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The Role of Fumonisins in the Biological Interaction between *Fusarium verticillioides* and *Sitophilus zeamais*

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Abstract

The aim of the current study was to investigate the entomopathogenic capacity of the mold *Fusarium verticillioides* and the effect of its mycotoxins fumonisins, on the grain beetle *Sitophilus zeamais*. We evaluated the capacity of this fungus to infect live insects, the antifungal activity of constituents of the insect's epicuticle, and the effect of a fumonisin extract on the fitness of the insects. We found that *F. verticillioides* could not penetrate the cuticle of *S. zeamais* and that the fumonisin extract had no negative effects on the fitness of the insects. However, the progeny of the insects increased, and the fumonisin extract had repellent effects. This is the first report about the effects of fumonisins on the relationship between *F. verticillioides* and *S. zeamais,* which may provide useful information about interactions between pathogenic microorganisms and insects, especially on stored product pests.

Keywords *Fusarium verticillioides* · *Sitophilus zeamais* · Fumonisins · Biological interaction · Integrated pest management

Introduction

Maize (*Zea mays*), one of the most cultivated cereals worldwide, is usually stored in grain bins until its commercialization (Mendoza et al. 2017; OECD-FAO 2018). However, when storage conditions are not optimal, a large number of biological interactions occur, which may cause great economic losses (Abass et al. 2014; Coyle et al. 2005; Cox 2004; Tefera et al. 2011). Some of the most important interactions in grain bins take place between the maize kernels, the insect *Sitophilus zeamais* (Coleoptera: Curculionidae), and the mycotoxicogenic fungus *Fusarium verticillioides* (Sacc) Nirenberg (Abebe et al. 2009; Chulze 2010; García-Lara et al. 2019). Usually, the insect-fungus-maize tritrophic interaction is studied as a dual system, focusing mainly on the insect-kernel and/or fungus-kernel relationships.

Sampietro et al. (2009) studied the relationship between *F. verticillioides* and maize kernels mediated by the kernel pericarp and its wax content, while Usseglio et al. (2018) reported on the regulatory role of chemical constituents of the maize kernel epicuticle on the relationship between the maize kernel and *S. zeamais*. Nevertheless, the relationship between *S. zeamais* and *F. verticillioides* and the role of the insect's cuticle in this interaction are still unknown. Other interactions between insects and fungi, mediated by the insect cuticle, have been widely studied. For example, Pedrini et al. (2009, 2013) investigated the relationship between the fungus *Beauveria bassiana* and *Triatoma infestans*. In their study, they found that the cuticular hydrocarbons of this insect increased the fungal virulence because of an increase in the cytochrome P450 enzyme production in the fungus, which oxidized these compounds. This may have allowed the fungus to overcome the insect's first protective barrier and start infection. In addition, several reports have associated the insecticidal effect of *B. bassiana* with beauvericin (BEA), the main mycotoxin produced by this fungus (Al Khoury et al. 2019; Arboleda Valencia et al. 2011; Genthner et al. 1994;). Although the effect of other mycotoxins on insect behavior and the entomopathogenic capacity of several fungi have been

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previously studied, to our knowledge there are no reports on the entomopathogenic capacity of *F. verticillioides* and the effects of its mycotoxins fumonisins against *S. zeamais*. Fumonisins B (FB) are the principle mycotoxins produced by *F. verticillioides*, and their ingestion is associated with the incidence of several diseases such as cancer and hepatitis (Theumer et al. 2010; World Health Organization 2018). In maize, FB represent up to 75% of the metabolites produced by *F. verticillioides,* with these mycotoxins being most commonly found in maize tissues throughout the world (Marín et al. 2004; Proctor et al. 2006; Sánchez-Rangel et al. 2012; Thompson and Raizada 2018). In mammals, the toxic effect of FB is related to the inhibition of ceramide synthetase activity and consequently, an imbalance in the cell lipid metabolism (IPCS-WHO 2000). Although this mechanism and the effect of this group of mycotoxins are unknown in invertebrates, FB may be important in some biological interactions, since they could act as a virulence factor by preparing organisms for fungal infection or by promoting its spread (Arias et al. 2016).

In this context, the aim of our investigations was to study the relationship between *F. verticillioides* and *S. zeamais* by focusing on the entomopathogenic capacity of the fungus, the effect of the insect epicuticle against *F. verticillioides,* and the role of fumonisins on insect behavior using an FB extract. A better understanding of this relationship and its chemical modulators could help achieve a more effective method for the joint management of *S. zeamais* and *F. verticillioides* during the storage of maize kernels.

