Microbiol. Res. (2003) **158,** 29–35
http://www.urbanfischer.de/journals/microbiolres

Microbiological Research
© Urban & Fischer Verlag

Fusarium graminearum and deoxynivalenol contamination in the durum wheat area of Argentina

Gladys A. Lori[1], Marina N. Sisterna[1], Miriam Haidukowski[2], Inés Rizzo[2]

[1] CIDEFI (Centro de Investigaciones de Fitopatología) – CIC, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Calle 60 y 119, CC31, (1900) La Plata, Prov. Buenos Aires, Argentina
[2] INAME (Instituto Nacional de Medicamentos), ANMAT, Mrio de Salud de la Nación, Avda Caseros 2161, (1264) Buenos Aires, Argentina

Accepted: October 22, 2002

Abstract

Fusarium graminearum head blight of wheat is a destructive disease of the world's wheat–growing areas. This work was performed to analyze the distribution and contamination of deoxynivalenol (DON) and its relationship with *F. graminearum* kernel invasion in Argentina durum wheat area during two consecutive harvests. A total of 147 samples (cultivars and lines) of durum wheat from 5 locations of the major cropping area (Southern Buenos Aires Province) were analyzed. Percentage of *F. graminearum* kernel infection was evaluated following the blotter test (ISTA method) and fusarotoxins were analyzed by thin layer chromatography. None of the varieties and lines were free of *F. graminearum* infection. In the first harvest fungal invasion was very low. From 40 samples, 55% showed DON contamination but only 4 samples (10%) were higher than 2 ppm. In the second harvest, a crop year conducive to scab development, the highest level of *F. graminearum* kernel invasion observed was 42% on a sample from the humid area (eastern Buenos Aires Province) DON was detected in 47 (78.2%) of 60 samples analyzed and 19 (31.6%) showed levels of DON higher than those established in the guidelines in Canada and USA for food and feedstuff. In both years all locations situated in the humid area showed levels ranging from 0 to > 8 ppm. Within the durum wheat area differences among locations were found. This analysis indicates the need for more information on the problem and distribution of *Fusarium* mycotoxins in durum wheat grown in Argentina.

Key words: head blight – fusarotoxins – macaroni wheat – mycotoxin contamination – trichothecene

Introduction

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe, is one of the most important fungal diseases affecting wheat in all cropping areas of the world. Scab results from infection of individual spikelets at/or soon after flowering, when they are more susceptible. Infected spikelets are killed, and the fungus then may girdle the rachis so that the head above that point dies. A distinct salmon-pink ring of fungus develops at the base of the glumes. Damage from head blight or scab includes shrunken and discoloured (pink or chalky white "tombstone") kernels, reductions in yield and seed quality, and toxin contamination. These factors also reduce test weight and lower market grade (Trigo-Stockli *et al.* 1998).

Deoxynivalenol (DON) is one of the most common trichothecene toxins produced by *F. graminearum.* This water-soluble toxin, also known as vomitoxin, is responsible for emesis and feed refusal in non-ruminant animals (Forsyth *et al.* 1997; Vesonder *et al.* 1976). Zearalenone (ZEA), an estrogenic metabolite, commonly occurs with DON in cereal crops. ZEA induces feminization, interferes with conception, ovulation, implantation, fetal development and viability of newborn animals (CAST, 1989). DON has been detected in cereal grains grown in Canada, the US (Benett *et al.* 1993), the UK (Gilbert *et al.* 1984) and Japan (Yoshizawa 1983). In Argentina, Quiroga *et al.* (1995), Dalcero *et al.* (1997) and Rizzo *et al.* (1997) recorded the natural occurrence of this toxin from the common wheat (*Triticum aestivum* L.) cropping area.

