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# *Fusarium* Species Colonizing Spears and Forming Mycotoxins in Field Samples of Asparagus from Germany and Poland

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## Abstract

The occurrence of *Fusarium* spp. and associated mycotoxins in asparagus spears was evaluated in Poland in 2002 and 2003 and in Germany in 2002. Spears of two cultivars, Eposs and Gijnlim, were collected from two locations in Poland, Swidwowiec and Poznan, on sandy and sandy loam soil, respectively. *Fusarium oxysporum* and *F. proliferatum* were detected at an average incidence of 38.3% and 15.8% in the spear sections sampled, respectively. In stands of 11 (tested) cultivars of asparagus sampled in Germany on sandy soil, the same species dominated, however, they were less frequent than in Poland (26.6% and 5.6% of the spears infected with *F. oxysporum* and *F. proliferatum*, respectively). Chemical analyses revealed that fumonisin $B_1$ ($FB_1$) and moniliformin (MON) were present in some of the spears sampled in Poland. $FB_1$ was not found and MON was not assessed in spears sampled in Germany in 2002, but *F. proliferatum* was able to form the toxin *in vitro* in the range from 101.4 up to 205.8 μg/kg maize kernel substrate. Asparagus samples in Poland contained $FB_1$ at up to 5.6 μg/kg spear fresh weight. The highest MON concentration (1350 μg/kg) was detected in cultivar Eposs in Marcelin, Poland, in 2002. MON and $FB_1$ were found in spears infected by both *F. oxysporum* and *F. proliferatum*, however, only the latter fungus was able to synthesize both toxins.

## Introduction

Asparagus (*Asparagus officinalis*), an important vegetable that originated from the Mediterranean (Rubatzky and Yamaguchi, 1997), has recently received increased worldwide interest because of its unique taste, high nutritional value and the presence of biotic compounds (Shao et al., 1999). Variation in susceptibility of asparagus cultivars (cv.) to *Fusarium oxysporum* Schl. f. sp. *asparagi*, *F. proliferatum* (Matsushima) Nirenberg and other *Fusarium* spp. (Elmer et al., 1996) has been reported, and cultivars with partial resistance to the asparagus stem and crown rot are known. These fungi are responsible for asparagus decline (Elmer et al., 1996). In addition, the fungi can produce mycotoxins which affect food quality. Thus, asparagus spears should be analysed for toxigenic fungi and the presence of mycotoxins (Elmer, 2000).

Fumonisins are mycotoxins formed by one of the most common fungi associated with maize (*Zea mays* L.), *F. verticillioides* (Sacc.) Nirenberg, as well as *F. proliferatum* (Ross et al., 1990; Doko and Visconti, 1994; Visconti and Doko, 1994). The occurrence of the toxins in maize and maize-based feeds has been recognized as causing equine leucoencephalomalacia and porcine pulmonary oedema (Vesonder et al., 1989; Kellermann et al., 1990; Thiel et al., 1991). Maize contaminated with fumonisins and consumed by humans has been associated with a high incidence of oesophageal cancer in South Africa (Thiel et al., 1992). A risk of the latter disease is a cause for concern for consumers of maize in north-eastern Italy (Franceschi et al., 1990). *Fusarium proliferatum* is one of the most common *Fusarium* species causing stem and crown rot of asparagus, and this fungus also produces fumonisins (Logrieco et al., 1998). This suggests that fumonisins may be present in harvested asparagus spears and that fumonisin formation could increase during the transport of spears to markets. Gossmann et al. (2001) detected fumonisins in freeze-dried asparagus spears.

Further studies (Seefelder et al., 2002) investigated contamination of spears from other fields and the effects of this contamination on food quality.

The ability of species in the genus *Fusarium* to produce moniliformin (MON) has been reported (Vesonder and Golinski, 1989). In the years, when weather conditions are favourable for head, cob or panicle infection of cereals by *F. avenaceum*, the pathogen that causes fusarium head blight, scab incidence – similarly as in the case of asparagus stem and crown rot caused by the same pathogens – can increase rapidly with economically significant losses in cereal production (Golinski et al., 1996, 1999, 2002; Kiecana et al., 2002). As has been described in the literature, *Fusarium* toxins present in feeds can cause significant aberrations in animal health state, including death. The sudden death syndrome in chickens, with clinical signs described as cardiac dysfunction (cyanosis, depression), was induced by a diet containing MON (Reams et al., 1997). Reductions in body weight gain and low feed conversion rates resulting in significant economic losses in animal production have also been reported (Vesonder and Golinski, 1989; Vesonder et al., 1989).

