Journal of Food Protection, Vol. 70, No. 1, 2007, Pages 151–156

Natural Occurrence of *Fusarium* Species, Fumonisin Production by Toxigenic Strains, and Concentrations of Fumonisins B_1 and B_2 in Conventional and Organic Maize Grown in Spain

AGUSTÍN ARIÑO,[1]* TERESA JUAN,[2] GLORIA ESTOPAÑAN,[2] AND JOSÉ F. GONZÁLEZ-CABO[1]

[1]*Veterinary Faculty, University of Zaragoza, c/Miguel Servet 177, 50013 Zaragoza, Spain; and* [2]*Agri-Food Research and Technology Center, Avda. Montañana 930, 50059 Zaragoza, Spain*

MS 06-222: Received 18 April 2006/Accepted 29 July 2006

ABSTRACT

Sixty samples of corn from both conventional and organic farms were tested for internal fungal contamination. Molds were identified to genus, and those belonging to the genus *Fusarium* were identified to species. Twenty isolates of *Fusarium verticillioides* were tested with a high-performance liquid chromatography–naphthalene dicarboxaldehyde–fluorescence method for their ability to produce fumonisins B_1 and B_2. The internal fungal infection in organic maize (63.20%) was significantly higher than that in conventional maize (40.27%) ($P < 0.05$). However, the distribution of fungal genera indicated a significantly higher prevalence of *Fusarium* in conventional (34.93%) than in organic (18.15%) maize, making *Fusarium* the predominant fungus in conventional maize. This difference in mold distribution between organic and conventional maize was attributed to the difference in cultivation system. The dominant *Fusarium* species in both conventional and organic samples was *F. verticillioides.* There were no significant differences in the ability of 20 selected isolates of *F. verticillioides* to produce fumonisins on conventional or organic corn. Up to 13.3% of the conventional corn samples contained fumonisins B_1 and B_2 at mean concentrations of 43 and 22 ng/g, respectively. Organic corn samples had somewhat lower levels of contamination: 35 ng/g fumonisin B_1 and 19 ng/g fumonisin B_2 ($P > 0.05$). The organic farming system, with well-balanced crop rotation, tillage, and compost fertilization, produced corn that was less likely to be contaminated with *Fusarium* species, although no significant difference in fumonisin concentrations was found between the two types of contaminated corn.

Fungal contamination of foods and feeds causes considerable economic losses due to direct damage to crops, discoloration, off-odors, taints, off-flavors, reduced yields, and loss of nutritive value. Fungal contamination of foods is associated with production of mycotoxins, which have toxic effects on both humans and animals. In the temperate climatic conditions prevailing in Europe, *Fusarium* fungi are important in the cereal food chain and can reduce crop yields and contaminate grain with mycotoxins *(29). Fusarium* includes many species that are pathogenic to plants, responsible for a broad range of diseases, and mycotoxigenic. In Aragón (Spain), maize diseases caused by *Fusarium* have been reported since the early 1980s *(21).*

Members of the *Gibberella fujikuroi* complex (e.g., *Fusarium verticillioides,* a synonym of *Fusarium moniliforme*) are generally regarded as the most important colonizers of cereal grains and can produce fumonisins *(27, 30).* Fumonisins have been associated with leukoencephalomalacia in equine species, pulmonary edema in pigs, and esophageal cancer in humans *(33).* Fumonisin B_1 (FB_1) is classified as possibly carcinogenic to humans (group 2B) by the International Agency for Research on Cancer *(12).* The European Commission Scientific Committee for Food *(27)* has evaluated fumonisins and established a provisional maximum tolerable daily intake of 2 μg/kg of body weight per day for the total of FB_1, FB_2, and FB_3, either alone or in combination. The European Union recently regulated fumonisins (as the sum of FB_1 and FB_2) in maize-based products and unprocessed maize so that if no specific concentration is fixed before 1 October 2007, maximum concentrations from 200 to 2,000 μg/kg will apply thereafter *(10).*