Corresponding author: G. A. Lori
e-mail: galori@infovia.com.ar

0944-5013/03/158/01-029 $15.00/0

In 1982, the Food and Drug Administration (FDA) issued an advisory to federal and state officials recommending a "level of concern" for DON of 2.0 µg/g (2 ppm) for wheat entering the milling process and 4.0 µg/g for wheat and wheat milling by-products used in animal feed. New guidelines emphasized maximums for DON in bran, flour, and germ intended for human consumption at 1.0 µg/g. (Jones and Mirocha 1999). *F. graminearum* contamination on grain quality for milling, baking, and pasta making has increased during the past few years. Tanaka *et al.* (1986) reported that 60% of DON in naturally contaminated wheat remained after the wheat flour milling process, and no appreciable loss of toxins occurred as a result of baking process.

In Argentina, durum wheat (*Triticum durum* Desf.) is only grown in the southern area of the province of Buenos Aires, *F. graminearum* is an important pathogen in this cereal species (González *et al.* 1999; Lori and Sisterna 2001). Epidemics produced by *F. graminearum* were registered in the 1960s, 1970s, 1980s and 1990s (Moschini and Fortugno 1996). For durum wheat the epidemics of 1977–78 and 1984–85 caused an important reduction in the cropping area but has increased considerably in the last years. Natural occurrence of Fusarium toxins is recorded for common wheat but there is few data about durum wheat in our country (González *et al.* 1999). The objective of this work was to analyze the distribution and contamination of DON and its relationship with *F. graminearum* kernel invasion in durum wheat cropped in Argentina.

Materials and methods

Wheat samples. During two consecutive harvests durum wheat samples from varietal trials were analized. They were carried out in five localities: Balcarce, Miramar, La Dulce, Barrow and Bordenave (Lat. 37–39° S, long 57–64° W) in Buenos Aires Province. The total number of samples were 145 (20 cultivars and lines), 85 in the first year and 60 in the second (Table 1). To determine whether differences existed among wheat cultivars grown in different locations, comparisons were made on six of the twenty cultivars and lines collected: Bonaerense Quilacó, B. Valverde, B. INTA Cumenay, Buck Topacio, B. Cristal and B. 14. These cultivars were grown in all locations for both years.

Meteorological data such as temperature, rainfall and relative humidity (RH) were registered from the weather station located nearest each experimental field.

Determination of fungal invasion. To determine the percentage (%) of kernel infection by *F. graminearum,* 200 kernels per sample were subjected to the blotter test following the ISTA rules (Neergaard 1974). After 7 days of incubation, fungi growing from the kernels were identified and counted with the aid of a stereoscopic microscope. The presence of *F. graminearum* was confirmed on the base of cultural features and the micromorphology on potato dextrose agar (PDA) (Booth 1971). The data for *F. graminearum* invasion were categorized in three levels: low (< 1.5), medium (1.5–10) and high (> 10) (Table 2).

Mycotoxins analysis. A total of 100 samples (40 in the first year and 60 in the second) were analyzed for fusarotoxins. Samples from Balcarce were not included in the first year. Trichothecenes were determined by a modified Trucksess method (Rizzo *et al.* 1995) with acetonitrile: ethylacetate: water (50:41:9) as extraction solvent, cleanup column packed with charcoal: alumina: celite (0.7:0.5:0.3) and thin-layer chromatography (TLC) for detection and quantification. This method allows detection of Group A toxin-2 (T-2), neosolaniol, (NEO) and Group B (DON, nivalenol (NIV). This method has an average recuperation (mean of means): for DON 100%, T-2 92.3% and for NEO 95.7%. These percentages were determined over artificially contaminated wheat flour from 100 to 1000 ng/g, each concentration by quintuplicated. The coeficients of variation were among 0.10 to 0.14. The limit of detection, with spike wheat flour at 50–75 and 100 ng/g was

Table 1. Cultivars and lines evaluated from different localities and years

Cultivars and lines	1st year[a]	2nd year[a]
Bonaerense Valverde	×	×
Bonaerense Quilacó	×	×
Bonaerense INTA Cumenay	×	×
Bonaerense INTA Facón	×	×
Buck Cristal	×	×
Buck Ambar	×	×
Buck Topacio	×	×
Buck 12	×	–
Buck 14	×	×
CBW 32	×	—
CBW 33	×	—
CBW 40	×	—
CBW 53	—	×
CBW 54	—	×
VF 002	×	—
VF 003	—	×
VF 008	×	—
VF 010	×	×
VF 012	×	—
MAR 989	×	—
Total samples	85	60