Methods and Materials

Biological Material

The *Zea mays* kernels were obtained from the Manfredi Experimental Station (INTA, Córdoba, Argentina) and kept in closed containers at −20 °C. The variety used was Illinois CV:1767MG Rep 2. N° station: 222, harvested in 2014. These maize kernels contained a basal concentration of fumonisins of 30.4 ± 6.8 μg/ kg of kernels, representing 3% of the upper limit established by the FAO to guarantee safe consumption (4000 μg/ kg) (Van Egmond and Jonker 2004).

A nourseothricin resistant *Fusarium verticillioides* strain was used in this investigation, provided by Dr. María Dolores García Pedrajas of the Subtropical and Mediterranean Horticulture "La Mayora" Institute (IHSM-UMA-CSIC, Spain), which was kept at −20 °C until use. The conidia suspension (1×10^6 conidia/ml) was made according to Dambolena et al. (2008).

Unsexed adults of *Sitophilus zeamais* (Coleoptera: Curculionidae) were used in the experiments. These insects were kept at 28 ± 2 °C and 70 ± 5% relative humidity (RH) in containers with whole maize kernels without insecticide.

The maize extract enriched in FB (FB extract) used in this work was a mixture of fumonisins FB_1, FB_2 and FB_3, the chemical structures of which are shown in Fig. 1 (FB_1: $C_{34}H_{59}NO_{15}$; FB_2 and FB_3: $C_{34}H_{59}NO_{14}$). The use of maize extract as a source of fumonisins is a widely used strategy in chronic and subchronic inmunotoxic studies in mammals (Voss et al. 1990; Marin et al. 2006; Theumer et al. 2008, 2010; Rudyk et al. 2019). Also, the use of FB extracts produced by liquid fermentation has been commonly used as subchronic models in plants (Arias et al. 2012, 2016; Otaiza-González et al. 2020).

Fumonisin Production in Maize Kernel Substrate

In a glass container, 25 g of maize kernels and 8 ml of distilled water were autoclaved. Then, each container was inoculated with five 10-mm diameter mycelial discs of a 7 days-old *F. verticillioides* culture on PDA agar. These inoculated maize kernels were maintained at 28 ± 2 °C for 28 days to favor mycotoxin production. Subsequently, the maize kernels were autoclaved and dried at 60 °C to obtain a powder, from which the mycotoxins were extracted (Voss et al. 1990). The maize powder was mixed with distilled water (1:3 *w*/*v*) in a plastic container, which was then placed in an orbital shaker for 2 h at 200 rpm. The supernatant was centrifuged for 15 min at 5000 rpm and stored at −20 °C until use.

To determine the concentration of FB, a method described by Shephard et al. (1990, 1994) was used. Briefly, an aliquot of the supernatant (0.5 ml) was mixed with 0.5 ml of acetonitrile: water (1:1 *v*/v), and quantification was carried out by using a Perkin Elmer HPLC. The instrument was equipped with a fluorescence detector and a C18 analytical reverse phase column (150 mm × 4.6 mm internal diameter and 5 μm particle size). The mobile phase consisted of methanol (HPLC grade) and NaH_2PO_4 0.1 M (3:1 *v*/v) at a pH of 3.35 ± 0.20 and was calibrated using orthophosphoric acid and a flow rate of the mobile phase of 1.5 mL/min. Each sample was derivatized by means of a derivatizing solution made of 5 ml of an aqueous solution of sodium tetraborate (0.1 M), 50 μL of 2-mercaptoethanol and 1 mL of methanol with 40 mg of o-phthaldialdehyde (OPA) (Sigma-Aldrich). The samples were then mixed with 50 μL of the FB solution and 200 μL of derivatizer for 3.5 min in the dark. Wavelengths used for excitation and emission were 335 nm and 440 nm, respectively, and quantification was performed by comparing the area of detected peaks with that obtained with an FB_1 analytical standard (≥90%; Sigma-Aldrich). For the control solution (0 mg/ml), the same procedure (without inoculation of maize kernels) was carried out.

