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Mycoflora and Occurrence of Alternariol and Alternariol Monomethyl Ether in Brazilian Sunflower from Sowing to Harvest

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The present study aimed to analyze the mycoflora and the occurrence of alternariol (AOH) and alternariol monomethyl ether (AME) in grain samples of sunflower during different stages of plant development in Nova Odessa, State of São Paulo, Brazil. The data obtained were correlated with the presence of fungi in soil, wind-dispersed fungi, and the predominant climatic conditions of the region where the experiment was carried out. Analysis of the mycoflora revealed the presence of *Fusarium verticillioides* and *Alternaria alternata* in 70% and 46% of the samples, respectively. The profile of wind-dispersed fungi also showed *F. verticillioides* as the most frequently isolated fungus (68%), although *A. alternata* was detected in 28% of samples. In soil, *Penicillium* was the most frequent species (49.9%), followed by *F. verticillioides* (47.7%) and *A. alternata* (10.9%). Regarding water activity, sunflower grains presenting a high frequency of isolation of *F. verticillioides* and *A. alternata* had a water activity ranging from 0.92 to 0.96, and statistical analysis revealed a negative linear correlation between the isolation of fungi and water activity. HPLC analysis showed that 18% of the sunflower grains were contaminated with alternariol (24.9−170.9 ng/g) and 10% with alternariol monomethyl ether (14.1−108.6 ng/g). The contamination of sunflower grains with AOH and AME in the field was low when compared to the LD_{50} necessary to cause toxicity to animals. However, the contamination with other toxigenic fungi such as *F. verticillioides* may indicate the presence of other mycotoxins in sunflower grains and a possible synergistic effect between them. This is the first report of the natural occurrence of alternariol and alternariol monomethyl ether in sunflower grains in Brazil.

KEYWORDS: sunflower; alternariol; alternariol monomethyl ether; mycoflora

INTRODUCTION

Sunflower is among the main oleaginous plants cultivated in the world. In Brazil, its production has been expanding over the past few years to attend the growing internal market for oil consumption and animal feeding. Despite the increase in production, the price paid to the producer by agroindustrial companies is low, and the most relevant factor in terms of culture consolidation for grain processing is the quality of the raw material.

Contamination with different *Alternaria* species is observed during all stages of development, but the plants show a higher susceptibility from the appearance of the anthers to grain filling (*1*). *Alternaria alternata* produces various secondary metabolites, including alternariol (AOH) and alternariol monomethyl ether (AME), which exerts cytotoxic effects mainly in reproductive organs (*2*).

In Brazil, few studies regarding the mycoflora present in sunflower (*3*) and the occurrence of mycotoxins are available. Therefore, a better understanding of the process of contamination of sunflower grains with *Alternaria* is necessary to develop control strategies that reduce the risks of human exposure to mycotoxins. The present study aimed to analyze the mycoflora and the occurrence of alternariol (AOH) and alternariol monomethyl ether (AME) in grain samples of sunflower during different stages of plant development in Nova Odessa, State of São Paulo, Brazil. It also aimed to correlate the results with abiotic factors (water activity, temperature, and rainfall) and the incidence of wind-dispersed fungi and soil.

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MATERIAL AND METHODS

Sunflower Samples. The sunflower cultivar (Catissol, seeds from CATI) of the 2002 harvest was cultivated at the Experimental Station of Zootechny, Nova Odessa, State of São Paulo, situated at 22° 42′ S latitude, 47° 18′ W longitude and at an altitude of 550 m. Fifty sunflower grain samples were analyzed. The area selected for the trial consisted of 10 uniform plots measuring 80 m^2. Random sampling was performed in each plot every 10 days from the 80th day of cultivation on (*4*). Five capitulum samples were collected from the same seeding row during each collection period, and a 1-kg subsample of grains was removed and analyzed regarding the mycoflora, water activity, and occurrence of mycotoxins (AOH and AME).

Soil Samples. Fifty soil samples were analyzed. All samples were obtained from the surface (depth of 0−2 cm) around each plant, and a subsample of 1 kg was removed and analyzed regarding the mycoflora and water activity.