The selection of asparagus cultivars resistant to *Fusarium* pathogens and mycotoxin formation, followed by monitoring of asparagus planting for the stem and crown rot and the toxin residues – for the same reasons as feeds, foodstuffs and their cereal components are monitored for toxigenic fungi and toxic secondary metabolites – is of prime concern for human health, as well as economics in agricultural production.

The aim of this study was to identify *Fusarium* spp. present in asparagus spears using morphological characteristics and to determine fumonisin $B_1$ ($FB_1$) and MON concentrations found in spears of two (most) common asparagus cultivars planted in Poland. In Germany, the main goal was collecting relevant data of *Fusarium* spp. present in spears of 11 asparagus cultivars and analyses of $FB_1$ in six cultivars.

## Materials and Methods

### Asparagus samples

Two asparagus cultivars, Eposs and Gijnlim, were analysed for the presence of *Fusarium* spp. and mycotoxins in 2002 and 2003. Asparagus spears were collected in Poland from a farm in Swidwowiec 100 km west of Poznan and the Marcelin Experimental Station of the August Cieszkowski Agricultural University, located in the suburbs of Poznan. In Swidwowiec, cv. Gijnlim was planted in 1993 on sandy soil, and Eposs was planted in 1997 on the same soil type. Therefore, spear samples were collected from this location in the 8th and 9th year of harvest and the 4th and 5th year of harvest, respectively. In Marcelin, both cultivars were planted on a sandy loam soil in 1993 and spears were sampled in the 7th and 8th years of harvest. The total yearly precipitation was 500 mm in both locations, no irrigation was used. Plants of asparagus cv. Ariane, Andrea's, Backlim, Eposs, Gijnlim, Grolim, Horlim, Huckel's Alpha, Ramos, Ravel and Thielim, grown in 2002 in the 4th year of harvest in an asparagus planting in a location with sandy soil in Germany, were sampled for microbiological analysis and only six of them (Backlim, Eposs, Grolim, Ramos, Ravel and Thielim) for chemical analysis. The asparagus field was established in 1998 and samples (17–25 per cv.) were taken in the first week of June 2002. A total of 248 samples, each with a modal length of 22 cm, were randomly selected.

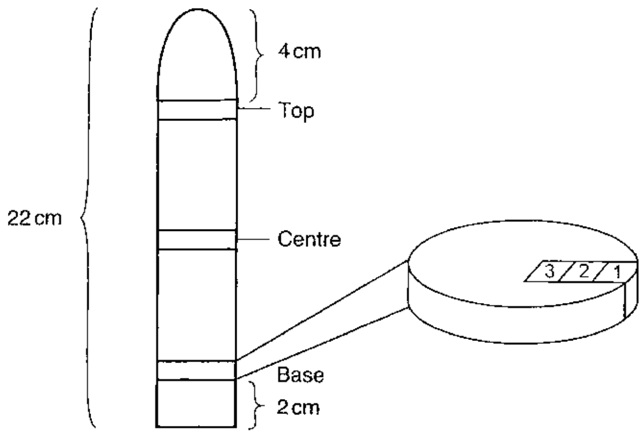

Fig. 1 Three pieces (1 = epidermal, 2 = pericambium, 3 = vascular tissue) of each disk (0.3–0.5 cm thickness) were sampled from the top, centre and basal part of each asparagus spear and transferred onto synthetic nutrient agar medium for isolation of *Fusarium* spp.

