JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

*J. Agric. Food Chem.* **2006,** *54,* 6113−6117 **6113**

# Maize (*Zea mays* L.) Genetic Factors for Preventing Fumonisin Contamination

ANA BUTRÓN,*,† ROGELIO SANTIAGO,† PEDRO MANSILLA,‡ CRISTINA PINTOS-VARELA,‡ AMANDO ORDÁS,† AND ROSA ANA MALVAR†

Misión Biológica de Galicia, Spanish Council for Scientific Research, Apartado 28, 36080 Pontevedra, Spain, and Estación Fitopatolóxica do Areeiro, Deputación de Pontevedra, Subida a la Robleda s/n, 36153 Lourizán, Pontevedra, Spain

*Fusarium moniliforme* and *Fusarium proliferatum* are the most frequently isolated fungi from maize (*Zea mays* L.) in Spain. Both *Fusarium* species produce toxins potentially dangerous for animals and humans, the fumonisins being the most significant of those toxins. White maize is preferred for human consumption, and extra care should be taken to avoid kernel mycotoxin contamination. The objectives of this study were to identify and quantify kernel infection by *Fusarium* spp. and contamination by fumonisin on white maize hybrids, to search for white maize sources of resistance to infection by *Fusarium* spp. and mycotoxin contamination, and to preliminarily study the genetics involved in such resistances. Ten $F_1$ single crosses derived from a diallel mating design among five white maize inbreds were evaluated in a randomized complete block design with three replications in 2002 at two locations. *Fusarium verticilloides* and *F. proliferatum* were detected on kernels of white maize hybrids cultivated in northwestern Spain. No differences in fungal infection were found among maize genotypes, but differences in fumonisin contamination were significant and could be related, in part, to differences in husk tightness. Among the genotypes studied, general combining ability (GCA) effects were the most important for resistance to fumonisin contamination. Inbreds EP10 and EC22 showed the most favorable GCA effects for husk tightness and fumonisin content, and the cross between them, EP10 × EC22, had the most favorable specific combining ability (SCA) effect for husk tightness. Inbreds EP10 and EC22 showed favorable GCA effects for fumonisin contamination and husk tightness, and the cross EP10 × EC22 was the only one with an average fumonisin level below 1 μg/g. Although this should be confirmed with more extensive studies, white maize inbreds developed from white maize landraces could be sources of resistance to fumonisin contamination.

**KEYWORDS: Fumonisin; *Fusarium verticilloides*; *Fusarium proliferatum*; plant resistance; *Zea mays***

## INTRODUCTION

*Fusarium moniliforme* Sheldom [syn.: *Fusarium verticilloides* (Sacc.) Niremberg] and *Fusarium proliferatum* (Matsushima) Niremberg are the most frequently isolated fungi from maize (*Zea mays* L.) and maize-based feeds from Spain (*1*−*5*). Both *Fusarium* species produce toxins potentially dangerous for animals and humans, the fumonisins being the most significant of those toxins (*6*, *7*). In Spain, 100% of *F. proliferatum* and >70% of *F. verticilloides* isolates are toxigenic (*8*, *9*). Many fumonisin analogues have been identified, but the most abundantly found are $B_1$, $B_2$, and $B_3$ (*10*). Reference *11* gives detailed information on the most frequent fumonisin, $B_1$. In Switzerland, a tolerance level of 1 μg/g of fumonisins in dry maize products for human consumption has been proposed (*6*), whereas the U.S. Food and Drug Administration has recommended that the fumonisin levels should be below 4 μg/g in whole or partially demerged dry milled corn products for human consumption (*12*). The Joint FAO/WHO Expert Committee on Food Additives allocated a group provisional maximum tolerable daily intake of 2 μL/g (*13*). In the European Union, legal regulation of maximum contents of fumonisins will commence on October 1, 2007, and establishes that the threshold fumonisin contents will be 2000 μg/kg in nonprocessed maize, 1000 μg/kg in maize flour, 50 μg/kg in maize snacks and flakes, 400 μg/kg in maize-based food for adults, and 200 μg/kg in maize-based products for baby food (*14*).

