates in two times per concentration, and control treatments were kept under same conditions without pure compounds. DDVP was used as a positive control due to its high vapor pressure and known insecticide activity. Insect mortality was checked after 24 h, with the mortality percentages and LC_{50} values being calculated according to Finney.[38]

Repellent/Attraction activity bioassay

The behavioral response of *S. zeamais* adults to individual VOCs was tested in two-choice olfactometer bioassay described by Herrera et al.[35] Briefly, two flasks (250 mL) were connected with a glass tube of 30 x 1 cm of diameter. In the middle (15 cm from the two flasks), a small hole was made of 1 x 1 cm. The connections between the two flasks and the tube were sealed with rubber plugs, which were covered with parafilm to prevent gas leakage. A filter paper of 2 cm diameter was placed within each flask where the compounds were added. Twenty insects, deprived of food for at least 4 h, were placed in the hole of the glass tube. These were then released and tested for 2 h in a climatic chamber, the experiments being carried out between 10:00 and 16:00 hours. The position of the flasks was changed at every replication, and insects that did not show any response in the experiment were not used to calculate response index. Insects were given a choice between a specific dose of the test compound and the solvent (n-hexane) used as a control. The experiments were performed five times for each assay, with insects only being used once. For each experiment, an independency control (without any compound) showed that the movement of the beetles towards either flask was random (RI= -2.1 ± 7.5). Propionic acid was used as positive control for repellent.[39]

In each trial, a response index (RI) was calculated by using the equation $RI = [(T-C)/Tot]\times100$, where T is number of insects responding to the treatment, C is number of insects responding to the control, and Tot is the total number of insects released.[40] Positive values of RI indicate attraction to the treatment, while negative ones indicate repellence.

Statistical analysis

Data were analyzed using InfoStat/Professional 2010p. [41] at $p = 0.05$. Randomized complete block design (RCBD) was used to the experimental designs and a one-way analysis of variance (ANOVA) to study the experimental data. The Shapiro-Wilk test was utilized to test the normality of the experimental data, and comparisons between treatments were carried out using the Duncan test. Experimental data without a normal distribution were statistically analyzed by the Kruskal-Wallis non-parametric test (at $p<0.05$). The pairwise comparison was used to compare means among treatment ranges. The lethal concentrations (LC_{50} and LC_{95}) were calculated from dose-mortality values, using probit regression analysis by POLO-PLUS Software.[42] The significance of the mean RI in each treatment of the two-choice olfactometer bioassay was evaluated by the Student's t-test for paired comparisons.[40] The chemical properties lipophilicity (Log P: Logarithm of the octanol/water partition coefficient) and vapour pressure, of the VOCs compounds, were obtained from ChemSpider database.[43]

RESULTS

Antifungal and antimicotoxicogenic activities

The inhibitory effects mediated by the VOCs on *F. verticillioides* growth was dose-dependent, with the most active compounds being the aldehydes: (2E)-2-nonenal, (2E)-2-hexenal, (2E, 6Z)-2,6-nonadienal and pentanal, which exhibited MIC values of <0.03 mM, 0.06 mM, 0.06 mM and 0.53 mM, respectively (Table 1). Of the alcohols tested, 1-hexanol revealed the highest activity, while of the alkyl ketones, 3-decanone had a greater inhibitory effect on fungal growth than 2-decanone, at several concentration. Treatments such as (2E)-2-hexenal (0.06mM), 1-pentanol (4.24mM), 1-hexanol (2.12 and 4.24 mM), pentanal (0.53 and 1.06 mM), 2-decanone (4.24 mM) and 3-decanone (2.12 and 4.24mM) all caused a delay in the fungal growth, with no growth being observed on the seventh day. However, on the 28th day post-inoculation fungal growth was apparent and the FB_1 concentration was determined**.** On the other hand, 1-pentanol showed a slight stimulatory effect on fungal growth at lower concentrations. The VOC effects on FB_1 production are presented in Table 2, where it can be observed that (2E)-2-hexenal, (2E)-2-nonenal and (2E, 6Z)-2,6-nonadienal caused a total inhibition of mycelium growth, implying an absence of FB_1 production. The 1-hexanol (4.24 mM) and 1-butanol (0.53 mM and 4.24 mM) effectively inhibited fumonisin production by *F. verticillioides*.

