Effect of Maize Hybrid Maturity and Grain Hardness on Fumonisin and Zearalenone Contamination

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Abstract

The level of resistance in commercial hybrids for Fusarium ear rot is still not in general adequate to prevent unacceptable toxin concentrations in field. The purpose of this experiment was to verify the behaviour of commercial dent maize hybrids for fumonisin and zearalenone contamination and to identify the variety traits that influence the production of these toxins.

Field experiments were carried out in 2000, 2001 and 2002 to evaluate the effect of maize hybrid maturity and endosperm hardness on European Corn Borer (ECB) incidence, fungal ear rot incidence and severity and on fumonisin B_1 and zearalenone contents. Nineteen yellow soft commercial hybrids, from the 500, 600 and 700 FAO maturity groups, were compared in 4 sites in NW Italy. Hybrid were grouped in 3 endosperm hardness categories (hard, intermediate, soft) in function of Hard/Soft (H/S) endosperm ratio.

No effect due to endosperm hardness or hybrid maturity on the ECB infestation or fungal ear rot incidence and severity was observed.

Grain hardness significant influenced fumonisin B_1 content: hard endosperm hybrids showed 50% lower contamination than soft hybrids. The presence of fumonisin B_1 in the grain of different maturity hybrids only resulted to be significantly different in 2001 experiment, with a mean concentration 2 times higher in the later hybrids (FAO rating 700) compared to the medium and medium-late hybrids.

The zearalenone content never resulted to be significantly different in function of the endosperm hardness, while, late maturing hybrids, in which grain moisture content decreases slowly below 30%, are more susceptible to zearalenone contamination.

This research has highlighted the presence of variety traits that can influence mycotoxin contamination. An accurate choice of hybrid, considering the territorial and cultivation context, could contribute to achieve products, that contain mycotoxins, which do not exceed the maximum international and UE regulation levels.

Key-words: fumonisin, Fusarium ear rot, hybrid, maize, mycotoxins, zearalenone.

Introduction

Mycotoxins, secondary metabolites of several fungi, are toxic to animals and humans because they can be found in a large variety of foods and feeds (D'Mello and Mac Donald, 1997; Bilgrami and Choudhary, 1998; Chelkowski, 1998). Among cereals, maize is the most commonly contaminated, mainly through ear infections during crop cultivation (Logrieco, 2001; Moretti et al., 2003; Pietri et al., 2004).

In South and Central Europe, the most common toxigenic field fungi, that are able to accumulate toxic metabolites in the grain before harvesting, belong to the *Fusarium* genus. This genus include in particular *F. verticillioides* (Saccardo) Nirenberg (= *F. moniliforme* Sheldon, teleomorf = *Gibberella subglutinans* (Edwards) Nelson, Toussoun et Marasas) producers of fumonisins (Bacon and Nelson, 1994; Dutton, 1996; Visconti et al., 1999) and *F. graminearum* Schwabe (teleomorf = *Gibberella zeae* (Schwein.) Petch), producers of zearalenone and trichothecenes (Sutton, 1982; Manka et al., 1989; Visconti et al., 1990).

At present time two approaches are known to reduce infections and mycotoxin accumulation in the field: pre-harvesting control strategies, which consist of crop practices designed to reduce the infection and the development of

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toxingenics fungi (Brown et al., 1998; AA.VV., 2004; Nicholson et al., 2004) and the utilization of genetically resistant hybrids or the identification of variety traits that are unfavourable for fungal development and toxinogenes (Duvick, 2001; Munkvold, 2003a).

Hybrid selection during the breeding process usually eliminates very susceptible genotypes, therefore commonly grown hybrids are not excessively susceptible (Munkvold, 2003a). Unfortunately hybrids with a high resistance to this pathology are not yet available on the market, as the process involved in the identification of natural resistances is complicated and costly, due to the necessity of carrying out inoculations of the pathogens and the analysis costs of the toxin content (Miedaner, 2004). In spite of these difficulties, much progress has been made in the identification of genetic resistance characteristics, especially against Gibberella and Aspergillus ear rot (Gorman et al., 1992; Brown et al., 1999; Hamblin and White, 2000; Vigier et al., 2001).

