Occurrence of Fumonisin B_1 and B_2 in *Fusarium proliferatum* Infected Asparagus Plants

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Fusarium proliferatum, a fumonisin producing fungal species, is a causal agent of a destructive disease of asparagus called *Fusarium* crown and root rot. The occurrence of fumonisin B_1 (FB_1) and B_2 (FB_2) in asparagus plants heavily infected by *F. proliferatum* has been investigated together with the capability of producing FB_1 and FB_2 by the fungus. Both fumonisins were detected in asparagus plants colonized by *F. proliferatum* at levels of 7.4 and 0.83 μg/g (FB_1) and 0.46 μg/g and 0.06 μg/g (FB_2) in crown and stem samples, respectively. Eight out of nine isolates of *F. proliferatum* tested for sexual compatibility proved to be members of *Gibberella fujikuroi* mating population D. All nine isolates produced high amounts of FB_1 and FB_2 (up to 2504 and 946 μg/g, respectively) when cultured on autoclaved maize kernels for 4 weeks at 25 °C. This is the first report of the natural occurrence of fumonisins in asparagus plants.

Keywords: *Fumonisins; Fusarium proliferatum; Liseola section; toxins; asparagus*

INTRODUCTION

Fusarium proliferatum (Matsushima) Nirenberg, a member of the *Liseola* Woll section, is considered a severe pathogen of various economically important plants, including asparagus (*Asparagus officinalis* L.). It can be colonizer of both vascular and epidermal asparagus tissues (La Mondia and Elmer, 1989) and cause crown and root rot, alone or together with *F. oxysporum* f. sp. *asparagi* (Damicone and Manning, 1985; Elmer, 1991; Schreuder et al., 1995). Despite the importance as pathogen of asparagus, limited information exist on the capability of producing toxins by *F. proliferatum* isolated from this host.

Fusarium proliferatum is a toxigenic species (Marasas et al., 1984) and various strains of the species, mostly isolated from maize plants, were reported as capable of producing several toxins, including fumonisin B_1 (FB_1) and B_2 (FB_2) (Ross et al., 1990; Nelson et al., 1992; Visconti and Doko, 1994). Although fumonisins were first studied as mycotoxins with cancer-promoting activity (Gelderblom et al., 1988), recently the toxic activities of fumonisins have been also shown in plant systems (Lamprecht et al., 1994) hyphostatizing their important role in the disease. To date, the production of FB_1 by *F. proliferatum* has been mainly shown for isolates from maize (Ross et al., 1990; Visconti and Doko, 1994; Chulze et al., 1996), wheat, sorghum (Leslie et al., 1992), and rice (Desjardins et al., 1997), and fumonisins have been detected in infected maize plant as a natural contaminant (Sydenham et al., 1990; Logrieco et al., 1995; Chulze et al., 1996).

Isolates of *F. proliferatum* can sexually cross to produce perithecia of *Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura mating population D (Leslie, 1991), which proved to be highly pathogenic toward asparagus (Elmer and Ferrandino, 1992). Recently, Elmer (1995) studied a large number of isolates of *Fusarium Liseola* section from asparagus in the United States and confirmed the predominance of the mating population D of *G. fujikuroi*.

In the course of a large investigation on the most occurring *Fusarium* species causing stem and crown rot of asparagus plants from all around Italy, we focused our attention on a single field of southern Italy (Potenza) for the fumonisin occurrence in infected asparagus plants. Strains of *F. proliferatum* isolated from infected tissues were also studied for their capability to produce FB_1 and FB_2 as well as for sexual compatibility.

MATERIALS AND METHODS

Twenty-five asparagus plants affected by crown rot were randomly collected from a field of an asparagus producing area of southern Italy (Potenza). Internal crown and stem tissues of each plant were surface-disinfected for 1 min in 3% NaOCl. After rinsing twice with sterile water, the tissues were placed on plates (three pieces per plate) containing a modified pentachloronitrobenzene medium selective for *Fusarium* (Nash and Snyder, 1962; Nelson et al., 1983) and incubated in the dark at 25 °C for 1 week. *Fusarium* colonies were transferred to potato−dextrose agar (PDA) and carnation leaf agar (Nelson et al., 1983) plates and incubated at 25 °C for 10 days under fluorescent and black-light lamps (2700 lux) with a 12-h photoperiod. The identification of the *Fusarium* species was made according to the taxonomic system of Nelson et al. (1983). Nine single-conidial strains of *F. proliferatum* isolated from these plants were used for fertility and toxin production studies and then deposited in the culture collection of the Istituto Tossine e Micotossine da Parassiti Vegetali (ITEM), Bari, Italy.