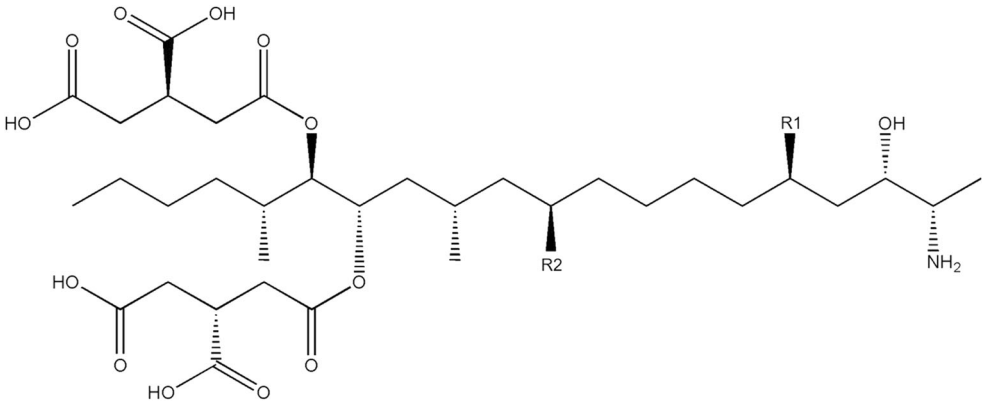

Fig. 1 Chemical structures of fumonisins. R1 and R2 indicate the position of substituents in FB_1, FB_2, and FB_3. Fumonisin B_1: R1 and R2 = OH. Fumonisin B_2: R1 = OH; R2 = H. Fumonisin B_3: R1 = H; R2 = OH

Extraction of the Epicuticle of *S. zeamais* and Influence on its Thickness

Epicuticular extraction of *S. zeamais* adults was carried out according to Pedrini et al. (2009, 2015) with some modifications. Three insects were extracted with 2 ml n-hexane for 15 s to remove the epicuticle; conditions, which had been optimized in previous investigations to guarantee the survival of the insect. In order to determine the reduction in the epicuticle after the extraction, confocal photographs were taken with an Olympus LEXT OLS4000 confocal microscope at the laboratory of Electron Microscopy and X-Ray Analysis (LAMARX-CONICET), Faculty of Mathematics, Astronomy, Physics and Computing, National University of Córdoba (FAMAF-UNC). The diameter of the depressions in the epicuticle was processed with ImageJ (Abramoff et al. 2004); five photographs were used to obtain the measurements.

Antifungal Activity of *S. zeamais* Epicuticle Extracts

The in vitro antifungal activity of the epicuticular extracts of adults of *S. zeamais* was investigated according to Pizzolitto et al. (2020). Petri dishes were prepared with PDA agar, and increasing concentrations of epicuticular n-hexane extract (100, 500, 1000, 5000 μL/L) were added. Each plate was inoculated with 10 μL of conidial suspension of *F. verticillioides* (1×10^6 conidia/mL) and placed in an incubator at 28 ± 2 °C. The radial growth was measured daily, and the growth rate was calculated. Seven final replicates were performed twice.

Infective Capacity of *F. verticillioides*

To determine, whether *F. verticillioides* can penetrate the cuticle of *S. zeamais* adults, an infective assay was carried out. First, live insects, control (with epicuticle) and treated insects (after the extraction process, without epicuticle) were disinfected twice with an aqueous solution of sodium hypochlorite (4% *v*/v). The infection process was then performed using one of two approaches. For one group, 10 *S. zeamais* adults of each treatment were submerged in a conidia suspension of *F. verticillioides* (1×10^6 conidia/ml) for 1 min, while for the other group 10 insects of each treatment were placed in contact for 1 min with the mycelium of a 7 days-old *F. verticillioides* culture (grown in a petri dish). The controls were insects submerged in sterile water for 1 min or placed in a petri dish with PDA agar. After treatment, the insects were placed in sterile glass petri dishes without food for 20 days at 28 ± 2 °C. Subsequently, the insects were freeze-killed, superficially disinfected with aqueous sodium hypochlorite (4% *v*/v) twice (for half of the insects of the assay), and placed in petri dishes with PDA agar and nourseothricin (NTC) (100 mg/ml). To favor development of the fungus, the insects were crushed using a sterile mortar, and the percentage of infected insects was determined. This experiment was performed twice, with six final replicates.

Toxicity of the FB Extract in Contact Bioassays

The contact toxicity of the FB extract was investigated by two series of experiments. In the first series, 10 adults of *S. zeamais* were placed in a petri dish (Ø = 4.5 cm) containing a filter paper at the base. On the paper, increasing concentrations of the FB extract (25.25, 50.50, 126.26 and 252.53 μg/cm^2 of filter paper) were added, and the mortality was recorded at 24, 48, and 72 h (Zaio et al. 2018). In the second series, 10 insects were anesthetized in a cold environment, and 10 μL of FB extract were topically applied at 0.011, 1.79, 17.9 and 1000 μg/insect on the dorsal site of the abdomen. The mortality was then recorded at 24, 48, and 72 h after treatment. Both experiments were carried out twice with five final replicates and 50 experimental individuals per concentration. For the controls of both experiments, the same concentrations were tested using the control extract (0 mg/ml of FB).