Organic products of plant origin are grown without the aid of chemical synthetic pesticides and largely without the use of readily soluble mineral fertilizers within a diverse system of crop rotation and extensive soil tillage. One hypothesis is that organic foodstuffs are more prone than conventional foodstuffs to contamination by mycotoxins because organic products are not treated to the same extent with antifungal agents. However, this hypothesis was not supported by a literature review conducted in 2000 by the Food and Agriculture Organization of the United Nations *(11).* In a recent study, organic farming systems had lower rates of *Fusarium* ear blight infection and lower mycotoxin contamination in winter wheat (*Triticum aestivum*) than did conventional farming systems *(4).* However, fungal attack and mycotoxin contamination in organically and conventionally grown produce is still an extremely controversial issue *(15).*

With the steady and rapid growth of the European and U.S. markets for organic foods, there is a need for quality control, including safety evaluation of the products *(8).* In organic cereals, the main concern is the growth of myco-

* Author for correspondence. Tel: +34-976-761543; Fax: +34-976-761612; E-mail: aarino@unizar.es.

toxin-producing fungi that can cause toxic syndromes in humans and animals. For maize, not all factors involved in the formation of *Fusarium* toxins, in particular FB_1 and FB_2, are known. Therefore, there is need for investigations of the formation of these mycotoxins and the identification of the management measures needed to prevent their presence in foodstuffs. The present study was carried out to gain information on fungal infection and the presence of the mycotoxins FB_1 and FB_2 in conventional and organic corn grown in Spain.

MATERIALS AND METHODS

Corn samples. Thirty samples of corn were selected from farms using conventional cultivation methods, and another 30 samples were collected from organic farms; no samples were moldy in appearance. These corn samples from various harvesting years (2001 to 2003) were collected 1 to 4 weeks after harvest, October to November, at farms in Aragón. The objective was to obtain pairs of samples (conventional and organic) from farms at neighboring sites. These sites differed in agricultural practices and fertilizer and pesticide usage, but environmental factors such as climate and soil conditions were as comparable as possible. At the conventional farms, the maize seeds had been treated with fungicides (approved treatments included 70% himexazol at 2 kg/Tm and 40% maneb at 3 liters/Tm), and insecticides and herbicides were sprayed as needed during the vegetation period. Mineral fertilization of soil also was used on the conventional farms. On the organic farms, by contrast, no pesticides and fungicides had been used, and agronomic techniques included tilling and crop rotation with legumes and alfalfa plus fertilization with compost.

To prepare representative samples, up to 10 250-g subsamples were collected from each lot of cereal. The subsamples were pooled, and a single 0.50-kg composite sample was taken to the laboratory and stored at −21°C until analyzed for fungi and mycotoxins.

Isolation and identification of fungi. Fifty kernels from each sample were surface sanitized in a 5% aqueous solution of sodium hypochlorite (NaOCl) for 1 min, rinsed twice with sterile distilled water, and then dried with sterile paper towels. Fifty kernels were then plated (five kernels per plate) on dichloran rose bengal chloramphenicol agar. The plates were incubated in the dark at 25°C for 5 to 7 days, and the fungal colonies that developed from the kernels were identified and counted. The colonies that developed were streaked for isolation and maintained on potato-dextrose agar (PDA) slants. *Fusarium* isolates were identified according to the method of Nelson et al. *(17)* using carnation-leaf agar (CLA) and PDA. Slide mounts were prepared and observed under a phase contrast microscope. *Alternaria, Aspergillus, Penicillium,* and other less often isolated genera were identified by microscopic morphology and culture characteristics in malt-extract agar, PDA, and Czapek agar according to the method of Samson et al. *(26).* The frequency distribution of the fungal genera is defined as the ratio of the number of kernels infected with each genus to the total number of infected kernels.