Climatic Conditions of the Region. The main climatic factors were determined with specific instruments such as thermometers, rain gauges, anemometers, heliographs, and class A tanks.

Water Activity Determination. Water activity of the sunflower grains and soil was determined automatically with a Testo 650 apparatus (Testo do Brasil Ltda). Each sample was measured five times.

Recovery, Identification, and Enumeration of the Soil and Sunflower Mycoflora. *(a) Isolation of the Mycoflora from Sunflower Grains*. A subsample of ∼30 g was obtained from each sunflower sample, disinfected by immersion in 2% sodium hypochlorite for 3 min, and washed three times in sterile distilled water. Fifty-one grains of this subsample were then selected randomly and directly seeded onto Petri dishes containing Malt agar (17 grains per plates) (*5*), and the plates were incubated at 25 °C for 5 days. The results are reported as percent infected grains per fungi. The recovered colonies were identified as recommended for each genus (*6*−*9*).

(b) Isolation of the Mycoflora from Soil Samples. The soil samples were analyzed by the method of Swanson et al. (*10*). Martin medium was used for the isolation of the mycoflora (*11*). The plates were incubated at 25 °C for 7 days and observed daily. The recovered fungal colonies were identified using standard methods (*6*−*9*).

Determination of Wind-Dispersed Fungi. Sampling of the wind-dispersed fungi was started before grain filling. For each period, 10 Petri dishes containing Sabouraud Dextrose agar (*12*) were kept open for 15 min to allow the deposition of wind-dispersed fungi spores. The plates were then incubated for 7 days at 25 °C, and the colonies were identified using standard methods recommended for each genus (*6*−*9*). The analysis of wind-dispersed fungi was carried out throughout the periods of soil and sunflower grain sampling.

Determination of Alternariol and Alternariol Monomethyl Ether in Sunflower Grain Samples. Triturated sunflower grains (50 g) were mixed with 150 mL of methanol and shaken for 5 min. After shaking, the content was filtered through Whatman no. 1 filter paper, and 50 mL of methanol was added. A 200 mL aliquot was mixed with 60 mL of 10% ammonium sulfate solution, and the extract was filtered again. Then, this solution was transferred to a separation funnel, and 50 mL of water at 8 °C was added. Two extractions with 40 mL of chloroform, shaking for 2 min each time, were conducted.

After concentration, the extract was settled until reaching 2 mL with methanol, passed through anhydrous sodium sulfate, and cleaned up with a Bond-Elute C_{18} SPE cartridge (500 mg, Varian Harbor City, CA) preconditioned with methanol followed by Milli-Q water (Millipore, Bedford, MA), using 2 mL of each. The column was then washed with portions of 2 mL each of water followed by acetonitrile−water (1:3 v/v), and the toxins were finally eluted with 4 mL of acetonitrile−acetic acid (99:1 v/v).

AOH and AME were analyzed with a HPLC system (Shimadzu LC-10AD with a SPD-M10Avp diode array detector) suited with a Spherisorb ODS-2 reversed phase column (5 μm, 250 mm × 4.6 mm; Phenomenex, Ultracarb) at a flow rate of 0.7 mL/min at scan mode. The mobile phase used was methanol/water (80:20) with 300 mg of $ZnSO_4 \cdot H_2O$.

For quantification purposes, the calibration curve was constructed using mycotoxin standards (Sigma, St. Louis, MO). For the AOH calibration curve (5.30 min), the concentration used ranged from 40 to 160 ng/mL ($r^2 = 0.989$), and for AME (9.11 min), the concentrations ranged from 20 to 80 ng/mL ($r^2 = 0.995$); all injections were done in duplicate. The identity of the peak was confirmed comparing the retention times of the standard with the samples on the chromatograms (*13*).

Statistical Analysis. The data were analyzed by multiple linear regression analysis using the SAS version 6.11 statistical software to determine the influence of the independent variables (water activity of soil and sunflower, percentage of fungi in soil and air, temperature, relative air humidity, and rainfall) on the dependent variable (*A. alternata* in sunflower grains) (*14*, *15*).

