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# Occurrence and toxicity of *Fusarium subglutinans* from Peruvian maize

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**Abstract.** Twenty-five samples of maize kernels collected at harvest time from geographically different corn fields in Peru, were examined for the occurrence of toxigenic *Fusarium* species. The most frequently recovered species were *F. subglutinans* (48%), *F. moniliforme* (46%), and *F. equiseti* (5%). Other *Fusarium* species isolated (up to 1%) included *F. graminearum*, *F. acuminatum*, *F. solani*, *F. oxysporum*, and *F. culmorum*. Assays of *Fusarium* culture extracts using *Artemia salina* larvae, showed *F. subglutinans* as one of the most toxigenic species, and its toxicity was mostly correlated to the capability to produce beauvericin (BEA). All eight tested isolates of *F. subglutinans* grown on autoclaved corn kernels produced BEA (from 50 to 250 mg/Kg) as well as moniliformin (M) (from 70 to 270 mg/Kg). This is the first report on BEA and M production by maize isolates of *F. subglutinans* from South America.

**Key words:** Beauvericin, *Fusarium subglutinans*, *Fusarium moniliforme*, Maize, Moniliformin

## Introduction

*Fusarium* species are common pathogens of the maize plant, causing stalk and ear rot [1, 2]. Several phytopathogenic isolates are also capable of producing potent mycotoxins implicated in human and animal diseases [3]. The most common *Fusarium* species isolated from maize ears is *F. moniliforme* Sheldon as well as *F. graminearum* Schwabe and *F. subglutinans* (Wollenw. & Reiking) Nelson Toussoun Marasas [2]. The relative incidence and the geographical distribution of *F. moniliforme* and *F. subglutinans*, both belonging to the Section *Liseola* Woll., is not well-documented, probably due to taxonomic misidentification [1, 2].

Most reports on the occurrence of *Fusarium* species on maize are from South Africa [4, 5], Australia [6, 7], Asia [8], Europe [9–11], North America [12], and only a few from Central and South America, even though the climate there is ideal for corn ear rot and mycotoxin formation [13]. In South America, *F. moniliforme* has been reported as the major maize colonizing *Fusarium* species [14–16]. Cuero et al. [17], who compared the toxigenic fungi on maize harvested in two different areas in Columbia, found that *F. graminearum* was the most toxigenic *Fusarium* species. In addition, maize samples from Chile were most commonly contaminated with *Fusarium* species although *Fusarium* toxins were not detected [18].

The aim of this study was to investigate the occurrence of *Fusarium* species in Peruvian maize and to determine their toxigenicity with special emphasis on *F. subglutinans* isolates. *Fusarium subglutinans* has been reported as one of the most prevalent fungi associated with home-grown corn in areas of high incidence of human oesophageal

cancer in South Africa [3]. This species is also reported to be toxic in animal bioassays [19, 20]. Moniliformin is the major toxin produced by this species, although not all isolates are able to produce it [3, 21]. *Fusarium subglutinans* also produces beauvericin [22, 23], a cyclodepsipeptide toxin known for its insecticidal properties [24].

## Materials and methods

*Source of samples*. During the 1987–1988 harvest season, twenty-five maize kernel samples (ca. 500 g) representing different genotypes and hybrids were collected from seven geographical locations throughout the major maize-producing areas of Peru (Table 1). A subsample of kernels (ca. 100 g) from the original sample (ca. 500 g) were surface-disinfected for 1 min in 3% NaOCl. After 2 sterile water rinsings, 100 kernels were placed on plates (5 kernels per plate) containing a modified pentachloronitrobenzene medium selective for *Fusarium* [25, 26], and incubated at 25 °C for one week in the dark. The *Fusarium* colonies that developed were transferred to a potato sucrose agar plate (PSA) and incubated at 25 °C for 10–14 days under fluorescent and black light lamps (12 h photoperiod). Single-spore cultures were maintained on PSA and carnation leaf agar under the conditions described above and identified as to species [26].

*Toxin production*. Thirty-four single-conidia isolates belonging to five *Fusarium* species were cultured on maize to detect toxin production [27]. Two hundred g of yellow corn kernels var 'Plata' at 45% moisture in 500 ml Erlenmeyer flasks were autoclaved for 20 min at 120 °C, and inoculated with approximately $10^7$ conidia in a water suspension. The cultures were incubated at 25 °C in the dark for 4 weeks and then dried at 60 °C for 48 h. Uninoculated corn was used as control.

*Toxin extraction*. A 20 g sample of inoculated maize was extracted in a blender with 100 ml of methanol-1% NaCl (55 + 45) for 3 min, filtered through filter paper (Whatman No. 1), and 50 ml of the filtrate were transferred into a separatory funnel and defatted twice using 50 ml of n-hexane. The upper n-hexane layer was discarded and the methanol layer extracted with methylene chloride (3 × 30 ml). The methylene chloride extracts were collected, evaporated to dryness, dissolved in 1 ml of methanol and analyzed and bioassayed for toxins.

