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Mycoflora and Fumonisin Contamination in Brazilian Corn from Sowing to Harvest

ADRIANA P. ALMEIDA,† HOMERO FONSECA,‡ ANTÔNIO LUIZ FANCELLI,‡ GLÓRIA MARIA DIREITO,§ EDWIN M. ORTEGA,# AND BENEDITO CORRÊA*,†

Instituto de Ciências Biomédicas, USP, Avenida Prof. Lineu Prestes, 1374 São Paulo, Brazil 05508-900; Escola Superior de Agricultura Luiz de Queiroz, USP, Piracicaba, Brazil; Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Rio de Janeiro, Brazil; and Instituto de Matemática, USP, São Paulo, Brazil

The present study aimed to analyze the mycoflora and potential mycotoxin contamination of soil and corn samples collected at different plant maturity stages in Capão Bonito and Ribeirão Preto, two regions of the State of São Paulo, Brazil. In addition, the data obtained were correlated with the occurrence of wind-dispersed fungi and the predominant climatic conditions of the two regions studied. Corn mycoflora profiles showed that *Fusarium verticillioides* prevailed in 35% of the samples from Capão Bonito and in 49% of the samples from Ribeirão Preto. Examination of wind-dispersed fungi also revealed a high incidence of *F. verticillioides*. Soil mycoflora analyses showed that *Penicillium* was the most prevalent genus, although *F. verticillioides* was present in 55.5% of Capão Bonito's samples and in 26.7% of Ribeirão Preto's samples. With respect to water activity, the corn kernels most contaminated with *F. verticillioides* had water activity levels of 0.70−0.80. HPLC analysis of fumonisins revealed that 88.5% of Capão Bonito's kernels were contaminated with fumonisin B_1 (FB_1) (0.09−10.87 μg/g) and 53.8% with fumonisin B_2 (FB_2) (0.05−0.52 μg/g); Ribeirão Preto's kernels presented contamination levels of 93.5% for FB_1 (0.11−17.69 μg/g) and 61.3% for FB_2 (0.05−5.24 μg/g). No aflatoxins were detected by thin-layer chromatography in corn grains of either region. The concomitant occurrence of *F. verticillioides* and fumonisins in most of the field corn assayed demonstrates the importance of an effective control of cultivation throughout the plant maturity stages.

KEYWORDS: Mycoflora; aflatoxin; fumonisin; corn

INTRODUCTION

Corn is one of the main crops grown in Brazil. This cereal has an important role in both human and animal nutrition, and Brazil has been ranked as the third largest world producer, following the United States and China. There have been significant advances in most areas of experimental agronomy, ecology, and ethnobiology regarding corn grains, which have given rise to a more comprehensive understanding of the relationship between corn and the environment. Due to the demand for increased productivity worldwide, corn has been continuously submitted to intense genetic improvement, resulting in the current existence of several commercial hybrids especially developed for specific cultivation and utilization settings.

The preharvest contamination of corn grains by *Fusarium* species, the most frequent fungal contaminants of field corn (*1*), has posed serious problems in several countries due to *Fusarium*'s ubiquity in nature and ability to produce toxic secondary metabolites called mycotoxins. According to estimates from the *World Bank Report—Investing in Health* (1993), diseases caused by mycotoxins lead to a reduced life expectancy in developing countries (*2*). This fact emphasizes the need to understand the evolution of fungal contamination of corn grains with *Fusarium verticillioides*, the main producer of fumonisins, to develop efficient control strategies that will reduce economic losses and health hazards. In view of these considerations, the present study aimed to evaluate the contamination risk of corn cultivated in the State of São Paulo, Brazil, by first studying the mycoflora of the air, soil, and corn in the regions of Capão Bonito and Ribeirão Preto, then determining the occurrence of aflatoxins and fumonisins in the corn grains, and, finally, correlating the results with the incidence of wind-dispersed fungi and abiotic factors (water activity, temperature, and rainfall).