[a] Localities: Balcarce, Miramar, La Dulce, Barrow and Bordenave (Lat. 37–39° S, long 57–64° W), Buenos Aires Province, Argentina

Table 2. Categories of *F. graminearum* occurrence related to infection level in the second year

Locations	Level infection (%) of *F. graminearum*					
	Low (< 1.5)		Medium (1.5–10)		High (> 10)	
	N° samples	Distribution[a]	N°samples	Distribution[a]	N°samples	Distribution[a]
Balcarce	0	0.0	2	8.0	10	47.6
Miramar	0	0.0	1	4.0	11	52.3
La Dulce	1	7.14	11	44.0	0	0.0
Barrow	1	7.14	11	44.0	0	0.0
Bordenave	12	8.57	0	0.0	0	0.0
Total of samples	14		25		21	

[a] Expressed in percentage

Table 3. Comparison of *F. graminearum* infection (% kernels) of six durum wheat cultivars during two years

Location	Bonaerense Quilacó		Buck Topacio		Buck Cristal		Bonaerense Valverde		Bonaerense INTA Cumenay		Buck 14	
	1st yr	2nd yr	1st yr	2nd yr	1st yr	2nd yr	1st yr	2nd yr	1st yr	2nd yr	1st yr	2nd yr
Balcarce	19.0[a]a	22.5a	1.0a	5.5b	17.0a	12.0b	22.5a	17.0a	8.0a	12.5a	7.5a	5.5b
Miramar	0.5b	29.5a	0.0b	14.5a	0.0b	25.0a	0.0b	18.5a	1.0b	13.5a	0.5b	12.0a
La Dulce	0.0b	3.0b	0.0b	4.0b	0.0b	4.5c	1.0b	6.0b	0.0b	3.5b	0.0b	3.0b
Barrow	0.0b	2.0b	0.0b	6.5b	0.0b	10.0b	0.0b	6.0b	0.0b	3.0b	0.0b	4.0b
Bordenave	1.0b	0.0c	0.0b	0.0c	1.0b	0.0c	1.0b	0.0c	1.0b	0.5c	0.0b	0.0c
Mean	5.12c	11.4a	0.2f	6.1bc	3.6d	10.3a	4.9c	9.5a	2.0e	6.6b	1.6e	4.9c

[a] Percentage of kernel invaded. Means within a year followed by common letters are not significantly different at $P < 0.05$.

made. The results were: 50 ng/g for DON and 100 ng/g for T-2 and NEO. For NIV the mean recovery was 69.6%. For this reason this method was not appropriate for quantification, so it can only be used to determine presence or absence of this toxin.

DON was confirmed using a spray of aluminum chloride solution (AOAC 2000a). Toxin after heat at 120 °C for 5 min was observed as blue fluorescent spot under UV-wavelength. T-2 and NEO were confirmed using a spray of 30% methanol sulfuric acid solution followed by heating at 120 °C for 5 min. Toxins were observed as greyish blue fluorescent spot under UV-wavelength (Mirocha 1982).

The modified BF method was employed to detect ZEA (AOAC 2000b). Protein interferences were removed by previous chloroform extraction and instead CH_3Cl, toluene was used for mycotoxin extraction into a separator (IRAM 1997). This qualitative method allows to detect ZEA with detection limit of 50 ppb and a recovery of 95% +/– 25%. This toxin was confirmed by using bis-diazotized benzidine sensitive spray (Malaiyandi *et al.* 1976), ZEA and Zearalenol (ZOL) became in red colour spot.