Several aspects of Gibberella ear rot resistance have been studied: Koehler (1959) and Enerson and Hunter (1980) demonstrated that correlations exist between Gibberella ear rot resistance and certain physical traits, such as husk tightness and the tendency of ears to remain upright. Assabgui et al. (1993) observed that there is a lower incidence of *F. graminearum* infection in hybrids with high concentrations of E-ferulic acid in the grain, Reid et al. (1992; 1994) identified a major gene which is responsible for the resistance to infection of *F. graminearium* through the silks, while Chungu et al. (1996) showed that resistance to silk infections and resistance to the subsequent diffusion of the pathogen in the kernels are under different genetic controls. Commercial hybrids that present these resistance mechanisms are not yet available, but some hybrids offered on the market show a partial resistance to Gibberella ear rot (Schaafsma et al., 1997; Payne, 1999).

The search for resistance to Fusarium ear rot, whose principal agent is *F. verticillioides*, is not so well documented in literature. Different susceptibility to Fusarium ear rot and different tendencies to accumulate fumonisins among commercial dent hybrids have been observed by Abbas et al. (2002), Pascale et al. (2002) and Kleinschmidt et al. (2005). The resistance sources that have been identified are polygenic and are therefore more difficult to incorporate in

hybrids (Snijders, 1994, Clements et al., 2003). Scott and King (1984) and Headrick and Pataky (1991) determined that the resistance factors of grain to *F. verticillioides* are neither in the endosperm nor in the embryo, but are in the pericarp and that they are inherited from the female parent. Duvick et al. (1992) isolated a peptide of the maize grain that inhibited germination and hyphal development in vitro, while Warfield and Davis (1996) and Butròn et al. (2006) showed how hybrids with adherent and tight husks and less open apical parts of the ears, present more resistance to Fusarium ear rot. The presence of the opaque-2 gene (o2), which increases the lysine and tryptophan contents in the endosperm, has shown more susceptibility to infection from this pathogen (Warren, 1978), while *waxy* maize obtained by introducing the *wx* gene is more prone to fumonisin accumulation compared to the counterpart hybrids (Blandino and Reyneri, 2007).

A recent approach to the search for hybrids that are resistant to mycotoxin contamination consists in the obtaining of genetically modified hybrids which create the resistance action through transgenes. At present it is possible to indirectly control contamination from fumonisins using Bt hybrids that are able to synthesise the transgenetic protein of *Bacillus thuringiensis* and which are active against European corn borer larvae (Munkvold et al., 1997; Munkvold et al., 1999; Masoero et al., 1999).

Genetic engineering for direct protection from mycotoxigenic fungi and their mycotoxins could be a valuable management strategy in the future, with the introduction of genes that codify according to protein or secondary metabolite with antifungal actions or genes that regulate the production of enzymes which are able to detoxificate the toxins. The most recent application concerns the insertion, into the maize genome, of genes that decodify for a fumonisin esterase, produced by a yeast, *Exophiala spinifera* (Blackwell et al., 1999), that is able to degrade the fumonisins; of a trichothecenes 3-O-acetyltransferase gene that is present in the *F. graminearum* genome (Okubara et al., 2002) and of α-amylase, from another plant, *Lablab purpureus* (a legume) inhibited aflatoxin production, spore germination and hyphal growth by *A. flavus* (Woloshuk and Fakhoury, 2000).

The level of resistance in commercial hybrids

for Gibberella and Fusarium ear rot is still not in general adequate to prevent unacceptable toxin concentrations in field (Snijders, 1994, Munkvold, 2003a). While waiting for hybrids with specific genetic resistance against these pathologies to appear on the market, and considering the actual necessary requirements of preventive systems that can guarantee a higher hygiene-health quality of the productions, it is however necessary to identify which morphological, physiological and physical characteristics of the grain of hybrids at present available on the market could be the most interesting for the containment of mycotoxins.

The purpose of this experiment was to verify the behaviour of some commercial dent maize hybrids with different maturity and grain hardness for fumonisin and zearalenone contamination and to identify the influence of this traits on the production of these toxins.