### *Fusarium* spp. detection

From each asparagus planting in Poland a subset of five spears with brown spots observed on the base were collected for both Eposs and Gijnlim on 10 June 2002 and 9 June 2003. All spears were divided into three parts of equal length (base, centre, top) (Fig. 1) and each part (in five replicates) was analysed for *Fusarium* spp. by isolation onto potato dextrose agar (PDA) amended with streptomycin at 100 $\mu$g/ml. The boundary between discoloured and healthy tissues of the base of symptomatic spears, or 1 cm$^2$ pieces of asymptomatic tissues from the centre or the top of each spear were used for isolation. After disinfection with 1.05% sodium hypochlorite, five sections of tissue (2 mm in diameter) were cut from each of the three pieces (base, centre, top) of each spear (five spears = replicates per cv. x five sections = 25 samples per each part of the spear) and transferred onto separate Petri dishes containing PDA and streptomycin. Colonies of fungi growing from the sections were transferred onto standard media and identified according to the methods described by Booth (1971), Gerlach and Nirenberg (1982), Kwasna et al. (1991), Barnett and Hunter (1998) and Nirenberg (1976). The material remaining after *Fusarium* isolation (not disinfected) was frozen within 3 h and stored at −30°C until chemical analyses were performed. Asparagus spears from Germany were surface disinfected by immersion in 2.1% sodium hypochlorite. Because of the disinfection of whole spears sampled in Germany vs. pieces of spears sampled in Poland, a higher concentration of the compound solution was used for the former samples. Spears were surface disinfected for 2 min and then washed with sterile water. From each sample, 0.3–0.5 cm thick slices were cut from the base, centre and top part of the spear (Fig. 1). Small pieces of the epidermal tissue, as well as parts of the pericambium and vascular bundles, were incubated separately on a synthetic nutrient agar according to Nirenberg (1976) for 7–10 days at 20°C under a 14-h near UV light/10-h dark cycle. Fungi were subsequently examined microscopically to identify the species using morphological parameters as described by Booth (1971), Gerlach and Nirenberg (1982) and Nirenberg (1976). The remaining plant material was immediately (within 3 h) frozen in liquid nitrogen and lyophilisized for $FB_1$ analysis.

### Mycotoxin potential of *F. proliferatum* and *F. oxysporum* isolates

Two isolates of each of *F. proliferatum* and *F. oxysporum* isolated from spears in Poland, that had amounts of $FB_1$ in the range of 0.8–5.2 μg/kg, were selected to assess the ability to synthesize $FB_1$ and MON mycotoxins. Samples (350 g) of wheat kernels (in triplicates) were sterilized for 0.5 h at 120°C at 40% water content and were inoculated separately in 750-ml Erlenmeyer flasks using a PDA disk (5 mm in diameter) colonized by each isolate. The cultures of *F. oxysporum* and *F. proliferatum* were grown at 20°C and shaken daily to prevent clumping of the colonized wheat kernels. After 2 weeks, the cultures were dried at 25°C, ground and analysed for $FB_1$ and MON using the protocol described below.

Eighteen isolates of *F. proliferatum* isolated from asparagus spears in Germany in 2002 were tested for their ability to produce $FB_1$ *in vitro*. Each isolate was cultured on 20 g of maize kernels in 20 ml of water. A spore suspension was used to inoculate the kernels ($2 \times 10^4$ conidia/ml), which were then maintained in the dark for 19 days at 25°C. The medium was filtered trough cheesecloth and toxins were extracted with methanol and water and subjected directly to an enzyme-linked immunosorbent assay (ELISA) test kit (Fumonisin Kit; Neogen Veratox, Lansing, MI, USA) following the manufacturers' protocol for $FB_1$ quantification.

### Mycotoxin standards

For the samples collected and assayed in Poland, $FB_1$, commercial samples of F-2643 (Lot 93H0628) and MON, M-5269 (Lot 116H4075) were used as standards (Sigma Chemical Co., St Louis, MO, USA).

### Extraction and purification of mycotoxins

Samples (10 g) of frozen asparagus tissue from Poland and/or fungal cultures were homogenized for 3 min in 20 ml of methanol–water (3 : 1, v/v) and filtered through Whatman no. 4 filter paper according to the method described by Sydenham et al. (1990). The supernatant was then divided into two equal subsamples for $FB_1$ and MON analyses. The fraction used for $FB_1$ analysis was adjusted to a pH of 5.8–6.5 using 0.1 M KOH. A SAX cartridge was attached to the SPE manifold unit (Supelco, Bellefonte, PA, USA) and conditioned at a flow rate of 2 ml/min successively with 5 ml of methanol followed by 5 ml of methanol–water (3 : 1, v/v). An aliquot (10 ml) of the filtered subsample extract was applied at the top of the conditioned cartridge at a flow rate of 2 ml/min, washed with 8 ml methanol–water (3 : 1, v/v), immediately followed by 3 ml of methanol. $FB_1$ was eluted at a flow rate of 1 ml/min from the column to a glass collection vial, with 10 ml of 1% acetic acid in methanol. The eluate was evaporated to dryness at 40°C under a stream of nitrogen. Dry residue was stored at 4°C until high-performance liquid chromatography (HPLC) analyses were performed.