Table 1. Concentration of Alternariol (AOH) and Alternariol Monomethyl Ether (AME) and Water Activity (A_W) in Sunflower Grains at Various Stages of Maturation Collected in Nova Odessa, SP, Brazil

days after flowering[a]	A_W[b]	*A. alternata* RF[b,c] (%)	AOH concn (ng/g) mean[b] (positive)	AME concn (ng/g) mean[b] (positive)
kernels	0.54–0.57	25.00	*d*	*d*
80	0.98–0.99	19.50	*d*	*d*
90	0.94–0.96	18.10	25.3 (3)	12.3 (2)
100	0.96–0.99	3.00	5.4 (2)	9.0 (1)
110	0.94–0.96	4.70	6.6 (2)	4.4 (2)
120	0.89–0.95	42.00	6.2 (2)	*d*

[a] 80 days after flowering = first collection; 90 days after florescence = second collection; 100 days after flowering = third collection; 110 days after florescence = fourth collection; 120 days after flowering = fifth collection; [b] Mean values of 10 samples. [c] Relative frequency. [d] Not detected.

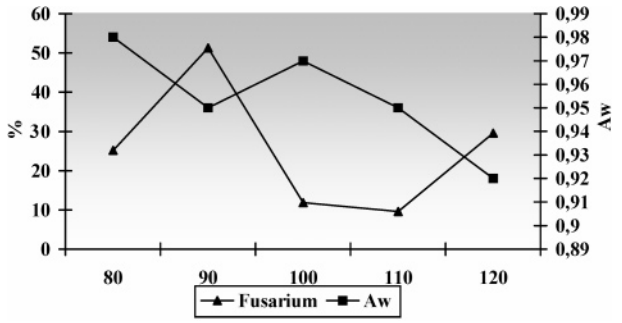

Figure 1. Mean frequency of *F. verticillioides* in 50 samples of sunflower grain at different maturity stages collected at Nova Odessa, State of São Paulo, Brazil.

RESULTS AND DISCUSSION

The sunflower grain samples collected during the different stages of development exhibited the following mycoflora: *Fusarium verticillioides* (70%), *Alternaria alternata* (46%), yeast (22%), *Cladosporium* spp. (18%), *Rhizopus* spp. (10%), *Aspergillus flavus* (8%), *Epicoccum* spp. (6%), *Penicillium* spp. (4%), and *Scopulariops* spp. (2%).

Fusarium verticillioides species presented frequency variation from 6% to 100%; this result was the major detectable percentage after 100 days after flowering (third period of collection), see **Table 1** and **Figure 1**. However, for *A. alternata*, the variation ranged from 3% to 42% with a major level obtained after 120 days of flowering (fifth period of collection) (**Table 1** and **Figure 2**). The reduced frequency obtained for *A. flavus* was 8% at the final stage of grain maturation (fifth period of collection).

The predominance of *F. verticillioides*, *A. alternata*, and *Cladosporium* spp. agrees with research of other investigators that isolated these fungi from sunflower seeds (*16*−*18*). These

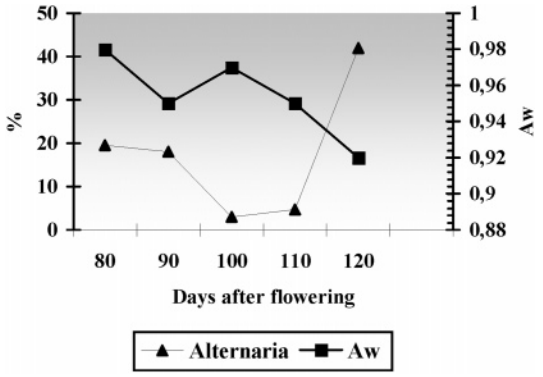

Figure 2. Mean frequency of *A. alternata* in 50 samples of sunflower grain at different maturity stages collected at Nova Odessa, State of São Paulo, Brazil.

fungi, which invade the tissues of growing plants, prefer to grow on substrates with elevated levels of humidity. The minimal water activity for their growth is 0.88 and the optimum at 0.98. In Brazil, high frequencies of *F. verticillioides* are reported in crop grains such as corn and sorghum (*19*, *20)*.

