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# REGIONAL DISTRIBUTION OF *Fusarium* STRAINS IN CORN FROM THE PROVINCE OF SANTA FE, ARGENTINA

*(Distribución regional de cepas de **Fusarium** en maíz en la provincia de Santa Fe, Argentina)*

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**Palabras clave:** ***Fusarium***, maíz, distribución, Argentina.
**Key words:** ***Fusarium***, corn, distribution, Argentina

## SUMMARY

*Strains of **Fusarium** isolated from corn harvested in different fields of the northern-central area of the province of Santa Fe, Argentina, were identified to the species level. Their distribution in the samples was evaluated and studies of prevalence had to be carried out in corn grains from this region.*

*Samples of recently harvested and post-harvest dried grains were examined. The isolation of **Fusarium** strains was performed by direct plating of the grains on potato-dextrose-chloramphenicol agar (PDA+CAF). After incubation at room temperature, percentages of grains colonised by the different species were recorded. For the identification of the species, conidial suspensions of monosporic cultures were plated simultaneously on PDA and synthetischer nährstoffarmer agar (SNA). Macro- and microscopic observations were performed after 4, 8 and 14 days of incubation at room temperature (22°-25°C).*

*The prevalence of **Fusarium** species in the samples was 100 %, and 29 % of them was colonized by more than one species. **F.moniliforme** was identified in 52.6% of the samples, **F.subglutinans** and **F.graminearum**, in 29% and **F.oxysporum**, in 2.6 %. The first species presented the highest levels of colonized grains (7.5 to 52.5 %).*

*According to the literature, reports for the different isolated species, we considered convenient to focus future prevalence surveys of **Fusarium** toxins on type B trichothecenes, zearalenone, moniliformin and fumonisin, in corn grains of this region.*

## RESUMEN

*Se identificaron a nivel de especie cepas de **Fusarium** aisladas de granos de maíz provenientes de diferentes campos del área centro-norte de la provincia de Santa Fe, Argentina, estudiandose su distribución y prevalencia en esta región.*

*Se examinaron muestras de granos secos de reciente cosecha y almacenados de post-cosecha. El aislamiento de las cepas de **Fusarium** se llevó a cabo mediante plaqueo directo de los granos en agar papa-dextrosa-cloranfenicol (PDA+CAF) y se registraron los porcentajes de granos colonizados por las diferentes especies. Para su identificación, se efectuaron cultivos monospóricos en agar PDA y SNA para inducir la formación de esporodoquios . Se realizaron observaciones macro y microscópicas a los 4, 8 y 14 días de incubación a temperatura ambiente (22°-25°C).*

*La prevalencia de **Fusarium** en las muestras fue del 100 %. El 29 % de las mismas se colonizó por más de una especie. Se identificaron **F.moniliforme**, en el 52.6 % de las muestras, **F.subglutinans - F.graminearum**, en el 29 %, y **F.oxysporum** en el 2.6 %. La primera especie, presentó los niveles más altos de granos colonizados (7.5-52.5%).*

*Según los reportes en la literatura, referente a las diferentes especies aisladas, consideramos conveniente orientar posteriores estudios de prevalencia de micotoxinas de **Fusarium** en granos de maiz de la región, hacia los tricotecenos tipo B, zearalenona, moniliformina y fumonisina.*

## INTRODUCTION

The genus ***Fusarium*** has attracted more attention from a broader range of scientists than possibly any other group of fungi, probably due to the cosmopolitan condition of the group, which is responsible for numerous plant diseases and storage rots (4, 24). ***Fusarium*** species are widely distributed in soil and on subterraneous and aerial plant parts, plant debris, and other organic substrates (3, 7, 19). They are common in tropical and temperate regions but also found in desert, alpine and arctic areas (8, 14, 18). The ability of ***Fusarium*** species for dispersion in atmosphere making them common colonizers of aerial plant parts, and their ability to cause vascular wilts, root rots and other diseases on a wide range of cereal grains and horticultural crops, explain the reason why they should be considered a threat of economical importance in agriculture (6, 11, 13, 22, 23). Under certain environmental conditions in the field as well as during post-harvest stages, the damage caused by certain species of the genus can affect technological properties and nutritional value of grains for human and animal consumption (11, 12, 13, 19, 20, 23).

In addition to the economical impact of ***Fusarium*** infections in agriculture, certain species have been studied extensively in the last 20 years in relation to the possible role as causative agents of mycotoxicoses. Therefore, foods and feeds contaminated with their toxins constitute a known risk for human and animal health and have importance in some diseases of unknown ethiology, specially demonstrated for ***Gibberella zeae*** **(*F.graminearum*)** and species in the Section **Liseola** in relation with rice and corn (1, 16, 26, 27, 34, 36).