Natural differences among maize genotypes for fumonisin accumulation have been found (*15*−*18*). As the water available for fungal growth plays a key role (*19*), late-maturing maize cultivars in which grain moisture content decreases slowly are more susceptible (*6*). It is thought that upright cobs (*20*) and thin grain pericarp increase susceptibility to *Fusarium* infection (*21*). Tight husks have been described as an unfavorable

* Corresponding author (telephone 34986 854800; fax 34 986 841362; e-mail abutron@mbg.cesga.es).
† Spanish Council for Scientific Research.
‡ Deputación de Pontevedra.

10.1021/jf0611163 CCC: $33.50 
Published on Web 06/23/2006

**Table 1.** Pedigree, Kernel Type, and Source of 10 White Corn Inbred Lines Crossed in a Diallel

| inbred | pedigree | kernel type | source |
|---|---|---|---|
| EP10 | northern white dent | dent | Misión Biológica de Galicia |
| EP64 | PB98 × PB261 | flint | Misión Biológica de Galicia |
| EP65 | PB98 × PB261 | flint | Misión Biológica de Galicia |
| EP71 | PB260 × PB261 | flint | Misión Biológica de Galicia |
| EC22 | local landrace | flint | Centro de Investigaciones Agrarias de Mabegondo |

characteristic (*20*) because they slow kernel drying or as a favorable characteristic (*22*) because they protect the ear from insect damage. Therefore, many questions remain to be answered about mechanisms for resistance of maize to fumonisin contamination. Clements et al. (*18*) suggested that several dominant genes are involved, and two quantitative trait loci (QTLs) located on chromosome 5 were associated with resistance to fumonisin contamination and *Fusarium* ear rot (*23*).

White corn is preferred for human consumption, and extra care should be taken to avoid drought stress or damage by insects that could contribute to fungal growth on kernels. Looking for sources of resistance to mycotoxin contamination among white maize genotypes would also contribute to reducing human hazard. The present study was focused on maize grown in northwestern Spain. A previous study made with maize from this area showed the presence of *Fusarium graminearum* Schwabe associated with appreciable levels of deoxynivalenol (DON) (*1*). However, *F. verticilloides* was the most frequently found fungus, and no test for fumonisin was made. In the present study, contamination with DON was also considered to compare its incidence with incidence of fumonisins. The objectives were to identify and quantify kernel infection by *Fusarium* spp. and contamination by fumonisin on white maize hybrids, to search for white maize sources of resistance to infection by *Fusarium* spp. and mycotoxin contamination, and to study the genetics involved in those resistances.

## MATERIALS AND METHODS

Five maize inbred lines with white kernels were used as parents of a diallel set of crosses without reciprocals (**Table 1**). In 1999 and 2000, 10 hybrids were obtained from the diallel design. The 10 $F_1$ single crosses were evaluated in a randomized complete block design with three replications in 2002 at Pontevedra (42° 25′ N, 4° 57′ W, and 20 m above sea level) and Barrantes (42° 30′ N, 8° 46′ W, and 50 m above sea level). Each variety was planted in a single-row plot, 0.80 m between rows and 0.21 m between two-kernel hills within rows. After thinning, the final density was ≈60000 plants/ha.

Before harvesting, husk tightness was evaluated on a visual scale from 0 (loose husks with visible cob) to 5 (tight husks) (*24*). At harvest, yield, ear damage by corn borers on a visual rating from 1 (completely damaged) to 9 (no damage), and kernel moisture were recorded. In each plot, harvested ears were shelled, and kernels were dried at 35 °C for 1 week and maintained at 4 °C and 50% humidity until analysis were performed. The kernel germination rate in each plot was determined on a sample of 100 dried kernels that were watered and maintained at 25 °C for 6 days.

