Incidence and distribution of *Fusarium* species associated with feeds and seeds from Spain

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Summary

Samples of seeds and feeds (corn-based and mixed) were collected during surveys in 1991-92 and 1992-93 from two regions of Spain, one in northern Spain where the annual rainfall is over 900 mm, and the other in southeastern Spain where the annual rainfall is about 400 mm. The level of *Fusarium* contamination was determined in the 657 samples analysed, and results were analysed statistically to assess the effects of type of sample and meteorological conditions on *Fusarium* proliferation. The predominant *Fusarium* species was *Fusarium moniliforme*, which represented 92.2% of the total *Fusarium* strains isolated. Other species isolated were *Fusarium oxysporum* (5.9%), *F. oxysporum* var. *redolens* (0.6%), *Fusarium poae* (0.6%) and *Fusarium sporotrichioides* (0.6%).

Key words

Feeds, *Fusarium moniliforme*, *Fusarium oxysporum*, *Fusarium* spp., Seeds, Spain

Incidencia y distribución de especies de *Fusarium* asociadas a piensos y semillas en España

Resumen

Se recogieron muestras de semillas y piensos (granulado y con maíz) durante las campañas 1991-92 y 1992-93 procedentes de dos regiones de España, una en el norte con una precipitación media anual superior a los 900 mm y la otra en el sudeste con una pluviosidad anual de unos 400 mm. Se determinó el nivel de contaminación por *Fusarium* spp en las 657 muestras recogidas y los resultados obtenidos se analizaron estadísticamente para determinar los efectos del tipo de muestra y condiciones climatológicas en la proliferación de *Fusarium* spp. Las principales especies aisladas fueron *Fusarium moniliforme* (92,2% del total de cepas de *Fusarium* aisladas), *Fusarium oxysporum* (5,9%), *F. oxysporum* var. *redolens* (0,6%), *Fusarium poae* (0,6%) y *Fusarium sporotrichioides* (0,6%).

Palabras clave

España, *Fusarium moniliforme*, *Fusarium oxysporum*, *Fusarium* spp, Piensos, Semillas

Fusarium Link is one of the most important fungal genera, which includes many species that are pathogenic to plants and responsible for a broad range of diseases [1], others that are highly mycotoxigenic [2-5] and some that cause opportunistic infections in humans and in farm animals [6].

The presence of *Fusarium* spp. is an indicator of grain storage conditions, which also suggests the possibility of mycotoxin formation [7,8]. Heavy contamination of maize, wheat and other cereals by *Fusarium* spp., resulting in mycotoxin production, has been associated with climatic factors such as drought or excessive rain [9, 10]. Several *Fusarium* species are important pathogens of cereals, causing severe crop yield loss [11]. Maize seems to be the staple diet in which *Fusarium*-mycotoxins are most likely to be produced, commonly causing mycotoxicoses when ingested [12,13]. *Fusarium moniliforme* Sheldon is the principal *Fusarium* species associated with maize; it has a wide geographic distribution and is one of the first known species to be implicated in animal and human toxicoses [14]. Geographical differences in the natural distribution of *Fusarium* species and their mycotoxins are apparent, resulting from environmental and storage conditions [11].

The object of this study carried out in Spain was determine the incidence and distribution of *Fusarium* species causing postharvest losses of grains and foodstuffs and producing mycotoxins harmful to humans and/or animals. In addition, the effects of type of sample and meteorological conditions on *Fusarium* contamination were evaluated.

MATERIALS AND METHODS

Samples. Samples of feeds and seeds (cereals, pulses and dried fruits) were collected in local stores, markets, agricultural cooperatives and farms located both in southeastern Spain (Comunidad Valenciana), representing an arid region, and in northern Spain (País Vasco), representing a humid region. Samples were taken between September 1991 and February 1992 in southeastern Spain and from December 1991 to January 1992 in northern Spain, and again from September 1992 to April 1993 in

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southeastern Spain, and from September 1992 to February 1993 in northern Spain.