Analysis of 55 soil samples collected in Nova Odessa revealed the presence of the following mycoflora: *Penicillium* spp. (49.9%), *Fusarium* spp. (47.7%), *Cladosporium* spp. (29.1%), *Geotrichum* spp. (27.1%), *Rhizopus* spp. (10.9%), *Alternaria* spp. (10.9%), *Aspergillus* spp. (10.9%), *Mucor* spp. (7.3%), yeast (3.6%), *Epicoccum* spp. (3.6%), *Scopulariops* spp. (3.6%), *Neurospora* spp. (1.8%), and *Trichoderma* spp. (1.8%).

The high frequency of *Fusarium* and *Cladosporium* and the presence of *Alternaria* spp. in soil has also been described by other investigators (*19*−*21*). The number of colony forming units of the main toxigenic genera ranged from 1×10^4 to 500×10^4 (*Penicillium* spp.), from 1×10^4 to 400×10^4 (*Fusarium* spp.), from 1×10^4 to 200×10^4 (*Alternaria* spp.), and from 1×10^4 to 100×10^4 (*Aspergillus* spp.). The soil samples obtained during the different phases of maturation of sunflower grains showed water activity values ranging from 0.94 to 0.99.

The following airborne contaminants were detected: *Fusarium* spp. (68%), *Cladosporium* spp. (66%), *Neurospora* spp.- (38%), yeast (15%), *Alternaria* spp. (28%), *Penicillium* spp. (12%), *Rhizopus* spp. (12%), *Mucor* spp. (12%), *Aspergillus* spp. (8%), *Geotrichum* spp. (6%), *Trichoderma* spp. (6%), *Epicoccum* spp. (6%), and nonsporulated fungi (2%). Among the fungi isolated in the present study considered to be "universal dominant" (*12*), the genera *Fusarium* and *Alternaria*, which are important in the contamination of sunflower, were isolated from atmospheric air during all phases of grain maturation. The presence of *Alternaria* spp., *Penicillium* spp., and *Aspergillus* spp. in atmospheric air and the high frequency of *Fusarium* spp. have also been reported by Gambale (*12*) and Almeida et al. (*20*). According to Lacey (*22*), *Alternaria* spp., especially *A. alternata*, are some of the most isolated air fungi species after *Cladosporium* spp. around the world.

The isolation from sunflower grains, soil, and air in our experiment of fungi belonging to the genera *Fusarium* and *Alternaria* is in agreement with theories proposed by other investigators who postulated that soil and air infection routes are important vehicles of fungal dissemination in the field (*21*−*25*).

Analysis of 50 sunflower grain samples during different phases of development in the field revealed the presence of AOH in nine (18%) of the samples analyzed with levels ranging from 24.9 to 170.9 ng/g. The highest concentrations of AOH were detected in grains at 90 days of growth during the second collection, which corresponds to December 28, with the grains showing water activities ranging from 0.94 to 0.96.

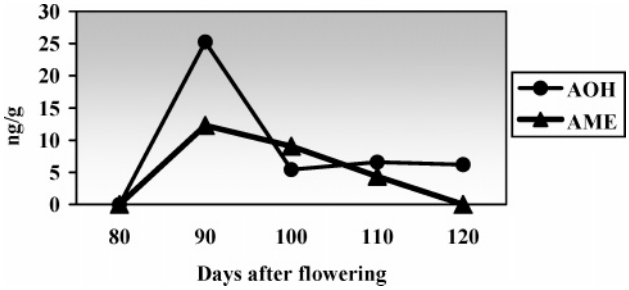

Figure 3. Mean levels of alternariol and alternariol monomethyl ether in sunflower grain at different maturity stages collected at Nova Odessa, State of São Paulo, Brazil.