For determining fungal infection proportion, 100 dried kernels from each plot were placed onto Komada's medium plates and incubated in the dark at 24 °C for 7−10 days. When fungal growth was observed, individual cultures were transferred to Spezieller−Nahrstoffarmer agar (SNA) medium plates and incubated in the dark for 10 days. Then, *Fusarium* species on each kernel were identified by examining colony and conidial morphology according to the method of Nelson et al. (*25*).

Total fumonisin (fumonisins $B_1$, $B_2$, and $B_3$) and DON quantification was made by the technical service of the Food Technology Departament of the University of Lleida using specific commercial ELISA kits (R-Biopharm Rhône Ltd., Glasgow, Scotland). This is a competitive enzyme immunoassay for the quantification of fumonisin residues in corn. The recovery rate of the test is ≈60% with a mean coefficient of variation of ≈8%, and specifities for $B_1$, $B_2$, and $B_3$ are 100, ≈40, and ≈100%, respectively. The ELISA kit used for the quantification of DON (R-Biopharm Rhône Ltd.) is a competitive enzyme immunoassay for the quantitative analysis of DON in cereals, malt, feed, beer, and wort and has a recovery rate of 85−110%; its specifity for DON is 100%. Extraction and preparation of samples, as well as test performance, were carried out as described in the kits.

Individual and combined analyses of variance were computed. Location and replication were considered random factors, and genotype was considered a fixed factor. Variation among hybrids of the diallel was partitioned into general combining ability (GCA) and specific combining ability (SCA) effects using Griffing's method 4, model I (fixed effects) (*26*). The diallel was analyzed using a program developed by Zhang and Kang (*27*). Simple correlation coefficients among traits were calculated. Comparisons of means were accomplished using Fisher's protected least significant difference method (LSD). All analyses were made using SAS, version 9.1 (*28*).

## RESULTS AND DISCUSSION

The *Fusarium* spp. detected were *F. proliferatum* and *F. verticilloides*, agreeing with previous studies that established that, in Spain, both species are the most frequent fungi in maize (*3*−*5*). The average infection rates by *F. proliferatum* and *F. verticilloides* were 6.2 (ranging from 0 to 23.4%) and 64.6% (ranging from 47.5 to 91.4%), respectively. Therefore, the most abundant species was *F. verticilloides*. Bakan et al. (*5*), analyzing kernel infection by *Fusarium* ssp., found that *F. proliferatum* was more abundant in northeastern Spain. Our experimental plots were located in northwestern Spain, where climatic characteristics during kernel filling are very different from northeastern Spain conditions, and those climatic differences could be responsible for differences in the predominant *Fusarium* species (*29*).

The high incidence of fumonisin contamination agreed with the expectation based on the mycoflora found on maize kernels because *F. verticilloides* and *F. proliferatum* are fumonisin producers (*30*). Sanchís et al. (*8*) had already pointed out the potential fumonisin contamination in many Spanish corn-based products containing both *Fusarium* species, although fumonisin levels detected were lower than in the present study.

Although kernel infection by *F. graminearum* and *F. colmorum* (W.G. Smith) Sacc. was not detected, DON levels in all samples from the Pontevedra experiment were >5 ppb (data not shown), the maximum recommended level in some countries (*31*). However, average levels of fumonisins across locations were much higher, being >1 ppm for all genotypes, except EC22 × EP10. Negative correlations between the presence of *F. verticilloides* and other *Fusarium* species have been reported (*32*, *33*). Then, the high kernel colonization by *F. verticilloides* and *F. proliferatum* could limit the presence of *F. graminearum* or *Fusarium colmorum* to a very low level that could not be detected by the infection experiment involving 100 kernels. It has been pointed out that *F. verticilloides* has at least one competitive advantage over *F. graminearum*, that is, a broader response to temperature (*34*).