MATERIALS AND METHODS

Corn Samples. The corn cultivar (hybrid XL-251, Braskalb seeds) of the 1999 crop was cultivated in two regions of the State of São Paulo: Capão Bonito (24° 02′ S, 48° 22′ W, 702 m altitude) and Ribeirão Preto (47° 27′ S, 47° 27′ W, 621 m altitude). Fifty-seven

* Author to whom correspondence should be addressed [telephone (+5511) 30917295; fax (+5511) 30917354; e-mail correabe@usp.br].
† Instituto de Ciências Biomédicas.
‡ Escola Superior de Agricultura Luiz de Queiroz.
§ Insituto de Veterinária.
# Instituto de Matemática.

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samples were analyzed, 26 of which were from Capão Bonito and 31 from Ribeirão Preto. This slight difference in the total of samples from each region was due to the fact that corn ear development and, consequently, ear sampling, started earlier in Ribeirão Preto, because of the hot weather and high temperatures that prevailed at that location; such ambient conditions favored plant growth and sped the development of corn ears. The area selected for sowing in either region was divided into 10 uniform parcels of 80 m^2. Five parcels were chosen for each collection, and 10 ears of corn from the same sowing line were sampled from each parcel. In this way, five samples were collected for every period, each containing 10 ears of corn. A 1 kg subsample was taken from each of these samples and assayed for mycoflora, aflatoxin and fumonisin contents, and water activity. Samples were collected at 15 day intervals, from the 75th day after flowering until harvest (*3*).

Soil Samples. Ninety samples were analyzed, 45 of which were from Capão Bonito and 45 from Ribeirão Preto. All soil samples were collected from the surface (0−2 cm depth), being five for each period; 1 kg subsamples taken from each sample were homogenized and assayed for mycoflora and water activity (*3*).

Climatic Conditions of the Production Regions. The determination of prevailing climatic factors was done with specific equipment such as thermometers, rain gauges, anemometers, heliographs, and class A tanks.

Water Activity Determinations. The water activity of corn grains and soil samples was determined by automatic analysis, using Aqualab CX-2 (Decagon Devices Inc., Pullman, WA). Each sample was measured five times.

Recovery, Identification, and Enumeration of the Mycoflora from Soil and Corn Samples. *(a) Isolation of Mycoflora from Corn Kernel Samples.* From each corn sample, one subsample of ∼30 g was taken and then disinfected by immersion in 2% sodium hypochloride solution for 3 min, followed by three rinses with sterile distilled water. From this subsample, 33 grains were randomly taken and sown on Petri dishes containing agar DRBC (11 kernels per dish) (*4*). Incubation was carried out at 25 °C for 5 days. Results are expressed as percentage of grains infected by fungi. The fungal colonies recovered were identified according to methods recommended for each genus (*5*−*7*).

(b) Isolation of Mycoflora from Soil Samples. Soil samples were analyzed according to the method described by Swanson et al. (*8*). For mycoflora isolation, Martin medium was used (*9*). The plates were incubated at 25 °C for 10 days and observed daily. The fungal colonies recovered were identified according to methods recommended for each genus (*5*−*7*).

Determination of Wind-Dispersed Fungi. Sampling of the wind-dispersed fungi began before silk emergence. For each period, 10 Petri dishes had the lid removed at 1.50 cm above the ground and were kept open for 15 min, to allow deposition of wind-dispersed fungal spores on the sterile Sabouraud agar (*10*). After quickly replacing the lids, the plates were incubated at 25 °C for 7 days, and fungal colonies were identified according to methods recommended for each genus (*5*−*7*). The analysis of wind-dispersed fungi was carried out throughout the periods of soil and corn sampling.

Aflatoxin Determination in Corn Samples. The procedure used involved extraction with methanol/4% KCl (9:1), followed by clarification of the extract with ammonium sulfate and partitioning to chloroform. The content was filtered through filter paper (Whatman No. 1) and evaporated to dryness (*11*). Suspended extracts were quantified by thin-layer chromatography (TLC) using aflatoxin standards (*12*). Confirmation of the identified aflatoxins was carried out by derivatization using trifluoroacetic acid (*13*). The TLC detection limit was 2 μg/kg.