Production of fumonisins by *F. verticillioides* isolates. For farm site comparisons of *F. verticillioides* isolates, we selected 10 isolates from different conventional corn samples and 10 isolates from matched organic corn samples. These 20 selected isolates were grown on autoclaved corn following the technique described by Ross et al. *(23).* Fifty grams of yellow corn and 50 g of water were added to a 250-ml beaker and left at room temperature for 1 h. The beakers were then covered with aluminum foil, autoclaved for 1 h, and cooled, and the corn was stirred to separate the kernels. The beakers were then covered with a cotton plug, sealed, and autoclaved again for 1 h. After the cooling period, 1 ml of phosphate-buffered saline inoculum (a suspension of conidia from single spore cultures on CLA) was introduced with a syringe. The cultures were incubated in the dark for 4 weeks at ambient temperature. The culture material was then autoclaved for 5 min, dried at 60°C, ground with a laboratory mill (IKA, Wilmington, N.C.), and stored at 4°C until analyzed.

Apparatus and reagents for mycotoxin analysis. High-performance liquid chromatography (HPLC) grade acetonitrile and methanol were purchased from Lab-Scan (Dublin, Ireland), and HPLC grade acetic acid was obtained from Merck (Darmstadt, Germany). Ultrapure water was obtained with a Milli-Q Plus apparatus (Millipore, Milford, Mass.). The solid-phase extraction columns were Multisep 211 Fum columns (Romer Labs, Union, Mo.). Fumonisins standards for FB_1 and FB_2 were provided by Sigma (St. Louis, Mo.), and stock solutions (1.0 mg/ml) were prepared in acetonitrile-water (1:1, vol/vol) and stored at 4°C. NDA reagent (2,3-naphthalene dicarboxaldehyde; Sigma) was prepared in methanol at 0.5 mg/ml and stored at 4°C. Reagents for sodium borate buffer (0.05 M), phosphate buffer (0.05 M), and sodium cyanide solution (0.13 mg/ml) were provided by Panreac (Barcelona, Spain).

The liquid chromatography (LC) system consisted of a Kontron model 322 pump, a model 360 autosampler, and an SFM 25 fluorescence detector at 420 nm (excitation) and 500 nm (emission). The LC column was a Kromasil RP C_{18} (250 by 4.6 mm, 5-μm particle size; Varian Inc., Lake Forest, Calif.). The LC mobile phase was a gradient of acetonitrile–acetic acid (solution A; 99:1, vol/vol) and water–acetic acid (solution B; 99:1, vol/vol) with the following protocol: 60% A + 40% B for 8 min, 80% A + 20% B for 16 min, hold for 4 min, and reequilibrate to initial conditions. The flow rate was 1 ml/min.

Analysis of fumonisins in corn and laboratory cultures. The technique for extraction and determination of fumonisins was based on the work of Bennett and Richard *(3).* A representative corn sample was ground in the laboratory mill and mixed. Twenty-five grams (corn) or 5 g (cultures) were extracted with 100 ml of acetonitrile-water (50:50, vol/vol) by shaking for 60 min on a wrist-action shaker. The extract was filtered through Whatman no. 1 filter paper, and the pH was adjusted to 6 to 7 with 0.5 M sodium hydroxide. Cleanup was carried out with Multisep 211 Fum columns according to the instructions of the manufacturer (Romer). The fumonisin-containing eluate was evaporated to dryness in a heating block under a gentle stream of nitrogen, and the residue was redissolved in 1 ml of methanol. The content of this vial was derivatized by adding 1 ml of 0.05 M sodium borate buffer, 0.5 ml of sodium cyanide reagent, and 0.5 ml of NDA reagent. The mix was heated at 60°C for 15 min in a heating block, cooled, and diluted with 7 ml of 0.05 M phosphate buffer–acetonitrile (40:60, vol/vol). Aliquots of 20 to 100 μl were injected into the LC fluorescence detection system.