Statistical analysis. Data taken as percentage were arcsin-transformed prior to analysis. The transformed data were subjeted to analysis of variance (ANOVA) and the treatment means were compared by LSD ($P < 0.05$). The analysis was performed with the STATGRAPHICS program.

Results

Determination of fungal invasion

F. graminearum infection of durum wheat was observed during the two years, but the distribution and severity of infection varied, depending on location and year of harvest. In the first year fungal invasion was very low for almost all locations. The samples were categorized in level < 1,5. Nevertheless, in Balcarce only 11.7% samples were in the first level and 47.0 and 41.0% of the samples fell into medium and high level respectively. The mean of kernel invasion was 10.2%. In the second year samples from Balcarce and Miramar reached a high percentage of infection (in average 15.1 and 22.5

Table 4. Frecuency distribution of DON contamination in durum wheat samples from five locations in Buenos Aires Province

DON level (ppm)	1st yr		2nd yr	
	N° of samples with indicated toxin level	Distribution (%)	N° of samples with indicated toxin level	Distribution (%)
0	18	45.0	13	21.6
0.1–0.5	5	12.5	1	1.6
0.5–1.0	8	20.0	9	15.0
1.0–2.0	5	12.5	18	30.0
>2.0	4	10.0	19	31.6
Total	40	100	60	100

Table 5. Contaminated samples and DON levels in the first and the second year

1st yr				
Location	N° of samples		DON level (ppm)	
	DON contaminated/ analyzed	>2 ppm	Mean	(Range)
Balcarce	NT[a]	NT	NT	NT
Miramar	10/10	2	1.3	(0.38–3.04)
La Dulce	9/10	2	0.9	(0.0–2.4)
Barrow	3/10	0	0.1	(0.0–0.57)
Bordenave	0/10	0	0.0	(0.0)
2nd yr				
Location	N° of samples		DON level (ppm)	
	DON contaminated/ analyzed	>2 ppm	Mean	(Range)
Balcarce	12/12	5	2.6	(0.38–8.44)
Miramar	12/12	5	2.0	(0.57–4.22)
La Dulce	12/12	4	1.6	(0.80–3.20)
Barrow	11/12	5	2.1	(0–5.33)
Bordenave	0/12	0	0.0	(0.0)

[a] Not tested

respectively). Most of the samples were in the higher level. On the contrary, *F. graminearum* infection in Bordenave samples was low. Except in this location, none of the varieties and lines were free of this fungus presence.

Considering the six cultivars in common in both harvests, the cultivars behaviour showed a similar tendency for the levels of *F. graminearum* invasion (Table 3). In Balcarce and Miramar higher percentages of kernels invaded with *F. graminearum* were observed while not so high values were registered in samples from La Dulce, Barrow and Bordenave.

Distribution and levels of mycotoxins

In both harvests DON contamination was present. Except in Bordenave, all locations showed levels ranging from 0 to > 8 ppm. DON levels were higher in the second than in the first year (Fig. 1). In the first harvest, 22 of 40 samples were possitive for DON (55%) (Table 4). Only 4 samples (10%) were higher than 2 ppm. In the second year, 47 samples of 60 (78%) were possitive for the same toxin. But 19 samples (31.6%) showed levels of DON greater than 2 ppm. Also for this year, Balcarce, Miramar and Barrow had mean values higher than 2 ppm (Table 5). The level

Table 6. Comparison DON levels in six durum wheat cultivars in the first and the second year

Location	Bonaerense Quilacó		Buck Topacio		Buck Cristal		Bonaerense Valverde		Bonaerense INTA Cumenay		Buck 14	
	1st yr	2nd yr	1st yr	2nd yr	1st yr	2nd yr	1st yr	2nd yr	1st yr	2nd yr	1st yr	2nd yr
Balcarce	NT[a]	4.2	NT	0.4	NT	4.2	NT	1.6	NT	0.8	NT	1.6
Miramar	0.8[b]	4.2	0.4	0.6	1.1	4.2	1.1	2.7	0.8	1.8	0.8	1.8
La Dulce	0.4	2.1	0.4	0.9	2.4	3.2	2.4	1.2	0.8	1.8	0.8	1.0
Barrow	0.6	1.7	0.0	0.0	0.6	5.3	0.4	2.4	0.0	1.6	0.0	2.4
Bordenave	0.0[c]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	0.4	2.4	0.2	0.4	1.0	3.3	0.7	1.6	0.4	1.2	0.4	1.4