To this respect, there seem to be some geographical differences in the natural distribution of ***Fusarium*** species, as well as of their corresponding toxins, which are influenced primarily by environmental conditions, crop production and post-harvest handling and storage methods (5).

Biotic, primarily rainfall, abiotic factors, type of cereal cultivar and fungal isolate, may be responsible for the extent of grain infection by ***Fusarium*** (2, 21).

Corn grains have proved to be commonly invaded and colonized by ***Fusarium*** species in vast areas of the world under temperate, subtropical and tropical climate conditions. This crop constitutes an important portion of human and animal diet in Argentina.

National reports concerning the distribution of ***Fusarium*** species present in corn crops are scarce (9, 10, 17, 28, 33). Notwithstanding, these surveys do confirm the presence of the **Liseola** section species (mainly ***F.moniliforme*** and ***F. graminearum***, as well as their teleomorphic states). In these surveys, references is made to these species as being responsible fo a fairly significant diseases which affects grains of corn in their cobs. Moreover the possible association of corn plant infected with ***G. zeae*** with cases of intoxication in pigs is put forward (17, 28). More recent papers (25, 26), in the province of B.Aires, revealed the presence of several species and varieties of ***Fusarium*** in arid soils and different cultures of the zone. In corn ***F.graminearum, F. moniliforme*** and ***F. oxysporum*** were isolated.

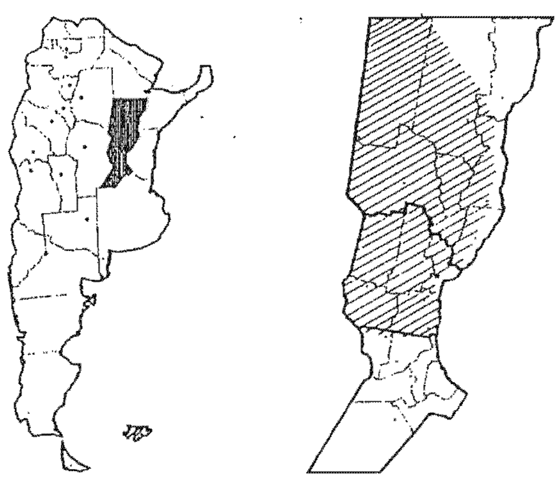
Previous data on the distribution of ***Fusarium*** species in grains of corn harvested in our region have not been made available.

In the present study, ***Fusarium*** strains isolated from corn grains were identified to the species level. Their prevalence and distribution in samples of Santa Fe province, Argentina, were also carried out as a preliminary study focused on future surveys for ***Fusarium*** mycotoxins in cereal of the region.

## MATERIALS AND METHODS

**1.- Samples:** A total of 38 samples of approximately 1.000 -2.000 g of freshly harvested and recently post-harvest dried corn grains were collected from different fields representing the northern-central geographical area of the province of Santa Fe, Argentina. Samples were subdivided in order to obtain portions of analysis of approximately 100 g, and kept in waterproof containers at

**Figure 1: Geographical area sampled for *Fusarium* species distribution in corn grains.**



**The sampling area is shown as a striped portion in the map.**

10 °C, until examined. Samples were collected during 1993 crop, and conserved for a maximum period of 6 months. Sampling geographical area is shown in Fig. 1.

**2.- Isolation of *Fusarium* strains:** Fifty seeds of each sample were surface sterilized for 2 minutes in 10 % commercial hypochloride, and subsequently washed in four 50 ml sterile water portions. After being dried with sterile paper filter, they were plated on potato-dextrose agar containing 100 µg of chloramphenicol/ml (PDA+CAF) to suppress bacteria. Five treated seeds were placed on the surface of 10 plates and incubated at room temperature(22°-25°C). *Fusarium* characteristic colonies growing out from the seeds after 5-7 days were subcultured on PDA slants, simultaneously recording the percentages of colonized seeds.

**3.- Single-spore isolation:** Single-spore isolation was carried out following Nelson et al. (31). A suspension of conidia was prepared in a 10 ml sterile water portion for each isolation so that it contained a few conidia per drop when observed under low-power microscope field. Each suspension was poured on the surface of a thin layer of solidified 2% water agar contained in a Petri dish, and the excess of water was drained off. After incubation at room temperature, small squares containing single germinating conidia were cut out and transferred to desired growth media in order to obtain monosporic cultures for subsequent identification.