Insecticidal and repellent/attraction effects

The results of fumigant insecticidal activity of VOCs tested on *S. zeamais* are shown in Table 3. After 24 h exposure, the most active compounds were 2- and 3-decanone, with LC_{50} values of 50.4µL/L and 54.6 µL/L, respectively. 1-hexanol, 1-pentanol, 1-butanol and (2E)-2-hexenal showed insecticide LC_{50} values between 224.1 µl/L and 306.6 µl/L, while (2E)-2-nonenal and pentanal did not show any insecticidal activity in the range of the evaluated concentrations (20 to 600 µl/L). The LC_{50} values of (2E, 6Z)-2,6-nonadienal and 3-methyl-1-butanol could not be determined because they did not show a dose-dependent relationship. However, at dose 150 µl/L the mortality was 98.0% (± 4.5) for 3-methyl-1-butanol and 28.7% (± 19.4) for (2E, 6Z)-2,6-nonadienal (data not shown).

The behavioral responses of *S. zeamais* adults to VOCs are shown in Figure 1. All the compounds showed repellent effect at 4 µl/L. Moreover, only (2E, 6Z)-2,6-nonadienal showed repellent effects at 0.05 µl/L (0.31µM), with a response index of -37.3 ± 14.0. On the other hand, 3-methyl-1-butanol and 1-butanol showed attractant effects at 0.4 µl/L.

DISCUSSION

The results obtained in the present work show the capacity of 10 natural VOCs present in the headspace volatiles of several cereal kernels [26, 27, 29, 39, 44] to affect the fungal growth of *F. verticillioides* and FB_1 production. Besides, these VOCs showed insecticidal and repellent effects against its insect vector *S. zeamais*. Our findings revealed that aldehydes had higher levels of antifungal activity than alcohols or alkyl ketones. In agreement, a previous report by Mita *et al.* [24] showed antifungal activity of C6 and C9 aldehydes against *Aspergillus carbonarius* and *Fusarium proliferatum*, with (2E)-2-nonenal being the most effective compound. In addition, other studies reported high antifungal activity of (2E)-2-hexenal, (2E)-2-nonenal and (2E,6Z)-2,6-nonadienal.[26-28, 45] Moreover, the results presented here suggest that a relationship between the antifungal activity and molecular properties, such as lipophilicity (Log P) and vapour pressure may exist in compounds with the same functional group. In the present work, the most active compound against *F. verticillioides* was (2E)-2-nonenal, which is the aldehyde with the highest lipophilic property. The relationship between Log P and antifungal activity of plant phenolic compounds against *F. verticillioides* has been previously reported by Dambolena *et al.*[46] In the present study, (2E)-2-hexenal, (2E)-2-nonenal and (2E, 6Z)-2,6-nonadienal, also prevented FB_1 production because these compounds inhibited completely the fungal growth, at the tested concentrations.

Previous investigations have reported aflatoxin B_1 being inhibited by (2E)-2-hexenal.[26, 27] However, this compound did not have any effect on FB_1 production.[28]

Kernels fed on by insects provide a favorable environment for *F. verticillioides* growth and FB_1 production,[43] and contribute to the dispersal of fungal spores. Hence, insect control could be considered a key strategy for controlling fungal growth in stored maize kernels. So, the repellent and insecticidal effects of VOCs against *S. zeamais*, an insect vector of *F. verticillioides* in stored maize, were also determined. The VOCs emitted by cereal grains are detected by the antennal sensila of the granary weevil and induce behavioral responses at different doses.[39] All the evaluated VOCs show repellent effects against *S. zeamais* at 4 µL/L, however at very low concentrations (0.4 µL/L and 0.05 µL/L) the repellent effect was only shown by (2E, 6Z)-2,6-nonadienal (one of the most antifungal compound). In agreement with our results, Germinara *et al.*[39] demonstrated a repellent effect of diolefinic aldehydes, alkyl ketones and the aliphatic alcohol 1-hexanol, and the attractive effects of butyl alcohols on *S. granarius*. 2-decanone and 3-decanone revealed strong fumigant activities against *S. zeamais*. On the other hand, the most antifungal and repellent compounds, (2E, 6Z)-2,6-nonadienal and (2E)-2-nonenal, did not show strong fumigant toxicity against *S. zeamais*, at the tested concentrations. However, Hubert *et al.* [30] reported insecticidal activity of (2E, 6Z)-2,6-nonadienal and (2E)-2-nonenal (LD_{50} ranging from 0.44 to 2.76 mg g^{-1}) against *Sitophilus granarius* and *Sitophilus oryzae*, in fumigant assays.