Materials and methods

Experimental sites and treatments

Mycotoxin contamination was evaluated in 19 commercial and commonly cultivated dent type maize hybrids from 2000 to 2002: 10 medium maturity hybrids (FAO rating 500), 6 medium-late maturity hybrids (FAO rating 600) and 3 late maturity hybrids (FAO rating 700).

The yellow dent maize hybrids used for the experiment were grouped in 3 categories of grain hardness (soft, intermediate and hard) based on the hard to soft endosperm ratio (H/S endosperm ratio), as reported by Li et al. (1996).

The H/S endosperm ratio in the grain samples was estimated by sectioning the kernels and measuring the areas of hard and soft endosperm presented at the cut surface. Dried kernels were sectioned just above the top of the embryo region using a pair of secateurs. The H/S ratios for 10 kernels of each sample were calculated by measuring the area of the cut surface and the soft endosperm region, using their scanned images and a digital image processing (ImageJ software, version 1.38). Four hybrids showed a mean H/S endosperm ratio higher than 4 and were classified as hard, while 6 hybrids showed values between 1 to 4 and were classified as intermediate. Hybrids with H/S endosperm ratio value lower than 1 were classified as soft.

The experiments were carried out in the following sites:
– Carmagnola (44° 50' N, 7° 40' E; altitude 245 m., in a sandy-medium textured soil (Typic Udifluvents);
– Giarole (45° 04' N, 8° 34' E; altitude 98 m, in a loamy and clayey soil (Alfic Eutrochrepts);
– Morozzo (44° 24' N, 7° 43' E; altitude 431 m, in a medium textured soil (Alfic Dystochrepts);
– Verolengo (45° 11' N, 7° 58' E; altitude 169 m, in a shallow and sandy soil (Typic Hapludalfs).

The hybrids were strip-test sowed in all the sites. The plot size was 100 m by 8 rows, and the row spacing was 0.75. The information relative to the crop practices of the experiment sites is given in table 1.

Three repetition of 100 ears (including the ears used for the evaluation of fungal ear rot incidence and severity and European corn borer damage incidence) were collected in each plot by hand at the end of maturity (moisture content of grains between 20-26%) and shelled using an electric sheller. The kernels were mixed thoroughly to obtain a random distribution of the kernels and samples (5 kg) were taken to analyze the mycotoxin content. These samples were frozen at a temperature of -18 °C.

Entomological and micological measurements

The European corn borer (ECB – *Ostrinia nubilalis* Hb.) damage incidence was evaluated on 3 repetitions of 30 ears from each plot at harvest time. This parameter was calculated as the percentage of ears per plot with injury and kernel damage or apical and basal tunnels in the cob due to larvae activity.

Fungal ear rot incidence and severity were calculated on 3 repetitions of 30 ears from each plot at harvest time. Fungal ear rot incidence was calculated as the percentage of ears per plot with symptoms, while fungal ear rot severity was calculated as the percentage of kernels per ear with symptoms. A scale of 1 to 7 was used in which each numerical value corresponds to a percentage interval of surfaces exhibiting symptoms of the disease according to the following schedule: 1 = 0%, 2 = 1-3%, 3 = 4-10%, 4 = 11-25%, 5 = 26-50%, 6 = 51-75%, 7 > 75% (Reid et al., 1999).

Table 1. General field trial information.

Site	Soil type	Fertilization unit	Irrigation (number and type)	Sowing	Harvest
Carmagnola (TO)	medium-textured	N = 200 P_2O_5 = 80 K_2O = 80	2000 = 3 2001 = 3 2002 = 2 flowing irrigation	2000 = April 23 2001 = April 21 2002 = April 23	2000 = October 5 2001 = Sept. 25 2002 = October 9
Giarole (AL)	muddy-medium textured	N = 180 P_2O_5 = 55 K_2O = 130	2000 = 3 2001 = 4 2002 = 3 flowing irrigation	2000 = May 17 2001 = April 15 2002 = April 24	2000 = October 27 2001 = October 2 2002 = October 6
Morozzo (CN)	medium-textured	N = 260 P_2O_5 = 100 K_2O = 150	2000 = 4 2001 = 4 2002 = 4 flowing irrigation	2000 = May 4 2001 = April 24 2002 = April 27	2000 = October 20 2001 = October 11 2002 = October 16
Verolengo (TO)	sandy	N = 350 P_2O_5 = 110 K_2O = 230	2000 = 4 2001 = 6 2002 = 4 flowing irrigation	2000 = April 26 2001 = April 19 2002 = April 20	2000 = October 4 2001 = Sept. 18 2002 = October 2

Mycotoxin analyses

A 5 kg representative sample of grain from each plot was freeze-dried and milled. A 50 g representative sub-sample of the milled material was analysed for mycotoxin content. Mycotoxin analysis was carried out using appropriate HPLC methods for each toxin. All the samples were analysed for fumonisin B_1 and zearalenone content.