**4.-Identification of *Fusarium* strains:** Identification of *Fusarium* strains to the species level was performed following the taxonomic schemes of Gerlach & Nirember g (15), Nelson et al. (31), and Pascoe (32). Conidial suspensions of monosporic cultures were inoculated on both PDA and SNA (31), and incubated at room temperature following day-light periods of 12 hours. After 4, 8, and 14 days of incubation, cultures were observed and data of colony pigmentation, texture and growth rate were recorded, as well as microscopic characteristics such as conidiogenesis, conidigenous cell types (mono- and polyphialides, and polyblastic cells), conidium types (macro, micro, meso and chlamydoconidia), conidia grouping types, as well as the presence or absence of sporodoquia and pinnote formations were recorded .

## RESULTS AND DISCUSSION

*Fusarium* was detected in all the 38 corn samples ( 100 %) and 29 % of them were colonized by more than one species. Figures 2 and 3 show the prevalence of the different species and their distribution in the grains, respectively.

***Fusarium moniliforme*** and ***F.subglutinans*** (Section ***Liseola***), ***F.graminearum*** (Section ***Discolor***), and ***F.oxysporum*** (Section ***Elegans***), were identified. The firstly named species was prevalent, being present in 71.1% of the total samples, and ***F.oxysporum*** was detected in only one sample (Table 1).The poor presence of ***F.oxysporum*** is an incommon situation because it is a soilborne fungus found in agricoltural soils throughout the world, and represent an attractive model for the ecological studies of fungal populations.This species also includes pathogenic and non pathogenic strains capable of persisting through asymptomatic colonization of plant roots and saprophytic growth on non living organic matter. Actually the sapro-phitic strains of ***F. oxysporum*** are considered success-full biocontrol agents in economically important crops (7, 37)

***F.subglutinans*** also exhibited rather high levels of colonized grains though its prevalence was lesser than 20% as it was for ***F.graminearum*** (Table 1). Present data for ***F.moniliforme*** are similar to those observed in other agricultural regions concerning maize crop and corn kernel infection (2, 7, 10, 17, 25, 28, 33). ***F.moniliforme*** is among the most common ***Liseola*** species isolated from the seed of corn. A range of 9 % to 91 % infection is usually reported for some seed lots of corn (2). In some years, corn kernels may be 100 % infected by this fungus indicating abiotic factors, primarily rainfall, might be responsible (21). The association of the fungus on corn kernel is both external and systemic. The nature of the systemic kernel infection is such that seed germination is not necessarily affected, but rather seedling vigour and growth may be reduced (2).

**Table 1: Percentage of corn grains colonized by *Fusarium* species**

| Species | % Colonized Grains (*) | (Mean) |
|---|---|---|
| ***F.moniliforme*** Sheldon | **8 - 52.5** | **(20)** |
| ***F.subglutinans*** (Woll.&Rein.) Nels., Touss. & Mar. | **9.8 - 40** | **(17.4)** |
| ***F.graminearum*** Schwabe | **5 - 10** | **( 5.5)** |
| ***F.oxysporum*** Schlecht. em. Snyd. & Hans. | **< 5** | |

(*) **Maximum and minimum values**

With reference to ***F. graminearum***, the mechanism of invasion and colonization of corn kernels seems to be