Summing up, our results demonstrate that the different biological activities are mainly related with the functional group of the compounds tested, with the most active antifungal and insect repellent compounds being the aldehydes, while the most insecticide compounds were the ketones. (2E, 6Z)-2,6-nonadienal demonstrated a complete inhibition of *F. verticillioides* growth and a repellent activity against its insect vector *S. zeamais,* thus preventing FB_1 occurrence, dispersion of fungal spores and broken grains. These results reveal the strong potential for this compound to be used as a natural alternative to synthetic fungicides. In addition, lethal dosis (LD_{50}) values of aldehyde VOCs show slight toxicity ($\leq$ 5g/kg) in rats.[48] On the other hand, other evaluated VOCs showed a potential capacity to be used as a natural insecticidal (ketones) or as a lure for *S. zeamais* (alcohol). The future use of VOC therefore opens up possibilities for a safer and economically viable option for the conservation of stored kernels and pest management.

ACKNOWLEDGMENTS

This work was supported by grants from the CONICET PIP 11220120100661CO, FONCYT-PICT 2012-2146 and SECyT Universidad Nacional de Cordoba. We would like to thank native speaker, Paul Hobson, for revision of the manuscript, and Dra. Marcela Palacio for technical support. MPZ, RPP, JSD and JAZ are Career Members of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and JMH has a fellowship from CONICET. The authors thank Robert H Proctor for supplying a sample of *F. verticillioides* M3125 from the United States Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research, Peoria, IL, USA.

REFERENCES

1. SIIA. Sistema Integrado de Información Agropecuaria. Ministerio de Agricultura, Ganaderia y Pesca. http://old.siia.gov.ar/index.php/series-por-tema/usda. Accessed 28 Febrary 2014.

2. Oerke, E. C. Crop losses to pest. *Journal of Agricultural Science.* **2006**, 144, 31-43.

3. Aulakh, J.; Regmi, A. Improving methods for estimating post harvest losses/Final PHLs Estimation. http://www.fao.org/ fileadmin/templates /ess /documents/meetings and_workshops/GS_SAC_2013/ _6-13-13.pdf.

4. Abass, A.B.; Ndunguru, G.; Mamiro, P.; Alenkhe, B.; Mlingi, N.; Bekunda, M. Post-harvest food losses in a maize-based farming system of semi-arid savannah area of Tanzania. *J. Stored Prod. Res.* **2014**, *57*, 49-57.

5. Stockmann-Juvala, H.; Savolainen, K. A review of the toxic effects and mechanisms of action of fumonisin B_1. *Hum. Exp. Toxicol.* **2008,** *27*, 799–809.

6. Theumer, M.G.; Canepa, M.C.; Lopez, A.G.; Mary, V.S.; Dambolena, J.S.; Rubinstein, H.R. Subchronic mycotoxicoses in Wistar rats: Assessment of the in vivo and in vitro genotoxicity induced by fumonisins and aflatoxin B_1, and oxidative stress biomarkers status. *Toxicol.* **2010,** *268*, 104 -110

7. Munkvold, G.P.; Desjardins, A.E. Fumonisins in maize: can we reduce their occurrence? *Plant. Dis.* **1997**, *81*, 556–565.

8. Martinez, M.; Moschini, R.; Barreto, D.; Bodega, J.; Comerio, R.; Forjan, H.; Piatti, F.; Presello, D.A.; Valentinuz, O. Environmental factors that affect the fumonisin content in maize grain. *Trop. Plant. Pathol.* **2010***, 35***,** 277-284.

9. Lamboni, Y.; Hell, K. Propagation of mycotoxigenic fungi in maize stores by post-harvest insects. *Int. J. Trop. Insect Sci.* **2009,** *29***,** 31–39.

10. Nesci, A.; Barra, P.; Etcheverry, M. Integrated management of insect vectors of *Aspergillus flavus* in stored maize, using synthetic antioxidants and natural phytochemicals. *J. Stored Prod. Res.* **2011**, *47*, 231-237.

11. Ferreira-Castro, F.L.; Potenza, M.R.; Rocha, L.O.; Correa, B. Interaction between toxigenic fungi and weevils in corn grain samples. *Food Control.* **2012**, *26*, 594-600.