The fraction used for MON analysis was defatted with *n*-hexane (3 × 50 ml), concentrated and later purified in glass columns containing 1.5 g of the Florisil gel (Merck 60–100 mesh, No 12,994; Merck, Darmstadt, Germany) according to the method described by Kostecki et al. (1995). The gel was activated for 1.5 h at 110°C prior to column preparation and the columns were conditioned with 5 ml acetonitrile and washed with 5 ml chloroform. The extract was applied at the top of the column and washed with 5 ml chloroform followed by 5 ml acetonitrile. Finally, MON was eluted using 5 ml water. After solvent evaporation, the toxin residue was dissolved in 5 ml methanol to be quantified. All solvents used for toxin extraction and purification were of analytical grade and were supplied by Sigma-Aldrich (Stenheim, Germany).

### Quantitative analysis of mycotoxins

Solvents used for mycotoxin determination by HPLC were also from Sigma-Aldrich, but were of HPLC grade. Phthaldialdehyde (OPA), Sigma-Aldrich, was used for $FB_1$ analyses.

For the samples from Poland (in triplicate) $FB_1$ was quantified according to the method described by Shepard et al. (1990) and Sydenham et al. (1990). The $FB_1$ standard (1 ng/μl in methanol–water at 1 : 1, v/v) was prepared and stored at 4°C. The OPA reagent (20 mg per 0.5 ml methanol) was prepared and diluted with 2.5 ml 0.1 M disodium tetraborate ($Na_2B_4O_7 \times 10H_2O$), then combined with 25 μl 2-mercaptoethanol. The mixture was stored up to 1 week at room temperature in a dark, capped amber vial. The $FB_1$ standard (25 μl) or spear extracts (200 μl) were derivatized with 225 or 200 μl of the OPA reagent. After 3 min, the reaction mixture (10 μl) was injected in an HPLC column. Methanol–sodium dihydrogen phosphate (0.1 M in water) solution (77 : 23, v/v) was adjusted to pH 3.35 with *o*-phosphoric acid after filtration through an 0.45 μm Waters HV membrane and used as the mobile phase with the flow rate of 0.6 ml/min (Waters Division of Millipore, Milford, MA, USA). A Waters 2695 apparatus, with a C18 Nova Pak column (3.9 × 150 mm) and a Waters 2475 fluorescence detec-

Table 1
Mean squares from the analysis of variance for number of asparagus spear sections infected by *Fusarium* fungi

| | | *F. oxysporum* | | | *F. proliferatum* | | | *F. solani* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Source of variation | df | Base | Centre | Top | Base | Centre | Top | Base | Centre | Top |
| Location (L) | 1 | 0.45 | 0.01 | 0.05 | 0.45* | 0.05 | 0 | 0.11 | 0.05 | 0 |
| Cultivar (C) | 1 | 0.20 | 0.31 | 0.20 | 0.05 | 0.05 | 0 | 0.11 | 0.20* | 0.05 |
| L × C | 1 | 0.05 | 0.01 | 0.05 | 0.45 | 0.05 | 0.45 | 0.01 | 0.05 | 0 |
| Residual | 16 | 0.06 | 0.08 | 0.06 | 0.12 | 0.065 | 0.10 | 0.43 | 0.03 | 0.03 |

*Significant at $P \leq 0.05$ level.