Fumonisin Determination in Corn Grain Samples. Briefly, 10 g of ground corn grains was added to 50 mL of a 1:1 solution of acetonitrile/water and stirred for 30 min. The extract was filtered through filter paper (Whatman No. 1). Next, 2 mL of the filtrate was added to 5 mL of water, and the mixture was applied to a Bond-Elut cartridge C_{18} (Varian, Harbor City, CA), preconditioned with 2 mL of methanol and 2 mL of Milli-Q water (Millipore, Bedford, MA). The cartridge was washed with 2 mL of a 20:80 mixture of acetonitrile/water and the toxin eluted with 2 mL of a 70:30 mixture of acetonitrile/water. The final extract was collected in Eppendorf tubes and kept at −20° C until use.

After derivatization with 200 μL of *O*-phthaldialdehyde solution (OPA) (40 mg of OPA, 1 mL of methanol, 5 mL of 0.1 M sodium tetraborate, and 50 μL of 2-mercaptoethanol), HPLC injections were made within 2 min. Fumonisins were analyzed by a reversed-phase isocratic HPLC system (Shimadzu LC-10AD, pump and RF-10AXL fluorescence detector), using a 5 ODS-20 C_{18} column (150 × 4.6 mm, Phenomenex, Ultracarb). Excitation and emission wavelengths were 335 and 440 nm, respectively. The eluent was acetonitrile/water (1:1)/acetic acid (0.5%). The flow rate was 1.0 mL/min at 22−23 °C (room temperature) and 24 °C column temperature (*14*). Quantitation was accomplished according to ref *15*. The detection limit of the method was 50 ng/g (50 μg/kg) for both FB_1 and FB_2.

Statistical Analysis. The data were analyzed through four stages: (a) simple and partial correlation analysis; (b) multiple linear regression analysis; (c) parallelism test; and (d) residual analysis using statistical software (SAS, version 6.11) to determine the influence of the independent variables (water activity of soil and corn, percentage of fungi in soil and air, temperature, and rainfall index) on the dependent variables (*F. verticillioides* in corn kernels) (*16*, *17*).

RESULTS AND DISCUSSION

Occurrence of Fungi. The corn samples collected at different maturity stages in Capão Bonito exhibited the following mycoflora: *F. verticillioides* (35%), yeasts (32%), *Penicillium* spp. (27%), *Aspergillus flavus* (1%), *Cephalosporium* spp. (1%), *Nigrospora* spp. (1%), and *Mucor* spp. (0.5%).

In Ribeirão Preto, the prevalences detected were as follows: *F. verticillioides* (49%), *Penicillium* spp. (21%), yeasts (10%), *A. flavus* (5%), *Cephalosporium* spp. (4%), *Cladosporium* spp. (0.8%), *Mucor* spp. (0.7%), *Trichoderma* spp. (0.7%), *Nigrospora* spp. (0.6%), nonsporulated fungi (0.5%), *Alternaria* spp. (0.2%), *Fusarium proliferatum* (0.1%), and *Rhizopus* spp. (0.1%). *F. verticillioides* was also detected in 30% of the corn kernels tested.

The high frequency of *F. verticillioides* agrees with data reported by other workers (*14*, *18*−*20*), who describe this genus as the most prevalent filamentous fungus in freshly harvested Brazilian corn.

In Capão Bonito, the evolution of *F. verticillioides* contamination at different stages of corn maturity showed that this fungus was the most frequent at the eighth collection (12%), that is, 150 days after flowering. Corn grain contamination with *F. verticillioides* in Ribeirão Preto reached the highest level at the seventh collection (13%), that is, 135 days after flowering (**Figure 1**).

The species *A. flavus*, another important toxigenic fungus, contaminated different plant maturity stages of corn at prevalences that ranged from 0 to 1% in Capão Bonito and from 0 to 5% in Ribeirão Preto The highest number of recoveries occurred at the eighth collection (1%), that is, 150 days after flowering, in Capão Bonito, and at the sixth collection (4%), that is, 120 days after flowering, in Ribeirão Preto.

Soil analysis of the 45 samples from Capão Bonito revealed the following mycoflora: *Penicillium* spp. (91.1%), *Geotrichum* ssp. (84.4%), *Aspergillus terreus* (60.0%), *F. verticillioides* (55.5%), *Mucor* ssp. (44.4%), *Trichoderma* spp. (37.8%), *F. oxysporum* (28.9%), *Cladosporium* spp. (26.7%), *Rhizopus* ssp. (24.4%), yeasts (20.0%), *Aspergillus tamarii* (11.1%), *Cephalosporium* spp. (11.1%), *Aspergillus niger* (6.7%), *Nigrospor* spp. (6.7%), *F. anthophilum* (4.4%), nonsporulated fungi (4.4%), *Fusarium avacenum* (2.2%), and *A. flavus* (2.2%).