[a] Not tested [b] DON levels in ppm [c] 0.0 = Not detected

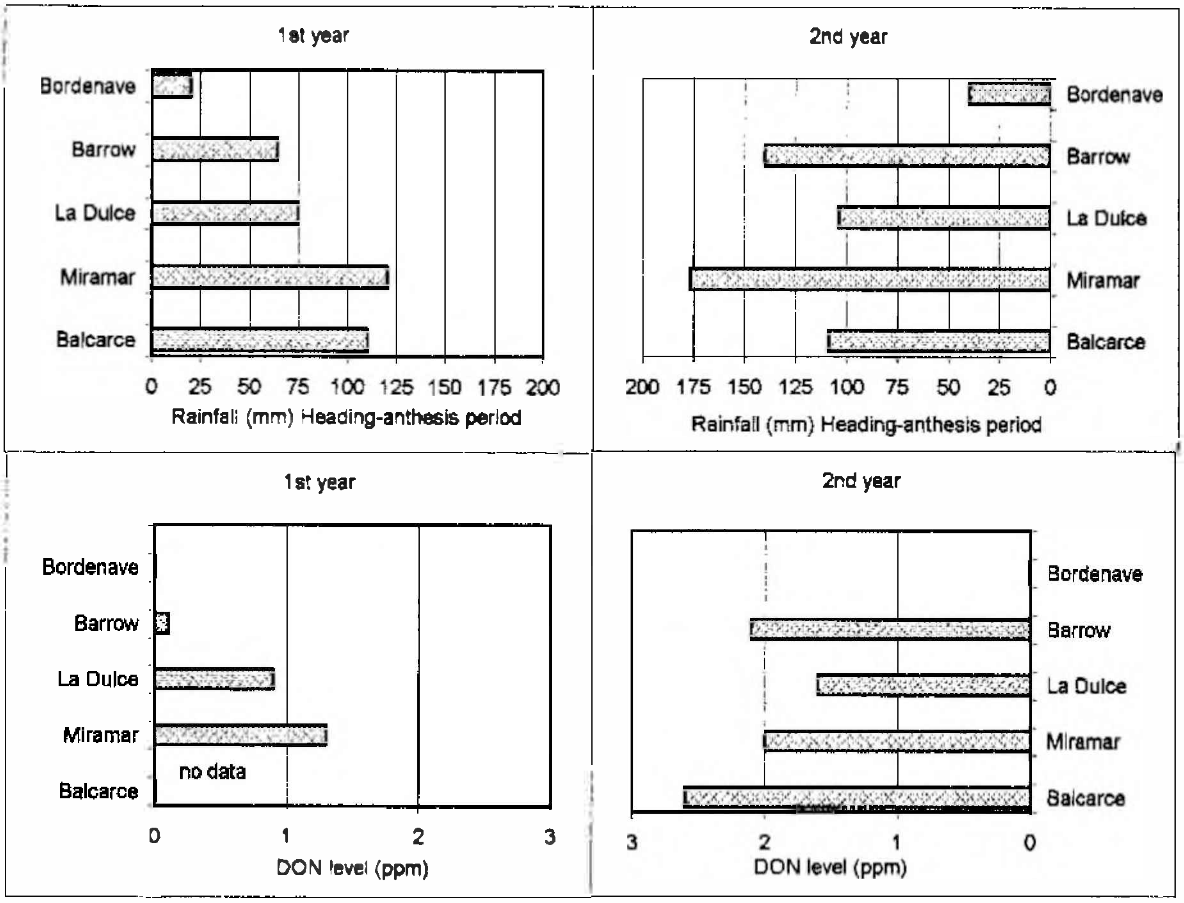

Fig. 1. Rainfall (mm) during heading periods and DON contamination in durum wheat samples from different locations (eastern to western) of Southern Buenos Aires Province (Argentina)

in Balcarce was very high, reaching in one sample 8.44 ppm. Bonaerense Quilacó and Buck Cristal showed, in average, high levels of both, *F. graminearum* and DON (Tables 3 and 6).