Feeding Assay

The effect of the maize extract enriched in FB on the feeding activity of adults of *S. zeamais* was mesured according to Usseglio et al. (2018), with some modifications. Ten insects, previously placed for 48 h in petri dishes with FB extract or control extract (25.25, 50.50, 126.26 and 252.53 μg/cm^2) were transferred to amber bottles (30 ml) containing 14 maize kernels without visible damage or fungal infection. After 20 days, the weight loss, grain damage (number of grains with holes) and insect mortality were determined. In parallel, 20 insects were placed in amber bottles (30 ml) with maize kernels treated with 0.24 μg/ml of each extract, FB or control (0.02 μg/cm^2 of grain or 240 ppm). After 20 days, the same variables as those described above were determined. Five replicates were performed twice.

Behavioral Assays

Repellent/Attraction Activity Bioassay

The effect of the FB extract on the preference of *S. zeamais* adults was determined using half filter paper discs, according to Wagan et al. (2018) with some modifications. A filter paper was placed in a plastic petri dish (Ø = 4.5 cm). Then, in the middle of the paper disk, a line was drawn, and different concentrations of the maize extract enriched in FB (0.02, 0.04, 0.07, 0.13, 0.27, 1.26, 12.63 and 25.25 μg/cm^2) were placed on one half of the discs, while on the other half, the same concentrations of the control extract were positioned. Ten adult insects starved for 24 h were released into the petri dishes, and their choice was recorded 2, 4, 6, and 24 h after the start of the trial, using a response index (*RI*) calculated from the following equation:

$$RI = \frac{(T-C)}{Tot} \times 100$$

where T is the number of insects in the treatment, C the number of insects in the control and *Tot* is the total number of insects in the experiment (Phillips et al. 1993). Positive values of the index indicate attraction whilst negative ones indicate repellency of the treatment. Independence tests (without mycotoxin) were carried out to ensure that there were no positional effects influencing the choice of the insects (indicated by a value of $p > 0.05$).

Walking Activity Bioassay

To evaluate the effect of FB extract on the walking behavior of adults of *S. zeamais,* an assay was performed according to Fussnecker et al. (2006) with some modifications. Briefly, in a circular arena (Ø = 4.5 cm) the extract enriched with FB (12.63 μg/cm^2) was placed in one half of a filter paper, and the control extract was placed in the other half. In the center of this arena one *S. zeamais* adult, starved for at least 24 h, was released, and the behavior was filmed over 1200 s. The parameters evaluated were: permanence time in each treatment, movement pattern (idle time and travel time) and grooming time, considering the latter as movement of the palps around the chewing apparatus. For this experiment, the concentration that provided the strongest effects in the repellent/attraction activity bioassay was chosen, and the experiment was performed 20 times with each insect used only once ($N = 20$). The arena was changed between replicates. ToxTrac Free Software was used to analyze the data (Rodriguez et al. 2017).

Progeny Assays

To study the effect of the FB extract on the reproduction or development of *S. zeamais* adults, a progeny assay was carried out according to Usseglio et al. (2018), with some modifications. First, 40 healthy maize kernels without visible damage or fungal infection were put in an amber glass bottle (30 ml) and mixed with 2 ml of FB extract (20 ppm = 0.27 μg/cm^2). This concentration is equivalent to the concentrations used in other chronic assay models (Arias et al. 2016; Otaiza-González et al. 2020). In each bottle, 2 adult couples of *S. zeamais* were released. The treated insects were placed in an incubator at 28 ± 2 °C for 60 days, after which the number of insects, their size, and their mortality rate were determined. The insect size and the measurements of the thorax, abdomen and the right 3rd leg were obtained using ImageJ (Abramoff et al. 2004), and 10 photographs of each body segment were utilized to obtain the measurements. This experiment was carried out three times with 15 final replicates.

Statistical Analyses

The statistical significance of the data in experiments concerning the thickness of the *Sitophilus zeamais* epicuticle (section 2.3), infective capacity of *F. verticillioides* (section 2.5), toxicity of FB extract (section 2.6), feeding and progeny assays (section 2.7 and 2.9, respectively) was evaluated using an analysis of variance (ANOVA) ($p < 0.05$). For the behavioral assays (section 2.8.1 and 2.8.2), the statistical differences were evaluated with a Student's t- test for paired comparisons ($p < 0.05$). The statistical analysis of the antifungal activity of the *S. zeamais* epicuticle (section 2.4) was carried out by using a linear regression to obtain the growth rate, followed by an ANOVA ($p < 0.05$) to evaluate the difference in this rate between treatments. These analyses were carried out with the statistical software Infostat (Di Rienzo et al. 2010). The assumptions of normality and homogeneity of variance were tested.