Table 2. Rainfall and Mean Relative Humidity (RH) and Minimum and Maximum Temperatures Observed during the Experimental Period in the Region of Nova Odessa, SP, Brazil

days after flowering[a]	rainfall (mm)	RH (%)	min temp (°C)	max temp (°C)
80	68.2	77.0	19.9	30.6
90	54.8	85.7	18.4	28.2
100	102.8	87.3	19.0	30.0
110	142.4	95.3	19.6	29.5
120	11.4	86.8	18.5	26.8

[a] 80 days after flowering = first collection; 90 days after flowering = second collection; 100 days after flowering = third collection; 110 days after flowering = fourth collection; 120 days after flowering.

AME was detected in five (10%) of the samples analyzed between the second and fourth collection with levels ranging from 14.1 to 108.6 ng/g. The water activity of the grains ranged from 0.94 to 0.99. The highest concentration of the mycotoxin was also observed during the second collection, 90 days after flowering (**Table 1** and **Figure 3**). Studies regarding the contamination of sunflower grains with AOH and AME conducted in Argentina have shown a high percentage of samples contaminated with AOH (76−85%) and AME (47−62%) at levels ranging from 35 to 792 (AOH) and from 90 to 630 μg/kg (AME) (*26*, *27*).

In our experiment, the highest mean concentrations of mycotoxins were observed 90 days after flowering, a period characterized by lower rainfall (54.8 mm), a mean relative air humidity of 85.7%, and minimum and maximum temperatures of 18.4 and 28.2 °C, respectively (**Table 2**). The quantification limit of the method was determined as the minimum quantity of toxin detected in the fortified samples, which permitted confirmation using the diode array detector. The detection limit of pure toxin using the diode array detector was measured as three times the variation of baseline noise under the same conditions used for all the samples, including the blank (*13*). The detection limits of the method for AOH and AME were 5 and 2.5 ng/mL, respectively.

The quantification limit for AOH obtained after fortification of negative samples using six different concentrations ranging from 12.5 to 400 ng/mL was 25 ng/mL with a recovery of 78.9% and relative standard deviation (RSD) of 8.5%. For AME, the quantification limit found also using six different concentrations ranging from 5 to 200 ng/mL was 10 ng/mL with a recovery of 86.8% and RSD of 6.2%.

Fungi were isolated from sunflower grains at water activity levels ranging from 0.94 to 0.99. The highest frequencies of isolation of *F. verticillioides* were obtained when the water

activity was lower (0.94−0.96) at 90 days after flowering, and a peak of isolation was also observed at 120 days after flowering at a water activity of 0.92 (**Figure 3**).

The isolation of *F. verticillioides* from sunflower grains with lower water activity (0.92−0.96) and from soil with elevated water activity levels (0.94 to 0.99) is consistent with data on fungal isolation from corn samples in different regions of the State of São Paulo, Brazil (*20*).

A. alternata was isolated from sunflower grains with water activities ranging from 0.94 to 0.96 at 90 days after flowering and from sunflower grains with a water activity of 0.92 at 120 days after flowering. The highest concentrations of AOH and AME were observed at 90 days after flowering (**Table 1**), when the water activity of grains was between 0.94 and 0.96, and rainfall during the period was 54.8 mm. The low rainfall (11.4 mm) and low water activity of the grains after 120 days of maturation may have contributed to the lower concentrations of AOH (**Tables 1** and **2**). The fungus needs a minimal water activity of 0.85 to germinate and a water activity of 0.90 at a temperature of 25 °C for the production of AOH and AME (*28*). In the present study, the mean temperature at peak mycotoxin production was 23.3 °C.

Statistical analysis of the data revealed that water activity influenced the development of *F. verticillioides* and *A. alternata* as shown by the negative linear correlation ($p < 0.05$; $r = -0.61$) between the isolation of fungi and the water activity of sunflower grains in the region of Nova Odessa. Several studies have demonstrated the influence of water activity and temperature on the growth of fungal species (*28*−*32*) with the optimal water activity for the growth of *F. verticillioides* ranging from 0.87 to 0.98 and the temperature from 25 to 30 °C (*29*). In the present study, *A. alternata* showed a negative correlation with the maximum temperature ($p < 0.05$; $r = -0.52$), the fungus being more frequently isolated at milder maximum temperatures. Regarding the presence of *F. verticillioides* and *A. alternata* as the fungi most frequently isolated from sunflower grains, some studies have described these fungi as competitors for the same substrate and have reported that *F. verticillioides* grows faster than *A. alternata*. Compared to other fungal strains, *A. alternata* has been shown to be a faster competitor (*30*).

