Alternaria alternata prevalence in cereal grains and soybean seeds from Entre Ríos, Argentina

Leticia Elvira Broggi, Héctor Horacio Lucas González, Silvia Liliana Resnik y Ana Pacin

Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina

Summary

A mycological survey was carried out at Entre Ríos province, Argentina, on sorghum grain, maize, rice, soybean seeds and on freshly harvested and stored wheat. The isolation frequencies and relative densities of species belonging to genera *Alternaria*, *Aspergillus*, *Fusarium, Penicillium* and other fungi were calculated. *Alternaria alternata* was the major fungal species isolated from sorghum, rice, soybean seeds and on freshly harvested wheat, and a low incidence of *Fusarium* species was observed on the same substrates. In maize the major fungal species isolated was *Fusarium verticillioides*. The high incidence levels of *A. alternata* observed, suggest that it may be necessary to determine, among other mycotoxins, if *Alternaria* toxins occur in these commodities.

Key words *Alternaria alternata*, Wheat, Sorghum, Rice, Maize, Soybean

Predominancia de *Alternaria alternata* en cereales y soja de la provincia de entre Ríos, Argentina

Resumen

En la provincia de Entre Ríos, Argentina se llevo a cabo un estudio micológico en granos de sorgo, maíz, arroz y semillas de soja y en muestras de trigo recién cosechado y con un año de almacenamiento. Se calcularon las frecuencias de aislamiento y las densidades relativas específicas de especies pertenecientes a los géneros *Alternaria*, *Aspergillus*, *Fusarium, Penicillium* y otros. *Alternaria alternata*, fue la especie más aislada en sorgo, arroz, soja y en el trigo recién cosechado. En los mismos sustratos se observó una incidencia menor de especies de *Fusarium*. En maíz, la especie fúngica predominante fue *Fusarium verticillioides*. Los altos niveles de incidencia de *A. alternata* observados, indicarían la necesidad de determinar, entre otras micotoxinas, la presencia natural de toxinas de *Alternaria* en estos sustratos.

Palabras clave *Alternaria alternata*, Trigo, Sorgo, Arroz, Maíz, Soja

Maize, soybean, wheat, rice and sorghum grain are the main commodities produced in Argentina. Wheat and rice are used for human consumption, and maize, soybean and sorghum grain are grown mainly for export [21]. A number of fungi that have been associated with cereal grains and soybean seeds include species of the genera *Fusarium, Aspergillus* and *Penicillium*, have been reported to produce toxic metabolites that cause mycotoxicoses of animals and human beings [22]. Information about fungi associated to wheat, maize, rice, sorghum grain, black bean and soybean seeds collected from different production areas of Argentina has been obtained in recent years [2,3,5,9-14,17,18]. Entre Ríos province is an important cereal grain and soybean producing area in Argentina [21] and little information is available on the fungi and mycotoxins associated with cereals and oil seeds produced in this region. Wheat, rice, maize, sorghum and soybean production represents more than 90% of the agricultural activity in the province and a knowledge of fungal contamination of these commodities is important in assessing the risk of mycotoxin occurrence [3,14,17]. Entre Ríos is bordered by two big rivers, the Paraná to the West and the Uruguay to the East, and both flow into the La Plata river. The environmental conditions of this province are considered to be temperate. The average temperature in the Southern departments reaches 23 °C in summer and 10 °C in winter and the presence of lagoons and swampy lands raises the humidity. In the North the climate is subtropical and warm with temperatures of 42 °C in summer and mild winters with an average temperature of 14 °C [21].

The aim of this work was to study the contaminant mycoflora of cereal grains and soybean seed in Entre Ríos province, in order to use this information to consider possible mycotoxin occurrence in this area.

Corresponding Author:
Dr Héctor H. L. González
Departamento de Industrias, Facultad de Ciencias Exactas y Naturales
Universidad de Buenos Aires
Ciudad Universitaria, C1428EHA
Buenos Aires, Argentina
Tel./Fax: +54 11 4576-3366
E-mail: glucas@speedy.com.ar

Aceptado para publicación el 6 de abril de 2006

Apdo. 699, E-48080 Bilbao (Spain)
1130-1406/01/10.00 €

Materials and methods

Sampling. Subsamples of wheat, rice, maize, sorghum or soybean were taken in six different truck positions during harvest. Samples of 10 kg of randomized kernels or seeds were processed and subsamples of 1 kg were taken for mycological analysis [1].