Results

FB Extract

The final concentration of the fumonisins in the maize extract was 1.79 mg/ml, while the concentration of the control extract was 0 mg/ml. The chromatograms are presented in the Electronic Supplementary Material 1 (ESM. 1). In these chromatograms, the presence and the absence of fumonisins in the FB (ESM. 1, c; d) and control (ESM. 1, b) extracts, respectively, are shown. When the peak areas of the control chromatogram (0–2 min) were subtracted from those of the FB extract, the FB peak area represented 40% of the total.

Thickness of the Epicuticle of *Sitophilus zeamais*

To determine the modulating effect of the insect epicuticle on *F. verticillioides,* surface extracts were prepared using n-hexane. Depressions in the epicuticle increased in size, indicating a reduction in the epicuticle of *S. zeamais* after extraction. In treated insects, the mean diameter of the depression increased significantly compared to control insects (24.0 ± 2.3 μm and 41.8 ± 4.8 μm, respectively) ($p = 0.0103$). The extraction yielded 1.33 ± 0.10 mg of n-hexane soluble epicuticular compounds per insect.

Antifungal Effect of *Sitophilus zeamais* Epicuticular Extract

When the effect of the epicuticular extract of *S. zeamais* on *F. verticillioides* growth was tested, no inhibitory effects were observed. The highest tested concentration of the epicuticular extract (5000 μL/L) showed a reduction in the growth rate compared to the control (9.9 ± 0.3 mm/day and 10.0 ± 0.2 mm/day, respectively), but there was no statistically significant difference ($p = 0.93$). In addition, no statistical difference was found in the lag phase, which was 17.5 ± 3.2 h for controls and 15.8 ± 4.4 h for the highest concentration tested (5000 μL/L) ($p = 0.9638$).

Entomopathogenicity of *Fusarium verticillioides*

To evaluate the infective capacity of *F. verticillioides* on *S. zeamais* and to determine whether the epicuticle of the insect had a protective effect against this fungus, the relationship between *S. zeamais* and *F. verticillioides* was investigated in vivo. After 20 days of infection with *F. verticillioides,* no insects had died. The percentages of infection recorded for the two methods tested are shown in Table 1.

The results shown in Table 1 reveal that the conidial suspension was not an effective agent to cause insect infection. Moreover, for the method that used the 7-day-old mycelium, the infection was only superficial since the insects that had been superficially disinfected did not show any fungal development inside their bodies. This result was observed both for insects with or without an epicuticle, suggesting that *F. verticillioides* cannot penetrate the cuticle of the insect and achieve tissue infection and fungal dispersion.

Toxic and Antifeedant Effects of FB Extract on *S. zeamais*

To determine if the maize extract enriched in FB would show lethal effects on *S. zeamais*, assays of contact toxicity were carried out. Using the contact filter paper test, none of the tested concentrations caused mortality in *S. zeamais* at 72 h. Moreover, when the FB extract was topically applied dorsally, results were inconsistent between the different repetitions performed. No insect mortality was observed in the control treatments. For 0.011 and 17.9 μg/insect of FB extract, the mortality percentage varied between 2 and 17% (2.5 ± 2.5% and 16.7 ± 6.7%, respectively), while for 1.79 and 1000 μg/insect, a 0 % mortality was obtained. Thus, these results exclude a lethal effect of fumonisins on *S. zeamais*.

Next, the effect of the FB extract on the feeding behavior of *S. zeamais* was evaluated. When the insects were pre-exposed (48 h) to the FB extract, the feeding rate (as determined by the weight loss of the kernels) and the percentage of grain damage did not show any statistical differences between control and treated insects ($p > 0.05$, Table 2). Furthermore, when the extract enriched with FB was mixed with the maize kernels, no statistical differences between the feeding rates were observed either ($p > 0.05$, data not shown). These results show that neither pre-exposure to FB extract nor its addition to the maize kernels affected the feeding behavior of *S. zeamais*. For 253.53 μg/cm^2, although the weight loss of the kernels was higher than that of the other treatments, there were no differences to the control. This same pattern was observed in the percentage of grain damage in both experiments ($p > 0.05$), with insect mortality not exceeding 5% in all cases (control and treatments). These results show that increase of FB in the insect environment did not affect their feeding behavior.