The average recoveries and relative standard deviations (RSDs) obtained by the described method for FB_1 and FB_2 were 95.0% (RSD = 4%) and 85.0% (RSD = 4%), respectively. The performance characteristics for the analytes FB_1 and FB_2 were within the acceptable margins indicated in Commission Directive 2005/38/EC *(9).* The study of sensitivity indicated that the limit of quantification (LOQ) for FB_1 and FB_2 was 25 ng/g. The linear range was from LOQ to 25 times the LOQ. The samples were diluted with phosphate buffer–acetonitrile as needed to fit within the linear range. The incidences of FB_1 and FB_2 were expressed

TABLE 1. *Total fungal infection and* Fusarium *infection in conventionally and organically grown corn by year of harvest*[a]

Infection	Growing conditions	% positive samples (mean ± SE)[a]		
		2001	2002	2003
All fungi	Conventional	38.80 ± 7.55 A	40.20 ± 7.79 A	41.80 ± 10.78 A
	Organic	70.00 ± 7.48 A	56.60 ± 7.31 A	63.00 ± 7.64 A
Fusarium	Conventional	32.70 ± 2.22 A	38.44 ± 1.46 A	33.64 ± 3.13 A
	Organic	15.65 ± 2.90 A	18.95 ± 3.31 A	19.85 ± 4.00 A

[a] $n = 10$ for each type of farm for each harvest year. Within each row, means with different letters are significantly different ($P < 0.05$, ANOVA).

as the percentage of samples containing concentrations above 25 ng/g. The mean was calculated using one half of the LOQ for results lower than the LOQ.

Statistical analysis. The data were analyzed with an analysis of variance for significance among groups according to the method of Sachs *(24).* Calculations were performed on StatView SE+Graphics (1988, Abacus Concepts, Inc., Berkeley, Calif.) for Macintosh personal computers.

RESULTS AND DISCUSSION

The incidences of total fungal infection and *Fusarium* infection in conventional and organic corn samples during the 3 harvest years (2001 through 2003) are shown in Table 1. Total infection was 38.80 to 41.80% on conventional farms and 56.60 to 70% on organically managed farms. Similarly, *Fusarium* infection remained essentially unchanged through the harvest seasons, 32.70 to 38.44% on conventional farms and 15.65 to 19.85% on organic farms. There were no significant differences between harvesting years in the mycoflora of the crops ($P > 0.05$). This finding allowed for a multiyear comparison between conventional and organic samples from farms at neighboring sites.

The farm site comparison revealed distinct differences between conventionally and organically managed farms in terms of total fungal infection and distribution of fungal genera. The fungal infection of conventional and organic maize samples is presented in Table 2. Generally, the percentage of infected corn samples originating from organic farms (63.20%) was significantly higher ($P < 0.05$) than that from conventional farms (40.27%). The distribution of fungal genera found in the samples is shown in Table 3. Identical fungal genera were isolated from the maize grown in both farming systems. However, the predominant genus identified in conventional samples was *Fusarium* (34.93% infection) followed by *Alternaria* (25.19%). In contrast, the predominant genus isolated from organic maize was *Alternaria* (40.29% infection) followed by *Fusarium* (18.15%). This shift in dominance between *Fusarium* and *Alternaria* could have been caused by the differences in the two farming systems. *Fusarium* and *Alternaria* are generally considered field fungi, infecting growing plants in the field, although both fungi also can cause problems in stored grains. Changes in agricultural practices can affect the incidence of infection by field pathogens and the genera present in cereal crops. Tilling the soil between crops is indispensable in organic systems as a weed control technique because use of herbicides and fungicides is prohibited *(15).* Compared with no tillage, plough tillage reduces the incidence of *Fusarium* attack and the concentration of deoxynivalenol in cereal crops *(14).* Because the use of fungicides differentially affects the *Fusarium* species that infect the ears, resistance to some fungicides may selectively remove dominant but susceptible nontoxigenic species, allowing more active colonization by toxigenic species *(28).*

TABLE 2. *Incidence of internal fungal infection detected in conventional and organic corn grown in Spain*

Growing conditions	No. of samples	% positive samples	
		Mean ± SE[a]	Range
Conventional	30	40.27 ± 4.92 A	4–92
Organic	30	63.20 ± 4.29 B	16–94
Total	60	51.73 ± 3.56	4–94

[a] Means with different letters are significantly different ($P < 0.05$, ANOVA).