*Toxin identification*. The identification and quantitative analyses of beauvericin (BEA), diacetoxyscirpenol (DAS), T2 toxin (T2) and moniliformin (M) by thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) were performed as previously described [27]. BEA production by *F. subglutinans* isolate 739 was confirmed by $^1$H NMR spectra. Detection limits for BEA, DAS and T-2 plus M were 5 μg/g, 1 μg/g and 5 μg/g of maize culture, respectively. Toxin reference standards were purchased from Sigma Chemical Co. (St. Louis, USA).

*Bioassay*. The toxicity of culture extracts was tested using brine shrimp (*Artemia salina* L.) according to Harwing & Scott [28]. *Artemia salina* eggs (Euraquarium, Bologna, Italy) were incubated for 36 h in one sector of a four – sector Petri dish filled with seawater (3.3% sea salt solution). Bioassays were performed in 24-well culture plates (Corning, New York), containing about 30–40 larvae in 500 μl seawater with 1% methanolic extract of fungal culture per well (4 replicates per extract). The number of dead shrimps was recorded after incubation at 27 °C for 36 h and compared to the total number of shrimps per well after freezing at −20 °C for 12 h.

## Results

The occurrence and distribution of *Fusarium* species identified is summarized in Table 1. Eight *Fusarium* species were isolated from the 25 samples and included, in percentage, *F. subgluti-*

*Table 1.* Distribution of *Fusarium* species in maize kernels from Peru

| Sample origin | Mean % of *Fusarium*-infected kernel | | | Other | Total *Fusarium* spp. in sample |
|---|---|---|---|---|---|
| | *F. subglutinans* | *F. moniliforme* | *F. equiseti* | | |
| Ancash | | | | | |
| 1 | 50 | 18 | 2 | – | 70 |
| 2 | 4 | 100 | 2 | – | 106 |
| 3 | 86 | 24 | – | 2 *F. graminearum* | 112 |
| a | 12 | 88 | 6 | – | 106 |
| Cusco | | | | | |
| 5 | 28 | 36 | 14 | – | 78 |
| 6 | 30 | 6 | 2 | – | 38 |
| 7 | 95 | 4 | – | – | 99 |
| 8 | 8 | – | – | 2 *F. acuminatum** | 100 |
| 9 | 22 | 56 | 50 | 2 *F. solani* | |
| | | | | 2 *F. oxysporum* | 132 |
| Puno | | | | | |
| 10 | 80 | 2 | 2 | 2 *F. graminearum* | 86 |
| 11 | 60 | – | 6 | 1 *F. solani* | 67 |
| 12 | 50 | 12 | – | 2 *F. graminearum* | |
| | | | | 3 *F. acuminatum** | |
| | | | | 1 *F. culmorum* | 68 |
| Ica | | | | | |
| 13 | 16 | 96 | – | 2 *F. graminearum* | 114 |
| 14 | – | 100 | – | – | 100 |
| 15 | – | 56 | – | – | 56 |
| La Merced | | | | | |
| 16 | – | 32 | 3 | – | 35 |
| 17 | 8 | 16 | 4 | 2 *F. solani* | 30 |
| 18 | – | 100 | – | – | 100 |
| Iquitos | | | | | |
| 19 | 96 | 31 | – | – | 127 |
| 20 | 80 | 4 | 3 | – | 87 |
| 21 | 100 | – | 3 | – | 103 |
| 22 | 95 | 2 | 1 | – | 98 |
| Huaraz | | | | | |
| 23 | 4 | – | – | 1 *F. acuminatum** | 5 |
| 24 | – | 69 | – | 1 *F. solani* | 70 |
| 25 | – | 100 | – | – | 100 |
| Total | 1014 | 952 | 98 | 23 | 2087 |

* All isolates of *F. acuminatum* proved to be slow-growing and carmine red pigmentation type [37].

*nans* (48%), *F. moniliforme* (46%), and *F. equiseti* (Corda) Sacc. (5%). Other *Fusarium* species isolated, but less than 1%, included *F. graminearum*, *F. acuminatum* Ell. & Ev., *F. solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans., *F. oxysporum* Schlecht. emend. Snyd. & Hans., and *F. culmorum* (W.G. Smith) Sacc. Overall, samples highly contaminated by *F. subglutinans* had a low level of infection by *F. moniliforme*, and vice versa.