Effect of FB Extract on the Behavior of *S. zeamais*

The aim of these bioassays was to determine the effect of maize extract enriched in FB on the behavior of *S. zeamais*. For the first experiment (repellency/ attraction), using the half filter paper choice test, there was no defined pattern related to the effect of the extract. At the four times tested, the intermediate concentrations (0.13 and 1.26 μg/cm^2) did not reveal any response by the insects ($p > 0.05$). However, the lowest (0.02 and 0.04 μg/cm^2) and the second highest (12.63 μg/cm^2)

Table 1 Entomopathogenic capacity of *F. verticillioides* expressed as the percentage of insects infected after 20 days

Insect condition	Infection method	Superficial disinfection after 20 days	Percentage of Infection (%)
WITH epicuticle (control insects)	Mycelium	–	100.0 ± 0.0 [a]
		√	0.0 ± 0.0 [b]
	Conidial suspension	–	0.0 ± 0.0 [b]
		√	0.0 ± 0.0 [b]
WITHOUT epicuticle (treated insects)	Mycelium	–	100.0 ± 0.0 [a]
		√	0.0 ± 0.0 [b]
	Conidial suspension	–	0.0 ± 0.0 [b]
		√	0.0 ± 0.0 [b]

(√): superficial disinfection. **(–):** no superficial disinfection. Different letters indicate statistical differences between treatments for the *ANOVA* and *LSD Fisher* posteriori tests ($p < 0.05$).

concentrations caused repellent effects at 2 h of exposure ($p < 0.05$), with the highest response index (−62.9 ± 17.7%) being obtained at 0.02 μg/cm^2. Moreover, at 4 and 6 h, the concentration of 0.07 μg/cm^2 revealed increasing effects, with response indices of 42.2 ± 9.7% and 47.8 ± 14.5%, respectively. At 24 h, only one of the higher concentrations was tested (12.63 μg/cm^2), which showed a significant repellent effect ($p = 0.0192$), with an RI higher (−45.1 ± 14.2%) than that obtained at 2 h.

A similar effect was observed in the walking activity behavior, when the beetles were exposed to 12.63 μg/cm^2 of FB extract. In this experiment, the insects were found on the control side 66.84 ± 7.55% of the time ($p = 0.05$, Fig. 2). Of individuals tested, only 4 were observed to be immobile during the experiment (4.5 ± 3.0% of the experimental time), mainly in the control sector (49.7 ± 32.8 s on control side vs 3.8 ± 3.8 s on the treatment side) ($p < 0.05$). On the other hand, grooming behavior was observed during 4.7 ± 3.1% of the time (60% of the tested insects). In all, 40.3 ± 15.1% of the grooming time was spent on the control side, while 19.7 ± 11.7% took place on the treatment side ($p < 0.05$).

The results obtained in both behavioral bioassays may also confirm a repellent effect of the maize extract enriched with fumonisins on *S. zeamais*.

Effects of FB Extract on Reproduction and Development of *S. zeamais*

To determine whether the extract enriched in FB could affect the reproduction or development of *S. zeamais,* progeny assays were performed. The FB extract concentration used to evaluate the chronic effects was 20 ppm (Arias et al. 2016; Otaiza-González et al. 2020). After 2 months of exposure, the number of emerging insects from the treatments was significantly higher than that of the controls ($p = 0.039$, Fig. 3). In agreement with this result, a higher number of damaged kernels were found in treatments with 90% of kernels being damaged ($p = 0.0071$, Fig. 3).

To evaluate the effect of FB extracts on the development of *S. zeamais,* the size of the progeny of the insects was determined. Figure 4 shows that the addition of FB extracts to the environment of developing *S. zeamais* (maize kernels) did not cause changes in the size of the progeny ($p > 0.05$). Thus, our results indicate that treatment with extracts enriched in FB increased the number of descendants of *S. zeamais* by modifying parameters related to the reproduction numbers, but not those concerning the development of its progeny.

Table 2 Effect of FB extract on *S. zeamais* alimentation after 20 days

FB extract concentration (μg/cm^2)	Grain weight loss (g)	Grain damage (%)
Control	0.14 ± 0.05[a]	15.72 ± 2.67[A]
25.25	0.20 ± 0.02 [a]	9.52 ± 2.38 [A]
50.50	0.22 ± 0.01 [a]	14.29 ± 0.00 [A]
126.26	0.22 ± 0.02 [a]	7.14 ± 0.00 [A]
253.53	0.23 ± 0.02[a]	14.29 ± 0.00 [A]

Different letters in the same column indicate statistical differences between treatments for ANOVA and LSD-Fisher posteriori tests ($p < 0.05$). Values are expressed as the mean ± the standard error.