The other investigated mycotoxins (NIV, neosolaniol, and T-2 toxin) were not detected. ZEA was only found in one sample from La Dulce and its precursor ZOL was present in the first year in three samples from La Dulce and three samples from Barrow.

Discussion

Our data showed that although none of the varieties and lines were free of *F. graminearum* infection, differences found among genotypes could be due to heading at a period unfavourable to infection. Factors such as RH and rainfall affect *F. graminearum* invasion and occurrence of DON. Trigo-Stockli *et al.* (1995) cited that the weather was an important factor affecting infection levels by *F. graminearum* in 1993 in Kansas wheat. Love and Seitz (1987) stated that *F. graminearum* grows ideally at or above 92–94% RH. In the second harvest, a crop year conducive to scab development, samples from Balcarce and Miramar reached a high percentage of infection (24.5 and 42.0 respectively). In these locations the heading period coincided with RH values higher than 90% (96 and 95, respectively). In Argentina, Moschini and Fortugno (1996) for Pergamino (Buenos Aires Province) and Moschini and Carmona (1998) for Balcarce, observed that the meteorological conditions have an influence from the beginning of heading to early grain development. In this sense, after flowering, most wheat cultivars continue to be receptive to scab infection as long as the required moisture and temperature conditions are present. Considering DON contamination, Hart *et al.* (1984) reported that longer exposure of the wheat heads to wetness increased the severity of infection as well as DON production. Our results showed that high DON levels were related with Fusarium kernel contamination, in agreement with the findings of González *et al.* (1999). These parameters decreased from eastern to western locations (Balcarce to Bordenave), this is correlated with the decrease of RH and rainfall in the region as shown in Fig. 1. Balcarce and Miramar are situated in a humid area while Bordenave has dry climate (68% mean RH during heading) that does not favour *F. graminearum* infection.

Comparing both years, in the first harvest only 4 samples from Miramar and La Dulce were higher than 2 ppm (maximum value 3.04 ppm). There are few references about DON contamination on argentinian durum wheat. González *et al.* (1999) from samples harvested in 1996 in south-east of Buenos Aires province found DON with maximum values of 6.4 ppm but without differentiating locations within the durum wheat area. During the second year, with conducive environmental conditions to scab development, our data showed that near 60% of the samples had DON levels ranging from 1 ppm to > 2 ppm. This information is important, because the FDA has set an advisory level of 1 ppm for finished wheat products for human consumption.

Potential toxic effects of mycotoxins associated with fusarium head blight, particularly DON have resulted in numerous investigations on levels in infected wheat, flour and processed products (Dexter *et al.* 1997). DON is stable during wheat milling, although it becomes partitioned in varying concentrations among screening, mill feed and flour streams and also stable during baking (Tanaka *et al.* 1986; Tkachuk *et al.* 1991). Seitz *et al.* (1985) mentioned that DON was found in all mill fractions of flour wheat and they observed that the first reduction flour exhibited a higher mean DON concentration than subsequent reduction flour. The presence of DON in common wheat is undesirable due to its known toxicological effects in humans and animals. In the case of durum wheat, where the intended end use is the human consumption, DON levels are reduced in cooked pasta because of leaching into the cooking water (Nowicki *et al.* 1988), however in the case of noodles soup the cooking water is not discarded.