**Figure 2: Prevalence of *Fusarium* species in corn grains**

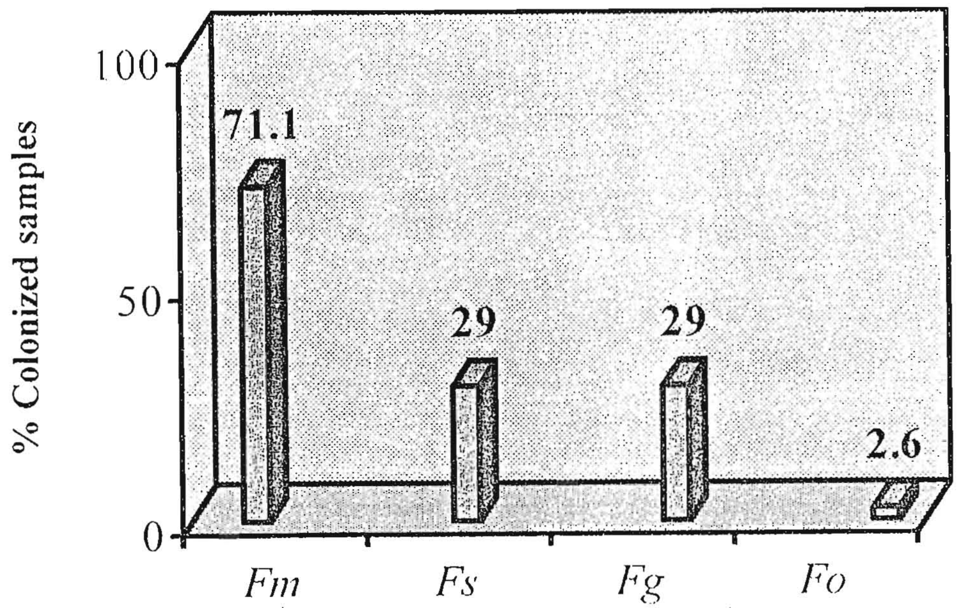
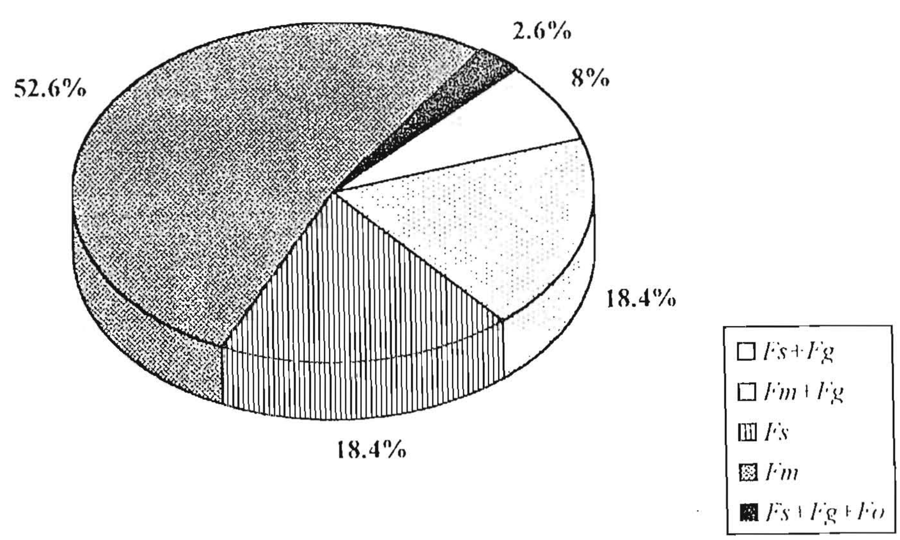



**Figure 3. Distribution of *Fusarium* species in corn grains**




*Fm*: ***Fusarium moniliforme*** Sheldon. *Fs*: ***Fusarium subglutinans*** (W. & R.) Nel., Tous. & Mar. *Fg*: ***Fusarium graminearum*** Schwabe. *Fo*: ***Fusarium oxysporum*** Schlecht. emend. Snyd. & Hans.

different from that observed for species in the Section **Liseola** (4, 7, 9, 21, 22, 28). Infection is again closely related to rainfall, but insect and bird damage can enhance infection. It could be interesting to point out that there may be a relationship between the occurrence of wheat head blight and maize ear rot epidemics (22).

In reserch works carried out in Argentina , on the presence of ***Fusarium*** species in several habitats and vegetals, results relate mainly to the phytopathological character of the studies, whereas our study takes in to account the relative range of association of the species in the grain lots that enter in to the processing chains.

Reports related with toxigenic ability of ***Fusarium*** isolates from different regions in the world suggest a high probability that the crops infected with ***Fusarium*** can be contaminated with its toxins (31). This has been particularly demonstrated for corn, barley and wheat (2, 22).

Mycotoxicosis caused by a plant pathogenic fungus produces different secondary metabolites harmful to animals or humans (26). Characteristic profiles of this toxic compound, have been pointed out to be produced by the different Sections of the genus, and even by the different species in a Section (1, 22, 29, 33, 36).

With reference to the type of mycotoxins expected to be present, ***F.graminearum*** strains from different geographical areas in the world have been shown to produce type B trichotheccenes, and occasionally zearalenone (1, 7, 9, 16, 19, 25, 33, 34, 35). Fumonisins and moniliformin have been found in cultures of strains belonging to Section ***Liseola***, as well as in the original samples of cereals and cereal by products (2, 5, 26, 27, 31). The last toxins occur frequently in naturally contaminated grains, particularly corn. Although firstly considered field fungi, at present ***Fusarium*** species are known to be active during storage, and possibly after some stages of cereal technological processing. From this point of view, monitoring of ***Fusarium*** toxins should not be restricted to the field nor to the first stages involved in handling crops after harvest.

It should be important to emphasise the prevalence and high percentage of colonization by ***F.moniliforme***.

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