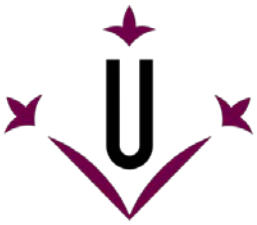

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Critical environmental and genotypic factors for *Fusarium verticillioides* infection, fungal growth and fumonisin contamination in maize grown in northwestern Spain.

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Keywords: maize; *Fusarium verticillioides*; fumonisin; ergosterol; environment

ABSTRACT

In northwestern Spain, where weather is rainy and mild throughout the year, *Fusarium verticillioides* is the most prevalent fungus in kernels and a significant risk of fumonisin contamination has been exposed. In this study, detailed information about environmental and maize genotypic factors affecting *F. verticillioides* infection, fungal growth and fumonisin content in maize kernels was obtained in order to establish control points to reduce fumonisin contamination. Evaluations were conducted in a total of 36 environments and factorial regression analyses were performed to determine the contribution of each factor to variability among environments, genotypes, and genotype × environment interactions for *F. verticillioides* infection, fungal growth and fumonisin content. Flowering and kernel drying were the most critical periods throughout the growing season for *F. verticillioides* infection and fumonisin contamination. Around flowering, wetter and cooler conditions limited *F. verticillioides* infection and growth, and high temperatures increased fumonisin contents. During kernel drying, increased damaged kernels favored fungal growth, and higher ear damage by corn borers and hard rainfall favored fumonisin accumulation. Later planting dates and especially earlier harvest dates reduced the risk of fumonisin contamination, possibly due to reduced incidence of insects and accumulation of rainfall during the kernel drying period. The use of maize varieties resistant to *Sitotroga cerealella*, with good husk coverage and non-excessive pericarp thickness could also be useful to reduce fumonisin contamination of maize kernels.

1. INTRODUCTION

F. verticillioides (Saccardo) Nirenberg is one of the most common fungal species associated with maize worldwide, and is, in particular, the most prevalent species in maize and maize foodstuffs in Spain (Aguín et al., 2013; Ariño et al., 2007; Jurado et al., 2006; Sala et al., 1994). *F. verticillioides* infection can occur asymptomatically or cause rots. Its major consequence is maize kernel contamination with fumonisins, which cause several disorders in humans and animals (Voss et al., 2007). Fumonisin B_1 is classified as possibly carcinogenic to humans by the International Agency for Research on Cancer (IARC, 2002) and maximum levels for fumonisin B_1 and B_2 in food and feed have been set by the European Union (Commission Regulation 1126/2007; Commission Recommendation 2006/576/EC).

Climatic conditions during the growing season, insect damage, and plant characteristics are determinant factors for *F. verticillioides* infection and fumonisin accumulation in maize in the field. Higher temperatures and drier weather during flowering, a key moment for ear infection by *F. verticillioides*, higher temperatures during kernel maturation, and more rainfall before harvest were observed to increase ear rot levels and fumonisin content at harvest (de la Campa et al., 2005; Fandohan et al., 2003; Pascale et al., 1997; Shelby, 1994). Insects have been associated with fumonisin contamination as their activity disperses the fungus and provides routes of entry into the ear and kernels (Alma et al., 2005; Avantaggiato et al., 2003; Fandohan et al., 2003). Special attention has been placed on corn borers but there are other insects to be considered such as thrips (*Frankliniella* spp.) (Parsons and Munkvold, 2010) or the Angoumois grain moth (*Sitotroga cerealella* Olivier) (Cao et al., 2013). Kernel and ear characteristics such as kernel humidity, pericarp thickness, and husk tightness can also affect fungal development and fumonisin production, by providing or not a suitable environment for the fungus or acting as a barrier against the fungal arrival (Fandohan et al., 2003). All these factors can act in combination, making it difficult to establish the contribution of each to the levels of infection and fumonisin contamination of maize kernels.

As *F. verticillioides* infection and fumonisin contamination begin in the field, suitable agronomic practices can reduce fumonisin accumulation in kernels. Early planting and harvest, moderate plant density and fertilization, control of crop residues, and insecticide treatments are proposed practices to reduce fungal infection and fumonisin accumulation (Ariño et al., 2009; Battilani et al., 2008;

Blandino et al., 2008; Maiorano et al., 2009a), while fungicide treatments are not effective in reducing fumonisin in kernels, except when combined with an insecticide treatment (De Curtis et al., 2011).

Most of the above mentioned factors have been evaluated and included in several ear rot or fumonisin risk assessment models (Barrier-Guillot et al., 2007; Battilani et al., 2008; de la Campa et al., 2005; Maiorano et al., 2009b; Stewart et al., 2002). However, while general relevance of specific climatic conditions (e.g. high temperature during flowering) has been acknowledged, environmental and agricultural conditions may had different effects on *F. verticillioides* infection and fumonisin contamination in different crop areas depending on many other environmental conditions (local weather, insect species and pressure, amount of inoculum in the environment, geographical location, etc.) (Battilani et al., 2008; Maiorano et al., 2009a; Schjøth et al., 2009; Torelli et al., 2012). Therefore, recommended guidelines to reduce fumonisin contamination could be effective in a general way but might not have the same importance in all the environments.

In northwestern Spain, *F. verticillioides* is the most abundant fungal species in maize kernels and a noteworthy risk of fumonisin contamination has been exposed (Aguín et al., 2013; Butrón et al., 2006; Cao et al., 2013). The particular conditions in this area are characterized by abundant rainfall throughout the year and mild summers and winters, and the insect damage is frequently produced by corn borers and the Angoumois grain moth (Cao et al., 2013; Cordero et al., 1998). Higher occurrence of *F. verticillioides* in northern than in southern Spain was previously reported, and it was attributed to the wetter climate in northern regions (Cantalejo et al., 1998; Muñoz et al., 1990). However, information about the critical factors (weather conditions, insect damage or agronomic practices) affecting *F. verticillioides* infection and growth in maize kernels in Spain, and specifically in northwestern Spain, was insufficient. The available information to date about fumonisin contamination has seasonal or geographical limitations, and an extensive approach is needed to determine the environmental and genotypic variables influencing fumonisin accumulation in maize kernels under these conditions.

Therefore, the objectives of this study were: i) to determine the relationship between *F. verticillioides* infection and growth and fumonisin content in maize kernels at harvest, and ii) to identify environmental and genotypic factors, along the growing season, affecting variability for *F.*

verticillioides infection, fungal growth and fumonisin concentration in maize kernels at harvest in diverse humid and temperate environments in order to establish control points to reduce fumonisin contamination.