For fumonisin B_1, 50 g samples were extracted by shaking for at least 15 min with 100 ml of methanol/water 80/20 containing 5 g of NaCl. The supernatant was filtered through filter paper (0.45 μm), diluted with 40 ml of PBS (8.0 g of NaCl + 1.2 g of Na_2HPO_4 + 0.2g of KCl + 0.2 g of KH_2PO_4 in 1 l of water), and was cleaned using a FumoniTest column (Vicam®) by rinsing with 10 ml of PBS. The fumonisin B_1 was then eluted using 1.5 ml of methanol and 200 μl of methanol/water 50/50. Fluorescent derivates of the fumonisin were formed using 225 μl of OPA (40 mg of o-phthaldialdehyde in 1 ml of methanol, and a solution 0.1 M of disodium tetraborate and 50 μl of 2-mercaptoethanol). 10 μl was injected into an HPLC column (Hypersil C18 column, 100 x 2.1 mm, 0.2 ml min^{-1}; fluorescence detector, with an excitation wavelength of 250 nm and an emission wavelength of 440 nm). Toxin quantification was performed using external standards and peak height measurements. The detection limit was 10 μg kg^{-1}.

As far as zearalenone is concerned, 50 g samples were extracted by shaking for at least 15 min with 250 ml of chloroform containing 25 g of diatomaceous earth. The extraction was completed using the AOAC 985.18 method (2005). A 2 μl test solution was injected into an HPLC column (Hypersil C18 column, 100 x 2.1 mm, 3 μm; 0.45 ml min^{-1}; mobile phase water/(methanol/acetonitrile 4/1) 55/45, fluorescence detector, with an excitation wavelength of 235 nm and an emission wavelength of 458 nm). Toxin quantification was performed as above.

Statistical analysis

An analysis of variance (SPSS Inc., Chicago, IL, Version 12.0) was utilized to compare European corn borer (ECB) damage incidence, fungal ear rot incidence and severity and the fumonisin B_1 and zearalenone content, according to Gomez and Gomez (1984). Multiple comparison tests were performed according to the Tukey test on treatment means.

The values of ECB damage incidence, fungal ear rot incidence and severity were previously transformed using y' = arcsen$\sqrt{x}$*180/π, as percentage data derived from counting.

Results

The comparison between the different types of commercial hybrids was carried out in years and sites with remarkably different meteorological trends (Tab. 2). The first year was characterised

Table 2. Rainfall and mean temperature from July to October 2000-2002 in the sites[z] of experimental fields.

	2000				2001				2002			
	CA	GI	MR	VE	CA	GI	MR	VE	CA	GI	MR	VE
Rainfall (mm)												
July	34.8	29.2	20.8	81.4	25.0	14.8	58.0	109.2	108.0	71.6	125.6	95.2
August	68.8	16.6	31.8	64.4	36.4	20.0	18.4	38.8	211.2	88.8	107.0	165.8
September	168.0	27.2	106.8	186.6	37.2	43.2	38.6	50.6	204.6	40.5	87.6	108.2
October	148.0	24.2	181.2	175.4	45.4	33.8	33.4	65.8	57.8	69.4	78.0	83.2
July-October	419.6	97.2	340.6	507.8	144.0	111.8	148.4	264.4	581.6	270.3	398.2	452.4
Mean temperature (°C)	18.1	19.0	18.6	18.8	18.6	19.4	18.8	19.2	17.6	18.4	17.6	18.1

(*) Data from: Carmagnola, Giarole, Morozzo, Verolengo.

by mild temperatures and limited rainfall from the early milk to dough stage (Growth Stage 73-83, according to Weber and Bleiholder, 1990; Lancashire et al., 1991) while it was later characterised by heavy rainfall during the final stages of ripening and during harvesting. The second year had higher mean temperatures and more limited rainfall during ripening. The last year was in general cooler and there was frequent rainfall from anthesis to the complete ripening. Carmagnola and Morozzo were the coolest sites with heavier rainfall, while Giarole had higher average temperatures and limited rainfall.