tor ($\lambda_{Ex} = 335$ nm and $\lambda_{Em} = 440$ nm) were used to quantify the metabolite. The $FB_1$ retention time was 7.35 min. MON content was preliminarily estimated on a Merck 5554 silica gel thin-layer chromatography plate (Merck) with 2-propanol–butanol–water–ammonium hydroxide (12 : 4 : 1 : 1, v/v/v/v) as the developing solvent, according to the method described by Golinski et al. (1999). The colour of spots was developed with 3-methyl-2-benzo-thiazolinonehydrochloride (MBTH) (Chelkowski et al., 1990). The intensity of dark spots on the chromatogram was compared with that of the metabolite standard. A more precise quantification was made by HPLC using a Waters 501 apparatus (Waters Division of Millipore) with a C18 Nova Pak column (3.9 × 300 mm) and a Waters 486 UV detector ($\lambda_{max} = 229$ nm). Acetonitrile–water solvent (15 : 85, v/v) buffered with 10 ml 0.1 M $K_2HPO_4$ in 40% *t*-butyl-ammonium hydroxide in 1 l of solvent (Sharman et al., 1991) was used as the mobile phase at a flow rate of 0.6 ml/min. The MON retention time was 11.5 min with 90% recovery and detection of 25 ng/g.

Table 2
Occurrence of *Fusarium* spp. in asparagus spears (mean values for 2002 and 2003)

| | | | Average number of sections infected by[a] | | |
|---|---|---|---|---|---|
| Cultivar | Location | Part of spear | *F. oxysporum* | *F. proliferatum* | *F. solani* |
| Eposs | Swidwowiec | Base | 1.0 | 2.5 | 0.0 |
| | | Centre | 1.5 | 1.5 | 0.0 |
| | | Top | 0.5 | 1.5 | 0.0 |
| | | Mean | 1.0 | 1.8 | 0.0 |
| | Marcelin | Base | 4.0 | 0.5 | 0.5 |
| | | Centre | 1.5 | 1.5 | 0.0 |
| | | Top | 2.0 | 0.5 | 0.0 |
| | | Mean | 2.5 | 0.8 | 0.2 |
| Gijnlim | Swidwowiec | Base | 3.0 | 1.0 | 0.5 |
| | | Centre | 2.5 | 0.5 | 0.5 |
| | | Top | 2.0 | 0.0 | 0.5 |
| | | Mean | 2.5 | 0.5 | 0.5 |
| | Marcelin | Base | 3.0 | 0.0 | 1.5 |
| | | Centre | 1.5 | 0.0 | 1.5 |
| | | Top | 0.5 | 0.0 | 0.5 |
| | | Mean | 1.7 | 0.0 | 1.2 |

[a] Calculated for 25 sections of each part of the particular cultivar spear in each planting and year.

### $FB_1$ quantification in F. proliferatum-infected asparagus

Lyophilized asparagus samples from Germany that tested positive for *F. proliferatum* were assayed for $FB_1$ concentration by liquid-chromatography–electrospray ionization–mass spectrometry (LC-ESI-MS) as described by Seefelder et al. (2002). LC/MS was performed using a 1050 gradient LC pump (Hewlett-Packard Co., Palo Alto, CA, USA) and a hydrophobic polymeric (etylvinylbenzene-divinylbenzene) NS1. No 0.8114 column (4 × 250 mm, 10 micron; Dionex Co., Sunnyvale, CA, USA). A solvent system consisted of acetonitrile and aqueous ammonium acetate (25 mM, pH 3.7). Mass spectrometry was performed using a VG Platform single quadrupole benchtop instrument (Fisons Instruments, Altrincham, UK) equipped with a standard electrospray interface. Continuous infusion, full-scan spectra – over a mass range of 100–900 Da – were obtained by injecting a 10 $\mu$l aliquot containing 5 ng/$\mu$l. Determination of the peak for $FB_1$ was performed by selected ion monitoring of respective protonated molecules.

### Statistical analysis

Two-way analysis of variance (Bogartz, 1994) was carried out to determine the effects of locations (L), cultivars (C) and the locations × cultivars (L × C) interaction on the variability of examined traits. Least significant differences for each trait were calculated. To compare the percentages of base, centre and top parts of spears infected by a particular *Fusarium* sp. (the mean for a location and an asparagus cultivar) Duncan's test was carried out (Duncan, 1955). The relationship between traits was calculated using correlation coefficients at different levels (Bobko, 2001).