2. MATERIALS AND METHODS

2.1. Field experiments

Six maize hybrids obtained from crosses among experimental inbred lines EP39, CM151, EP42 and EP47 were used in this study. As corn borer attack has been associated to increased kernel damage by fungus and fumonisin content (Smith and White, 1988; Avantaggiato et al., 2003), two inbred lines, EP42 and EP47, were susceptible to ear and stem attack by the Mediterranean corn borer (*Sesamia nonagrioides* Lefèbvre), CM151 was resistant to stem attack and susceptible to ear attack, and EP39 was resistant to both ear and stem attack (Butrón et al., 1998, 1999; Santiago et al., 2003). Hybrids were grown in three consecutive years (2007, 2008, and 2009) in three locations in northwestern Spain. Locations were Pontevedra (42° 24′ N, 8° 38′ W, 50 m above sea level), Barrantes (42° 30′ N, 8° 46′ W, 50 m above sea level), both placed close to the coast, and Valongo (42° 26′ N, 8° 27′ W, 500 m above sea level), situated in inland. At each year and location, hybrids were evaluated at two planting dates, early (mid-late April) and late (early-mid May), and at two harvest dates. Therefore, hybrids were evaluated in a total of 36 environments (year x location x planting x harvest). A split-plot design with three replications was used for each trial (year-location-planting combination). Hybrids were assigned to main plots and harvest dates to sub-plots. Main plots consisted in two rows with 13 plants each, rows being 0.80 m apart from each other and hills 0.21 m apart. After thinning, the final density was around 60.000 plants/ha. Within each plot, all ears from one row (sub-plot) were harvested at late September or early October (early harvest) and all ears from the other row one month later (late harvest). Thus, 216 samples were obtained each year. Husks were removed manually and ears were dried at 35 °C for one week. Ears were maintained at 4 °C and 50 % humidity, and subsequently kernels were shelled and maintained at the same conditions until analyses were performed.

2.2 Environmental and genotypic variables

A meteorological station was installed at each location for recording climatic data every 12 minutes throughout the growing season. The following climatic variables were computed based on recorded climatic data: average of daily mean temperatures (°C), average of daily maximum temperatures (°C), average of daily minimum temperatures (°C), rainfall (mm), average of daily relative humidity

(%), number of days with minimum temperature ≤ 15 °C, number of days with maximum temperature ≥ 30 °C, number of days with mean temperature ≥ 10 °C and < 15 °C, ≥ 15 and < 20 °C, ≥ 20 and < 25 °C, ≥ 25 and < 30 °C, and number of days with rainfall ≥ 2 mm. These climatic variables were selected according to previous reports on the influence of climatic factors on fungal development in wheat and maize (de la Campa et al., 2005; Maiorano et al., 2009b; Marín et al., 2004). They were calculated for the following periods: the entire maize growing period, from planting to harvest; the maize vegetative period, from planting to silking; the maize reproductive period, from silking to harvest; the flowering period, from 15 days before silking to 15 days after silking; critical period 1 (C1), from 10 to 4 days before silking; critical period 2 (C2), from 4 days before silking to 2 days after silking; critical period 3 (C3), from 2 to 8 days after silking; critical period 4 (C4), from 8 to 14 days after silking; milk-dough kernel stage, from 16 to 30 days after silking; dent kernel stage, from 31 to 45 days after silking; kernel developing period, from silking to physiological maturity; kernel drying period, from physiological maturity to harvest.

Mid-silking and mid-tasseling dates were recorded for each plot. Ear height and plant height of five random plants per plot were measured. At harvest, husk coverage was evaluated at each subplot by a visual scale from 1 (loose husks with visible cob) to 5 (tight husks). The following variables were also recorded in five random ears or plants: ears were evaluated for kernel and rachis damage by corn borers (ear damage) on a visual rating scale from 1 to 9, where 1 > 90% damaged, 2 = 81 to 90% damaged, 3 = 71 to 80% damaged, 4 = 61 to 70% damaged, 5 = 41 to 60% damaged, 6 = 31 to 40% damaged, 7 = 21 to 30% damaged, 8 = 1 to 20% damaged, and 9 = no damage; stem damage by borers was measured as tunnel length; percentage of kernel humidity was measured using a humidimeter; after drying, the number of kernels damaged by *S. cerealella* per ear was recorded. The percentage of kernels with damaged pericarp was calculated from a random 100 kernel sub-sample per sub-plot. For a better damage detection, kernels were stained in a 0.1% Brilliant Blue (Sigma St. Louis, MO, USA) solution for 30 s according to Henry and Kettlewell (1996). Finally, pericarp thickness was measured in at least 10 random kernels per sub-plot using a micrometer and following Wolf et al., (1969) and St. Martin et al. (1980) protocols with some modifications. Two pericarp thickness measures per kernel, at germinal and abgerminal kernel sides, were taken.

2.3. *F. verticillioides* **isolation and identification**

F. verticillioides infection was estimated from 50 kernels per sub-plot in 2008 and 2009. Kernels were surface disinfected in a solution of sodium hypochlorite, incubated, and both morphological and molecular identifications of the *F. verticillioides* isolates were performed as described in Aguín et al. (2013). Results are given as the percentage of kernels infected by *F. verticillioides*. *F. verticillioides* infestation was assessed in kernels that were not surface disinfected in 2007, 2008 and 2009 following the same procedure.

2.4. Ergosterol analyses

Ergosterol is a sterol exclusively found in fungal cell membranes, so ergosterol concentration was used as an indirect measure of the fungal growth in kernels. A representative dried kernel subsample of 25 g from each sub-plot was ground through a 0.75 mm screen in a Pulverisette 14 rotor mill (Fritsch GmbH, Oberstein, Germany). Analyses were carried out as previously described (Reid et al., 1999) with slight modifications: 100 mg of ground sample was placed in culture tubes closed with teflon-lined caps along with 2 ml of methanol and 0.5 ml of 2 M sodium hydroxide, placed inside capped 1 L plastic bottles, and irradiated in a microwave oven (Teka, model MW-219) at 80% power (2450 MHz, 800 W maximum output) for 20 s and, after 5 min, for an additional 20 s. After cooling, samples were neutralized with 1 ml of 1 M hydrochloric acid and treated with 2 ml of methanol. The samples were partitioned with 3 × 4 ml of pentane, and the extracted top pentane layers were combined and evaporated in a rotary evaporator at 50 °C. The extracts were redissolved in 0.5 ml of methanol HPLC grade and passed through a 13 mm nylon syringe filter, 0.45 μm pore size, into HPLC vials, and stored at -20 °C until HPLC analysis. Ergosterol was quantified with a Shimadzu HPLC-system equipped with a diode array detector set at 282 nm. HPLC separation was carried out at room temperature by injection of 50 μL of sample onto an ACE C18 column (150 × 4 mm i. d., 5 μm particle size) at a flow rate of 2 ml/min with acetonitrile - methanol (90% - 10%) as eluent under isocratic conditions. Quantification was performed using external calibration with ergosterol standard solutions (Sigma, St. Louis, MO, USA). Results were given as μg/g of dry maize flour.