The ECB infestation progressively increased over the three year experimental period, and involved almost all the ears that were collected in 2002 in all the sites. Neither the grain hardness nor the hybrid maturity showed any significant influence of insect activity, in any of the years that were considered (Tab. 3 and 4).

As for ECB incidence, no significant differences were observed for fungal ear rot incidence or severity in function of the grain hardness or the hybrid maturity (Tab. 3 and 4).

The fumonisin B_1 and zearalenone contamination for the different hybrid categories is reported in tables 5 and 6. Fumonisin B_1 contamination above the instrumental detection limits concern all the samples; this toxin ranged be-

Table 3. Effect of grain hardness on ECB incidence, Fusarium ear rot incidence and severity, field experiments conducted in 2000-2002 in 4 Piedmont sites[v].

Year	Grain hardness[z]	ECB incidence[w]	Fusarium ear rot incidence[x]	Fusarium ear rot severity[y]
		%	%	%
2000	soft	84.4	56.9	2.8
	intermediate	73.3	55.0	2.8
	hard	81.7	59.2	3.0
	P - value	0.126	0.910	0.980
2001	soft	95.9	80.7	7.1
	intermediate	90.8	72.3	7.7
	hard	87.0	69.3	4.5
	P - value	0.160	0.406	0.186
2002	soft	96.7	94.7	6.4
	intermediate	98.6	92.8	5.1
	hard	95.6	90.0	4.8
	P - value	0.151	0.309	0.202

[v] Carmagnola, Giarole, Morozzo, Verolengo. Data reported for each year are mean values of 4 sites.
[w] ECB incidence was calculated as the percentage of ears with symptoms, based on 30 ears per plot.
[x] Fusarium ear rot incidence was calculated as the percentage of ears with symptoms, based on 30 ears per plot.
[y] Fusarium ear rot severity was calculated as the mean percentage of kernel with symptoms per ear, based on 30 ears per plot.
[z] based on the H/S endosperm ratio: soft (values < 1); intermediate (values between 1 and 4), hard (values > 4).
The values in the same column followed by the same letter are not significantly different according to the Student-Newman-Keuls test (ANOVA, P < 0.05).

Table 4. Effect of hybrid maturity on ECB incidence, Fusarium ear rot incidence and severity; field experiments conducted in 2000-2002 in 4 Piedmont sites[w].

Year	Maturity (FAO rating)	ECB incidence[X]	Fusarium ear rot incidence[Y]	Fusarium ear rot severity[Z]
		%	%	%
2000	500	80.8	52.8	2.6
	600	76.9	59.7	3.0
	700	81.7	57.5	3.2
	P - value	0.745	0.643	0.520
2001	500	89.4	69.8	6.1
	600	91.1	78.2	6.1
	700	95.6	78.3	7.0
	P - value	0.457	0.465	0.935
2002	500	97.2	93.0	5.8
	600	95.7	91.0	5.4
	700	99.3	96.0	5.1
	P - value	0.190	0.456	0.768

[W] Carmagnola, Giarole, Morozzo, Verolengo. Data reported for each year are mean values of 4 sites.
[X] ECB incidence was calculated as the percentage of ears with symptoms, based on 30 ears per plot.
[Y] Fusarium ear rot incidence was calculated as the percentage of ears with symptoms, based on 30 ears per plot.
[Z] Fusarium ear rot severity was calculated as the mean percentage of kernel with symptoms per ear, based on 30 ears per plot. The values in the same column followed by the same letter are not significantly different according to the Student-Newman-Keuls test (ANOVA, $P < 0.05$).

tween 145 and 5079 µg kg^{-1} in 2000, between 380 and 6559 µg kg^{-1} in 2001 and between 1262 and 5357 µg kg^{-1} in 2002. Zearalenone was found in concentrations above the instrumental detection limits in 39.3, 12.5 and 91.4% of the samples taken in 2000, 2001 and 2002, respectively. The maximum value of contamination recorded each year was 118 µg kg^{-1} in 2000, 40 µg kg^{-1} in 2001 and 340 µg kg^{-1} in 2002.