F. graminearum is an aggressive invader of wheat kernels destroying starch granules, storage proteins and cell walls. Also a decrease in the proportion of glutenins is observed, showing affecting kernels a higher ratio of gliadins to glutenins. Nevertheless, this alteration of the ratio does not affect pasta texture (Dexter *et al.* 1997). When marketing semolina, millers generaly must meet semolina refinement indices, most commonly ash content, number of bran specks and color (brightness and yellowness). A higher yellow pigment content in semolina is a parameter associated to quality and to cleaned samples. Fusarium damage has significant durum wheat proccesing quality implications. In this sense semolina yield is reduced and became duller and more red.

In terms of food quality, this study confirms that DON occurrence and the widespread distribution of FHB in the major durum wheat cropping area would implicate both, a downgrade in the commercial parameters and a risk for the health of human beings.

Acknowledgements

The authors acknowledge Chacra Experimental de Barrow (Ministerio de la Producción de la Provincia de Buenos Aires) for submit the samples and the partial financial support from Agencia Nacional de Investigaciones Científicas y Técnicas and Industrias Fideeras Argentinas.

References

Association of Official Analitycal Chemists (AOAC). (2000a): Natural Toxins. 49.4.01 Deoxynivalenol in wheat. Official Method of Analysis. Chapter 49, 42.

Association of Official Analitycal Chemists (AOAC). (2000b): Natural Toxins. 49.4.01 Zearalenone in corn. Official Method of Analysis. Chapter 49, 55.

Benett, G. A., Stubblefield, R. D., Shannon, G. M., Shotwell, O. L. (1983): Gas chromatographic determination of deoxynivalenol in wheat. J. Assoc. Off. Anal. Chem. **66,** 1478–1480.

Booth, C. (1971): The genus *Fusarium.* Commenwealth Mycological Institute. Kew, Surrey, England, 237.

Council for Agricultural Science and Technology (CAST). (1989): Mycotoxins: Economics and health risk. Report 116. Ames, IA.

Dalcero, A., Torres, A., Etcheverry, M., Chulze, S., Varsavsky, E. (1997): Occurrence of deoxynivalenol and *Fusarium graminearum* in Argentinian wheat. Food Add. and Cont. **14,** 11–14.

Dexter, J. E., Marchylo, B. A., Clear, R. M., Clarke, J. M. (1997): Effect of Fusarium head blight on semolina milling and pasta making of durum wheat. Cereal Chem. **74,** 519–525.

Forsyth, O. M., Yoshizawa, T., Morooka, N., Tuite, J. (1997): Emetic and refusal activity of the deoxynivalenol to swine. Appl. Environ. Microbiol. **34,** 547–552.

Gilbert, J., Shepherd, M. J., Startin, J. R. (1984): The analysis and occurrence of Fusarium mycotoxins in the United Kingdom and their fate during food processing. In: Toxigenic fungi – Their toxins and health hazard. (Eds: Kurata, Y., Ueno, Y.), Elsevier, Amsterdam, 209–216.

González, H. H. L., Martínez, E. J., Pacin, A., Resnik, S. L. (1999): Relationship between *Fusarium graminearum* and *Alternaria alternata* contamination and deoxynivalenol occurrence on Argentinian durum wheat. Mycopathologia **144,** 97–102.

Hart, L. P., Pestka, J. J., Liu, M. T. (1984): Effect of kernel development and wet periods on productionof deoxynivalenol in wheat infected with *Gibberella zeae.* Phytopathology **74,** 1415–1418.

Instituto Argentino de Normatización (IRAM). (1997): Micotoxinas, determinación de aflatoxinas y zearalenona en maíz y maní. Norma IRAM 14803.

Jones, R. K., Mirocha, C. J. (1999): Quality parameters in small grains from Minnesota affected by Fusarium head blight. Plant Dis. **83,** 506–511.

Lori, G., Sisterna, M. (2001): Occurrence and distribution of *Fusarium* spp. associated with durum wheat seed from Argentina. J. Plant Pathol. **83,** 63–67.

Love, G. R., Seitz, L. M. (1987): Effects of locations and cultivar on Fusarium head bligth (scab) in wheat from Kansas in 1982 and 1983. Cereal Chem. **64,** 124–128.