12. Boyer, S.; Zhang, H.; Lemperiere, G. A review of control methods and resistance mechanisms in stored-product insects. *B. Entomol. Res.* **2012**, *102*, 213-229.

13. Brent, K. J.; Hollomon, D. W. Fungicide resistance: the assessment of risk. *FRAC Global Crop Prot. Fed. Brussels*. **1998**, 2, 1-48.

14. Pimentel , M.A.G.; Faroni, L.R.D.A.; Guedes, R.N.C.; Sousa, A.H.; Tótola, M.R. Phosphine resistance in Brazilian populations of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) *J. Stored Prod. Res*. **2009,** 45, 71–74.

15. Silva, L.B.; Reis, A.P.; Pereira, E.J.G.; Oliveira, M.G.A.; Guedes, R.N.C. Altered cysteine proteinase activity in insecticide-resistant strains of the maize weevil: Purification and characterization. *Comp. Biochem. Phys. B.* **2010**, *157*, 80–87.

16. Correa, A.S.; Pereira, E.J.G.; Cordeiro, E.M.G.; Braga, L.S.; Guedes, R.N.C. Insecticide resistance, mixture potentiation and fitness in populations of the maize weevil (*Sitophilus zeamais*). *Crop Prot.* **2011**, *30*, 1655-1666.

17. Sofos, J.N.; Beuchat, L.R.; Davidson, P.M.; Johnson, E.A. Naturally occurring antimicrobials in food, an interpretive summary. *Regul. Toxicol. Pharm.* **1998**, *28*, 71-72.

18. Cook, S.P. Beetle (Coleoptera) diversity in mixed pine-hardwood stands in the Ouachita highlands five years following tree harvests. *Southwest Entomol.* **2002**, *27*, 269-276.

19. Ukeh, D.A.; Woodcock, C.M.; Pickett, J.A.; Birkett, M.A. Identification of Host Kairomones from Maize, Zea mays, for the Maize Weevil, *Sitophilus zeamais*. *J. Chem. Ecol.* **2012**, *38***,** 1402–1409.

20**.** Boue, S. M.; Shih, B. Y.; Carter-Wientjes, C. H.; Cleveland,T. E. Effect of Soybean Lipoxygenase on Volatile Generation and Inhibition of *Aspergillus flavus* Mycelial Growth. *J. Agric. Food Chem*. **2005**, *53*, 4778-4783.

21. Cramer, A.C.J.; Scott Mattinson, D.; Fellman, J.K.; Baik, B.K. Analysis of Volatile Compounds from Various Types of Barley-Cultivars. *J. Agric. Food Chem*. **2005**, *53*, 7526-7531.

22. Germinara, G.S.; De Cristofaro, A.; Rotundo, G. Antennal olfactory responses to individual cereal volatiles in *Theocolax elegans* (Westwood) (Hymenoptera: Pteromalidae) *J. Stored Prod. Res*. **2009,** *45*, 195–200.

23. Mosblech, A.; Feussner,I.; Heilmann, I. Oxylipins: Structurally diverse metabolites from fatty acid oxidation. *Plant Physiol. Bioch.* **2009**.47,511–517.

24. Mita, G.; Fasano, P.; De Domenico, S.; Perrone, G.; Epifani, F.; Iannacone, R.; Casey, R.; Santino, A. 9-Lipoxygenase metabolism is involved in the almond/*Aspergillus carbonarius* interaction*. J. Exp. Bot.* **2007**, *58***,** 1803–1811.

25. Cox, P.D.; Collins, L.E. Factors affecting the behaviour of beetle pests in stored grain, with particular reference to the development of lures. *J. Stored Prod. Res.* **2002**, *38*, 95–115.

26. Cleveland, T.E.; Carter-Wientjes, C.H.; De Lucca, A.J.; Bou, S. M. Effect of soybean volatile compounds on *Aspergillus flavus* growth and aflatoxin production. *J. Food Sci.* **2009**, *74***,** 83-87.

27. De Lucca, A.J.; Carter-Wientjes, C.H.; Boue, S.; Bhatnagar, D. Volatile trans-2-hexenal, a soybean aldehyde, inhibits *Aspergillus flavus* growth and aflatoxin production in corn. *J. Food Sci.* **2011**, *76*, 381-386.