2.5. Fumonisin analyses

A representative dried sample of 200 g from each sub-plot was taken and ground for fumonisin determinations. Extraction was performed on 10 g of dried ground kernels with 50 ml of distilled water:methanol:acetonitrile (50:25:25) solution and 1 g of sodium chloride. The mixture was agitated for 20 minutes in a magnetic stirrer, filtered, and 10 ml of the filtered solution were suspended in 40 ml of PBS (8.0 g sodium chloride, 1.2 g disodium hydrogen phosphate, 0.2 g potassium dihydrogen phosphate, and 0.2 g potassium chloride in 1 L of distilled water, adjusted to pH 7.4 with hydrochloric acid). The resulting 50 ml were passed through an immunoaffinity column (Fumoniprep, R-Biopharm Rhône Ltd, UK) and fumonisins were recovered using 1.5 ml of methanol and 1.5 ml of ultrapure water (Milli-Q water system, Millipore Corporation, Billeca, MA, USA). Fumonisin quantification was performed in a Waters HPLC-system (Waters 2695, separations module, Waters Corporation, Milford, USA) equipped with fluorescence detector (Waters Multi λ Fluorescence Detector 2475, excitation λ at 335 nm and emission λ at 440 nm) and a C18 column (Waters Spherisorb ODS2, 150 mm x 4.6 mm, 5 μm) connected to a precolumn. One hundred μl were injected into the HPLC system after derivatization of fumonisins with *o*-phthaldialdehyde, at 30 °C and a flow rate of 1 ml/min. The mobile phase was methanol and 0.1 M sodium dihydrogen phosphate (77:23). Quantification was performed using external calibration with fumonisin B_1 (FB_1) and fumonisin B_2 (FB_2) standard solutions (Sigma, St. Louis, MO, USA). Results were converted into μg/g of dry maize flour.

2.6. Statistical analyses

Pearson correlation coefficients between FB_1, FB_2, total FB (FB_1+FB_2) concentrations, percentage of kernels infected by *F. verticillioides*, and ergosterol concentration in each environment (year x location x planting date x harvest date) were computed. Combined analyses of variance (ANOVA) were performed for all traits. Years, locations, planting date, harvest date, and hybrids were considered fixed factors. Replications and their interactions were considered random factors. Mean comparisons were made using the Fisher´s protected least significant difference (LSD) at 0.05 probability level. All analyses were performed with the SAS software (Version 9.2, SAS Institute Inc., Cary, NC, USA).

Factorial regression analyses were performed to find out environmental and genotypic factors contributing to variability among environments (E), genotypes (G), and genotype x environment

(GE) interactions for *F. verticillioides* infection, ergosterol concentration, and total FB concentration. The general form for a factorial regression model with genotypic (k) and environmental (h) covariates is (Denis, 1980):

$$Yij = \mu + [\Sigma \varrho_k \times G_{ik} + \alpha_i] + [\Sigma \delta_h \times E_{jh} + \beta_j] + [\Sigma G_{ik} \times \theta_{kh} \times E_{jh} + \Sigma \alpha'_{ih} \times E_{jh} + \Sigma \beta'_{jk} \times G_{ik} + \varepsilon_{ij}]$$

Where ϱ_k and δ_h are the regression coefficients of genotypic (G_{ik}) and environmental (E_{jh}) covariates, respectively; α_i and β_j are the residuals of genotype and environmental main effects, respectively; θ_{kh} is the regression coefficient of the cross-product of covariates G_{ik} and E_{jh}; and α'_{ih} and β'_{jk} are the genotype-specific (i) and environment-specific (j) regression coefficient of environmental covariate E_{jh} and genotypic covariate G_{ik}, respectively. ε_{ij} is the residual interaction effect. All sources of variation were considered fixed. Selection of the covariates and their order in the factor regression model were obtained by performing a stepwise regression on genotype covariates (n = 6) and a second one on environmental covariates (n = 36 for ergosterol and total FB concentrations; n = 24 for *F. verticillioides* infection), using genotypic and environmental means, respectively (Denis, 1988). After standardization of covariates, factorial regression analyses were performed with the INTERA software (Decoux and Denis, 1991). All terms were tested against the residual experimental error.

3. RESULTS

3.1. *F. verticillioides* infection and fungal growth in maize kernels

The average percentage of kernels infected by *F. verticillioides* was 18.6%, while the percentage of kernels infested by *F. verticillioides* (not surface disinfected kernels) reached 78.3%. Analysis of variance showed that differences for *F. verticillioides* infection were significant among years, locations, harvest dates, and hybrids, and non-significant differences were found between planting dates. Infection was lower at early harvests (16.9%) than at late harvests (20.3%) and varied among locations from around 5% (in the inland location) to 35% (Table 1). Hybrids showed significant differences for *F. verticillioides* infection, hybrids CM151 × EP39 and EP42 × EP47 having more kernels infected by *F. verticillioides* (27.0% and 22.7%, respectively). *F. verticillioides* infestation had a similar behavior than *F. verticillioides* infection, but showed higher percentages, and differences were only significant among locations and harvest dates (Table 1).

Differences for ergosterol concentration were significant among locations, planting dates, and hybrids (Table 1). Ergosterol concentration in kernels was lower after late plantings (2.29 μg/g) than early plantings (5.97 μg/g). Hybrids CM151 × EP39 and EP42 × EP47 had the lowest (1.71 μg/g) and the highest (6.13 μg/g) ergosterol concentrations, respectively.