Every year, the grain hardness has significantly influenced fumonisin B$_1$ content with a 50% lower mean contamination of the hard endosperm hybrids than the soft (Tab. 5). The intermediate endosperm hybrids always had values of this toxin higher than the hard grain varieties, but the differences were always not significant.

The zearalenone content never resulted to be significantly different in function of the grain hardness of the hybrids.

The presence of fumonisin B$_1$ in the grain of different maturity hybrids only resulted to be significantly different in 2001, with a mean concentration 2 times higher in the later hybrids (FAO rating 700) compared to the medium and medium-late hybrids (Tab. 6).

The influence of the hybrid maturity on the zearalenone contamination was instead clearly

Table 5. Effect of grain hardness on fumonisin B1 and zearalenone concentration; field experiments conducted in 2000-2002 in 4 Piedmont sites[Y].

Year	Grain hardness[Z]	Fumonisin B$_1$ (µg kg^{-1})	Zearalenone (µg kg)
2000	soft	2677 a	23
	intermediate	1420 b	29
	hard	872 b	25
	P - value	0.003	0.912
2001	soft	3573 a	8
	intermediate	2658 ab	8
	hard	1553 b	5
	P - value	0.045	0.510
2002	soft	4180 a	42
	intermediate	3693 ab	128
	hard	3141 b	114
	P - value	0.038	0.055

[Y] Carmagnola, Giarole, Morozzo, Verolengo. Data reported for each year are mean values of 4 sites.
[Z] based on the H/S endosperm ratio: soft (values < 1); intermediate (values between 1 and 4),hard (values > 4).
The values in the same column followed by the same letter are not significantly different according to the Student-Newman-Keuls test (ANOVA, $P < 0.05$).

observed in 2001 and 2002. Contamination of the class 500 hybrids was significantly lower (by 66%) than the class 700 hybrids. Contamination of the class 500 and class 600 hybrids was only significantly different in 2001 (+ 41% for the later hybrids).

Table 6. Effect of hybrid maturity on fumonisin B_1 and zearalenone concentration; field experiments conducted in 2000-2002 in 4 Piedmont sites[z].

Year	Maturity (FAO rating)	Fumonisin B_1 ($\mu g\ kg^{-1}$)	Zearalenone ($\mu g\ kg$)
2000	500	2152	23
	600	1699	27
	700	1609	30
	P - value	0.591	0.947
2001	500	2179 b	5 b
	600	2082 b	6 b
	700	4410 a	16 a
	P - value	0.023	0.003
2002	500	3223	63 c
	600	3686	102 b
	700	3960	173 a
	P - value	0.611	0.030

[z] Carmagnola, Giarole, Morozzo, Verolengo. Data reported for each year are mean values of 4 sites.
The values in the same column followed by the same letter are not significantly different according 5 to the Student-Newman-Keuls test (ANOVA, P < 0.05).

Discussion

The collected data highlighted the presence of differences between the maize hybrids tested for contamination to fumonisins and zearalenone, which resulted to be regulated by different variety characteristics.

No difference were observed for fungal ear rot incidence and severity. Several authors reported that mycotoxins are not clearly related to ear rot symptoms (Stewart et al., 2002; Munkvold et al., 2003b): toxins are very stable compounds and they could remain in grains also after the mould death. On the other hand not all the mould are able to produce mycotoxins (Bottalico, 1999).