Fusarium infection was nearly 50% higher in conventionally grown maize than in organically grown maize. In contrast, the occurrence and distribution of the postharvest fungi *Aspergillus* and *Penicillium* was similar in both conventional and organic corn samples. *Fusarium* species infect the grain preharvest, and several risk factors have been identified in connection with *Fusarium* infection and mycotoxin formation, e.g., climatic conditions and the competitiveness of different fungal species. For example, Marin et al. *(16)* reported that the activity of several *Aspergillus* and *Penicillium* species in corn reduced the presence of *F. verticillioides.* In our study, the concurrent presence of *Aspergillus* and *Penicillium* did not play a significant role as a control factor for *Fusarium* development on maize kernels.

Recently summarized data *(31)* provide evidence for greater bacterial, actinomycete, and fungal abundance and activity under organic management systems, and most research suggests that organic farming practices have a positive, stimulating influence on the soil microbial community. Knudsen et al. *(13)* reported that the microbial biomass and activity of the soils on organic farms were higher than those in soils on conventional farms, although high biomass and activity were not always correlated with suppression of brown foot rot disease, caused by *Fusarium culmorum.* In comparison, several organic soil amendments significantly reduced the incidence of *Fusarium* stalk rot disease of

TABLE 3. *Frequency distribution of different fungal genera identified in conventional and organic corn samples*

Corn type	% of total fungal infection[a]				
	Aspergillus	*Penicillum*	*Fusarium*	*Alternaria*	Others
Conventional	14.19 A	9.40 A	34.93 A	25.19 A	16.29 A
Organic	14.37 A	12.34 A	18.15 B	40.29 B	14.85 A
Total	14.28	10.87	26.54	32.74	15.57

[a] Within a column, percentages with different letters are significantly different ($P < 0.05$, ANOVA).

maize, caused by *F. verticillioides (18).* Postma et al. *(22)* reported that compost-amended soil was suppressive against several fungal diseases of plants. One of the factors behind these antagonistic effects has been studied by Pal et al. *(20),* who isolated several rhizobacteria from maize that were strongly antagonistic in vitro to *F. verticillioides* and might be involved in the biological suppression of maize root diseases.

A representative number of isolated strains of *Fusarium* were identified to species according to the method of Nelson et al. *(17).* For current names of *Fusarium* species, we have followed the *Index Fungorum (1).* The most frequently identified *Fusarium* species was *F. verticillioides.* The prevalence of *F. verticillioides* as the predominant species isolated from corn kernels has been previously reported in Spain *(5, 6, 25)* and in other parts of the world *(2, 32).*

Fumonisin production by cultures of 20 selected *F. verticillioides* strains is shown in Table 4. These results were obtained with the HPLC–naphthalene dicarboxaldehyde–fluorescence method. Of the 10 strains isolated from conventional maize, 80% produced FB_1 and FB_2. For the 10 strains isolated organic maize, 80% produced FB_1 and 70% produced FB_2. The occurrence of fumonisin producing strains was much higher than that reported by Sala et al. *(25)* but very similar to more recent data presented by Castella et al. *(6),* both in Spanish corn samples. There was a high degree of variability in the concentration of fumonisins produced by the *F. verticillioides* strains. In conventional isolates, FB_1 concentrations were 108 to 1,057 μg/g, with a mean value of 447.9 μg/g, and FB_2 concentrations were 59 to 410 μg/g, with a mean value of 140.3 μg/g. The fumonisin concentrations observed in organic isolates were somewhat lower, with mean values of 283.3 μg/g for FB_1 (range, 58 to 1,062 μg/g) and 98.5 μg/g for FB_2 (63 to 307 μg/g). However, these small differences in fumonisin production between conventional and organic isolates were not significant ($P > 0.05$).