Kernel damage by corn borers has been associated with *Fusarium* infection and mycotoxin contamination (*17*, *21*, *35*). The association between ear damage by corn borers and infection by *F. verticilloides* was corroborated in the present study as the simple correlation coefficient between both traits showed (**Table 2**), but the absence of differences among genotypes for ear damage by insects did not allow testing of

**Table 2.** Simple Correlation Coefficients among Traits Recorded on 10 Hybrids Resulting from a Diallel Mating Design among 5 White Maize Inbreds Evaluated in 2002 at Two Locations

| | ear damage[a] | husk tightness[b] | kernel moisture | germination rate | *F. verticilloides* | *F. proliferatum* | fumonisin content |
|---|---|---|---|---|---|---|---|
| husk tightness | –0.02 | | | | | | |
| kernel moisture | –0.18 | –0.40 | | | | | |
| germination rate | 0.07 | 0.75 | –0.76* | | | | |
| *F. verticilloides* | –0.75* | 0.21 | 0.11 | 0.12 | | | |
| *F. proliferatum* | –0.10 | –0.34 | –0.23 | –0.14 | –0.26 | | |
| fumonisin content | 0.01 | –0.72* | –0.28 | –0.20 | –0.22 | 0.39 | |
| DON content | 0.13 | –0.28 | 0.55 | –0.28 | –0.14 | –0.23 | –0.09 |

[a] Ear damage by corn borers was evaluated on a visual rating from 1 (completely damaged) to 9 (no damage). [b] Husk tightness was evaluated on a visual scale from 0 (loose husks with visible cob) to 5 (tight husks).

**Table 3.** Means of 10 Hybrids Resulting from a Diallel Mating Design among 5 White Maize Inbreds Evaluated in 2002 at Two Locations[a]

| hybrid | ear damage (1–9)[b] | husk tightness (0–5)[c] | kernel moisture (%) | germination rate (%) | *F. verticilloides* (%) | *F. proliferatum* (%) | fumonisin content ($\mu$g/g) | DON content (ng/g) |
|---|---|---|---|---|---|---|---|---|
| EP64 × EP65 | 5.7 a | 1.0 b | 38.7 ab | 82.2 a | 65.8 a | 8.3 a | 3.01ab | 302.1 a |
| EP64 × EP71 | 5.2 a | 1.2 b | 35.9 cd | 86.6 a | 60.2 a | 23.4 a | 3.49 ab | 27.1 a |
| EP64 × EP10 | 5.8 a | 1.2 b | 38.1 bc | 87.0 a | 56.7 a | 0.0 a | 2.85 ab | 295.0 a |
| EP64 × EC22 | 5.8 a | 1.5 b | 36.3 bd | 84.7 a | 47.5 a | 7.7 a | 2.92 ab | 268.3 a |
| EP65 × EP71 | 5.2 a | 1.0 b | 38.1 bc | 80.0 a | 64.2 a | 3.3 a | 3.60 ab | 30.9 a |
| EP65 × EP10 | 5.0 a | 1.2 b | 41.2 a | 79.8 a | 61.4 a | 6.6 a | 2.31 bc | 389.3 a |
| EP65 × EC22 | 5.5 a | 1.0 b | 37.8 bc | 82.8 a | 68.3 a | 4.2 a | 3.30 ab | 147.5 a |
| EP71 × EP10 | 4.5 a | 1.7 b | 37.6 bc | 87.2 a | 91.4 a | 2.6 a | 2.55 b | 221.9 a |
| EP71 × EC22 | 5.3 a | 1.7 b | 34.6 d | 94.0 a | 62.0 a | 5.5 a | 4.03 a | 196.5 a |
| EP10 × EC22 | 5.5 a | 3.0 a | 36.5 bd | 93.4 a | 68.6 a | 0.0 a | 0.99 c | 57.0 a |

[a] In each column, means followed by the same letter did not differ at the 0.05 probability level. [b] Ear damage by corn borers was evaluated on a visual rating from 1 (completely damaged) to 9 (no damage). [c] Husk tightness was evaluated on a visual scale from 0 (loose husks with visible cob) to 5 (tight husks).