Soil analysis of the 45 samples from Ribeirão Preto showed the presence of the following species: *Penicillium* spp. (71.1%),

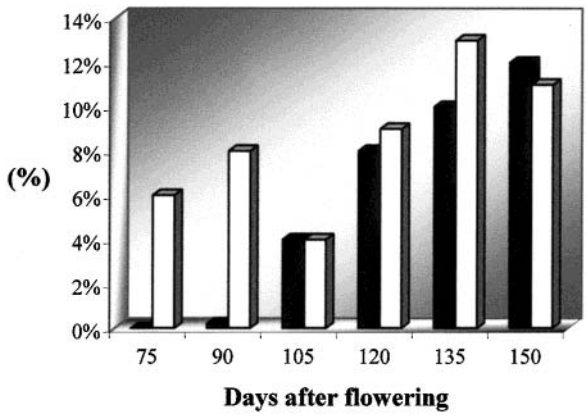

Figure 1. Incidence of *F. verticillioides* in 57 samples of corn grains at different maturity stages, collected at Capão Bonito (black bars) and Ribeirão Preto (white bars) (two regions of the State of São Paulo, Brazil) from November 1998 to April 1999.

Nigrosporum spp. (71.1%), *A. terreus* (48.9%), *Mucor* spp. (33.3%), *Cladosporium* spp. (33.3%), *F. verticillioides* (26.7%), *Trichoderma* spp. (20.0%), *A. tamarii* (17.7%), *A. flavus* (15.6%), *Paecilomyces* spp. (13.3%), *Rhizopus* spp. (13.3%), yeasts (13.3%), nonsporulated fungi (8.9%), *F. oxysporum* (6.7%), *Cephalosporium* spp. (4.4%), *A. niger* (4.4%), *Fusarium anthophilum* (2.2%), *Fusarium acuminatum* (2.2%), and *Aspergillus versicolor* (2.2%).

The high frequency of *Penicillium* spp. and the presence of *Fusarium* spp., mainly *F. verticillioides*, in soil samples have also been described by other authors (*21*, *22*).

The numbers of colony-forming units (CFU) for *Fusarium*, *Aspergillus*, and *Penicillium*, the three most important genera in terms of toxigenicity, ranged from 0.5×10^3 to 60×10^3 (*F. verticillioides*), from 0 to 2×10^3 (*A. flavus*), and from 1.5×10^3 to 155×10^3 (*Penicillium* spp.) in soil samples isolated from Capão Bonito. In Ribeirão Preto, soil colony-forming unitvalues for these genera varied from 0.5×10^3 to 100×10^3 CFU/g (*F. verticillioides*), from 5×10^3 to 100×10^3 CFU/g (*A. flavus*), and from 0.5×10^3 to 250×10^3 CFU/g (*Penicillium* spp.). In both Capão Bonito and Ribeirão Preto, soil a_w levels ranged from 0.86 to 1.00.

In Capão Bonito, the following wind-dispersed fungi were found: *Neurospora* spp. (80.0%), yeasts (75.7%), *Cladosporium* spp. (67.1%), *Fusarium* spp. (61.4%), *Rhizopus* spp. (51.4%), *Mucor* spp. (45.7%), *Geotrichum* spp. (27.1%), nonsporulated fungi (17.1%), *Aspergillus* spp. (11.4%), *Penicillium* spp. (7.1%), *Helminthosporium* spp. (7.1%), *Trichoderma* spp. (7.1%), and *Absidia* spp. (1.4%). In Ribeirão Preto, the airborne contaminants detected were *Fusarium* spp. (67.1%), yeasts (64.2%), *Mucor* spp. (61.4%), *Cladosporium* spp. (51.4%), *Neurospora* spp. (48.5%), *Geotrichum* spp. (37.1%), *Penicillium* spp. (18.6%), *Rhizopus* spp. (17.1%), *Aspergillus* spp. (10.0%), nonsporulated fungi (8.6%), *Helminthosporium* spp. (5.7%), and *Trichoderma* spp. (2.9%).

