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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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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

Fusarium 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

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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 abovementioned 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 with 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 attacks 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 attacks (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 × location × planting × 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 min 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; and 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.