whether ear resistance to corn borers reduces fumonisin contamination (**Table 3**). Significant associations were also found between germination rate and husk tightness and kernel moisture (**Table 2**). Although no relationship between percentage of infection by *Fusarium* spp. and germination rate was detected, a significant and negative relationship between mycelial growth and germination rate would be expected (*36*), giving support to the decreased germination rate observed when fungal growth is favored by unprotected ears and high water activity. Therefore, in future studies, other parameters besides proportion of kernels infected by *Fusarium* spp. should be recorded, such as ergosterol and fungal biomass quantification, to ascertain significant relationships between maize characteristics and *Fusarium* growth.

Maize was harvested in Barrantes (42.2% average kernel moisture) earlier than in Pontevedra (32.8% average kernel moisture), whereas fumonisin contamination was lower (2.7 ppm in Barrantes and 3.3 ppm in Pontevedra), agreeing with a previous study that pointed out that early harvest may help to reduce levels of contamination (*37*). These authors based that recommendation on data about the increases in infection and fumonisin contamination over time. Kernel infection by *F. verticilloides* and fumonisin contamination appeared as the kernel neared physiological maturity and increased up to the average harvest date.

Differences among maize hybrids were significant for fumonisin content, husk tightness, and kernel moisture (**Table 3**). There was not a significant location × genotype interaction for any trait, except for husk tightness (data not shown). On the basis of simple correlation coefficients (**Table 2**), only differences among genotypes for husk tightness could contribute to the differences for fumonisin content. The hybrid EP10 × EC22 had the lowest level of fumonisin contamination, although it did not differ from that of EP65 × EP10, and showed the tightest husks (**Table 3**). Therefore, husk coverage could protect the ear from fungal colonization, but not necessarily through reduction in insect damage, as was pointed out previously (*22*). As most kernels are infected through silks, husk tightness could act as a barrier to fungus entrance and delay fumonisin contamination. However, genotypes, such as EP65 × EP10, that differed from the best hybrid for husk coverage showed fumonisin levels as low as the least contaminated hybrid. Therefore, other mechanisms besides husk tightness should contribute to the lower level of fumonisin contamination in some genotypes. The hybrid EP10 × EC22 could be recommended for flour production because, besides its low fumonisin contamination level, it has shown high grain and flour yields and kernel density in a previous study (*38*).

The GCA effects were significant for fumonisin content and husk tightness, whereas SCA effects were significant only for husk tightness. The GCA effect is related to the mean performance of an inbred in crosses with other inbreds, whereas the SCA effect is related to the deviation of the performance of a particular cross from the expected performance based on the average general combining ability of the two inbreds (*39*). No significant interactions of location × GCA or location × SCA were found (data not shown), which means that the mean performances of inbreds and hybrids did not differ from one location to another. The non-strictly-additive inheritance of husk tightness was observed previously (*40*, *41*). Clements et al. (*18*) suggested that several dominant genes were involved in maize resistance to fumonisin contamination, but we did not find evidence for dominance among the genotypes evaluated. Inbreds EP10 and EC22 showed favorable GCA effects for husk tightness and fumonisin content, and the cross between them, EP10 × EC22, had the most favorable SCA effect for husk tightness (**Table 4**). Inbreds EP10 and EC22 were obtained from white maize landraces that could have been under selection for

