Dynamic of water activity in maize hybrids is crucial for fumonisin contamination in kernels

Paola Battilani[a,*], Silvia Formenti[a], Cesare Ramponi[b], Vittorio Rossi[a]

[a] Institute of Entomology and Plant Pathology, Faculty of Agriculture, Università Cattolica del Sacro Cuore, via Emilia Parmense 84, I-29122 Piacenza, Italy
[b] Pioneer Hi-Bred Italia, Via Pari Opportunita' 2, I-26030 Gadesco Pieve Delmona, Cremona, Italy

ABSTRACT

The dynamic of water activity (a_w) and humidity (h) of maize (*Zea mays* L.) kernels and their relevance for fumonisin (FUM) accumulation in kernels was studied in 10 commercial hybrids grown in 5 locations of North Italy, in 2007 and 2008. The dynamic of both a_w and h in maize kernels was different in diverse hybrids and was accurately described by monomolecular and linear regression, respectively, using degree-days (base 0 °C) accumulated between female flowering and harvest as an independent variable ($R^2 = 0.61-0.96$, depending on the hybrid). FUM contamination at harvest was predicted by using a_w as an independent variable in a logistic regression which provided 67% of correct prediction of cases with FUM $\geq$ 4000 μg/kg of kernel; accuracy of prediction increased to 72% by using both a_w and ECB severity as independent variables. The use of h as independent variable provided 71% correct predictions, but specific correction factors were necessary for each hybrid. Results showed that "slow dry down" hybrids were more prone to FUM accumulation, irrespective of their season length. The identification of factors able to drive a_w dynamic in maize kernels and their genetic bases may then provide a crucial contribution in breeding maize for resistance to FUM contamination.
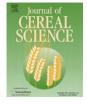
1. Introduction

Zea mays L. is a worldwide distributed crop and *Fusarium verticillioides* Sacc. (Nirenberg; sin. *Fusarium moniliforme*) and *Fusarium proliferatum* are commonly associated kernel pathogens in most growing areas, especially those with moderately hot and dry weather occurring after flowering (Logrieco et al., 2002; Munkvold, 2003). These fungi are the main cause of *Fusarium* ear rot or pink ear rot, a white or light pink mould which typically affects random kernels, groups of kernels or physically injured kernels (Miller, 1994); *F. verticillioides* is frequently found also in symptomless kernels (Munkvold et al., 1997).

F. verticillioides and *F. proliferatum* produce fumonisins (FUM), a group of compounds, the most prevalent being fumonisin B_1 (FB_1). Fumonisins are the main mycotoxin of concern in different maize-growing areas (Bottalico et al., 1995; Chulze et al., 1996; Doko et al., 1996; Kedera et al., 1994; Orsi et al., 2000; Pietri et al., 2004; Shetty and Bhat, 1997) where they represent a serious,

multifaceted economic problem related to grain yield reduction or production unfit for sale, and reduced animal productivity due to health problems and human health costs. Worldwide food legislation safeguards the health of consumers with mandatory or suggested limits on the amount of specific mycotoxins admitted in food and feed. In Europe, the maximum tolerated level for $FB_1 + FB_2$ in raw maize is 4000 μg/kg and lower levels are fixed for maize derived food for direct human consumption (European Commission regulation, 2007/1127). Recommendations for animal feed define the lowest concentration at 5 mg/kg for complete feeds destined for pigs and horses (European Commission recommendation, 2006/576).

Much effort has been devoted to define the factors able to influence, and the actions useful to mitigate, FUM contamination in maize kernels. Meteorological conditions are crucial (Battilani et al., 2003); i.e. temperature during flowering and rain/humidity in the last month before harvest explained the differences in FUM contamination in a Spanish study (Herrera et al., 2010). Relevant roles have also been attributed to the cropping system, the hybrids (Battilani et al., 2008; Blandino et al., 2009) and the pest borer attacks on ears (Dowd, 2003; Saladini et al., 2008). Protocols for good agricultural practices (Blandino et al., 2009) have been developed with encouraging results in terms of grain safety at harvest (Battilani et al., 2008).

The role of hybrids has not been clarified yet and transgenic approaches have recently been considered (Cary et al., 2009; Herrera et al., 2010). It is well known that both susceptibility to *F. verticillioides*, the most studied fungus, and FUM accumulation vary in commercial hybrids, but this variation has not been attributed to specific genetic traits (Lanubile et al., 2011). Since the production of FUM by *F. verticillioides* and *F. proliferatum* depends on water activity of the substrate (Marin et al., 1995), the dynamic of water availability during the ripening of different maize hybrids has been suggested as a possible genotype-related factor. The aim of this research was to study the dynamic of water in kernels in some maize commercial hybrids and the relationships between this factor and FUM production. The interaction between FUM and European corn borer (*Ostrinia nubilalis* Hübner, ECB) attacks on ears was also considered, as the trial was managed in natural conditions in the field.