3.2. Fumonisin content in maize kernels and relationship between fumonisin content, *F. verticillioides* infection, and fungal growth

Around 60% of the kernel samples analyzed showed detectable levels of fumonisin ranging from 0.02 to 27.8 μg/g. Differences for FB_1, FB_2 and total FB concentration were significant between years, locations, and harvest dates (except for FB_2 in the latter), and non-significant differences were found between planting dates and hybrids (Table 1). In 2008, total FB content reached 7.3 μg/g in one location, Pontevedra (data not shown), above the allowed levels for human consumption in the EU [4 μg/g in unprocessed maize (Commission Regulation 1126/2007)]. There were significant differences between locations with the inland location showing the lowest fumonisin concentrations (0.4 μg/g for total FB) (Table 1). Kernels harvested earlier had significant lower fumonisin content than those harvested one month later (1.4 μg/g and 2.2 μg/g, respectively, for total FB), while fumonisin accumulation tended to be lower after later plantings than after earlier plantings. Early

planting-late harvest combination reached the highest fumonisin concentration in kernels (2.7μg/g) which significantly differed from other planting-harvest combinations (data not shown).

FB_1, FB_2, and total FB concentrations were highly correlated with *F. verticillioides* infection (r = 0.76, $p \leq 0.01$, for total FB). However, fumonisins had a low correlation with *F. verticillioides* infestation (r = 0.34, $p \leq 0.05$, for total FB), and were not correlated with ergosterol contents ($p > 0.05$), except FB_2 (r = 0.41, $p \leq 0.05$).

F. verticillioides infection was significantly correlated with *F. verticillioides* infestation (r = 0.43, $p \leq 0.05$), but was not correlated with ergosterol concentration (r = 0.23, $p > 0.05$). Ergosterol concentration and *F. verticillioides* infestation showed a low correlation (r = 0.40, $p \leq 0.05$).

3.3. Ear traits

Results of the analyses of variance for ear traits such as ear damage by corn borers, *S. cerealella* damage, husk tightness, kernel moisture, pericarp thickness, and pericarp damage at harvest are also showed in Table 1. There were significant differences among all main factors for all traits, except between plantings and harvests for ear damage by corn borers and between plantings for kernel damage by *S. cerealella*. In the inland location, Valongo, husk tightness, kernel moisture, and pericarp thickness were higher and insect damage and pericarp damage were lower than in the coastal locations. This behavior was also observed after late plantings or early harvests compared to early plantings and late harvests, respectively, except for ear damage by corn borers.

3.4. Factorial regression analyses

Environment explained most of the variation for *F. verticillioides* infection (61.5%) and for total FB content in maize kernels (77.7%). The effect of the genotypic factors on the variation for *F. verticillioides* infection and ergosterol content had minor importance and for total FB content was not significant (Tables 2-4). However, GE interaction was significant for all characters. For this reason, we considered appropriate to include genotypic covariates in the factorial regression analysis for total FB concentration. GE interaction explained most of the variation for ergosterol content (57.7%).

3.4.1. *F. verticillioides* infection

Two environmental and three genotypic covariates were selected after stepwise regression analyses ($p \leq 0.15$ and $p \leq 0.25$, respectively) and included in the factorial regression model for *F. verticillioides* infection (Table 2). According to the model, environmental variation for *F. verticillioides* infection was most explained by the number of days with rainfall ≥ 2 mm during the flowering period (70.1%), and by relative humidity from 4 days before to 2 days after silking (C2) (13.5%). Both covariates had a negative effect on *F. verticillioides* infection. The residual environmental variation (variation not explained by the environmental covariates) was significant; therefore, environmental covariates not included in the model might have significant effects on *F. verticillioides* infection.

Genotypic variability for *F. verticillioides* infection was largely explained by the genotypic covariate ear damage by corn borers (66.9%), followed by plant height (21.4%). Thus, less ear damage (higher ratings) was related to less *F. verticillioides* infection. Husk tightness had a non-significant effect as individual covariate but it was included in the model because it had significant effects in the GE variability. The interactions between the genotypic covariates and the residual environmental variation significantly explained most of the GE interaction effect on *F. verticillioides* infection (ca. 68%), while the only significant cross-product was husk tightness × relative humidity during C2 (Table 2).

3.4.2. Ergosterol concentration

Three environmental and one genotypic covariates were selected and included in the factorial regression model after stepwise regression analyses ($p \leq 0.15$) (Table 3). The percentage of kernels with damaged pericarp explained most of the environmental variability (65%) and had a positive effect on ergosterol concentration. The number of days with minimum temperatures ≤ 15 °C during the flowering period and the daily minimum temperatures from 2 to 8 after silking (C3) had a negative effect on ergosterol concentration, and explained 15% of environmental variation.

Husk tightness explained most of the genotypic variation (79.5%) and had a negative effect on ergosterol concentration. GE variability was most explained by the interaction between husk tightness and the residual environmental variation (19.9%), followed by the interactions between genotypic residual variation and the percentage of kernels with damaged pericarp (5.8%) and days with minimum temperatures ≤ 15 °C during the flowering period (4.9%). The only significant cross-

product was percentage of kernels with damaged pericarp × husk tightness and accounted for 3.7% of ergosterol variability due to GE interaction.

3.4.3. Total FB concentration

Three environmental and two genotypic covariates were selected using stepwise regression analyses ($p \leq 0.15$ and $p \leq 0.25$, respectively) and included in the factorial regression model for total FB concentration (Table 4). Ear damage by corn borers explained most of the environmental variation (70.1%); less ear damage (higher ratings) was related to less total FB content in kernels. The number of days with maximum temperature ≥ 30 °C from 2 to 8 after silking (C3) and days with rainfall ≥ 2 mm during the drying period had a positive effect on total FB concentration, and both accounted for approximately 20% of the environmental variation. The residual environmental variation was significant meaning that environmental covariates not included in the model could have significant effects on fumonisin production.

The genotypic covariates included in the model were number of kernels damaged by *S. cerealella* and pericarp thickness. The interactions between environmental and genotypic covariates explained a low percentage of GE variability; only the cross-product pericarp thickness × number of days with rainfall ≥ 2 mm during the drying period was significant (Table 4). The interaction between the number of kernels damaged by *S. cerealella* and the residual environmental variation explained the higher percentage of GE variability (24.2%). The interaction between ear damage by corn borers and genotypic residual variation had also a significant effect on total FB concentration and explained approximately 3.8% of GE variability. The residual GE variation accounted for 51% of GE variation but its effect on total FB was not significant.

4. DISCUSSION

4.1. *F. verticillioides* infection, fungal growth and fumonisin content in maize kernels

This study corroborates the noteworthy risk of kernel contamination with fumonisin in northwestern Spain conditions, and brings extensive information about critical environmental and genotypic factors affecting *F. verticillioides* infection, fungal growth and fumonisin accumulation in maize kernels.