28. Menniti, A.M.; Gregori, R.; Neri, F. Activity of natural compounds on *Fusarium verticillioides* and fumonisin production in stored maize kernels. *Int. J. Food Microbiol.* **2010**, *136*, 304–309.

29. Germinara, G.S.; De Cristofaro, A.; Rotundo, G. Bioactivity of short-chain aliphatic ketones against adults of the granary weevil, *Sitophilus granarius* (L.). *Pest Manag. Sci.* **2012**, *68*, 371–377.

30. Hubert, J.; Munzbergova, Z.; Santino, A. Plant volatile aldehydes as natural insecticides against stored-product beetles. *Pest Manag. Sci.* **2008**, *64*, 57-64.

31. Leslie, J.; Summerel, B.A. Species concepts in *Fusarium*. Chapter 9. In *The Fusarium Laboratory Manual*, Leslie, J., Summerel, B.A., Eds.; Blackwell Publishing: USA, 2006; pp 88-95.

32. Leslie, J.; Plattner, R.; Desjardins, A.; Klittich, C. Fumonisin B_1 production by strains from different mating populations of *Gibberella fujikuroi* (*Fusarium* sectionLiseola). *Phytopathol.* **1992**, *82*, 341-345.

33. FAO. Boletín Fitosanitario de la FAO. Método provisional para gorgojos adultos importantes en cereals almacenados con malatión o lindano. *Método N°15 de la FAO* **1974**, *22*, 127-137.

34. Neri, F.; Mari, M.; Brigati, S.; Bertolini, P. Fungicidal activity of plant volatile compounds for controlling *Monilinia laxa* in stone fruit. *Plant Dis.* **2007**, *91*, 30-35.

35. Herrera, J.M; Pizzolitto, R.P.; Zunino, M.P.; Dambolena, J.S.; Zygadlo, J.A. "Effect of fungal volatile organic compounds on a fungus and an insect that damage stored maize". *J. Stored Prod.* **2015,** *62*, 74-80.

36. Shephard, G.S.; Marasas, W.F.O.; Leggott, N.L.; Yazdanpanah, H.; Rahimian, H.; Safavi, N. Natural occurrence of fumonisins in corn from Iran. *J. Agr. Food Chem.* **2000**, *48***,** 1860-1864.

37. Huang, Y.; Lam, S.L.; Ho, SH. Bioactivities of essentialoil from *Elletaria cardamomum* (L.) Maton. to *Sitophilus zeamais* Motschulsky and Tribolium castaneum (Herbst). *J. Stored Prod. Res.* **2000**, *36***,** 107-117.

38. Finney, D.J. Probit analysis. Cambridge University Press: London, UK, 1971.

39. Germinara, G.S.; De Cristofaro, A.; Rotundo, G. Behavioral responses of adult *Sitophilus granarius* to individual cereal volatiles. *J. Chem. Ecol.* **2008**, *34***,** 523–529.

40. Phillips, T.W.; Jiang, X.L.; Burkholder, W.E.; Phillips, J.K.; Tran, H.Q. Behavioral responses to food volatiles by two species of stored-product Coleoptera, *Sitophilus oryzae* (Curculionidae) and *Tribolium castaneum* (Tenebrionidae). *J. Chem. Ecol.* **1993**, *19***,** 723-734.

41. Di Rienzo, J.; Casanoves, F.; Balzarini, M. G.; Gonzalez, L.; Tablada, M.; Robledo, C. Infostat, versión; 2010. Grupo Infostat: FCA, Universidad Nacional de Córdoba, Argentina, 2010.

42. LeOra Software. POLO-PLUS: a user's guide to probit or logit analysis. LeOra Software, Berkeley, CA, 2002-2014.

43. Chemspider. Search and share chemistry. http://www.chemspider.com/.

44. Buśko, M.; Jeleń, H.; Góral, T.; Chmielewski, J.; Stuper, K.; Szwajkowska-Michałek, L.; Tyrakowska, B.; Perkowski, J. Volatile metabolites in various cereal grains. *Food Addit. Contam.* **2010**, *27*, 1574-1581.

45. Gardini, F.; Lanciotti, R.; Guerzoni, M.E. Effect of trans-2-hexenal on the growth of *Aspergillus flavus* in relation to its concentration, temperature and water activity. *Lett. Appl. Microbiol.* **2001**, *33*, 50-55.