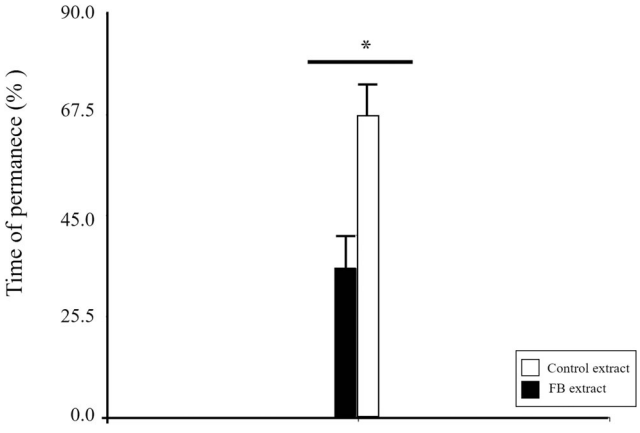

Fig. 2 Walking behavior of *S. zeamais* exposed to FB extract: time devoted to the exploration of the control and treatment areas. (*) Indicates a statistical difference for a paired t-Student's test ($p < 0.05$)

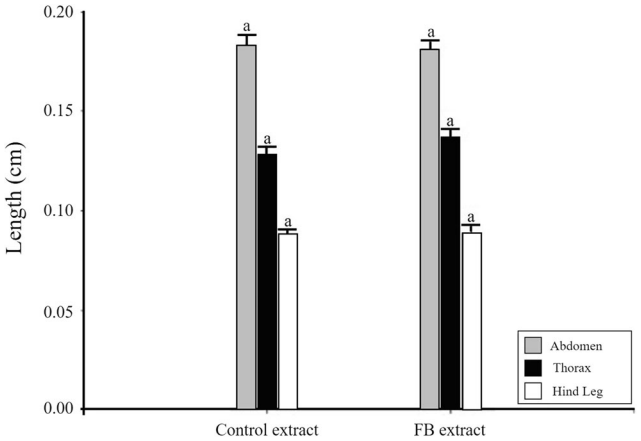

Fig. 4 Growth of *S. zeamais* progeny exposed to FB extracts. Different letters indicate statistical differences between treatments for ANOVA and LSD-Fisher posteriori tests ($p < 0.05$)

Discussion

Insects and fungi share a long co-evolutionary history, and their interactions have been widely studied from various perspectives, including ecological and evolutionary investigations and aspects of biological pest control. During recent decades, growing evidence has been observed concerning the participation of natural chemical compounds as modulators in these biological interactions. Tasin et al. (2011, 2012) reported the inhibitory effect of 3-methyl-1-butanol, emitted by *Botrytis cinerea,* on the oviposition of *Lobesia botrana*. In addition, it has been reported that chemical constituents of the insect cuticle can affect the development of the fungus *B. bassiana* (Forlani et al. 2015; Pedrini et al. 2013; Schama et al. 2016). Although the role of natural compounds in the insect-fungus interaction has been explored in different biological systems, to our knowledge, there are no studies dealing with chemical modulators in the biological interaction between *F. verticillioides* and *S. zeamais*, despite the fact that they share the same biological niche.

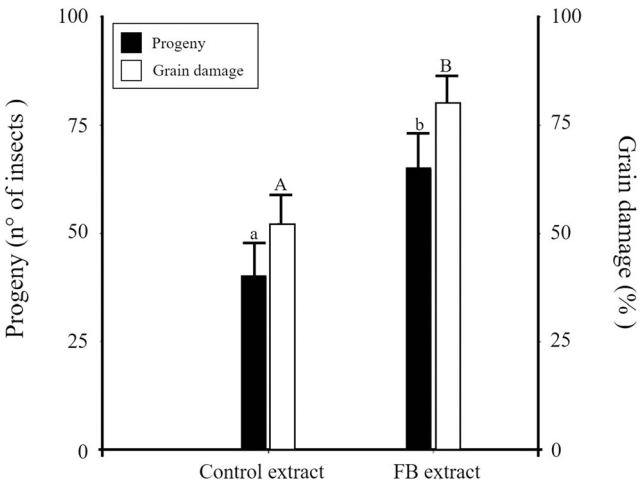

Fig. 3 Effect of FB extract on the progeny and feeding activities of *S. zeamais*. Different letters indicate statistical differences between treatments for ANOVA and LSD-Fisher posteriori tests ($p < 0.05$)