The contamination of sunflower grains with AOH and AME in the field was low when compared to the LD_{50} necessary to cause toxicity to animals (*33*). However, the contamination with other toxigenic fungi such as *F. verticillioides* may indicate the presence of other mycotoxins in sunflower grains and a possible synergistic effect between them.

LITERATURE CITED

(1) Davet, P.; Péres, A.; Regnault, Y.; Tourvielle, D.; Penaud, A. *Les Maladies du Tournesol*; Cetron: Paris, 1991.

(2) Visconti, A.; Logrieco, A.; Bottalico, A. Natural occurrence of *Alternaria* mycotoxins in olive-their production and possible transfer into the oil. *Food Addit. Contam.* **1986**, *3*, 323−330.

(3) Mentem, J. O. Diagnóstico de Patologia de Sementes de Girassol no Brasil. *Rev. Bras. Sementes* **1985**, 25−30.

(4) Delp, R. B.; Stwell, L. J.; Marois, J. J. Evaluation of field sampling techniques for estimation of disease incidence. *Phytopathology* **1986**, *76*, 1299−1305.

(5) Pitt, J. L.; King, A. D.; Hocking, A. D. Dichloran-rose Bengal medium for enumeration and isolation of molds from foods. *Appl. Environ. Microbiol.* **1979**, *37*, 954−964.

(6) Barnett, H. L.; Hunter, B. B. *Illustrated Genera of Imperfect Fungi*; Burguess Publishing Company: Minneapolis, MN, 1972, p 232.

(7) Ellis, M. B. *Dematiaceous Hyphomycetes*; CAB International: Wallinford, U.K., 1993, p 608.

(8) Pitt, J. I.; Hocking, A. D. *Fungi and Food Spoilage*; Blackie Academic & Professional: London, 1997, p 593.

(9) Nelson, E. P.; Touson, T. A.; Marasas, W. F. O. *Fusarium Species. An Illustrated Manual for Identification*; The Pennsylvania State University Press: London, U.K., **1983**, p 193.

(10) Swanson, K. M.; Busta, F. F.; Petterson, E. H.; Johnson, M. G. Colony Count methods. In *Compendium of Methods for the Microbiological Examination of foods*; Vanderzant, C., Splittoesser, D. S., Eds.; American Public Health Association: New York, 1992; Vol. 1, pp 75−95.

(11) Martin, J. P. Use of acid rose bengal and streptomycin in the plate method for estimating soil fungi. *Soil Sci.* **1950**, *69*, 215−232.

(12) Gambale, W.; Purchio, A.; Paula, C. R. Ação de fatores abióticos na dispersão aérea de fungos na cidade de São Paulo, Brasil. *Rev. Microbiol.* **1983**, *14*, 204−214.

(13) Motta, S.; Soares, L. M. V. A method for the determination of two *Alternaria* toxins, alternariol and alternariol monomethyl ether, in tomato products. *Braz. J. Microbiol.* **2000**, *31*, 315−320.

(14) Draper, N. R.; Smith, H. *Applied Regression Analyses*, 2nd ed; Wiley: New York, 1991.

(15) Searle, S. R.; Casella, G.; Mcculock, C. E. *Variance Components*; Wiley: New York, 1992.

(16) Zad, J. A note on the mycoflora of sunflower seeds in Iran. *Seed Sci. Technol.* **1978**, *6*, 953−956.

(17) Jimenez, M.; Mateo, R.; Querol, A.; Hverta, T.; Hernandez, E. Mycotoxins and mycotoxigenic moulds in nuts and sunflower seeds for human consumption *Mycopathologia* **1991**, *115*, 121−127.