46. Dambolena, J.S.; López, A.G.; Meriles, J.M.; Rubinstein, H.; Zygadlo, J.A. Inhibitory effect of 10 natural phenolic compounds on *Fusarium verticillioides*. A structure-property-activity relationship study. *Food Control.* **2012**, *28*, 163-170.

47. Trematerra, P.; Ianiro, R.; Athanassiou, C.G.; Kavallieratos N.G. Behavioral responses of *Sitophilus zeamais* Motschulsky adults to conditioned grain kernels. *J. Stored Prod. Res.* **2013**, *53*, 77-81.

48. Pest Management Regulatory Agency (PMRA) label search web page. http://www.hc-sc.gc.ca/cps-spc/pest/registrant-titulaire/tools-outils/label-etiq-eng.php.

Figure captions

Figure 1. Behavioural responses of *S. zeamais* adults to VOCs.

Footnote:

*P≤0.05; **P<0.01; ***P<0.001 (significant response to experimental stimulus; paired-sample T test).
Values having different letters in the same column are significantly different from each other according to Duncan's multiple range test at P ≤ 0.05 (n=5).
(+) values of RI indicate attraction.
(-) values of RI indicate repellence.

Table 1. Antifungal activity of VOCs against *Fusarium verticillioides* M3125 in maize meal extract agar (3%) at 28°C.

Compounds	**MIC**^{A}		**Inhibition of fungal growth**^{B} (%)							
	mM	**µl/L**	**0.03 mM**	**0.06 mM**	**0.13 mM**	**0.27 mM**	**0.53 mM**	**1.06 mM**	**2.12 mM**	**4.24 mM**
(2E)-hexenal	0.06	7.8	46.4 ± 2.6^{b}	100^{*a}	100^{*a}	100^{*a}	100^{*a}	100^{*a}	100^{*a}	100^{*a}
(2E)-nonenal	<0.03	<5.6	100^{*a}	100^{*a}	100^{*a}	100^{*a}	100^{*a}	100^{*a}	100^{*a}	100^{*a}
(2E,6Z)-nonadienal	0.06	10.6	53.9 ± 3.0^{b}	100^{*a}	100^{*a}	100^{*a}	100^{*a}	100^{*a}	100^{*a}	100^{*a}
pentanal	0.53	56.2	5.7 ± 4.3^{b}	26.3 ± 6.1^{b}	39.1 ± 0.2^{b}	42.3 ± 7.5^{b}	100^{*a}	100^{*a}	100^{*a}	100^{*a}
1-pentanol	4.24	460.7	2.8 ± 2.8 ^{b}	-42.0 ± 0.2^{b}	-42.0 ± 0.1^{b}	-64.7 ± 0.1^{b}	11.4 ± 0.2^{b}	49.6 ± 13.3^{b}	81.1 ± 8.8^{b}	97.8 ± 0.9^{*a}
1-hexanol	2.12	264.0	-0.1 ± 1.5 ^{b}	18.8 ±3.8^{b}	21.3 ± 6.3^{b}	34.6 ± 2.3^{b}	54.2 ± 0.2^{b}	89.5 ± 0.5^{b}	99.8 ± 0.3^{*a}	100*
3-methyl-1-butanol	>4.24	>460.7	-9.0 ± 4.6^{b}	1.4 ± 0.2^{b}	1.4 ± 0.1^{b}	6.9 ± 5.5^{b}	21.3 ± 2.7^{b}	18.6 ± 0.1^{b}	69.7 ±5.8^{b}	73.9 ± 1.5^{b}
1-butanol	>4.24	>393.2	-0.1 ± 4.4^{b}	28.8 ± 1.2^{b}	30.0 ± 2.4^{b}	26.4 ± 1.2^{b}	31.6 ± 0.2^{b}	32.8 ± 3.7^{b}	46.6 ± 7.7^{b}	78.3 ± 1.4^{b}
2-decanone	>4.24	>797.7	0.1± 1.5^{b}	42.8 ± 5.8^{b}	3.0 ± 1.5^{b}	13.3 ± 2.8^{b}	62.3 ± 9.3^{b}	81.9 ± 7.1^{b}	92.9 ± 0.4^{*a}	93.4 ± 2.7^{*a}
3-decanone	>4.24	>797.7	-7.8 ± 3.2^{b}	42.9 ± 1.2^{b}	20.2 ± 4.1^{b}	53.8 ± 1.0^{b}	61.8 ± 0.9^{b}	91.98 ± 1.3^{*a}	95.2 ± 2.6^{*a}	96.6 ± 1.1^{*a}

Values are expressed as means ± SD. ^{A}MIC: minimum inhibitory concentration. ^{B}Inhibition of fungal growth was determined after 7 days of incubation.