The results obtained in the present study demonstrate that the *S. zeamais* n-hexane extract epicuticle did not inhibit fungal growth. This is in agreement with Kurita and Koike (1983), who showed that some hydrocarbons, similar to this insect's epicuticle extract, did not affect fungal growth. Moreover, the results obtained in the current investigation also revealed the inability of *F. verticillioides* to infect live *S. zeamais*. Only a few investigations have focused on the entomopathogenic capacity of *F. verticillioides* (Batta 2012; Patel and Ghetiya 2019; Pelizza et al. 2011;), reporting fungal infections of dead insects. Thus, the saprophytic nature of *F. verticillioides* enhanced fungal growth on decomposing insect bodies. In contrast, in the present work, the entomopathogenic capacity of *F. verticillioides* was determined on living *S. zeamais* insects. Our results are in agreement with previous findings, suggesting that fungi with crop spoilage activity are not necessarily entomopathogenic (Teetor-Barsch and Roberts 1983). Moreover, the relationship between *Fusarium* spp. and beetles has been usually reported to be mutualistic: the insects benefit from the fungus and collaborate with their dispersion (Ferreira-Castro et al. 2012; Jayaraman and Parihar 1975; Kok et al. 1970; Teetor-Barsch and Roberts 1983). The predominance of this mutualistic relationship and the inability of *F. verticillioides* to colonize *S. zeamais*, as demonstrated in our study, may be attributed to the lack of vegetative structures to allow the fungus to penetrate the insect's cuticle or to chitin-degrading enzymes. This could explain why many *Fusarium* spp. become ectoparasites and only cause some gray spots on the cuticle (Teetor-Barsch and Roberts 1983). In our investigation, we demonstrate that *F. verticillioides* develops as an ectoparasite of living insects and colonize its body after death.

The mycotoxin fumonisins are the main secondary metabolites produced by *F. verticillioides* in maize, which maycause considerable problems due to their toxic effects on mammals (Theumer et al. 2010; Vila-Donat et al. 2018; Thompson and Raizada 2018). However, little is known about their role in natural biological interactions. Although positive correlations among insect damage, *Fusarium verticillioides* and FB content in maize have been previously reported (Madege et al. 2019), there are as yet no studies about the role of FB in the *S. zeamais-F. verticillioides* interaction. In the present study, we used a maize extract enriched in FB to evaluate the role of the fumonisins in the *S. zeamais-F. verticillioides* relationship and observed that the FB extract did neither negatively influence feeding nor cause mortality of *S. zeamais*. Despite the toxic effects of other mycotoxins such as destruxin and beauvericin against *Galleria mellonella, Spodoptera littoralis, S. litura,* and *Tetranychus urticae* that have been reported earlier (Al Khoury et al. 2019; Sowjanya Sree et al. 2008; Vey et al. 2002), there are no previous studies about the effect of pure FB or FB extract against any insects. Nevertheless, recent works on *S. frugiperda* (Sf9) cell lines have reported that pure FB trigger the programmed cell death process (Zhang et al. 2017, 2018). On the other hand, we demonstrated a repellent effect and progeny induction caused by the FB extract acting on *S. zeamais.* The exposure of *S. zeamais* to FB extract made the insects move to zones free of toxin, suggesting an escape response to the toxic compound or a signal indicating the fungal presence and deterioration of kernels, which may explain the absence of lethal effects caused by this mycotoxin (Cutler and Guedes 2017). In addition to the behavioral effects discussed above, the increase of insect progeny found in our study may be caused by two factors: 1) an egg laying stimulation exerted by the addition of the FB extract, or 2) a shortening in the life cycle of the insects. Zhang et al. (2017) determined that FB induce an overexpression in genes related to the hormonal regulation of the insect's life cycle in Sf9 cell lines. Complementary research should now be performed to try to elucidate the mechanism by which FB cause an increase in the population.

Summing up, it can be hypothesized that *F. verticillioides* can still become an ectoparasite of *S. zeamais* to ensure its dispersal, with fumonisin FB participating as a chemical modulator in this relationship. Concerning this, FB could have two ways of increasing fungal infection: by their ability to augment the progeny of the insect and also by their repellent effect at concentrations that can be found in grain bins. To our knowledge, this is the first investigation to report the role of fumonisins B as a chemical modulators of the *F. verticillioides-S. zeamais* relationship. The findings of the present investigation suggest that if *F. verticillioides* contamination is reduced using good management practices (hermetic conditions of the storage system and reduction of oxygen and humidity), the amounts of mycotoxins and the size of the *S. zeamais* population will be reduced.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that there are no conflicts of interest.

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