Meteorological conditions. Each time we took into account the climatic conditions prevailing in the regions under study (Table 1), and carried out the sample collection from the same places. Northern Spain has a mild oceanic climate, with average temperatures ranging from 8.5°C in January to 20°C in August and a mean annual rainfall of 1260 mm with more than 160 rainy days a year. Southeastern Spain has a Mediterranean climate, with an average temperature ranging from 9°C in January to 25°C in August and a mean rainfall of 400 mm, with extended dry seasons in the coarse of year. The climate may be cooler in the inner mountainous areas.

Table 1. Climatic data referred to both surveys in the regions under study.

MEAN CLIMATIC CONDITIONS	First Survey (1991-92)		Second Survey (1992-93)	
	Northern Spain	Southeastern Spain	Northern Spain	Southeastern Spain
Annual rainfall (mm)	928.65	453.66	1119.20	325.60
Annual Temperature (°C)	12.88	17.22	12.38	17.29
Annual Relative Humidity (%)	74.24	64.22	78.56	66.08

Dilution plating. The sample of feedstuffs was ground in a mortar and 10 g were suspended in 90 ml of sterile distilled water with 0.05% Tween 80 added. A 1 ml sample of this feed suspension was used to prepare a dilution series from 1:10 to 1:10 000. Based on preliminary tests, a 1 ml sample of the feedstuffs suspension of 1:100 dilution was uniformly dispensed over the surface of a potato dextrose agar (PDA), medium supplemented with 0.6% oxytetracycline hydrochloride and 0.01% Rose Bengal at pH 5.5, and cultures (five plates per suspension) were incubated at 28°C in darkness for 7 days.

Similarly, individual kernels from 50 g sample of grain were surface sterilized with commercial bleach (1% sodium hypochlorite), followed by two rinses in sterile distilled water. The sterilised material (10 grains per plate and five plates per sample) were plated on the same medium, and incubated under the same conditions.

Isolation and identification of* Fusarium *strains. At the end of the incubation period, the plates were examined for the presence of suspected *Fusarium* colonies and transferred individually to modified Nash-Snyder medium [15,16], modified Czapek-Dox medium [16], oatmeal agar (OA) [17], carnation leaf agar (CLA) [18] and PDA [1].

Fusarium species were identified according to Booth [19] and Nelson *et al.* [16]. Representative isolates of each species isolated were grown from single conidia on Petri dishes of CLA [16] and on PDA slants [16,19] at 23°C under a mixture of cool white and black fluorescent lamps located 42 cm above cultures with a 12-h photoperiod. Cultures were grown for 5 to 10 days and stored in a refrigerator.

Statistical analysis. The frequency of contamination of samples by *Fusarium* in the two areas under study in the two surveys (1991-92 and 1992-93) was compared statistically, using multivariance techniques such as analysis of variance and comparison means´ tests [20]. Regression analysis was used to determine the relationship between *Fusarium* contamination and climatic conditions. Additionally, residuals were analysed to detect possible anomalies in the data. The statistical programm Statgraphics Version 5.0 was used for the multifactorial analysis of the variance of the data.

As the variable "*Fusarium* contamination" followed a binomial distribution rather than a normal distribution, data were transformed using the algebraic expression $z' = \arcsin z1/2$, where "z" is the relative frequency expressed in percentage of samples containing *Fusarium* species with respect to the total number of analised samples corresponding to one sort of sample, in the hope of increasing the homogenicity of variances and the data normalization [21].

RESULTS AND DISCUSSION

Incidence of* Fusarium *and influence of their sample. In most samples, a mixed mould flora was present. Species of the genus *Penicillium* were prevalent (62.8% in total), followed by *Aspergillus*, *Fusarium*, *Rhizopus* and *Saccharomyces*, that contaminated the samples at an intermediate level (between 21% and 53% of the samples).

Fusarium contamination was more frequent in northern Spain (incidence, 24.8%) than in southeastern Spain (21.9%). These differences could be a consequence of the different climatic conditions in the two regions, especially the higher relative humidity in northern Spain.