(18) Robertson, J. A.; Roberts, R. G.; Chapman, G. W. An evaluation of "Heat damage" and fungi relation sunflower seed quality. *Phytopathology* **1985**, *75*, 143−145.

(19) Pozzi, C. R.; Corrêa, B.; Gambale, W.; Paula, C. R.; Chacon-Reche, N. O.; Meirelles, M. C. A. Post-harvest and stored corn in Brazil mycoflora interaction, abiotic factors and mycotoxins occurrence. *Food Addit. Contam.* **1995**, *12*, 313−319.

(20) Almeida, A. P.; Fonseca, H.; Fancelli, A. L.; Direito, G. M.; Ortega, E.; Corrêa, B. Mycoflora and fumonisin contamination in Brazilian corn from sowing to harvest. *J. Agric. Food Chem.* **2002**, *50*, 3877−3882.

(21) Asan, A. Microfungi flora occurrence in the corn field of the European part of Turkey. *Turk. J. Biol.* **1997**, *21* (1), 89−101.

(22) Lacey, J. Pre- and post-harvest ecology of fungi causing spoilage of foods and other stored products. In *Filamentous fungi in foods and feeds*; Moss, M. O., Jarvis, B., Skinner, F. A., Eds.; The Society for Applied Bacteriology, Symposium Series No. 18; Blackwell Scientific Publications: London, 1989, pp 11−25.

(23) Mills, J. T. Ecology of mycotoxigenic species on cereal seeds. *J. Food Prot.* **1989**, *52*, 737−742.

(24) Munkvold, G. P.; McGee, D. C.; Carlton, W. M. Importance of different pathways for maize kernel infection by *Fusarium moniliforme Phytophatology* **1997**, *87*, 209−217.

(25) Naik, D. M.; Nawa, I. N.; Raemacker, R. H. Absence of an effect from internally seed-born *Fusarium moniliforme* on emergence, plant growth and yield of maize. *Seed Sci. Technol.* **1982**, *10* (2), 347−356.

(26) Torres, A.; Chulze, S.; Varsavsky, E.; Rodriguez, M. *Alteraria* metabolites in sunflower seeds: incidence and effects of pesticides on their production. *Mycopathologia* **1993**, *121*, 17−20.

(27) Chulze, S.; Torres, A.; Dalcero, A.; Etcheverry, M.; Ramirez, M.; Farnochi, C. *Alternaria* mycotoxins in sunflower seeds: incidence and distribution of the toxins in oil and meal. *J. Food Prot.* **1995**, *58*, 1133−1135.

(28) Magan, N.; Cayley, G. R.; Lacey, J. Effect of water activity and temperature on mycotoxin production by *Alternaria alternata* in culture and on wheat grain. *Appl. Environ. Microbiol.* **1984**, *47* (5), 1113−1117.

(29) Marin, S.; Sanchis, V.; Teixido, A.; Sáenz, R.; Ramos, A. J.; Viñas, I.; Magan, N. Water and temperature relations and microconidial germination of *Fusarium moniliforme* and *F. Proliferatum* from maize. *Can. J. Microbiol.* **1996**, *42* (10), 1045−1050.

(30) Marin, S.; Companys, E.; Sanchis, V.; Ramos, A. J.; Magan, N. Effect of water activity and temperature on competing abilities of common maize fungi. *Mycol. Res.* **1998**, *120*, 959−964.

(31) Cahagnier, B.; Melcion, D.; Richard-Molard, D. Growth of *Fusarium moniliforme* and its biosyntesis of fumonisin B_1 on maize grain as a function of different water activities. *Lett. Appl. Microbiol.* **1995**, *20*, 247−251.

(32) Torres, M. R.; Ramos, A. J.; Soler, J.; Sanchis, V.; Marín, S. SEM study of activity and temperature effects on the initial growth of *Aspergillus ochraceus, Alternaria alternata* and *Fusarium verticillioides* on maize grain. *Int. J. Food Microbiol.* **2003**, *81*, 185−193.

(33) Watson, D. H. An Assessment of food contamination by toxic products of *Alternaria*. *J. Food Prot.* **1984**, *47*, 485−488.

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