The toxicity of *Fusarium* species culture extracts to *A. salina* is reported in Table 2. The

*Table 2.* Toxicity of *Fusarium* species on *Artemia salina* larvae*

| *Fusarium* spp. | No. of isolates tested | No. of isolates causing 100% of mortality | Percentage of toxic isolates |
|---|---|---|---|
| *F. subglutinans* | 8 | 6 | 75 |
| *F. moniliforme* | 12 | 0 | 0 |
| *F. equiseti* | 6 | 1 | 16 |
| *F. graminearum* | 5 | 2 | 40 |
| *F. acuminatum* | 3 | 2 | 67 |

* Four replicates per isolate.

*Table 3.* Toxicity of *F. subglutinans* isolates to *Artemia salina*

| Isolate (ITEM) | Mycotoxin produced (mg/Kg) | | % mortality of *A. salina* in 24 h* |
|---|---|---|---|
| | Beauvericin | Moniliformin | |
| 724 | 50 | 200 | 50 |
| 672 | 80 | 270 | 92 |
| 745 | 100 | 200 | 100 |
| 673 | 125 | 100 | 100 |
| 739 | 150 | 70 | 100 |
| 719 | 150 | 135 | 100 |
| 720 | 200 | 135 | 100 |
| 744 | 250 | 70 | 100 |
| Control + methanol | | | 4 |

* Four replicates per isolate.

most toxic species was *F. subglutinans* with 6 of 8 isolates tested (75%) causing 100% larvae mortality. The toxicity of other species tested was *F. acuminatum* (67%), *F. graminearum* (40%), *F. equiseti* (16%), and *F. moniliforme* (0%).

The toxin production of the eight toxigenic isolates of *F. subglutinans* is reported in Table 3. On autoclaved maize kernels, all isolates of *F. subglutinans* produced BEA (from 50 to 250 mg/Kg) and M (from 70 to 270 mg/Kg), but not DAS or T-2 trichothecenes. Overall, strains producing high levels of BEA produced relatively low M levels and vice versa. For example, isolate 724 produced 50 and 200 mg/Kg of BEA and M, respectively whereas isolate 744 produced 250 and 70 mg/Kg of BEA and M, respectively. Brine shrimp toxicity of *F. subglutinans* culture extracts was somewhat correlated to the levels of BEA produced.

## Discussion

Our findings indicate that *F. subglutinans* and *F. moniliforme* commonly occur in maize kernels produced in Peru. Although *F. subglutinans* is reported to be one of the most prevalent fungi associated with maize in Africa [4, 29], Australia [6], Asia [30], Europe [11, 31], North America [32], relatively little information is available on its occurrence in South America. This may be due to the fact that *F. subglutinans* is confused with *F. moniliforme*, a closely related species [1]. Previous investigations on *Fusarium* species occurring on maize in South America usually mention only *F. moniliforme* [14–16, 33]. Therefore, to our knowledge, this is the first report of the widespread distribution of *F. subglutinans* in maize from South America.

In our investigation the presence of high levels of *F. subglutinans* was normally associated with a low level of *F. moniliforme* and vice versa, which agrees with others reports [4, 7, 10–12]. A probable cause may be that the 2 species have different optimum climatic conditions for growth. For example, *F. subglutinans* has a lower optimum temperature for growth and predominates in more temperate areas than *F. moniliforme* [1, 4, 7]. In fact, in this study maize samples collected from humid-cool climate (e.g., Iquitos, Puno) were mostly infected by *F. subglutinans* whereas samples from dry-warm areas (e.g., Ica) were mostly colonized by *F. moniliforme*. Thus, climate appears to be the major factor in determining whether *F. subglutinans* or *F. moniliforme* predominates.

*Fusarium subglutinans* was the most toxigenic towards *A. salina*, and all isolates produced BEA and M but not T-2 toxin nor DAS. Other reports [3, 34] confirm that the production of T-2 and DAS is very rare in *F. subglutinans*.

Moniliformin producing isolates of *F. subglutinans* from cereals including maize have been reported in Europe [21, 34], South Africa [4, 20, 35], the United States [19] and New Zealand [36]. This is the first report of M production by South American isolates of *F. subglutinans* as well as BEA by isolates from maize. In addition, this is also the first report of BEA and M occurring together in maize samples. The previous report [3] that the toxicity of *F. subglutinans* isolates in mycotoxicoses could not be attributed solely to M production, suggests that BEA may be involved in these outbreaks as well.

In conclusion, the occurrence of toxigenic isolates of *F. subglutinans* and *F. moniliforme* in Peruvian maize indicates a potential toxicological risk in that area where maize is an important

human dietary staple. Furthermore, the high incidence of *F. subglutinans* suggests that further investigations are needed on the capability of isolates of this species from different geographical areas to synthesize both BEA and M and to study the importance of these toxins in mycotoxicosis outbreaks.

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