A total of 162 samples were obtained from different localities at Entre Ríos; during the 1999 harvest 37 samples of sorghum, 32 of rice, 58 wheat samples and 52 of maize were taken. In the 2002 harvest season 12 samples of maize; 23 of soybean and 24 of stored wheat were collected.

The agricultural practices during crop development and harvest time were similar in all sampled localities, including no fertilization, no irrigation and no zero tillage in any case.

Isolation of fungi. Wheat, maize, rice, sorghum kernels or soybean seed subsamples from each sample were surface-disinfected in a commercial 5% aqueous solution of sodium hypochlorite for 1 minute and rinsed twice with sterile distilled water. One hundred kernels or seeds per subsample were plated at 20 units per plate, on YGCA (Yeast Extract-Glucose-Chloramphenicol Agar, Merck No 16000). The plates were incubated in the dark at 25 °C for 4-7 days and the resulting fungal colonies were subcultured onto PDA (Potato-Dextrose Agar, Merk No 10130) and identified. Where several different fungi were isolated from a single kernel or seed, all were recorded.

Identification of fungi. Fungi were identified according to the following schemes: *Fusarium* spp. according to Nelson et al. [16]; Dematiaceous fungi according to Ellis [8], *Penicillium* spp., *Aspergillus* spp. and other fungi according to Pitt and Hocking [19]. The isolation frequency (*Fr*) and relative density (*RD*) of species were calculated as follows [9]:

$$Fr(\%) = (\frac{ns}{N}) \times 100 \qquad RD(\%) = (\frac{ni}{Ni}) \times 100$$

Where, *ns* is the number of samples where a genus or species of fungi occurred; *N* is the total number of samples; *ni* is the number of isolates of a genus or species; *Ni* is the total number of fungal isolates obtained.

Statistical analysis. Asymptotic tests for equality of proportions were used to compare the *RD* of fungal species [7], and the Fischer exact test was used to analyze possible differences in the *Fr* of fungal species. The analysis was performed with the Statistix 4.1 package [24].

Results and discussion

The *RD* and *Fr* of the potential toxigenic fungi present on wheat, sorghum and rice collected in 1999 and maize and soybean collected in 2002, are given in table 1. *Alternaria alternata* was the most common fungal species isolated. In soybean seeds harvested in 2002, *A. alternata* was also the most common species, and the second most common was *Fusarium semitectum* (syn. *F. pallidoroseum*, *F. incarnatum*). In maize collected in 2002, *Fusarium verticillioides* was the main fungal contaminant (Table 1) and this observation agrees with those from other Argentinean maize-producing regions [9,13,14,17,18]. Other fungi recovered from freshly harvested cereals and soybean in Entre Ríos in 1999 and 2002 are given in table 2.

With regard to the wheat mycoflora, a survey carried out on common wheat harvested in 1993 in the major wheat production areas in Argentina [10], shown that in the Southeast of Córdoba, South of Santa Fé and North and Southeast of Buenos Aires (region II) *Fusarium graminearum* was the most frequently isolated species (*Fr*: 100.0% and *RD*: 89.2%) and that *A. alternata* was recovered at low incidences (*Fr*: 47.1% and *RD*: 2.7%). On the other hand, in the same year in region V (Central, Northwest and South of Córdoba, Southwest of Buenos Aires and La Pampa) *F. graminearum* (44.4%) was found at a lower frequency than *A. alternata* (74.1%). *F. poae* was also relatively common in the current study (*Fr*: 13.8%). Table 3 gives the total rainfall and temperatures registered in Entre Ríos (region III) and in regions II and V. These meteorological data were registered at the stations nearest to the fields harvested.

In Eastern Oregon and Washington states Smiley and Patterson [23] reported *Fusarium* species associated with winter wheat. They found that *F. graminearum* was the most common species followed by *Fusarium avena-*

Table 1. Relative density (*RD*) and isolation frequency (*Fr*) of the toxigenic fungi isolated in freshly harvested cereal grains and soybean seeds in Entre Ríos, Argentina.