The comparison between hybrids with different maturity showed that the zearalenone contamination can be remarkably reduced with the cultivation of early maturing hybrids, thus confirming data by Reid and Sinha (1998) and Bata et al. (2001). The later hybrids, with the use of FAO class 700 hybrids for the production of maize grain in the Po Plain, have generally high zearalenone contamination, according to AA.VV. (2004). The differences between the late maturing hybrids (FAO rating 700) and the medium-early (FAO rating 500) or medium-late maturing hybrids (FAO rating 600) were higher in 2002, which was the year with the heaviest rain-

fall and coolest temperatures, and this caused a longer crop thriving and an extended duration of the ripening stages. The presence of zearalenone in the grain in the dryer 2000 and 2001, was rather reduced but it however always affected the later maturing hybrids in the different sites. This toxin was instead found in hybrids rated at FAO 500 and 600 maturity only in some hybrids in the wetter environments. The difference between late and early maturing hybrids is probably not related to the different conditions during infection, which for *F. graminearum*, is primary by silks during the first 6 days after silk emergence (Reid and Hamilton, 1996), but to the conditions in which ripening occurs. Gibberella ear rot development is favoured by moderate temperatures, high rainfall and humid conditions during the maturation period (Sutton et al., 1980; Munkvold, 2003b) and this occurs more frequently for late maturing hybrids. Moreover, Reid and Sinha (1998) suggest that *F. graminearum* may not spread to additional kernels once drying and hardening of the kernels has started, and this occurs before for earlier maturing genotypes.

Fumonisins, instead, were confirmed to occur ubiquitously in maize-producing areas (Shephard et al., 1996; AA.VV., 2005). These toxins do not seem to be primarily influenced by hybrid maturity, confirming data by Ramirez et al., (1996) and Camargos et al. (2001), but rather by grain hardness or by some other kernel characteristics that provide physical barriers to ear infection, according to Hoenisch and Davis (1994). The present data suggest that hard endosperm kernels, thanks to their texture, are more resistant than soft kernels to fumonisin contamination. The lower susceptibility of hard hybrids could be connected both to a greater hardness and density of the endosperm fraction that could be a less susceptible substrate to toxinogenesis and to a more compact pericarp which is a more effective physical barrier to fungal infection, as suggested by Costa et al. (2003).

Moreover, *F. verticillioides* infection and Fusarium ear rot severity are closely correlated to insect injury, primarily due to *O. nubilalis* (Bakan et al., 2002; Alma et al., 2005), and this appears to be the most important infection pathway in the Po Plain area. Kernels with hard pericarps may have the ability to avoid grain injury and reduce disease development and my-

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cotoxin production. De la Campa et al. (2005) reported that some hybrids are more susceptible to insect damage than others and a significant interaction exists between insect injury susceptibility of hybrids and fumonisin accumulation.

Apart from the lower severity of infection from fungal species in the field, it should also be considered that harder grains show less breaking and cracking after harvesting (Stroshine et al., 1986), thus ensuring lower contamination risks, even from other mycotoxins, during storage (Magan et al., 2003, Paulsen et al., 2003).

If some hybrids that showed a high contamination risk for both toxins are excluded, the analysed hybrids generally show an inverse relation due to the presence of the two toxins in the grain. There are therefore some hybrids that are more susceptible to contamination from zearalenone, but generally more resistant to fumonisins, while others are characterised by a higher susceptibility to contamination from fumonisin B_1 and a higher resistance to zearalenone.

In conclusion, this research stress the role of kernel endosperm and of the hybrid maturity class on mycotoxin contamination.

Prolonged ripening has proved to be important for zearalenone contamination; for this reason, late maturing hybrids (FAO 600 and 700) are potentially more at risk in the Po plain area. Most of the contamination of late maturing hybrids, compared to early-medium maturing hybrids, is more obvious in the environments and years that favour a greater vegetative growth of the crop. Grain endosperm hardness has not shown any effect on the presence of zearalenone.

The presence of fumonisins is not strictly correlated with hybrid maturity but rather with the physical properties of the endosperm of the grain. Soft hybrids are more prone to contamination from these toxins, while an increase in the endosperm hardness reduces the concentration of fumonisin B_1 in the grain.

In conclusion, the results that were obtained have shown clear differences in susceptibility to *Fusarium* toxin contamination among the yellow dent commercial hybrids.

In the near future it is essential to obtain, through genetic improvement, hybrids that can guarantee low risks of contamination for a wider range of toxins; however, at the moment the variety choices in the farm should be oriented towards privileging a reduced content of few mycotoxins or the one which results to be more critical with final use of the product.

In order to reach this objective, the choice of hybrid should always be coupled together with all those good agricultural practices that are able to reduce the risk of encountering a high mycotoxin content and therefore to increase the health quality of the production.

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