(-): Indicate fungal growth stimulation.

* Indicate significant difference with the control according to Kruskal-Wallis non parametric test (H= 249.27. P < 0.0001). All pairwise comparison was used to compare the means among treatments ranges.

$^{a, b}$ Values having different letters are significantly different from each treatments. The experiments were performed twice in triplicate.

Table 2. Effects of VOCs on FB_1 production in maize meal extract agar (3%) at 28°C.

Compounds	**Inhibition of FB_1 production** (%)							
	0.03 mM	**0.06 mM**	**0.13 mM**	**0.27 mM**	**0.53 mM**	**1.06 mM**	**2.12 mM**	**4.24 mM**
(2E)-hexen*l	19.3 ± 60.7^{b}	- 5.7 ± 82.1^{b}	ND	ND	ND	ND	ND	ND
(2E)-nonenal	ND	ND	ND	ND	ND	ND	ND	ND
(2E, 6Z)-nonadienal	57.5 ± 16.4^{b}	ND	ND	ND	ND	ND	ND	ND
pentanal	- 25.2 ± 17.2^{b}	47.8 ± 21.5^{b}	- 10.2 ± 15.6^{b}	9.1 ± 15.9^{b}	65.0 ± 10.5^{b}	50.0 ± 27.7^{b}	ND	ND
1- pentanol	38.3 ± 51.7^{b}	3.4 ± 32.4^{b}	63.6 ± 214.9^{b}	- 81.9 ± 79.6^{b}	- 3.8 ± 19.2^{b}	12.5 ± 15.5^{b}	- 0.4 ± 20.0^{b}	- 110.6 ± 23.4^{b}
1-hexanol	52.9 ± 86.0^{b}	59.9 ± 31.9^{b}	40.4 ± 51.6^{b}	8.1 ± 18.9^{b}	58.2 ± 37.4^{b}	57.5 ± 20.0^{b}	59.2 ± 8.2^{b}	100.0 ± 14.13^{*a}
3-methyl-1-butanol	64.3 ± 32.8^{b}	21.1 ± 13.7^{b}	34.2 ± 10.3^{b}	58.9 ± 4.7^{b}	64.3 ±13.0^{b}	36.3 ± 15.4^{b}	39.5 ± 59.2^{b}	- 39.3 ± 24.0^{b}
1-butanol	23.3 ± 9.5^{b}	8.5 ± 19.3^{b}	29.6 ± 11.8^{b}	55.2 ± 4.8^{b}	78.1 ± 4.1^{*a}	57.6 ± 7.5^{b}	26.5 ± 15.9^{b}	73.8 ± 3.1^{*a}
2-decanone	- 71.0 ± 40.9^{b}	16.1 ± 74.6^{b}	22.3 ± 9.6^{b}	27.1 ± 12.9^{b}	38.7 ± 14.1^{b}	- 241.6 ± 64.2^{b}	- 8.2 ± 12.5^{b}	- 56.3 ± 27.3^{b}
3-decanone	25.1 ± 5.4^{b}	56.9 ± 9.3^{b}	18.8 ± 27.3^{b}	37.7 ± 8.1^{b}	38.0 ± 23.8^{b}	30.1 ± 27.0^{b}	- 85.9 ± 30.5^{b}	- 169.1 ± 79.1^{b}

Values are expressed as medians ± SE. ND: No determined. FB_1 inhibition was not determined due to there was no fungal growth(-): Indicate FB_1 stimulation.

[*] Indicate significant difference with the control according to Kruskal-Wallis non parametric test (H= 249.27. $P < 0.0001$). All pairwise comparison was used to compare the means among treatments ranges.