**Table 4.** Significant General and Specific Combining Abilities for the Parental Inbreds of the Diallel Evaluated in 2002 at Two Locations

| | GCA | | SCA husk tightness[a] | | | |
|---|---|---|---|---|---|---|
| inbred | fumonisin content ($\mu$g g$^{-1}$) | husk tightness | EP65 | EP71 | EP10 | EC22 |
| EP64 | 0.2 ± 0.3 | –0.3 ± 0.1 | 0.4 ± 0.1 | 0.1 ± 0.1 | –0.4 ± 0.1 | –0.1 ± 0.1 |
| EP65 | 0.2 ± 0.3 | –0.5 ± 0.1 | | 0.2 ± 0.1 | –0.2 ± 0.1 | –0.4 ± 0.1 |
| EP71 | 0.7 ± 0.3 | –0.1 ± 0.1 | | | –0.1 ± 0.1 | –0.2 ± 0.1 |
| EP10 | –0.9 ± 0.3 | 0.4 ± 0.1 | | | | 0.7 ± 0.1 |
| EC22 | –0.1 ± 0.3 | 0.5 ± 0.1 | | | | |

[a] Husk tightness was evaluated on a visual scale from 0 (loose husks with visible cob) to 5 (tight husks).

kernel appearance for years because white maize is mostly dedicated to human consumption. It is supposed that such selection was done against any kind of ear rot and against contamination by fumonisin because severity of ear rot is associated with ear contamination by mycotoxins (*16*, *42*). The coefficient of correlation between GCA effects for fumonisin content and husk tightness was high (−0.63), although not significant. Therefore, other maize mechanisms besides husk tightness should be explored in order to determine why some maize genotypes show lower fumonisin contamination.

Although this should be confirmed with more extensive studies, white maize inbreds developed from white maize landraces could be a source of resistance to fumonisin contamination. Future studies should be focused on confirming the present results and looking for more maize materials resistant to fumonisin contamination under artificial inoculations with *Fusarium* spp.

## LITERATURE CITED


(1) Muñoz, L.; Cardelle, M.; Pereiro, M.; Riguera, R. Occurrence of corn mycotoxinns in Galicia (northwest Spain). *J. Agric. Food Chem.* **1990**, *38*, 1004−1006.

(2) Sala, N.; Sanchís, V.; Vilaro, P.; Viladrich, R.; Torres, M.; Viñas, I.; Canela, R. Fumonisin producing capacity of *Fusarium* strains isolated from cereals in Spain. *J. Food Prot.* **1994**, *57*, 915−917.

(3) Doko, M. B.; Rapior, S.; Visconti, A.; Schroth, J. E. Incidence and levels of fumonisin contamination in maize genotypes grown in Europe and Africa. *J. Agric. Food Chem.* **1995**, *43*, 429−434.

(4) Castellá, G.; Bragulat, M. R.; Cabañes, F. J. Surveillance of fumonisins in maize-based feeds and cereals from Spain. *J. Agric. Food Chem.* **1999**, *47*, 4707−4710.

(5) Bakan, B.; Melcion, D.; Richard-Molard, D.; Cahagnier, B. Fungal growth and *Fusarium* mycotoxin content in isogenic traditional and genetically modified maize grown in France and Spain. *J. Agric. Food Chem.* **2002**, *50*, 728−731.

(6) Fandohan, P.; Hell, K.; Marasas, W. F. O.; Wingfield, M. J. Infection of maize by *Fusarium* species and contamination with fumonisin in Africa. *Afr. J. Biotechnol.* **2003**, *2*, 570−579.

(7) Cavaglieri, L. R.; Andrés, L.; Ibáñez, M.; Etcheverry, M. G. Rhizobacteria and their potential to control *Fusarium verticillioides*: effect of maize bacterisation and inoculum density. *Antonie van Leeuwenhoek* **2005**, *87*, 179−187.

(8) Sanchís, V.; Abadías, M.; Oncins, L.; Sala, N.; Viñas, I.; Canela, R. Fumonisins B1 and B2 and toxigenic *Fusarium* strains in feeds from the Spanish market. *Int. J. Food Microbiol.* **1995**, *27*, 37−44.

(9) Abarca, M. L.; Bragulat, M. R.; Castellá, G.; Accensi, F.; Cabañes, F. J. Hongos productores de micotoxinas emergentes. *Rev. Iberoam. Micol.* **2000**, *17*, S63−S68.