Cross Condition. Tester strains of the six *G. fujikuroi* mating population (A, B, C, D, E, and F) were used to make crosses with the isolates from asparagus using carrot agar medium as reported by Klittich and Leslie (1988). All strains

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were crossed twice as male parents to tester strains of the six mating populations before assigning them to a mating population.

Toxin Production. The toxigenic potential of these strains was evaluated by culturing them on maize kernels, under the experimental conditions reported by Bottalico et al. (1983) for the production of other *Fusarium* toxins. Fifty grams of yellow maize kernels cv. Plata was brought to about 45% moisture, left overnight in a 250-mL Erlenmeyer flask, and autoclaved for 30 min at 120 °C. The maize was then inoculated with 2 mL of a conidial suspension containing approximately 10^7 conidia per milliliter, and it was shook once a day during the first 3 days to distribute the inoculum. The cultures were incubated at 25 °C in the dark for 4 weeks. The harvested culture material was dried in a forced draft oven at 60 °C for 48 h and then finely ground and stored at 4 °C until used. Uninoculated media treated in the same way were used as controls.

Extraction and Chemical Analysis of Fumonisins. The 25 asparagus plants highly affected by *F. proliferatum* crown rot were cut for obtaining two subsamples, one of stems and the other of crowns. These two subsamples were then cut into small pieces (about 1 × 1 cm), dried in a forced draft oven at 60 °C for 48 h, and finely ground.

Ten grams of asparagus stem/crown or maize samples (three replicates) was used for the analysis.

The extraction and HPLC analysis of FB_1 and FB_2 were performed according to the method previously described (Doko and Visconti, 1994). Briefly, samples were extracted with 50 mL of MeOH/H_2O (3:1) for 3 min using a laboratory blender. After filtration, 10 mL aliquots were applied to Bond-Elut strong anion exchange (SAX) cartridges, previously conditioned by the successive passage of 5 mL of MeOH and 5 mL of MeOH/H_2O (3:1). The cartridge was washed with 8 mL of MeOH/H_2O (3:1), then with 3 mL of MeOH, and fumonisins were eluted with 14 mL of 0.5% acetic acid in MeOH. The eluates were evaporated to dryness at 40 °C under nitrogen stream, and residues were reconstituted with 200 μL of CH_3CN/H_2O (1:1). A 50-μL aliquot of the purified extract was derivatized with 200 μL of *o*-phthaldialdehyde (OPA) solution obtained by adding 5 mL of 0.1 M $Na_2B_4O_7 \cdot 10H_2O$ and 50 μL of 2-CH_3CH_2SH to 1 mL of MeOH containing 40 mg of OPA. The analysis of the OPA derivatives was performed by reversed-phase HPLC with fluorescence detection. The HPLC apparatus consisted of a 2150 LKB pump (Bromma, Sweden) connected to an MPF-44 florometric detector (Perkin-Elmer, Norwalk, CT) with excitation and emission wavelengths set at 335 and 440 nm, respectively. A stainless steel column (25 cm × 3.2 mm i.d.) packed with Spherisorb ODS 2, 5 μm C_{18} (Merck & Co., Darmstadt, Germany) was used with mobile phase MeOH/0.1 M $NaH_2PO_4 \cdot 2H_2O$ (75:25) adjusted to pH 3.35 with H_3PO_4, at a flow rate of 1 mL/min. Fumonisin quantification was performed by peak height measurements, by comparison with reference standard solutions obtained by dissolving FB_1 and FB_2 (CSIR, Division of Food Science and Technology, Pretoria, South Africa) in CH_3CN/H_2O, at concentrations of 100 and 50 μg/mL, respectively.

RESULTS AND DISCUSSION

All nine strains of *F. proliferatum* used in this study except for ITEM-1481 belong to the D mating population, and produced FB_1 (from 744 to 2504 μg/g) and FB_2 (from 118 to 946 μg/g) on autoclaved maize kernels (Table 1). As far as we are aware, this is the first report on the production of these two toxins by *F. proliferatum* isolated from asparagus. It is noteworthy that strains with opposite mating types were isolated from the same field. This shows that there is a high possibility of genetic recombination in the sampled asparagus field and that this may improve the genetic pool available for the pathogenic population of *F. proliferatum*. Although on the basis of the limited number of strains, our data on the distribution of D^+ and D^- mating types are consistent with other reports. In particular six strains proved to be D^+ mating type and two strains D^- mating type confirming Elmer's (1995) larger investigation where he found that in each asparagus field examined there were roughly 2−22 times as many D^+ mating type as D^- mating type. Although limited information exists on the toxicity of *F. proliferatum* and/or *G. fujikuroi* mating population D from different geographic areas and hosts, it appears that the capability of producing fumonisins is a common trait of this species. Fumonisin B_1 was produced by strains belonging to mating population D isolated from maize, sorghum (Leslie et al. 1992), and rice (Desjardins et al., 1997). Nelson et al. (1992) and Thiel et al. (1991) tested for FB_1 production isolates of *F. proliferatum* from maize and soil from United States and Sierra Leone, wheat from Nepal, sorghum from Nigeria and South Africa, and most of them proved to be toxin producers. Subsequently, also European isolates of *F. proliferatum* from maize and sorghum were found to be fumonisin producers (Logrieco et al., 1995; Visconti and Doko, 1994). Overall, the strains examined in this investigation produced high level of FB_1 (>1000 μg/g, with the exception of ITEM-1491) similar to some populations isolated from maize and feeds (Ross et al., 1990; Visconti and Doko, 1994; Logrieco et al., 1995; Desjardins et al., 1997; Chulze et al., 1998). In contrast, members of *G. fujikuroi* mating population D isolated from sorghum and rice appear to be lower FB_1 producers, with a mean concentration of positive isolates between 400 and 580 μg/g of FB_1 (Leslie et al., 1992; Desjardins et al., 1997).