The evolution of corn contamination by wind-dispersed fungi at the different stages of plant maturity showed that, in both regions, *Fusarium* was the prevalent genus at the middle and late stages, that is, 105−135 days after flowering. It should be noted that the wind-dispersed fungi isolated in our experiment are those considered to be universal dominants.

In Brazil, the high frequency of *Fusarium* spp. and the presence of *Penicillium* spp. and *Aspergillus* spp. in air have been previously reported (*10*).

Two routes have been postulated for corn contamination with *Fusarium* spp. According to the first, spores originating from the previous harvest, weeds, grasses, and soil would fall upon the exposed seeds, which would eventually be planted on the ground, and fungal colonization would occur in the presence of adequate moisture and temperature. According to the second route, insects and birds would damage the seeds, and fungal spores brought by wind would fall upon the exposed seeds, thereby promoting contamination. In the latter case, moisture is a less important factor (*1*). Recent work suggests that the second route is the most likely one.

Table 1. Average Fumonisins Levels in 57 Samples of Corn Grains at Different Stages of Maturity, from the Regions of Capão Bonito and Ribeirão Preto, State of São Paulo, Brazil (1998/1999 Crop)

days after flowering[a]	Capão Bonito region		Ribeirão Preto region	
	FB_1 (μg/g)	FB_2 (μg/g)	FB_1 (μg/g)	FB_2 (μg/g)
kernels	0.91	ND[b]	0.91	ND
75	NF[c]	NF	0.82	0.52
90	0.29	0.05	0.24	ND
105	0.27	0.06	0.33	0.24
120	3.61	0.24	4.07	0.63
135	1.60	0.11	0.42	0.48
150	0.79	0.25	2.13	1.34

[a] 75 days after flowering = third collection; 90 days after flowering = fourth collection; 105 days after flowering = fifth collection; 120 days after flowering = sixth collection; 135 days after flowering = seventh collection; 150 days after flowering = eighth collection. [b] ND = fumonisin not detected. [c] NF = ear not formed (analysis not done).

Our results revealed the presence of *F. verticillioides* in corn and soil and of *Fusarium* spp. in atmospheric air. Such findings match the theory proposed by Mills (*1*), whereby fungi might utilize two infection routes for their dispersion. The presence of *F. verticillioides* in the seeds agrees with data reported by other authors (*23*, *24*). This finding confirms the classification of *F. verticillioides* as a seed-borne organism.

Occurrence of Mycotoxins. Our analysis of corn grains did not indicate the presence of aflatoxins in either region. On the other hand, almost all samples from both Capão Bonito and Ribeirão Preto presented detectable amounts of fumonisins. Of a total of 26 samples from Capão Bonito, 23 (88.5%) were contaminated with fumonisin B_1 (FB_1), at concentrations that ranged from 0.09 to 10.87 μg/g, and 14 (53.8%) were contaminated with fumosinin B_2 (FB_2), at concentrations that ranged from 0.05 to 0.52 μg/g. In Ribeirão Preto, of the 31 samples assayed, 29 (93.5%) were contaminated with FB_1 at 0.11−17.69 μg/g and 19 (61.3%) were contaminated with FB_2 at 0.05−5.24 μg/g.

Studies of fumonisin contaminants in corn grains from several countries have revealed levels of contamination lower (*15*, *25*−*27*) and higher (*28*−*31*) than those presently reported.

In both regions, the highest average levels of corn FB_1 were found at the sixth collection, that is, 120 days after flowering, whereas FB_2 peaked at the eighth collection, that is, 150 days after flowering (**Table 1**; **Figure 2**). Such findings, however, do not agree with those reported by Chulze et al. (*32*), who found higher fumonisin levels in corn from Cordoba, Argentina, at 75 days after flowering.