Fumonisins were detected above the LOQ (25 ng/g) in 13.3% of the samples of conventional corn and in 10.0% of the samples of organic corn. The concentrations of FB_1 and FB_2 were 43 and 22 ng/g, respectively, in conventional corn samples but somewhat lower in organic corn samples, 35 and 19 ng/g, respectively (Table 5). Again, these small differences in fumonisin concentrations between conventional and organic corn samples were not significant ($P > 0.05$). Similarly, the agricultural system did not have any significant effect on the fumonisin concentrations found in conventional and organic cornflakes from the Belgian market *(19).* However, in a study of the Italian market, Cirillo et al. *(7)* reported that the highest median concentration of FB_1 occurred in conventional maize-based foods ($P > 0.05$) whereas the highest median concentration of FB_2 was in organic maize-based foods ($P < 0.05$).

In our study, the fumonisin concentrations in the corn samples were very low, much lower than the maximum of 2,000 ng/g (for the sum of FB_1 and FB_2) proposed for unprocessed maize in a recent EU regulation *(10).* However, during this survey, a maize sample suspected to have caused an outbreak of equine leukoencephalomalacia resulting in two dead ponies came to the laboratory. This sample was heavily contaminated with *F. verticillioides* and had an extremely high concentration of fumonisins at 12.5 μg/g (the sum of FB_1 and FB_2). Thus, high concentrations of *Fusarium* mycotoxins can occur in cereal grown in Aragón, which is a concern because some of these cereals are intended for human consumption.

Maize from an organic farm had a 50% lower *Fusarium* infection rate than did maize from a conventional farm, probably mainly because of lower intensity of cultivation, different crop rotation, plough tillage, and the distinct biomass activity of organic soils. However, although microbial interactions can lead to decreased colonization by *Fusarium* in organically grown maize, a significant decrease in fumonisin concentration may not occur. The present study is

TABLE 4. *Fumonisin production by cultures of selected* F. verticillioides *isolates from conventional and organic maize*

Corn type	No. of isolates	Fumonisin B_1		Fumonisin B_2	
		% positive cultures	Concn (μg/g)[a]	% positive cultures	Concn (μg/g)
Conventional	10	80	447.90 ± 109.06 A	80	140.30 ± 37.97 A
Organic	10	80	283.30 ± 100.99 A	70	98.5 ± 30.48 A
Total	20	80	365.60 ± 74.76	75	119.4 ± 24.17

[a] Values are mean ± standard error. Within a column, means with different letters are significantly different ($P < 0.05$, ANOVA).

TABLE 5. *Total fumonisin in conventional and organic corn samples*

Corn type	No. of samples	Fumonisin B_1		Fumonisin B_2	
		% positive samples	Concn (ng/g)[a]	% positive samples	Concn (ng/g)
Conventional	30	13.33	43.19 ± 15.52 A	10.00	21.92 ± 5.28 A
Organic	30	10.00	35.36 ± 13.74 A	6.67	18.78 ± 4.90 A
Total	60	11.67	39.27 ± 10.29	8.33	20.35 ± 3.58

[a] Values are mean ± standard error. For values below the LOQ (25 ng/g), the mean was recorded as one half of the LOQ. Within a column, means with different letters are significantly different ($P < 0.05$, ANOVA).

only a partial risk assessment because other mycotoxins such as deoxynivalenol, zearalenone, aflatoxins, ochratoxin, and alternaria mycotoxins were not analyzed.

ACKNOWLEDGMENTS

This research was supported by the Government of Aragón (Spain) through projects DGA/Grupos de Investigación Consolidados and PIP065/2005. We thank L. Pitarch, M. Benito, and J. Guilmin for their assistance with the laboratory work and F. Villa and the Aragonese Committee for Organic Agriculture for the collection of samples.