(10) Shephard, G. S.; Thiel, P. G.; Stockenstrom, S.; Sydenham, E. W. Worldwide survey of fumonisin contamination of corn and corn-based products. *J. AOAC Int.* **1996**, *79*, 671−687.

(11) Marasas, W. F. O.; Miller, J. D.; Riley, R. T.; Visconti, A. Environmental Health Criteria 219, Fumonisin $B_1$; June 2006; http://whqlibdoc.who.int/ehc/WHO_EHC_219.pdf.

(12) U.S. Food and Drug Administration. June 2006; www.cfsan-.fda.gov.

(13) WHO. *Evaluation of Certain Mycotoxins in Food*; 56th report of the Joint FAO/WHO Expert Committee on Food Additives; WHO Technical Report Series 906; World Health Organization: Geneva, Switzerland, 2001.

(14) Legislación Comunitaria sobre Contenidos Máximos de Micotoxinas en Productos Alimenticios; June 2006; http://www.mcx.es/plaguicidas/Micotox.htm.

(15) Shelby, R. A.; White, D. G.; Bauske, E. M. Differential fumonisin production in maize hybrids. *Plant Dis.* **1994**, *78*, 582−584.

(16) Pascale, M.; Visconti, A.; Pronczuk, M.; Wisniewska, H.; Chelkowski, J. Accumulation of fumonisins in maize hybrids inoculated under field conditions with *Fusarium moniliorme* Sheldon. *J. Sci. Food Agric.* **1997**, *74*, 1−6.

(17) Avantaggiato, G.; Quaranta, F.; Desiderio, E.; Visconti, A. Fumonisin contamination of maize hybrids visible damaged by *Sesamia*. *J. Sci. Food Agric.* **2002**, *83*, 13−18.

(18) Clements, M. J.; Maragos, C. A.; Pataky, J. K.; White, D. G. Sources of resistance to fumonisin accumulation in grain and *Fusarium* ear and kernel rot of corn. *Phytopathology* **2004**, *94*, 251−260.

(19) Marín, S.; Magan, N.; Bellí, N.; Ramos, A. J.; Canela, R.; Sanchos, V. Two-dimensional profiles of fumonisin $B_1$ production by *Fusarium moniliforme* and *Fusarium proliferatum* in relation to environmental factors and potential for modeling toxin formation in maize grain. *Int. J. Food Microbiol.* **1999**, *51*, 159−167.

(20) Enerson, P. M.; Hunter, R. B. Response of maize hybrids to artificially inoculated ear mould incited by *Gibberella zeae*. *Can. J. Plant Sci.* **1980**, *60*, 1463−1465.

(21) Hoenish, R. W.; Davis, R. M. Relationship between kernel pericarp thickness and susceptibility to *Fusarium* ear rot in field corn. *Plant Dis.* **1994**, *78*, 517−519.

(22) Jeffers, D. P. Disease control. In *Corn: Origin, History, Technology, and Production*; Smith, C. W., Ed.; Wiley: New York, 2004; pp 669−716.

(23) Clements, M. J.; White, D. G. Identifying sources of resistance to aflatoxin and fumonisin contamination in corn grain: history and progress from the University of Illinois. In *Aflatoxin and Food Safety*; Abbas, H. K., Ed.; Taylor and Francis Group, CRC Press: Boca Raton, FL, 2005; pp 395−405.

(24) Wiseman, B. R.; Isenhour, D. J. Relationship of planting dates and corn earworm developmental parameters and injury to selected corn entries. *Maydica* **1992**, *36*, 149−156.

(25) Nelson, P. E.; Toussoun, T. A.; Marasas, W. F. *Fusarium Species: An Illustrated Manual for Identification*; Pennsylvania State University Press: University Park, PA, 1983.

(26) Griffing, B. Concept of general and specific combining ability in relation to diallel crossing systems. *Aust. J. Biol. Sci.* **1956**, *9*, 463−493.