Natural kernel infestation by *F. verticillioides* in northwestern Spain fields is very frequent, which suggests that local conditions are very favorable for *F. verticillioides* dispersal and growth. Natural infection by *F. verticillioides*, i.e. frequency of its occurrence inside kernels, is lower or much lower. This is consistent with the moderate level of aggressiveness of *F. verticillioides* (Farrar and Davis, 1991; Reid et al., 2002) and the difficulty of overcoming plant barriers to access into the kernel; documented *F. verticillioides* main pathways into kernels are through pericarp wounds and the stylar canal (Duncan and Howard, 2010; Parsons and Munkvold, 2010). The difference between infection and infestation frequencies suggests that it is possible to find environmental and genetic factors that impede *F. verticillioides* entry to kernels.

Ergosterol concentration was used as an indirect measure of the fungal biomass. In particular, we used it as a measure of *F. verticillioides* growth in maize kernels, on the basis of previous reports in the area that indicate that *F. verticillioides* is by far the most prevalent fungal species recovered from kernels (Aguín et al., 2013; Cao et al., 2013; Muñoz et al., 1990). Ergosterol content had a positive correlation with *F. verticillioides* infestation but it was not correlated with *F. verticillioides* infection. Thus, ergosterol content appears to estimate the fungal biomass related to kernel infestation and not the fungal biomass related to infection, that is, inside the kernel. The large difference in *F. verticillioides* infection and infestation could mean that the fungal growth on kernel surfaces exceeds the fungal growth within the kernels.

F. verticillioides disease progress, evaluated by visual ratings, symptomatic kernels, frequency of infected kernels, etc, is often used as an indirect measure of fumonisin content in maize kernels (Löffler et al., 2010; Parsons and Munkvold, 2010; Pascale et al., 1997; Presello et al., 2007), but in some cases this relation was found moderate or non-existent (Clements et al., 2003; Eller et al., 2008).

In agreement to previous reports, *F. verticillioides* infection was highly and positively correlated with fumonisin content. However, total fumonisin content had a low correlation with *F. verticillioides* infestation and was not correlated with ergosterol content. The spread of infection appeared to have a greater effect on fumonisin content than the fungal biomass. *F. verticillioides* mainly penetrates into the kernel through the pericarp or systemically through the pedicel (Zummo and Scott, 1990), which determines that the initial fungal growth inside the kernel often occurs in the pericarp and, to a lesser extent, in the germ, so these tissues tend to accumulate more fumonisin (Bullerman and Bianchini, 2007). Nevertheless, fumonisin production is determined by the substrate, and endosperm is the most conducive kernel tissue for fumonisin production (Bluhm and Woloshuk, 2005). If the infection spreads and the fungus reach the endosperm, fumonisin accumulation would increase significantly with respect to the amount of fungal biomass in the kernel.

4.2. Factors affecting variability for *F. verticillioides* infection, fungal growth, and fumonisin content in maize kernels

The results confirmed the determinant role of the environmental factors on *F. verticillioides* infection and fumonisin accumulation in maize kernels. The use of suitable agronomic practices can change the environmental conditions and, consequently, reduce fungal infection and fumonisin contamination (Battilani et al., 2008; Maiorano et al., 2009a). Early plantings and harvests are associated with less *F. verticillioides* infection and fumonisin contamination (Abbas et al. 2007; Blandino et al., 2009; Parsons and Munkvold, 2010). Early harvests reduce the exposure of kernels to insect attack and the accumulation of mycotoxins in the field. Early plantings reduce the risk of overlapping the flowering period with warmer and drier weather, which are more favorable conditions for *F. verticillioides* infection and fumonisin production (de la Campa et al., 2005; Shelby, 1994), avoiding harvest delays. Increased fumonisin occurrence after late plantings was also associated with higher incidence of thrips in immature ears (Parsons and Munkvold, 2010) and higher damage by borers (Blandino et al., 2008, 2009).

As expected, kernels harvested later had higher fumonisin contents and infection by *F. verticillioides* than those collected one month earlier. However, early planting tended to accumulate more fumonisin and fungal growth than late planting, confirming previous results in this area (Cao et al., 2013). Both early planting and late harvest resulted in the lowest husk tightness, pericarp thickness,

and kernel moisture, and consequently, the highest percentage of kernels with damaged pericarp, as well as the highest *S. cerealella* damage. Unlike other insect attack, *S. cerealella* is related to early plantings. The kernel moisture threshold which allows *S. cerealella* infestation to occur is reached quicker after early plantings (Weston, 1994), and the kernel damage produced by the attack facilitates the entry of fungi and favors their development by increasing local moisture content (Imura and Sinha, 1984). Thus, while corn borer damage, mostly produced by *S. nonagrioides* (Velasco et al., 2007), was not conditioned by the planting date, the high incidence of *S. cerealella* advises against early plantings under northwestern Spain conditions for preventing fumonisin contamination.

4.2.1. Environmental and genotypic factors affecting variability for *F. verticillioides* infection

By constructing factorial regression models, this study detailed the critical environmental and maize genotypic factors influencing *F. verticillioides* infection and growth and fumonisin accumulation in maize kernel, and their contribution to the variation of these traits.

Most of the variation for *F. verticillioides* infection was explained by environmental factors, specifically by conditions related to rain and relative humidity around silking, a critical time for ear infection. Hard rain during the flowering period and higher relative humidity around mid-silk reduced the percentage of *F. verticillioides* infection. In previous work, rain and humidity were favorable to the initation of plant infection by *F. verticillioides*, since water splashing disperses fungal propagules and higher moisture levels favor spore germination and mycelial growth (Marín et al., 2004; Ooka and Kommedahl, 1977). However, hard rain could limit spore dispersal and wash off inoculum reservoirs (Rossi et al., 2009), and high rain intensities during flowering have been related to reduced kernel contamination with fumonisin (Maiorano et al., 2009b).

Dry conditions during flowering may increase *F. verticillioides* infection at harvest, perhaps by causing hydric stress in maize plants and rending them more susceptible to insect and fungal attack (Ariño and Bullerman, 1994; Fandohan et al., 2003; Shelby, 1994). Alternatively, dry warm conditions during flowering could favor the movement of insects inside the ear and enable fungal infection via hitchhiking on insects or through the wounds created by the insect activity (Parsons and Munkvold, 2010; Sobek and Munkvold, 1999). In agreement to the latter, ear damage by corn

borers was the genotypic covariate that explained the highest proportion of the genotype effect, i. e. increased ear damage by corn borers was correlated with increased kernel infection by *F. verticillioides*. Maize genotypes resistant to corn borer attack usually have a lower incidence of *F. verticillioides* infection and ear rot severity (Bakan et al., 2002; Munkvold et al., 1997).