REFERENCES

1. Anonymous. 2006. Index fungorum. Available at: http://www.indexfungorum.org. Accessed 7 April 2006.
2. Ariño, A., and L. B. Bullerman. 1994. Fungal colonization of corn grown in Nebraska in relation to year, genotype and growing conditions. *J. Food Prot.* 57:1084–1087.
3. Bennett, G. A., and J. L. Richard. 1994. Liquid chromatographic method for analysis of the naphthalene dicarboxaldehyde derivative of fumonisins. *J. AOAC Int.* 77:501–506.
4. Birzele, B., A. Meier, H. Hindorf, J. Krämer, and H. W. Dehne. 2002. Epidemiology of *Fusarium* infection and deoxinivalenol content in winter wheat in the Rhineland, Germany. *Eur. J. Plant Pathol.* 108: 667–673.
5. Cantalejo, M. J., J. M. Carrasco, and E. Hernández. 1998. Incidence and distribution of *Fusarium* species associated with feeds and seeds from Spain. *Rev. Iberoam. Micol.* 15:36–39.
6. Castella, G., M. R. Bragulat, and F. J. Cabañes. 1999. Fumonisin production by *Fusarium* species isolated from cereals and feeds in Spain. *J. Food Prot.* 62:811–813.
7. Cirillo, T., A. Ritieni, M. Visone, and R. A. Cocchieri. 2003. Evaluation of conventional and organic Italian foodstuffs for deoxinivalenol and fumonisins B_1 and B_2. *J. Agric. Food Chem.* 51:8128–8131.
8. Dimitri, C., and L. Oberholtzer. 2006. EU and U.S. organic markets face strong demand under different policies. *Amber Waves* 4:12–19.
9. European Commission. 2005. Directive 2005/38/EC of 6 June 2005, laying down the sampling methods and the methods of analysis for the official control of the levels of *Fusarium* toxins in foodstuffs. *Off. J. Eur. Union* L143:18–26.
10. European Commission. 2005. Regulation (EC) no 856/2005 of 6 June 2005 amending regulation (EC) no 466/2001 as regards *Fusarium* toxins. *Off. J. Eur. Union* L143:3–8.
11. Food and Agriculture Organization. 2000. Food safety and quality as affected by organic farming. Presented at the 22nd Food and Agriculture Organization Regional Conference for Europe, Porto, Portugal, 24 to 28 July 2000.
12. International Agency for Research on Cancer. 2002. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. *IARC Monogr.* 82:301–366.
13. Knudsen, I. M. B., K. Debosz, J. Hockenhull, D. F. Jensen, and S. Elmholt. 1999. Supressiveness of organically and conventionally managed soils towards brown foot rot of barley. *Appl. Soil Ecol.* 12: 61–72.
14. Krebs, H., B. Streit, and H. R. Forrer. 2000. Effect of tillage and preceding crops on *Fusarium* infection and deoxynivalenol content of wheat, p. 13. *In* T. Alfoldi, W. Lockeretz, and U. Niggli (ed.), The world grows organic. Proceedings of the 13th International IFOAM Scientific Conference. IOS Press, Basel.
15. Magkos, F., F. Arvaniti, and A. Zampelas. 2006. Organic food: buying more safety or just peace of mind? A critical review of the literature. *Crit. Rev. Food Sci. Nutr.* 46:23–56.
16. Marin, S., V. Sanchis, F. Rull, A. J. Ramos, and N. Magan. 1998. Colonization of maize grain by *Fusarium moniliforme* and *F. proliferatum* in the presence of competing fungi and their impact on fumonisin production. *J. Food Prot.* 61:1489–1496.
17. Nelson, P. E., T. A. Toussoun, and W. F. O. Marasas. 1983. *Fusarium* species. An illustrated manual for identification. Pennsylvania State University Press, University Park.
18. Osunlaja, S. O. 1990. Effect of organic soil amendments on the incidence of stalk rot of maize caused by *Macrophomina phaseolina* and *Fusarium moniliforme*. *J. Basic Microbiol.* 30:753–757.
19. Paepens, C., S. De Saeger, L. Sibanda, I. Barna-Vetroä, M. Anselme, Y. Larondelle, and C. Van Peteghem. 2005. Evaluation of fumonisin contamination in cornflakes on the Belgian market by ''flow-through'' assay screening and LC-MS/MS analyses. *J. Agric. Food Chem.* 53:7337–7343.
20. Pal, K. K., K. V. B. R. Tilak, A. K. Saxena, R. Dey, and C. S. Singh. 2001. Suppression of maize root diseases caused by *Macrophomina phaseolina, Fusarium moniliforme* and *Fusarium graminearum* by plant growth promoting rhizobacteria. *Microbiol. Res.* 156:209–223.
21. Palazón, C. F., and M. L. Palazón. 1982. Estudio patológico de problemas de nascencia de maíz en la zona de Ejea de los Caballeros (Zaragoza). *Inf. Téc. Econ. Agraria* 46:47–53.
22. Postma, J., M. Montanari, and P. H. J. F. Van der Boogert. 2003. Microbial enrichment to enhance the disease suppressive activity of compost. *Eur. J. Soil Biol.* 39:157–163.
23. Ross, P. F., P. E. Nelson, J. L. Richard, G. D. Osweiler, L. G. Rice, R. D. Plattner, and T. M. Wilson. 1990. Production of fumonisins by *Fusarium moniliforme* and *Fusarium proliferatum* isolates associated with equine leukoencephalomalacia and a pulmonary edema syndrome in swine. *Appl. Environ. Microbiol.* 56:3225–3226.
24. Sachs, L. 1978. Estadística aplicada, 1st ed. Labor, Barcelona, Spain.
25. Sala, N., V. Sanchis, P. Vilaro, R. Viladrich, M. Torres, I. Viñas, and R. Canela. 1994. Fumonisin producing capacity of *Fusarium* strains isolated from cereals in Spain. *J. Food Prot.* 57:915–917.
26. Samson, R. A., E. S. Hoekstra, and J. C. Frisvad (ed.). 2004. Introduction to food- and airborne fungi. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.
27. Scientific Committee on Foods, European Commission. 2003. Updated opinion of the Scientific Committee on Foods on fumonisin B_1, B_2 and B_3. SCF/CS/CNTM/MYC/28 final. Directorate—scientific opinions. European Commission, Brussels.
28. Scientific Committee on Plants, European Commission. 1999. Opinion on the relationship between the use of plant protection products