Table 2. Distribution of *Fusarium* in the collected samples from northern and southeastern Spain classified by type of sample and survey.

Type of sample	Total No Samples / No Samples contaminated by *Fusarium* Sp.					
	Southeastern Spain		Northern Spain		TOTAL	P†
	1991-92	1992-93	1991-92	1993-93		
Total No.Samples (In %)	90/23 (25.5%)	229/47 (20.5%)	85/25 (29.4%)	253/59 (23.3%)	657/154 (23.4%)	NS
Total No. Feeds	45/11	93/26	47/16	166/ 41	351/94	NS
Corn-based feeds	26/10	49/21	27/16	65/21	167/68	(*)
Mixed feeds	19/1	44/5	20/0	101/20	184/26	NS
Total No. Seeds	45/12	136/21	38/9	87/18	306/60	NS
Cereals						
Corn (*Zea mays* L.)	18/12	17/6	9/7	18/6	62/31	(*)
Oats (*Avena sativa* L.)	4/0	9/1	6/1	9/4	28/6	NS
Wheat (*Triticum aestivum* L.)	6/0	19/5	8/1	7/1	40/7	NS
Barley *(Hordeum vulgare* L.)	5/0	4/0	9/0	14/3	32/3	NS
Rice (*Oryza sativa* L.)	3/0	9/1	1/0	1/0	14/1	NS
Others (birdseed, millet)	4/0	22/2	----	----	26/2	NS
Non-Cereals						
Beet, soya, peanut, etc.	5/0	56/6	5/0	38/4	104/10	NS

P†: Statistical differences; NS: non-significant differences; (*): P= 0.1.

However, another determining factor could be the storage conditions [22] whilst were not ideal in either area.

The frequency of *Fusarium* in about a quarter of the samples analysed appears contradictory to the much used classification of *Fusarium* as a field fungus, although Pelhate [23] suggested that *Fusarium* spp. could be classified as an intermediate group between field and storage fungi with the ability to grow at intermediate water availabilities.

Table 2 shows the distribution of *Fusarium* in samples. Altogether, 70.7% of *Fusarium* isolates came from maize or from maize-based feed samples. This high incidence in maize is in agreement with an earlier study of 116 maize samples collected from the 1980 corn crop in southeastern Spain [24].

***Identified species of* Fusarium** . The species of *Fusarium* most frequently isolated from samples was *F. moniliforme*, which represented 92.2% of the 154 isolates obtained in both surveys (Table 3). This species is cosmopolitan and has been observed in all climatic regions of the world in association with a wide variety of crop plants [25]. The importance of this contamination must be considered in relation to its toxigenic potential.

In this study, 85% of maize and maize-based feeds were contaminated with *Fusarium*, mostly *F. moniliforme* (94.5% of isolates), in agreement with a previous study of feeds in Spain [26], in which the incidence of *F. moniliforme* was significantly higher in maize-based feeds (poultry feeds) than in mixed feeds, probably because this species is one of the principal fungi in maize crops worldwide [14] and it is able to produce fusarin C [27,28], fumonisin B1 [29], and other metabolites [14, 30] which may cause animal and human toxicoses.

F. oxysporum Schlecht was the second most prevalent species isolated, accounting for 6.5% of total *Fusarium* isolates. Like *F. moniliforme*, this species was most frequently isolated from maize and maize-based feeds, in the first survey from northern Spain.

F. oxysporum is widely distributed in both temperate and tropical regions where it has been reported to be pathogenic to many crops [31].

According to Booth´s classification [19], *F. oxysporum* var. *redolens* differs from *F. oxysporum* in the size of macroconidia. Only one culture of this variety was isolated from maize collected in southeastern Spain during the second survey.

Also isolated, both from southeastern Spain in the second survey, were *Fusarium poae* (Peck) Wollenw. from earth-almond and *Fusarium sporotrichioides* Sherb from red sorghum. *F. poae* and *F. sporotrichioides* are both stable in culture and toxigenic [14,16].