The importance of ear damage by insects to kernel infection by *F. verticillioides* also can be seen in the significant GE interactions. Under low relative humidity conditions at silking, genotypes with less husk tightness became more susceptible to kernel infection by *F. verticillioides*, since husks are a known barrier to insect attack and insect incidence is favored by low humidity and high temperature (Parsons and Munkvold, 2010; Velasco et al., 2007). Much of the GE variation also was explained by the interactions between the covariates ear damage by corn borers and husk tightness and the residual environment variability of *F. verticillioides* infection. Thus, environment factors not included in the model could interact with these covariates to affect *F. verticillioides* infection.

4.2.2. Environmental and genotypic factors affecting variability for ergosterol concentration

Environment main effect and the GE interaction explained most of the variation for ergosterol concentration in kernels. The differences among environments for ergosterol content were largely due to the percentage of kernels with damaged pericarp, suggesting that environments with conditions conducive to further kernel damage, such as increased insect incidence, bird damage, or days with wide temperature fluctuations, which may cause cracks in kernels, favored fungal growth. Much of the kernel pericarp damage was produced by *S. cerealella,* reemphasizing the importance of insect incidence in the variation of fungal growth. Two climatic variables related to flowering also were involved in the variability for ergosterol concentration. Minimum temperatures were critical: More days with minimum temperatures ≤ 15 °C during the flowering period and lower minimum temperatures in the first days after mid-silk (from 2 to 8 days later) reduced ergosterol concentrations in kernels. Low temperatures are unfavorable for *F. verticillioides* sporulation and growth; *F. verticillioides* growth increases with temperature up to 25-30 °C, and no growth was detected at < 10 °C in maize kernels (Marín et al., 2004; Rossi et al., 2009). Therefore, low temperatures during flowering could be critical to reduce colonization by *F. verticillioides* and slow its growth.

Husk tightness explained most of the variation for ergosterol concentration due to genotype effects. Hybrids with tighter husk had less ergosterol in their kernels. Good husk coverage protects the kernels against external damage and reduces kernel colonization by windborne or waterborne spores. Husk tightness × percentage of kernels with damaged pericarp was the only significant genotype × environment cross-product. This result suggests that husk tightness was a more effective barrier against fungal growth in maize kernels in environments conducive to pericarp damage. Furthermore, an important fraction of the variation due to GE interaction was explained by the interaction between husk tightness and the residual environment variation for ergosterol concentration. Thus, other environmental covariates not included in the model also may be interacting with husk tightness to limit fungal growth.

F. verticillioides infection and ergosterol concentration were not correlated and they were affected by different factors, yet both characters were influenced by interactions between husk tightness and environmental covariates. Thus, the more favorable the environmental conditions are for *F. verticillioides* infection and fungal growth, the more beneficial higher husk tightness is for the reduction of both characters.

4.2.3. Environmental and genotypic factors affecting variability for fumonisin content

Environmental differences were extremely important for fumonisin contamination of maize kernels, and the conditions during flowering and kernel drying were critical. Most of environmental variation was due to ear damage by corn borers; environments more conducive to ear damage by corn borers increased fumonisin content in kernels. The relationship between corn borer attack and fumonisin contamination has been noted by several authors (Avantaggiato et al., 2003; Blandino et al., 2008; Munkvold et al., 1997). In northwestern Spain conditions, ear damage is caused by the larvae of the second generation of *S. nonagrioides*. Larvae initiate their activity in late August or September (Velasco et al., 2007), when the kernel drying begins, so much of the ear damage by corn borers is produced during this period. Additionally, during the kernel drying period fumonisin accumulation was affected by rainfall; more days with moderate-hard rainfall increased fumonisin contamination, probably because of slower drying which delay the decrease of water activity to unfavorable levels for fumonisin production (Marín et al., 2004). Rainfall before harvest has been

previously associated with increased *F. verticillioides* infection and fumonisin contamination (Fandohan et al., 2003; Munkvold, 2003).

During the flowering period more days with maximum temperatures ≥ 30 °C favored fumonisin contamination. As discussed above, high temperatures around silking increase *F. verticillioides* infection and fumonisin accumulation. Note that in the model developed by de la Campa et al. (2005), daily maximum temperatures ≥ 34 °C at flowering were also critical for fumonisin accumulation. In our conditions, temperatures never reached that value, but maximum temperatures were indeed determinant variables for fumonisin contamination.

The influence of genotype factors on fumonisin contamination of maize kernels resided in their interaction with the environmental factors. *S. cerealella* damage and pericarp thickness were the genotypic factors with a significant effect on fumonisin concentration. Higher *S. cerealella* damage in maize hybrids increased fumonisin content, probably because it favors fungal dispersal and growth. However, contrary to expectations, a thicker pericarp favored fumonisin accumulation in kernels. It is considered that a thicker pericarp offers more resistance to breaking and to insect damage, and it was related with less kernel infection by *F. verticillioides* (Hoenisch and Davis, 1994). However, a thicker pericarp could slow down the kernel drying, moisture conditions within the kernel being longer favorable for fungal growth and fumonisin production. A thicker pericarp could also increase the slow-down of kernel drying due to the moderate-high rainfall, extending the period of optimal conditions for fumonisin production.

In summary, attention should be paid to two critical periods throughout the growing season for maize kernel contamination with fumonisin: flowering and kernel drying. Around silking, hard rainfall and higher humidity conditions limited kernel infection by *F. verticillioides*, low temperatures limited fungal growth, and high temperatures increased kernel contamination with fumonisins. During the drying period, a higher percentage of damaged kernel favored fungal growth, and higher ear damage by corn borers and more days with hard rainfall increased fumonisin content in kernels.

Later plantings and especially earlier harvests had a lower risk of fumonisin contamination, possibly due to result in a lower incidence of insects such as *S. cerealella* and corn borers and a

reduced accumulation of rainfall during the kernel drying period. Although the hybrids used in this study had non-significant differences for fumonisin contamination, tighter husk coverage, high kernel resistance to insect attack by *S. cerealella* and non excessive pericarp thickness seemed favorable characteristics to reduce fumonisin contamination in maize kernels.