2. Experimental

2.1. Experimental fields

Maize crops were grown in North Italy, in Lodi (45° 18′ 46″ N, 9° 29′ 53″ E) and Venice (45° 26′ 09″ N, 12° 20′ 15″ E) in 2007 and 2008, and in Pavia (45° 11′ 09″ N, 9° 09′ 24′ E) in 2008. Hereafter, these crops grown in different locations and years are mentioned as locations and detailed as LO07, VE07, LO08, VE08, and PV08. Ten maize hybrids belonging to the FAO classes 500, 600 and 700 (medium to late season), were sown according to a strip plot design (8 rows per plot, 5 m width, 5 m long) with three replicate plots. Hybrids were selected to include the variability in kernel characteristics of the commercial hybrids currently available in the market (Supplementary Table 1). Maize was seeded on 9 April, 16 April, 28 March, 2 May and 2 April, respectively in LO07, VE07, LO08, VE08 and PV08, and harvested in early October in all the locations. Maize crops were managed according to the ordinary cropping system for the area, without artificial inoculation by *Fusaria* or pesticides application on the growing crop. The prevalent growth stage of maize plants was detected at weekly intervals in all the plots, using the BBCH decimal codes (Weber and Bleiholder, 1990), starting from the tassel emergence (BBCH 51).

Hourly data of air temperature, relative humidity and rain were collected, from March to October, in meteorological stations placed close (less than 2 km) to the experimental fields.

2.2. Ear and kernels analysis

Ten ears were randomly collected at weekly intervals from each plot, from early dough growth stage (BBCH 83) to fully ripe (BBCH89). These ears were de-husked and the ECB attack was scored according to a reference scale (Supplementary Table 2) which included six levels of attack based on the presence of visible signs in particular parts of the ear. The application of this scale has been tested in previous studies (Battilani et al., unpublished data). The assessment is quick, it has good repeatability and reproducibility, and the results are well correlated to the number of ECB larvae per ear, shown as a good parameter to study ECB severity in relation to FUM contamination in maize (Mazzoni et al., 2011).

Ears were then hand shelled and 250 g of kernels were randomly collected from each 10-ear sample; humidity (h), water activity (a_w), and the content of FB_1 and FB_2 have been determined in each sample. Water activity in kernels was measured using the AquaLab LITE (version 1.3 © Decagon devices Inc., WA, USA) equipment. A dielectric humidity sensor measures the a_w and temperature of sample and its accuracy is ±0.015 a_w. Twenty

kernels, about 6 g, were randomly selected from each sample and measured immediately after delivery to the laboratory. Kernel moisture content (h) was determined following the method reported in the EC regulation (European Commission regulation, 2009/152). Briefly, maize kernels were dried in an oven at 120 °C for 24 h; kernel samples with moisture content above 17% were predried. Mass loss was determined by weighing the sample before and after drying with an analytical balance.

For the analysis of FUM (Visconti et al., 2001), maize kernels were ground to 1 mm particle diameters with a Retsch ZM200 mill (Retsch Italia srl, Italy); 12.5 g of ground maize were transferred to a flask, added with 1.25 g of salt (NaCl) and FUM were extracted with 50 mL of methanol:water (80:20 v/v) for 45 min and filtered. Ten mL of the filtered extract were diluted with 40 mL of Phosphate Buffer Solution (PBS: 8 g/l NaCl, 1.2 g/L Na_2HPO_4, 0.2 g/L KH_2PO_4, 0.2 g/L KCl; pH 7.0 with HCl 1:1), mixed and filtered through a microfibre filter (Vicam part #31955, VICAM, Milford, MA, USA).

Ten mL of filtered extract was cleaned with a FumoniTestTM WB affinity column (VICAM) and the flow rate was 0.2 mL min^{-1} followed by 10 mL of PBS; 1.5 mL HPLC grade methanol was added and the column was eluted at a flow rate of 0.2 mL min^{-1} and dried by a vacuum pump. The eluate was diluted with 1.5 mL of ultrapure water. Four hundred μL of a solution with 100 mg ortho-phthalaldehyde (OPA), 2.5 ml methanol, 12.5 mL Na tetraborate 0.1 M, 125 μL mercaptoethanol was added to a vial, 100 μL of diluted eluate was added, and mixed for 1 min and injected into HPLC. The limit of detection was 0.02 mg/kg and the recovery value for FB_1 and FB_2 was higher than 90%. Analytical results were reported in mg of FUM per kg of maize flour. FUM analysis was managed in a single run. In all samples with differences between replicates higher than 30%, the analysis was repeated.

2.3. Statistical analysis

A complete randomized block design combined over locations was used for the analysis of variance by using MSTAT (MSTAT-C Michigan State University, ver. 1.3, 1991, East Lansing, MI, USA). The effect of locations, maize hybrids and their interaction on the level of FUM content in maize kernels, ECB attack, a_w, and h at harvest were considered. Data on ECB and FUM content were transformed before data analysis using the natural logarithm function to homogenize variances (Clewer and Scarisbrick, 2001). The coefficient of variation (CV, in %) was calculated as the ratio of the standard deviation to the mean as a measure of variability.

Pearson correlation analysis was used to evaluate the relationship between FUM contamination and ECB score.

Values of a_w and h of each hybrid were regressed against the degree-days (DD, base temperature 0 °C) accumulated between female flowering (stigmata fully emerged, stage 65 of the BBCH decimal code) and harvest. Based on a preliminary analysis, a monomolecular equation was used for a_w and a regression line for h; the monomolecular equation was used in the following form:

$$a_w = 1 - \exp(-a*\exp(-b*DD/100))$$

where *a* and *b* are the equation parameters. The monomolecular equation is a simple function based on exponentials, also named saturating exponential growth function. This function has two parameters, namely, the initial value *a* and the growth rate *b* (Seber and Wild, 2003). In the equation used in this work, *a* and *b* make possible, respectively, the estimation of a_w at female flowering and the reduction of a_w over time expressed in degree-days.

Hybrids were then grouped on the basis of the estimated values of the equation parameters using cluster analysis and the quadratic Euclidean distance was considered for cluster definition.