Study of the frequency of contamination due to* Fusarium *in feeds and seeds. A total of 657 samples of feeds and seeds, 175 from the first survey and 482 from the second were analysed statistically. The only significant association between *Fusarium* contamination and the three factors, sample, survey and region, was with sample type (p= 0.1) with maize and maize-based feeds being more frequently contaminated by *Fusarium* than other sample types (Table 2). The reason for this is likely to be the host range of F . moniliforme, which principally infects maize.

Study of the influence of climatic conditions on the* Fusarium *contamination. Climatic conditions analysed for their effect on *Fusarium* contamination included the annual average temperature, humidity and rainfall (Table 1).

A simple linear regression model was tested to determine whether there was any dependency (linear, multiplicative, exponential or inverse) between contamination by *Fusarium* and climatic conditions (temperature, relative humidity and annual average rainfall) during both surveys (1991-92 and 1992-93) and in either region, considering climatic conditions as an independent variable and *Fusarium* contamination as a dependent variable.

The correlation between climatic factors and fungal contamination was no statistically significant (p< 0.10); it was not possible to propose a model of behaviour for *Fusarium* in relation to the climatic conditions due to the lack of sufficient data.

Table 3. Main species of *Fusarium* isolated in feeds and seeds.

Type of sample	*Fusarium* species									
	Fusarium moniliforme					*Fusarium oxysporum*				
	1991-92		1992-93		Total	1991-92		1992-93		Total
	NS	SES	NS	SES		NS	SES	NS	SES	
In %	23 (50)	23 (50)	58 (60.4)	38 (39.6)	142 (92.2)	2 (100)	---	1 (14.3)	6 (66.7)	9 (6.5)
Maize & maize-based feeds	21	22	26	25	94	2	-	1	1	4
Mixed feeds	-	1	20	5	26	-	-	-	-	-
Wheat	1	-	1	4	6	-	-	-	1	1
Oat	1	-	4	1	6	-	-	-	-	-
Barley	-	-	3	---	3	-	-	-	-	-
Pulses	-	-	4	---	4	-	-	-	2	2
Other cereals	-	-	-	2	2	-	-	-	1	1
Dried fruits	-	-	-	1	1	-	-	-	1	1

NS: North Spain; SE S: Southeastern Spain

Climatic conditions during the growing period of the crop sampled in the first survey in northern Spain were adequate for *Fusarium* infection, with greater than usual springtime rainfalls, which caused late and bad sowing and even floods. During the growing season before the second survey in northern Spain, rainfall was irregular and temperatures were constantly below the seasonal average, delaying crop growth. The high humidity caused many fungal diseases and subsequent storage problems.

By contrast, conditions were dry in southeastern Spain before the first survey and temperatures were high, decreasing *Fusarium* contamination. Similar climatic conditions occurred before the second survey.

One plausible explanation to these facts is that rainfall determines the availability of water for fungi, to allow them germinate spores, etc. If there is little rain (less than about 400 mm per year, as in southeastern Spain), growth rate of *Fusarium* may be decreased and spore germination may be delayed, and the production of

mycotoxins by *Fusarium* decreased. This parameter also affects the physiological state of the plant, rendering more easily attacked by *Fusarium* if the rainfall at time of infection when plant susceptibility is high, as in northern Spain. In fact, it is possible an endophytic growth of *F. moniliforme* from seed borne infection through the stem to infect the new season's seed.

Another reason for the frequent occurrence of contaminated samples could be bad storage conditions and poor preservation of samples, since the main factors that determine the germination and growth of Fusarium, as well as the production of mycotoxins are not only humidity and temperature, but also strain, pH, and CO_2 and O_2 concentrations [32-34]. Because the environmental relative humidities are high (64-78% or even higher) and the temperatures are mild in both northern and southeastern Spain, the environmental conditions could directly affect fungal proliferation.

We can conclude that *Fusarium* contamination in feeds and seeds from both arid and humid regions of Spain was frequent and that the species isolated could be mycotoxigenic [12]. The presence of *Fusarium* in feeds and seeds, particularly in maize constitutes, potentially, hazard to human and animal health, which should be ruled by law in the near future.

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