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TABLES

Table 1. Means of the percentages of kernels infected and infested by *F. verticillioides*, ergosterol and fumonisin concentrations (μg/g) in kernels and traits related to ear and kernel characteristics of six maize hybrids, at three locations, two planting dates and two harvest dates in 2007, 2008 and 2009. The percentage of kernels infected by *F. verticillioides* was determined at two years (2008 and 2009)[a].

		F. verticillioides		Ergosterol (μg/g)	FB_1 (μg/g)	FB_2 (μg/g)	Total FB (μg/g)	Ear rating (1-9)[b]	*S. cerealella* damage (no.)	Husk tightness (1-5)	Kernel moisture (%)	Pericarp thickness (μm)	Damaged kernels (%)
		Infection (%)	Infestation (%)										
Year	2007	-	75.75 a	2.74 a	0.30 b	0.06 c	0.36 b	8.90 a	4.88 c	2.45 b	18.77 c	90.78 a	6.63 b
	2008	26.09 a	78.70 a	6.12 a	3.73 a	0.33 a	4.06 a	8.45 c	11.22 b	3.13 a	22.81 a	82.11 b	10.32 a
	2009	11.51 b	80.28 a	3.41 a	0.74 b	0.20 b	0.94 b	8.80 b	15.02 a	2.19 c	20.05 b	82.71 b	9.82 a
	LSD	3.97	-	-	0.74	0.08	0.78	0.08	3.11	0.22	0.30	1.81	1.21
Location	Pontevedra	35.32 a	83.76 a	2.79 b	2.71 a	0.26 a	2.97 a	8.61 b	10.28 b	2.34 b	17.91 c	83.93 b	7.23 b
	Barrantes	14.75 b	84.29 a	8.88 a	1.62 b	0.28 a	1.90 b	8.57 b	21.11 a	2.47 b	18.41 b	84.09 b	14.95 a
	Valongo	4.56 c	66.56 b	0.70 b	0.36 c	0.04 b	0.40 c	8.96 a	0.50 c	2.94 a	25.37 a	87.43 a	4.90 c
	LSD	4.86	5.06	3.06	0.74	0.08	0.78	0.08	3.11	0.22	0.30	1.81	1.21
Planting	Early	18.75 a	79.99 a	5.97 a	1.80 a	0.20 a	2.00 a	8.74 a	11.71 a	2.47 b	19.46 b	84.17 b	10.45 a
	Late	18.43 a	76.63 a	2.29 b	1.38 a	0.19 a	1.57 a	8.69 a	9.17 a	2.68 a	21.55 a	86.05 a	7.54 b
	LSD	-	-	2.50	-	-	-	-	-	0.18	0.25	1.48	0.99
Harvest	Early	16.93 b	74.75 b	3.34 a	1.20 b	0.20 a	1.40 b	8.70 a	5.57 b	2.67 a	22.96 a	87.98 a	6.32 b
	Late	20.27 a	81.83 a	4.83 a	1.96 a	0.19 a	2.16 a	8.73 a	15.18 a	2.50 b	18.12 b	82.29 b	11.59 a
	LSD	3.29	3.41	-	0.32	-	0.34	-	1.15	0.09	0.22	1.16	0.82
Hybrid	CM151×EP39	26.98 a	78.59 a	1.71 c	1.20 a	0.19 a	1.39 a	8.81 a	6.76 b	3.73 a	20.20 c	85.21 c	5.89 c
	CM151×EP42	14.51 c	78.50 a	5.11 ab	1.70 a	0.17 a	1.87 a	8.71 bc	12.34 a	2.37 d	18.53 d	74.34 e	8.96 b
	CM151×EP47	15.63 c	77.61 a	2.79 bc	1.60 a	0.19 a	1.79 a	8.69 c	12.20 a	2.73 c	20.75 b	68.30 f	9.23 b
	EP39×EP42	16.13 c	76.87 a	3.91 abc	1.82 a	0.24 a	2.06 a	8.80 ab	9.31 b	3.22 b	20.91 b	105.40 a	6.48 c
	EP39×EP47	16.86 bc	77.96 a	4.60 abc	1.50 a	0.18 a	1.68 a	8.67 c	8.97 b	1.96 e	22.03 a	95.91 b	10.36 b
	EP42×EP47	22.69 ab	80.15 a	6.13 a	1.64 a	0.20 a	1.84 a	8.63 c	12.47 a	1.61 f	20.75 b	82.18 d	12.36 a

LSD	6.11	-	3.05	-	-	-	0.09	2.60	0.23	0.41	2.30	1.56

[a] Within each column and factor means followed by the same letter did not differ at the 0.05 probability level.

[b] Ear rating: ear damage by borers on a visual rating scale from 1 (> 90% damaged) to 9 (no damage); Husk tightness: on a visual scale from 1 = loose husks with visible cob to 5 = tight husks.

Table 2. Analysis of variance of the factorial regression model including two environmental and three genotypic covariates for the percentage of kernels infected by *F. verticillioides*, in trials conducted with six maize hybrids at three locations, two sowing dates, and two harvest dates in 2008 and 2009.

Source of variation[a]	Sum of squares	DF	Mean of squares	*F*	% Sum of squares[b]	Regression coefficient
Environment (E)	**35283.00**	**23**	**1534.04**	**15.08****	**61.46**	
R2mmF	26187.31	1	26187.31	248.40**	70.09	-9.53
RHC2	5059.90	1	5059.90	48.00**	13.54	-7.39
Residual	6112.88	21	291.09	2.80**	16.36	
Genotype (G)	**1984.87**	**5**	**396.97**	**3.77****	**3.46**	
Plant height (PH)	410.78	1	410.78	3.90*	21.38	-7.05
Ear rating (ER)	1285.41	1	1285.41	12.20**	66.92	-9.11
Husk tightness (HT)	91.93	1	91.93	0.90	4.79	
Residual	132.80	2	66.40	0.60	6.91	
G × E	**20143.33**	**113**	**178.26**	**1.69****	**35.09**	
PH × R2mmF	150.59	1	150.59	1.40	0.70	
PH × RHC2	234.17	1	234.17	2.20	1.09	
PH × E	5196.64	21	247.46	2.30**	24.21	
ER × R2mmF	216.04	1	216.04	2.00	1.01	
ER × RhC2	6.23	1	6.23	0.10	0.03	
ER × E	4751.34	21	226.25	2.10*	22.14	
HT × R2mmF	0.03	1	0.03	0.00	0.00	
HT × HrC2	515.91	1	515.91	4.90*	2.40	-9.12
HT × E	4678.50	21	222.79	2.10**	21.80	
R2mmF × G	214.78	2	107.39	1.00	1.00	
RhC2 × G	302.74	2	151.37	1.40	1.41	
Residual	5197.00	42	123.74	1.20	24.21	

Error	**24035.40**	**228**	**105.42**

*, ** Significant at 0.05 and 0.01 probability levels, respectively.