on food plants and the occurrence of mycotoxins in foods. SCP/RESI/063–final. Directorate—scientific opinions. European Commission, Brussels.
29. Scientific Cooperation, European Commission. 2003. Task 3.2.10. Collection of occurrence data of *Fusarium* toxins in food and assessment of dietary intake by the population of EU member states. Final report. Directorate—general health and consumer protection. European Commission, Brussels.
30. Shephard, G. S., P. G. Thiel, S. Stockenström, and E. W. Sydenham. 1996. Worldwide survey of fumonisin contamination of corn and corn-based products. *J. AOAC Int.* 79:671–687.
31. Stark, C. 2005. Effects of long- and short-term crop management on soil biological properties and nitrogen dynamics. Ph.D. thesis. Lincoln University, Canterbury, New Zealand.
32. Torres, A. M., M. M. Reynoso, F. G. Rojo, M. L. Ramirez, and S. N. Chulze. 2001. *Fusarium* species (section *Liseola*) and its mycotoxins in maize harvested in northern Argentina. *Food Addit. Contam.* 18:836–843.
33. Visconti, A., W. F. O. Marasas, J. D. Miller, and R. Riley. 1999. Mycotoxins of growing interest: fumonisins. Third Joint FAO/WHO/UNEP International Conference on Mycotoxins, Tunis, Tunisia, 3 to 6 March 1999.