Table 1. Production of Fumonisin B_1 and B_2 by Strains of *Fusarium proliferatum* Isolated from Asparagus in Italy

		fumonisins (μg/g)[a]	
strain no.	mating type	FB_1	FB_2
ITEM-1475	D^+	1318	497
ITEM-1477	D^+	1717	665
ITEM-1478	D^+	2069	819
ITEM-1479	D^+	2266	946
ITEM-1480	D^+	1647	375
ITEM-1481	not fertile	1202	259
ITEM-1483	D^-	2504	118
ITEM-1484	D^+	1234	279
ITEM-1491	D^-	744	138

[a] Isolates were grown on autoclaved corn kernels at 25 °C for 4 weeks. Corn blank contained 0.04 μg/g FB_1.

The high percentage of asparagus plants infected by *F. proliferatum* and the capability to produce in vitro high amounts of FB_1 and FB_2 by representative isolates of this population led us to suspect the natural occurrence of the toxins in infected asparagus tissues. In particular, all 25 stems and crowns (100%) proved to be infected by *F. proliferatum* and, in addition, the crowns were also contaminated by *F. oxysporum* (8%). Fumonisin B_1 and FB_2 were detected in both samples, with higher amounts of both fumonisins in crown than in stem samples (7.4 and 0.46 μg/g of FB_1 respectively and 0.83 and 0.06 μg/g of FB_2, respectively) (Table 2). This is also the first report on the natural occurrence of FB_1 and FB_2 in asparagus tissues colonized by *F. proliferatum*. Previously, FB_1 was naturally found primarily in maize and in maize-based feeds and foods infected by *F. moniliforme* and *F. proliferatum* (Thiel et al., 1991; Logrieco et al., 1995; Chulze et al., 1996). The capability to produce high level of FB_1 and FB_2 by

Table 2. Occurrence of Fumonisin B_1 and B_2 and *Fusarium* Species in Asparagus Infected Plants

asparagus sample	*Fusarium* spp. (%)[a]	fumonisins (μg/g)[b]	
		FB_1	FB_2
stem	*F. proliferatum* (100)	0.46	0.06
crown	*F. proliferatum* (100)	7.4	0.83
	F. oxysporum (8)		

[a] Percentage are based on 25 plants. [b] Concentration referred to dry weight.

F. proliferatum and the natural occurrence of FB_1 and FB_2 in infected tissues led us to suspect an important role of these toxins in the disease. Recently, FB_1 was shown to induce a phytotoxic effect toward some plants such as maize and tomato (Lamprecht et al., 1994). The higher amount of fumonisins found in infected plant tissue and, in particular, in crown rot could be due to a major tissue colonization by *F. proliferatum*. A possible additive fumonisin accumulation due to the *F. oxysporum* cooccurrence can be excluded because this species has never been reported to produce fumonisins (Thiel et al., 1991). In addition, one strain of *F. oxysporum* (ITEM-1461) isolated from infected asparagus tissues failed in producing in vitro fumonisins.

In addition to fumonisins, the strains of *F. proliferatum* used in this study were also able to produce in vitro beauvericin and fusaproliferin (Moretti et al., 1996). In this respect, it would be interesting also to investigate the capability of *F. proliferatum* to produce and accumulate beauvericin and fusaproliferin in asparagus infected tissues. Recently, FB_1, fusaproliferin and beauvericin have been simultaneously found in maize in Italy, mainly infected by *F. proliferatum* (Ritieni et al., 1997).

Finally, although this report provides new information about the toxigenicity of *F. proliferatum* from asparagus and the natural occurrence of fumonisins, we believe that a deeper knowledge of the role that fumonisins have in the disease and the possible sinergistic effect with other toxins produced by this species should be warranted.

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