Species	wheat 1999		sorghum 1999		rice 1999		maize 2002		soybean 2002	
	RD	*Fr*	*RD*	*Fr*	*RD*	*Fr*	*RD*	*Fr*	*RD*	*Fr*
Alternaria alternata	79.2	100.0	58.1	100.0	22.0	100.0	1.2	16.7	21.2	95.7
Alternaria (Pleospora infectoria)	2.6	31.0	0.03	2.7	nd	nd	nd	nd	nd	nd
Alternaria tenuissima	0.3	5.2	nd	nd	nd	nd	nd	nd	nd	nd
Aspergillus flavus	nd	nd	1.9	2.7	0.7	15.6	23.7	83.3	0.1	4.4
Aspergillus niger	0.04	3.5	0.03	2.7	0.1	6.3	9.7	58.3	nd	nd
Fusarium graminearum	0.3	15.5	0.8	21.6	1.4	45.0	nd	nd	8.1	65.2
Fusarium heterosporum	nd	nd	0.03	2.7	0.6	6.3	nd	nd	nd	nd
Fusarium poae	0.7	13.8	nd	nd	nd	nd	nd	nd	nd	nd
Fusarium proliferatum	0.7	8.6	nd	nd	nd	nd	nd	nd	nd	nd
Fusarium semitectum	0.2	3.5	nd	nd	nd	nd	nd	nd	23.0	87.0
Fusarium subglutinans	nd	nd	0.3	8.1	nd	nd	nd	nd	nd	nd
Fusarium verticillioides	0.05	1.7	0.4	8.1	0.03	3.1	43.3	91.7	nd	nd
Microdochium nivale	0.04	1.7	nd	nd	1.2	21.9	nd	nd	nd	nd
Penicillium citrinum	0.02	1.7	1.0	8.1	0.9	12.5	nd	nd	2.0	17.4
Penicillium funiculosum	nd	nd	nd	nd	nd	nd	18.2	66.7	7.1	34.8

RD: Relative density (%); *Fr*: Isolation frequency (%); nd: not detected.

Table 2. Other contaminant fungi relative density (*RD*) and isolation frequency (*Fr*) in freshly harvested cereal grains and soybean seeds in Entre Ríos, Argentina.

Species	wheat 1999		sorghum 1999		rice 1999		maize 2002		soybean 2002	
	RD	*Fr*	*RD*	*Fr*	*RD*	*Fr*	*RD*	*Fr*	*RD*	*Fr*
Acremonium strictum	0.9	10.3	nd	nd	2.4	12.5	nd	nd	nd	nd
Arthrinium phaeospermum	0.2	1.7	0.2	10.8	nd	nd	nd	nd	11.5	60.9
Cladosporium cladosporioides	2.6	65.5	0.6	18.9	1.0	40.6	nd	nd	0.1	4.4
Curvularia lunata	0.02	1.7	3.3	62.2	7.7	78.1	nd	nd	6.6	69.6
Diplodia zeae	nd	nd	nd	nd	nd	nd	0.7	16.7	nd	nd
Dreschlera spp	2.1	22.4	0.1	8.1	nd	nd	nd	nd	nd	nd
Epicoccum nigrum	1.4	53.5	1.7	51.4	0.8	43.8	0.3	8.3	0.2	4.4
Mucor racemosus	0.2	3.5	1.8	10.8	0.3	18.8	2.4	16.7	nd	nd
Nigrospora oryzae	1.3	17.2	1.5	32.4	28.4	93.8	nd	nd	5.6	34.8
Phoma glomerata	0.8	31.0	nd	nd	23.9	84.4	nd	nd	nd	nd
Phoma sorghina	nd	nd	26.9	86.5	nd	nd	nd	nd	nd	nd
Rhizopus stolonifer	0.8	6.9	0.4	24.3	0.8	12.5	0.5	25.0	1.4	4.4
Sclerotinia sclerotiorum	nd	nd	nd	nd	nd	nd	nd	nd	13.1	65.2
Sordaria spp	3.8	20.7	0.2	2.7	7.6	22.0	nd	nd	nd	nd
Trichoderma harzianum	0.4	1.7	0.4	2.7	nd	nd	0.2	8.3	0.1	4.4