[a] R2mmF: number of days with rainfall ≥ 2 mm during the flowering period (from 15 days before to 15 days after mid-silk); RhC2: average of daily relative humidity (%) during critical period 2, from 4 days before silking to 2 days after silking; Ear rating (ER): ear damage by corn borers on a visual rating scale from 1 (> 90% damaged) to 9 (no damage); Husk tightness (HT) on a visual scale from 1 = loose husks with visible cob to 5 = tight husks.

[b] In bold, percentage of variation explained for *F. verticillioides* infection. Without bold, percentage of sum of squares of the corresponding main or interaction effect explained by each covariate and cross-product of covariates.

Table 3. Analysis of variance of the factorial regression model including three environmental and one genotypic covariates for the ergosterol concentration (μg/g) in kernels from trials conducted with six maize hybrids at three locations, two sowing dates, and two harvest dates in 2007, 2008 and 2009.

Source of variation[a]	Sum of squares	DF	Mean of squares	*F*	% Sum of squares[b]	Regression coefficient
Environment (E)	**5141.00**	**35**	**146.89**	**2.65****	**37.87**	
Damaged kernels (DP)	4350.38	1	4350.38	127.10**	64.99	3.80
Tmin15F	793.64	1	793.64	23.20**	11.86	-2.64
TminC3	214.72	1	214.72	6.30*	3.21	-1.22
Residual	1334.93	32	41.72	1.20	19.94	
Genotype (G)	**605.94**	**5**	**121.19**	**3.54****	**4.46**	
Husk tightness (HT)	494.33	1	494.33	14.40**	79.49	-1.66
Residual	127.52	4	31.88	0.90	20.51	
G × E	**7828.33**	**173**	**45.25**	**1.32***	**57.67**	
HT × DP	292.33	1	292.33	8.50**	3.36	-1.25
HT × Tmin15F	96.08	1	96.08	2.80	1.10	
HT × TminC3	54.02	1	54.02	1.60	0.62	
HT × E	1733.89	32	54.18	1.60*	19.94	
DP × G	504.56	4	126.14	3.70*	5.80	
Tmin15F × G	427.96	4	106.99	3.10*	4.92	
TminC3 × G	94.63	4	23.66	0.70	1.09	
Residual	5493.23	128	42.92	1.30	63.16	
Error	**11504.89**	**336**	**34.24**			

*, ** Significant at 0.05 and 0.01 probability levels, respectively.

[a] Damaged kernels (DP): percentage of kernels with damaged pericarp; Tmin15F: number of days with minimum temperature ≤ 15 °C during the flowering period (from 15 days before to 15 days after mid-silk); TminC3: average of daily minimum temperatures during critical period 3, from 2 to 8 days after silking; Husk tightness (HT) on a visual scale from 1 = loose husks with visible cob to 5 = tight husks.

[b] In bold, percentage of variation explained for *F. verticillioides* infection. Without bold, percentage of sum of squares of the corresponding main or interaction effect explained by each covariate and covariate cross-products.

Table 4. Analysis of variance of the factorial regression model which explains variation for total FB concentration (FB_1+FB_2) (μg/g) in kernels from trials conducted with six maize hybrids at three locations, two sowing dates, and two harvest dates in 2007, 2008, and 2009.

Source of variation[a]	Sum of squares	DF	Mean of squares	*F*	% Sum of squares[b]	Regression coefficient
Environment	**1290.24**	**35**	**36.86**	**9.22 ****	**77.70**	
Ear rating (ER)	698.47	1	698.47	490.7 **	53.54	-1.22
Tmax30F	168.60	1	168.60	118.5 **	12.92	1.06
R2mmS	96.64	1	96.64	67.9 **	7.41	0.71
Residual	340.75	32	10.65	7.5 **	26.12	
Genotype	**10.38**	**5**	**2.08**	**1.46**	**0.62**	
S. cerealella damage (SD)	5.59	1	5.59	3.9 *	45.03	0.30
Pericarp thickness (PT)	5.47	1	5.47	3.8 *	44.09	0.22
Residual	1.35	3	1.24	0.3		
Genotype x Environment	**360.02**	**173**	**2.08**	**1.46 ****	**21.68**	
SD x ER	3.55	1	3.55	2.5	0.98	
SD x Tmax30F	2.13	1	2.13	1.5	0.59	
SD x R2mmS	4.67	1	4.67	3.3	1.29	
SD x Environment	87.52	32	2.73	1.9 **	24.18	
PT x ER	3.65	1	3.65	2.6	1.01	
PT x Tmax30F	0.67	1	0.67	0.5	0.18	
PT x R2mmS	11.38	1	11.38	8 **	3.14	0.33
PT x Environment	39.71	32	1.24	0.9	10.97	
ER x Genotype	13.56	3	0.45	3.2 *	3.75	
Tmax30F x Genotype	2.78	3	4.52	0.7	0.77	
R2mmS x Genotype	7.85	3	2.62	1.8	2.17	
Residual	184.42	96	1.92	1.3	50.96	
Error	**482.48**	**339**	**1.42**			

*, ** Significant at 0.05 and 0.01 probability levels, respectively.

[a] Ear rating (ER): ear damage by corn borers on a visual rating scale from 1 (> 90% damaged) to 9 (no damage); Tmax30F: number of days with maximum temperature ≥ 30 °C during the flowering period; R2mmF: number of days with rainfall ≥ 2 mm during the flowering period; *S. cerealella* damage (SD): number of kernels per ear perforated by the insect; Pericarp thickness (PT) measured in μm.

[b] In bold, percentage of variation explained for the trait total FB concentration. Without bold, percentage of sum of squares within the corresponding main or interaction effect explained by each covariate or cross-product.

HIGHLIGHTS

- Factorial regression models detail critical factors for infection, ergosterol and fumonisin contamination
- *F. verticillioides* infection but not ergosterol was highly related to fumonisin content
- Environmental factors were determinant for infection and fumonisin contamination
- Flowering and kernel drying were the most critical periods for fumonisin contamination
- Later plantings and earlier harvests reduced infection and fumonisin contamination