(27) Zhang, Y.; Kang, M. S. DIALLEL-SAS: A SAS program for Griffing's diallel analyses. *Agron. J.* **1997**, *89*, 176−182.

(28) SAS. *The SAS System*, SAS Online Doc. HTML format ver. 8; SAS Institute Inc.: Cary, NC, 2000.

(29) Marín, S.; Sanchís, V.; Teixido, A.; Saenz, R.; Ramos, A. J.; Viñas, I.; Magan, N. Water and temperature relations and microconidial germination of *Fusarium moniliforme* and *Fusarium proliferatum* from maize. *Can. J. Microbiol.* **1996**, *42*, 1045−1050.

(30) Logrieco, A.; Bottalico, A.; Mulé, G.; Moretti, A.; Perrone, G. Epidemiology of toxigenic fungi and their mycotoxins for some Mediterranean crops. *Eur. J. Plant Pathol.* **2003**, *109*, 645−667.

(31) Sanchos, V.; Marín, S.; Ramos, A. J. Control de micotoxinas emergentes, situación legislativa actual. *Rev. Iberoam. Micol.* **2000**, *17*, S69−S75.
(32) Blaney, B. J.; Ramsey, M. D.; Tyler, A. L. Mycotoxins and toxigenic fungi in insect-damaged maize harvested during 1983 in Far North Queensland. *Aust. J. Agric. Res.* **1986**, *37*, 235−244.
(33) Rheeder, J. P.; Marasas, W. F. O.; van Wyk, P. S. Fungal associations in corn kernels and effects on germination. *Phytopathology* **1990**, *80*, 131−134.
(34) Reid, L. M.; Nicol, R. W.; Ouellet, T.; Savard, M.; Miller, J. D.; Young, J. C.; Stewart, D. W.; Schaafsma, A. W. Interaction of *Fusarium graminearum* and *F. moniliforme* in maize ears: disease progress, fungal biomass, and mycotoxin accumulation. *Phytopathology* **1999**, *89*, 1028−1037.
(35) Smith, D. R.; White, D. G. Diseases of corn. In *Corn and Corn Improvement*; Sprague, G. F., Dudley, J. W., Eds.; Agronomy 18; ASA: Madison, WI, 1988; pp 687−766.
(36) Murillo, I.; Cavallarin, L.; San Segundo, B. Cytology of infection of maize seedlings by *Fusarium moniliforme* and immunolocalization of the pathogenesis-related PRms protein. *Phytopathology* **1999**, *89*, 737−747.
(37) Bush, B. J.; Carson, M. L.; Cubeta, M. A.; Hagler, W. M.; Payne, G. A. Infection and fumonisin production by *Fusarium verticilloides* in developing maize kernels. *Phytopathology* **2004**, *94*, 88−93.
(38) Sotelo, J. Valor del maíz blanco para alimentación humana. Graduate Thesis, Universidad de Vigo, Spain, 2005.
(39) Falconer, D. S. *Introduction to Quantitative Genetics*; Ronald Press: New York, 1960.
(40) Brewbaker, J. L.; Kim, S. K. Inheritance of husk cover and ear insect damage in maize. *Crop Sci.* **1979**, *19*, 32−36.
(41) Butrón, A.; Li, R. G.; Guo, B. Z.; Widstrom, N. W.; Snook, M. E.; Cleveland, T. E.; Lynch, R. E. Molecular markers to increase corn earworm resistance in a maize population. *Maydica* **2001**, *46*, 117−124.
(42) Schaafsma, A. W.; Miller, J. D.; Savard, M. E.; Ewing, R. J. Ear rot development and mycotoxins production in corn in reations to inoculation method, corn hybrid and species of *Fusarium*. *Can. J. Plant Pathol.* **1993**, *15*, 185−192.

**Received for review April 20, 2006. Revised manuscript received June 9, 2006. Accepted June 9, 2006.**

JF0611163