RD: Relative density (%); *Fr*: Isolation frequency (%); nd: not detected.

ceum, F. verticillioides, F. semitectum and *Fusarium proliferatum*. These results agree with those observed in Entre Ríos for *Fusarium* species on wheat, but the incidence level was lower in this work than in the American study. In the Southeastern Buenos Aires (region IV), *A. alternata* was most commonly isolated (*RD*: 60.0% and *Fr*: 97.8%) at the 1996 durum wheat harvest [12]. That year *F. graminearum* reached 18.6% in *RD* and 69.5% in *Fr.* In Entre Ríos the *RD* and *Fr* of *F. graminearum* (0.3% and 15.5% respectively) were lower than in Southeastern Buenos Aires ($p < 0.01$).

Table 4 lists the contaminant mycoflora of wheat stored for a year in Entre Ríos in 2002. *Eurotium chevalieri* was the most frequent fungus among the potential toxigenic fungal species isolated followed by *Aspergillus flavus, Penicillium funiculosum, Chaetomium globosum* and *A. alternata*. In the comparison between freshly harvested and stored wheat samples it can be seen that *Alternaria* spp. as well as *Fusarium* spp. were prevalent in the freshly harvested wheat meanwhile in the stored samples the only *Fusarium* species isolated was *Fusarium napiforme*. This suggests that the environmental storage conditions allow the prevalence of *Aspergillus* group species, mainly *E. chevalieri* and *A. flavus* which are species that can grow at lower water activity [19]. From these results, it is possible that freshly harvested wheat in Entre Ríos could be contaminated by *Alternaria* toxins, ochratoxin A, fumonisins, zearalenone, trichothecenes group A and B and there is also a low probability of citrinin occurrence. In storage also might occur aflatoxins, cyclopiazonic acid and patulin.

In relation to fungi associated with sorghum, a study on freshly harvested sorghum at Pergamino, Buenos Aires province (humid pampean region) in 1991 and 1993 [11] showed that *RD* of species of *Fusarium* was higher than in Entre Ríos. In that work *F. semitectum, Fusarium equiseti, F. graminearum, F. verticillioides* and *F. proliferatum* were the most frequent *Fusarium* species isolated. In the same study in 1992, also in Pergamino, where there was similar rainfall and relative humidity to Entre Ríos in 1999, *A. alternata* and *F. verticillioides* were isolated at similar levels [11], but the *RD* observed in Entre Ríos was 58.1% and in Buenos Aires 22.5%. In a survey on sorghum samples collected in West Lafayette, Indiana [15] the predominant fungi isolated were *Alternaria* spp. and *F. verticillioides*. The *RD* of *Alternaria* spp. recovered in the American study of sorghum was 62.4% and 58.1% for Entre Ríos. In sorghum grain harvested at different places from South and North India [20] *F. verticillioides* was found to be the most prevalent *Fusarium* species isolated followed by *F. proliferatum*. In Sao Paulo, Brazil [6] *Phoma* spp. was the most frequent fungal species isolated from sorghum, followed by *Aspergillus* spp. and *Fusarium* spp., and *Alternaria* spp. occurred at low levels. In the Fusaria identified in the Brazilian samples *F. verticillioides* was the most frequent and *F. graminearum* was not isolated in any samples. *A. flavus* was the predominant *Aspergillus* species, as in Entre Ríos sorghum. As seen with the wheat

Table 3. Meteorological data[a] for three wheat crop seasons in different regions[b] in Argentina.

Region	Harvest season	Total rainfall (mm)	Maximum temperature (ºC)	Minimum temperature (ºC)
II	1993	87.5	18.4	7.3
V	1993	58.8	19.0	7.2
IV	1996	93.2	18.3	5.2
III	1999	38.1	20.2	10.0

[a] average data for period June-November
[b] data supplied by Servicio Meteorológico Nacional Argentino.

Table 4. Contaminant mycoflora of stored wheat in Entre Ríos province in 2002.