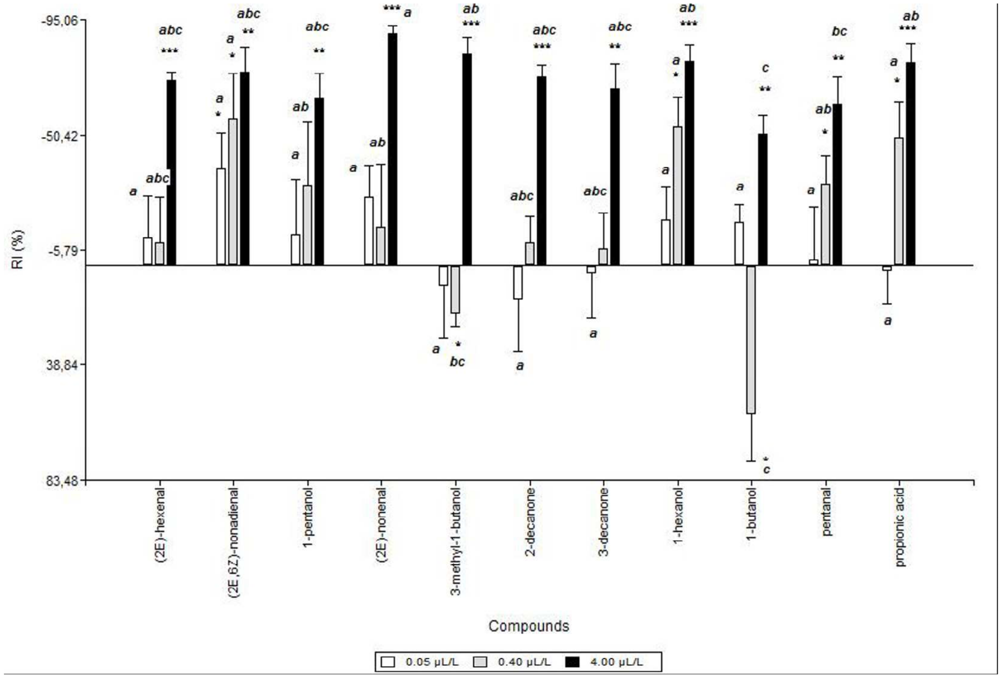
[a, b] Values having different letters are significantly different from each treatments. The experiments were performed twice in triplicate.

Table 3. Fumigant toxicity of VOCs against *S. zeamais* adults after 24 h exposure[a].

Compounds	**LC$_{50}$ (mM)**	**LC$_{50}$ (µl/L)**	**95% confidence interval (µl/L)**	**LC$_{95}$ (mM)**	**LC$_{95}$ (µl/L)**	**95% confidence interval (µl/L)**	**Slope ± SE**	**(χ^2)[b]**	**Log P[a]**	**VP (Pascal) 25°C[a]**
(2E)-2-hexenal	2.64	306.6	263.7 - 612.0	3.95	458.2	361.4 - 1678.2	5.44 ± 0.89	38.44	1.58	613.2
(2E)-2-nonenal	>3.62	>600							3.17	39.99
(2E. 6Z)-2.6-nonadienal	ND	ND							2.6	39.99
pentanal	>5.64	>600							1.44	4239.6
1-pentanol	2.49	271.2	241.8 – 321.4	3.71	403.2	343.6 - 572.5	7.32 ± 1.02	23.48	1.41	373.3
1-hexanol	1.78	224.1	199.0 - 252.6	3.44	431.6	375.6 – 531.1	2.53 ± 0.85	3.29	1.94	119.99
3-methyl-1-butanol	ND	ND							1.22	559.95
1-butanol	3.18	291.6	260.9 - 354.9	5.21	477.0	394.9 - 727.6	5.33 ± 1.08	1.49	0.88	1133.2
2-decanone	0.26	50.4	46.4 – 55.5	0.35	66.2	59.8 – 80.7	13.53 ± 1.95	16.95	3.56	26.6
3-decanone	0.28	54.6	49.9 – 59.6	0.46	86.6	78.6 – 99.2	5.76 ± 0.84	1.46	3.56	26.6
DDVP		<0.06								

ND: not determined. Each value represents the mean of five times/ concentration, each set up with 10 adults. [a]Values obtained from Chemspider 2013, Log P (Logarithm of the octanol/water partition coefficient) and VP (Vapor pressure). [b]X^2: chi-square value, significant at $P < 0.05$ level. LC: lethal concentration.

Figure 1.


RI (%)
-95,06
-50,42
-5,79
38,84
83,48
(2E)-hexenal
(2E,6Z)-nonadienal
1-pentanol
(2E)-nonenal
3-methyl-1-butanol
2-decanone
3-decanone
1-hexanol
1-butanol
pentanal
propionic acid
Compounds
0.05 µL/L
0.40 µL/L
4.00 µL/L

TOC graphic