The rise in FB_1 concentration at the sixth collection could be accounted for by the prevailing ambient conditions at that period, which would be ideal for fungal growth and fumonisin production (i.e., high a_w values, temperature within the 24.1−25.2 °C range, and declining rainfall index) (**Table 2**). Higher concentrations of FB_1 than FB_2, as is the case in this study, have been previously detected in similar surveys (*20*, *33*). Some authors report that FB_1 makes up 70% of the total of fumonisins

Table 2. Water Activity of Corn Samples, from the Regions of Capão Bonito and Ribeirão Preto, at Different Maturity Stages and the Environmental Data Registered in the Experimental Period

Capão Bonito region				Ribeirão Preto region			
days after flowering[a]	water activity (a_w)	av rainfall index (mm)	av temp (°C)	days after flowering	water activity (a_w)	av rainfall index (mm)	av temp (°C)
75	NF[b]	NF	NF	75	0.98–0.99	201.6	24.8
90	0.96	70.1	22.4	90	0.97–0.99	142.6	26.1
105	0.98–0.99	103.6	23.3	105	0.97–0.99	112.1	25.6
120	0.98–0.99	55.1	25.2	120	0.93–0.94	154.0	24.1
135	0.89–0.93	23.4	24.0	135	0.81–0.84	102.8	24.3
150	0.71–0.81	23.0	22.4	150	0.54–0.56	0.0	24.4

[b] NF = ear not formed (analysis not done). [a] 75 days after flowering = third collection; 90 days after flowering = fourth collection; 105 days after flowering = fifth collection; 120 days after flowering = sixth collection; 135 days after flowering = seventh collection; 150 days after flowering = eighth collection.

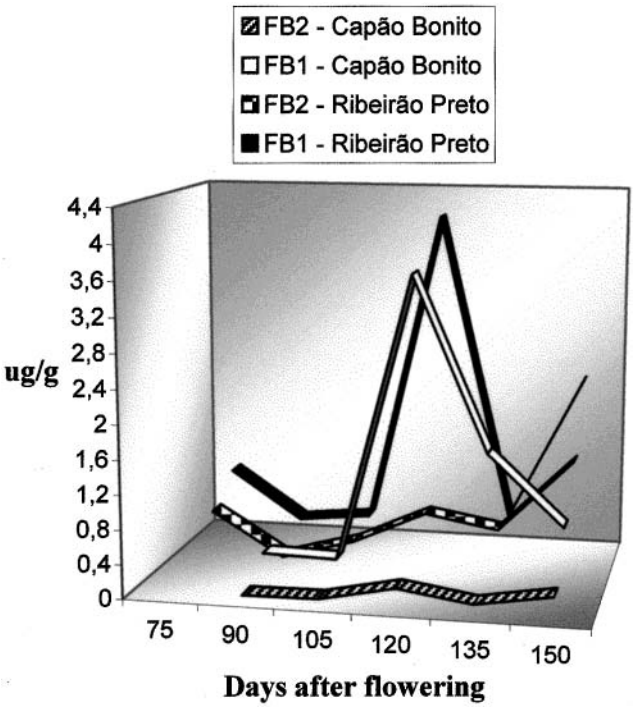

Figure 2. Average levels of fumonisins B_1 and B_2 in 57 samples of corn grains at different maturity stages, collected at Capão Bonito and Ribeirão Preto (two regions of the State of São Paulo, Brazil) from November 1998 to April 1999.

found in naturally contaminated corn. The concentrations of FB_2 and FB_3, when present, are ~10−20% less than those of FB_1 (*33*). Similar contamination profiles have been detected in corn harvested at different plant maturity stages in Argentina (*33*).

The highest average levels of contamination with FB_1 and FB_2 were found in corn samples from Ribeirão Preto (4.07 and 1.34 μg/g, respectively) (**Table 1**; **Figure 2**). This agrees with studies of freshly harvested corn grains (1997/1998 crop) from different regions of São Paulo, Brazil (*30*). It is interesting to note that, when *F. verticillioides* isolates from corn were tested, those from Ribeirão Preto also produced higher fumonisin levels.

Most samples contained FB_1 levels of <1.0 μg/g; however, 7% showed contamination >5.0 μg/g. There are no legal limits established for fumonisin contamination in the world, but the maximum levels recommended by the Mycotoxin Committee of the American Association of Veterinary Diagnosticians are 5, 10, and 50 μg/g for equine, swine, and bovine, respectively (*34*).