Species	*RD*	*Fr*
Alternaria alternata	3.79	20.83
Arthrinium phaeospermum	3.64	16.67
Aspergillus flavus	8.60	50.00
Aspergillus fumigatus	2.19	16.67
Aspergillus glaucus	0.44	4.17
Aspergillus ochraceus	0.15	4.17
Aspergillus tamarii	2.48	8.33
Chaetomium globosum	10.20	33.33
Cladosporium cladosporioides	3.94	41.67
Curvularia lunata	0.29	8.33
Epicoccum nigrum	0.44	4.17
Eupenicillium cinnamopurpureum	1.46	4.17
Eurotium chevalieri	29.74	62.50
Eurotium herbariorum	0.15	4.17
Fusarium napiforme	2.92	4.17
Mucor racemosus	8.89	20.83
Nigrospora oryzae	1.31	8.33
Penicillium citrinum	0.29	8.33
Penicillium funiculosum	10.64	41.67
Rhizopus stolonifer	2.62	29.17
Trichoderma harzianum	5.83	8.33

RD: Relative density (%)
Fr: Isolation Frequency (%)

harvested in Entre Ríos, *A. alternata* was the major fungal species isolated from sorghum. Therefore further studies on *Alternaria* toxins should be undertaken in sorghum. Other probable mycotoxins that could occur are aflatoxins, cyclopiazonic acid, zearalenone, trichothecenes group A and B, fumonisins and citrinin. Ochratoxin A could also occur in sorghum due to the presence of isolates of *Aspergillus niger*, a potential ochratoxin producer.

Analyzing the mycoflora associated with rice, this work confirms the findings of a previous survey carried out on rice samples harvested in different locations in Entre Ríos [3], which found that *A. alternata* was the most prevalent component of the internal seedborne mycoflora. *Phoma glomerata*, *F. graminearum, Microdochium nivale, Penicillium citrinum* and *A. flavus* were also isolated, as in this study. As a result, there is a potential for rice harvested in Entre Ríos to be contamined by Alternaria toxins, zearalenone, trichothecenes group A and B, citrinin, fumonisins, aflatoxins and cyclopiazonic acid.

With regard to the fungi associated with soybean, a previous study was carried out on freshly harvested soybean samples collected at an experimental field in Buenos Aires in 1999 [2], where *A. alternata* was the most frequently isolated fungus (*Fr*: 48.1) followed by *P. citrinum* and *F. graminearum*. In this first report for Entre Ríos, *A. alternata* gave a higher *Fr* than in Buenos Aires (95.7%), followed by *F. semitectum* (*Fr*: 87.0) and *F. graminearum* (*Fr*: 65.2).

Based on the fungal contamination, the following possible mycotoxins might be considered for occurrence studies in soybean collected in Entre Ríos: *Alternaria* toxins, zearalenone, trichothecenes group A and B, citrinin, patulin, aflatoxins and cyclopiazonic acid.

In maize collected in Entre Ríos the most probable mycotoxins that could occur are fumonisins, aflatoxins, cyclopiazonic acid and patulin. Ochratoxin A could also occur due to the presence of isolates of *A. niger* (Table 1).

In conclusion this is the first report of the mycoflora of freshly harvested wheat, sorghum and soybean in Entre Ríos. *A. alternata* was the most common fungal species associated with wheat, sorghum, rice and soybean in this province. These results, suggest that it may be necessary to study the toxigenic ability of *A. alternata* isolates and the occurrence of *Alternaria* toxins in these commodities. On the other hand, the prevalence of *F. verticillioides* in maize, could lead to this cereal being contaminated with fumonisins, but at lower levels than expected in other production areas of Argentina.

This work was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas, Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, Universidad de Buenos Aires, Universidad Nacional de Entre Ríos, Centro de Investigaciones en Micotoxinas - Universidad Nacional de Luján, Agencia Nacional para la Promoción Científica y Tecnológica, INCO-DEV Project Contract number ICA4-CT-2002-10043 and PICTO 2002 Código 12949 ANPCyT – INTA