Malaiyandi, M., Barrette, J. P., Wavrock P. L. (1976): Bisdiazotized benzidine as a spray reagent for detecting zearalenone on thin layer chromatoplates. J. Assoc. Off. Anal. Chem. **59,** 959–962.

Mirocha, C. J. (1982): Analytical method-analysis of T-2 and other trichothecenes in cereal grains. In: Environmental carcinogens selected methods of analysis. Vol 5, Some Mycotoxins. (Ed. H. Egan-IARC) 373–383.

Moschini, R., Fortugno, C. (1996): Predicting wheat head blight incidence using models based on meteorological factors in Pergamino, Argentina. European J. Plant Pathol. **102,** 211–218.

Moschini, R. C., Carmona, M. (1998): Fusariosis en trigo. Nuevo enfoque para su control para el área de Balcarce. ALEA Informa **8,** 16–20.

Neergaard, P. (1974): Report of fourth Regional Workshop on Seed Pathology for Developing Countries. (Eds: Danish Government Institute of Seed Pathology). Denmark.

Nowicki, T. W., Gaba, D. G., Dexter, J. E., Matsuo, R. R., Clear, R. M. (1988): Retention of the Fusarium mycotoxin deoxynivalenol in wheat during processing and cooking of spaghetti and noodles. J. Cereal Sci. **8,** 189–202.

Quiroga, N., Resnik, S., Pacin, A., Martínez, E., Pagano, A., Riccobene, I., Neira, S. (1995): Natural occurrence of trichothecenes and zearalenone in Argentine wheat. Food Control **6,** 201–204.

Rizzo, I., Kneteman, E., Salom, R., Frade, H. (1995): Thin layer chromatography for the determination of T-2, neosolaniol and DON in wheat flour and by-products. International Seminar on *Fusarium*, Martina Franca, Italy, 1995, 54.

Rizzo, I., Lori, G., Vedoya, G., Carranza, M., Haidukowski, M., Varsavsky, E., Frade, H., Chiale, C., Alippi, H. (1997): Sanitary factors and mycotoxin contamination in the argentinian wheat crop 1993/94. Mycotoxin Res. **13,** 67–72.

Seitz, L. M., Yamazaki, W. T., Clements, R. L., Mohr, H. E., Andrews, L. (1985): Distribution of deoxynivalenol in soft wheat mill steams. Cereal Chem. **62,** 467–469.

Tanaka, T., Hasegawa, A., Yamamoto, S., Matsuki, Y., Ueno, Y. (1986): Residues of Fusarium mycotoxins, nivalenol, deoxynivalenol, and zearalenone, in wheat and processed food after milling and baking. J. of Food Hyg. Soc. Japan. **27,** 653–655.

Tkachuk, R., Dexter, J. E., Tipples, K. H., Nowicki, T. W. (1991): Removal by specific gravity table of tombstone kernels and associated trichothecenes from wheat infected with Fusarium head blight. Cereal Chem. **68,** 428–431.

Trigo-Stockli, D. M., Curran, S. P., Pedersen, J. R. (1995): Distribution and occurrence of mycotoxins in 1993 Kansas Wheat. Cereal Chem. **72,** 470–474.

Trigo-Stockli, D. M., Sanchez-Mariñez, R. I., Cortez-Rocha, M.O., Pedersen, J. R. (1998): Comparison of the distribution and occurrence of *Fusarium graminearum* and deoxynivalenol in hard red winter wheat for 1993–1996. Cereal Chem. **75,** 841–846.

Vesonder, R. F., Ciegler, A., Jensen, A. H., Rohwedder, W. K., Weislander, D. (1976): Co-identity of the refusal and emetic principle from Fusarium-infected corn. Appl. Environ. Microbiol. **31,** 280–285.

Yoshizawa, T. (1983): Red-mold diseases and natural occurrence in Japan. In: Thichothecenes. Chemical, biological and toxicological aspects. (Ed: Ueno, Y.) Elsevier, Amsterdam, 195–209.