The absence of aflatoxins in corn samples could be related to the low incidence of *A. flavus* in both Capão Bonito (1%) and Ribeirão Preto (5%).

Abiotic Factors. The fungal isolates were recovered from corn grains with a_w levels that ranged from 0.54 to 0.99. The highest frequencies of *F. moniliforme* and *A. flavus*, two major toxigenic fungi, were detected at a_w values of 0.71−0.81 in Capão Bonito (eighth collection, i.e.,135 days after flowering) and 0.81−0.84 in Ribeirão Preto (seventh collection, i.e.,135 days after flowering).

Concerning corn kernel contamination with FB_1, the highest levels were found at the sixth collection (120 days after flowering) in both regions, a period when water activity levels ranged from 0.98 to 0.99 in Capão Bonito and from 0.93 to 0.94 in Ribeirão Preto (**Table 2**). Our findings are similar to those reported by Lacey et al. (*35*), who found that the highest FB_1 production occurred at water activity levels of ~1.0.

Regarding soil samples, *F. verticillioides* was most prevalent at a_w values of 0.99−1.00 in both regions. The a_w levels detected in corn and soil samples from both regions are considered to be adequate for corn trading in Brazil (*36*).

Statistical analysis of our data indicated that a_w did influence the prevalence of *F. verticillioides* in corn from both regions, as shown by the significant negative correlation ($p < 0.0006$; $r = 0.44$) between the occurrence of *F. verticillioides* and a_w values of Capão Bonito's corn kernel and the significant positive correlation ($p < 0.02$, $r = 0.41$) between the occurrence of *F. verticillioides* and a_w values of Ribeirão Preto's soil samples.

Furthermore, we also observed that corn kernels from Ribeirão Preto were significantly more contaminated with *F. verticillioides* (90% significance level). In this region, ambient temperature varied between 15.5 and 34.5 °C (average = 24.8 °C) throughout the experiment, whereas the temperature range at Capão Bonito was somewhat lower during the same period (11.2−34.2 °C; average = 23.5 °C) (**Table 2**). Several authors (*7*, *33*) have reported a greater production of fumonisins by strains of *F. verticillioides* at 25 °C; in this respect it is worth noting that, in Ribeirão Preto, where the average ambient temperature was 24.8 °C, we also found a greater prevalence of *F. verticillioides* and production of fumonisins by this species.

Concerning rainfall, higher contamination by *F. verticillioides* was registered during the periods of lower rainfall index. This can be particularly appreciated in the contamination data recorded around March, the period corresponding to the sixth, seventh, and eighth collections, with the lowest rainfall index in both regions (**Table 2**).

The reduction in rainfall after plant flowering correlates with *F. verticillioides* growth and consequent fumonisin production. Such facts could be explained by the arguments put forward by Schneider and Pendery (*37*), who noted that corn root senescence is accelerated during periods of low rainfall and reasoned that this may result in early aging of the plant, which,

in turn, increases the probability of contamination by *F. verticillioides*.

In our work, both temperature and rainfall index were closely related to the development of *F. verticillioides* in corn grains. Significant positive and negative correlations between corn contamination by this fungus and mean temperature were found in Capão Bonito ($p < 0.008$, $r = 0.44$) and Ribeirão Preto ($p < 0.002$, $r = 0.41$), respectively. A highly significant negative correlation ($p < 0.008$, $r = 0.41$) between corn contamination by *F. verticillioides* and average rainfall index was also found in Ribeirão Preto.

Significant fungal contamination during dry periods has been observed by other authors (*18*, *20*). Indeed, Marasas et al. (*38*) observed that, in South Africa, *F. verticillioides* was frequently isolated from regions with dry climate.

In a study on mycotoxin contamination of corn from different geographical areas of Colombia, Cuero et al. (*39*) reported a better adaptation of *Fusarium* spp. to the ecological conditions of the highlands. In our study, altitude was not a determining factor in the occurrence of *F. verticillioides* in corn; in fact, of the two regions surveyed, Ribeirão Preto was located at the lowest altitude (621 m) and yet was the most susceptible to *F. verticillioides* contamination of corn.

The concomitant occurrence of *F. verticillioides* and fumonisins in most of the field corn assayed demonstrates the importance of an effective control of cultivation throughout the plant maturity stages.

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