References

1. Apro N, Resnik SL, Ferro Fontán C. Representative samples for mycotoxins analysis. An Asoc Quím Arg 1987; 75: 501-510.
2. Boca R, Pacin A, González HHL, Resnik SL, Souza JC. Soja y micotoxinas: flora fúngica-variedades-prácticas agronómicas. Aceites y Grasas 2003; 4: 510-515.
3. Broggi LE, Moltó GA. Fungi associated with rice in Entre Ríos Province, Argentina. Toxigenic capacity of *Fusarium graminearum* and *Microdochium nivale* isolates. Mycotoxin Res 2001; 17: 96-107.
4. Broggi LE, González HHL, Resnik SL, Pacin A. Mycoflora distribution in dry-milled fractions of corn in Argentina. Cereal Chem 2002; 79: 741-744.
5. Castillo MD, González HHL, Martínez EJ. Pacin A, Resnik SL. Mycoflora of freshly harvested black bean (*Phaseolus vulgaris* L.) from the main production area of Argentina. Mycopathologia 2004; 158: 107-112.
6. Da Silva JB, Pozzi CR, Mallozzi MAB, Ortega EM, Correa B. Mycoflora and occurrence of aflatoxin B_1 and fumonisin B_1 during storage of Brazilian sorghum. J Agric Food Chem 2000; 48: 4352-4356.
7. Devore JL. Probability and Statistics for engineering and the sciences. Monterrey, California, Brooks-Cole Publishing Company, 1987.
8. Ellis M. Dematiaceous Hyphomycetes. Kew, England, Commonwealth Mycological Institute, 1971.
9. González HHL, Resnik SL, Boca RT, Marasas WFO. Mycoflora of Argentinean corn harvested in the main production area in 1990. Mycopathologia 1995; 130: 29-36.
10. González HHL, Pacin A, Resnik SL, Martínez EJ. Deoxynivalenol and contaminant mycoflora in freshly harvested Argentinean wheat in 1993. Mycopathologia 1996; 135: 129-134.
11. González HHL, Martínez EJ, Resnik SL. Fungi associated with sorghum grain from Argentina. Mycopathologia 1997; 139: 35-41.
12. González HHL, Martínez EJ, Pacin A, Resnik SL. Relationship between *Fusarium graminearum* and *Alternaria alternata* contamination and deoxynivalenol occurrence on Argentinean durum wheat. Mycopathologia 1999; 144: 97-102.
13. González HHL, Martínez EJ, Pacin A, Resnik SL, Sydenham EW. Natural co-occurrence of fumonisins, deoxynivalenol, zearalenone and aflatoxins in field trial corn in Argentina. Food Add Contam 1999; 16: 565-569.
14. González HHL, Resnik SL, Pacin A. Mycoflora of freshly harvested flint corn from Northwestern provinces in Argentina. Mycopathologia 2001; 155: 207-211.
15. Menkir A, Ejeta G, Butler LG, Melakeberhan A, Warren HL. Fungal invasion of kernels and grain mold damage assessment in diverse sorghum germ plasm. Plant Dis 1996; 80: 1399-1402
16. Nelson PE, Toussoun TA, Marasas WFO. *Fusarium* species: an illustrated manual for identification. Pennsylvania, The Pennsylvania State University Press, University Park, 1983.
17. Pacin A, Broggi LE, Resnik SL, González HHL. Mycoflora and mycotoxins natural occurrence in corn from Entre Ríos Province, Argentina. Mycotoxin Res 2001; 17: 31-38.
18. Pacin A, González HHL, Resnik SL, Boca R, Burak R, Broccoli A, de Souza JC. Natural occurrence of mycotoxins and mycoflora of Argentinean popcorn. Mycotoxin Res 2002; 18: 90-96.
19. Pitt JI, Hocking AD. Fungi and food spoilage. New York, Blackie Academic & Professional, 1997.
20. Prathapkumar HS, Ramesh VB. Natural occurrence of fumonisin B1 and its co-occurrence with aflatoxin B1 in Indian sorghum, maize, and poultry feeds. J Agric Food Chem 1997; 45: 2170-2173.
21. Secretaría de Agricultura, Ganadería, Pesca y Alimentación. Ministerio de Economía de la República Argentina. 2003. (http://www.sagpya.mecon.gov.ar).
22. Sinha K, Bhatnagar D. Mycotoxins in agriculture and food safety. New York, Marcel Dekker Inc, 1998.
23. Smiley RW, Patterson LM. Pathogenic fungi associated with *Fusarium* foot rot of winter wheat in the semiarid Pacific Northwest. Plant Dis 1996; 80: 944-949.
24. Statistix Version 7.0. User's manual. Analytical software. Borland International Inc and Fleming Software, Tallahassee, Florida, 2000.