## Results

*Fusarium oxysporum*, *F. proliferatum* and *F. solani* colonizing asparagus spears were found in asparagus grown in Poland (Tables 1 and 2). As interaction L × C was not significant, we analysed locations and cultivars individually. The incidence of *F. oxysporum* was similar in spears of two asparagus cultivars and at the locations (Table 1). In the base parts of spears the occurrence of *F. proliferatum* was significantly (at $\alpha = 0.05$) higher in Swidwowiec than in Marcelin (Tables 1 and 2). The occurrence of *F. solani* in the centre parts of spears was significantly higher at $\alpha = 0.05$ in asparagus cv. Gijnlim than in Eposs. The distribution of *F. oxysporum* and *F. proliferatum* on spears sampled in Poland was statistically similar in

2002 and 2003, while *F. solani* was detected in 2003 only. As expected, all the three species were detected most frequently (but statistically not significantly) in the base of spears, where brown spots were often visible on the surface of the spears.

In samples collected in Germany during the main harvest period at the beginning of June 2002, one-third of the sampled spears (80 of 248 samples) were infected with one or more *Fusarium* spp. *Fusarium oxysporum* was found in 26.6% of all spears examined, followed by *F. proliferatum* detected on 5.6% of the samples. *Fusarium subglutinans*, *F. redolens*, *F. merismoides*, *F. equiseti*, *F. dimerum* and *F. lateritium* were detected occasionally at the incidence rate of 0.4–2% of tested spears (Fig. 2).

As the results of a two-way analysis of variance (Table 3) show that the L × C interaction was not significant, we examined $FB_1$ and MON separately for locations and cultivars. The chemical analyses showed (Tables 3 and 4) that $FB_1$ and MON were present in asparagus spears, with the highest concentrations (up to 5.6 $\mu$g/kg for $FB_1$ and up to 1350 $\mu$g/kg for MON) both found in Marcelin in 2002. The level of $FB_1$ residue in spears did not depend on the location and asparagus cultivar. MON concentration was significantly higher (at $\alpha = 0.001$) than $FB_1$ and was dependent (Table 3) on the asparagus cultivar (in the base part) and on the location (in the top part of spears). This is the first report of the simultaneous occurrence of $FB_1$ and MON in asparagus spears. $FB_1$ was found mostly in the samples where the dominant species was *F. oxysporum*, but was also present in a few samples, on which *F. proliferatum* was the dominant species (Table 4). In a separate experiment, in this study, on mycotoxin potential, only *F. proliferatum* isolates were able to produce $FB_1$ (up to 760 $\mu$g/g) *in vitro*. *Fusarium oxysporum* is not known to produce $FB_1$ and MON. Therefore, although *F. proliferatum* was less prevalent in the spears than *F. oxysporum*, the former species may not have been detected in some spears because of its uneven distribution in the spears. It is also possible that the results (Table 2) reflect the distribution of *Fusarium* species at the time of sample collection and could differ at other times in the cropping

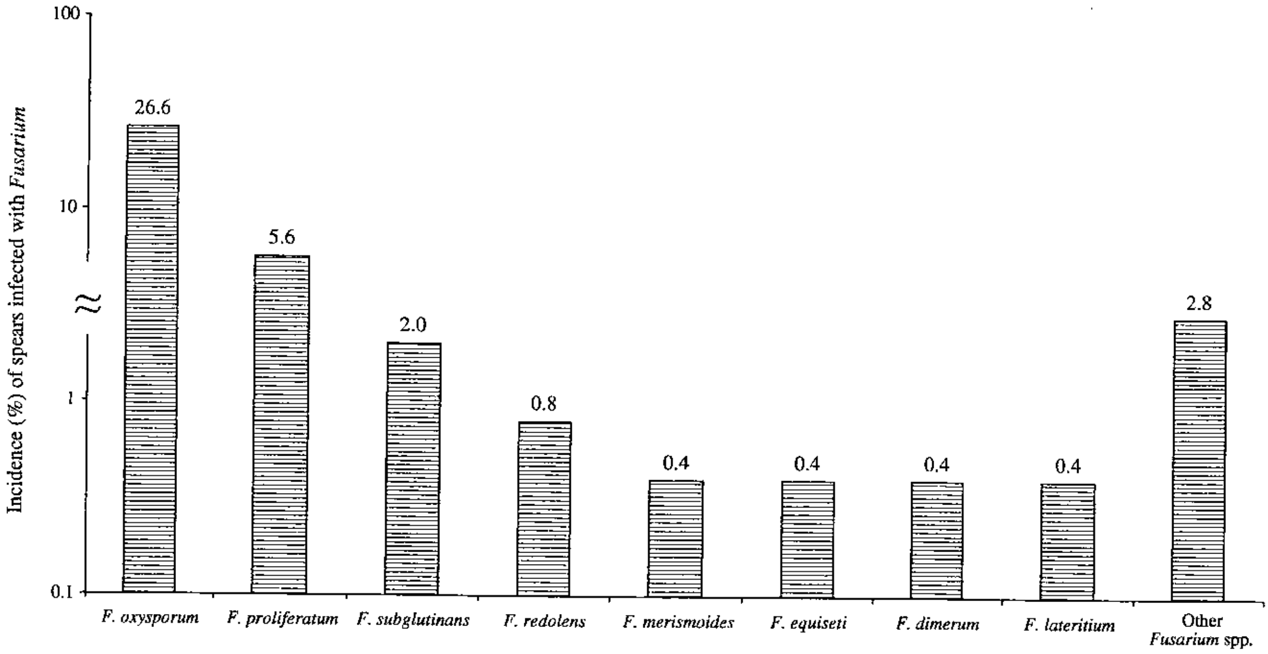

Fig. 2 *Fusarium* species isolated from asparagus spears ($n = 248$) of 11 cultivars collected in Germany in 2002

Table 3
Mean squares from analysis of variance for occurrence of fumonisin $B_1$ ($FB_1$) and moniliformin (MON) in spears

| Source of variation | df | $FB_1$ | | | | | MON | | |
|---|---|---|---|---|---|---|---|---|---|
| | | B02[a] | C02 | T02 | B03 | T03 | B02 | B03 | T03 |
| Location (L) | 1 | 3.784 | 0.392 | 0.242 | 1.458 | 5.202 | 3640 | 17 405 | 73 229* |
| Cultivar (C) | 1 | 5.940 | 0.288 | 0.008 | 0.512 | 0.722 | 458 984* | 31 205* | 4799 |
| L × C | 1 | 0.761 | 0.288 | 0.008 | 2.178 | 0.072 | 46 | 3645 | 5 |
| Residual | 16 | 3.406 | 0.2012 | 0.750 | 0.9703 | 1.481 | 99 124 | 4790 | 14 398 |

[a] Base (B), centre (C) and top (T) parts of spears in years of experiment: 2002 (02) and 2003 (03).
*Significant at $P \le 0.05$.

Table 4
Prevalent *Fusarium* species and average fumonisin $B_1$ ($FB_1$) and moniliformin (MON) concentrations in (tested parts of) asparagus spears (based on fresh weight) from Poland (Swidwowiec and Marcelin) in 2002 and 2003

| Experimental station | Cultivar | 2002 Dominating *Fusarium* spp. | 2002 $FB_1$ (μg/kg) Base | 2002 $FB_1$ (μg/kg) Centre | 2002 $FB_1$ (μg/kg) Top | 2002 MON (μg/kg) Base | 2003 Dominating *Fusarium* spp. | 2003 $FB_1$ (μg/kg) Base | 2003 $FB_1$ (μg/kg) Top | 2003 MON (μg/kg) Base | 2003 MON (μg/kg) Top |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Swidowiec | Eposs | *F.o.*, *F.p.*[a] | ND[b] | 2.1 | ND | 320 | – | 1.8 | 0.7 | ND | TR[c] |
| | | *F.o.* | 0.5 | ND | ND | 30 | *F.o.* | ND | ND | ND | ND |
| | | *F.p.* | ND | TR[c] | 0.8 | 320 | *F.p.*, *F.o.* | 1.7 | 1.6 | 70 | 360 |
| | | *F.p.* | ND | TR[c] | 0.5 | 650 | *F.p.* | ND | 0.9 | ND | 180 |
| | | *F.p.* | ND | ND | ND | 330 | *F.p.* | 3.8 | 4.9 | 90 | 270 |
| | Gijnlim | *F.o.* | 1.3 | ND | ND | 80 | *F.o.* | 2.0 | 2.5 | 290 | 150 |
| | | *F.o.* | ND | ND | 0.9 | ND | *F.s.*, *F.o.* | 1.1 | 2.3 | 190 | 250 |
| | | *F.p.* | ND | TR | ND | 70 | *F.o.* | 1.0 | 2.1 | 80 | 310 |
| | | *F.o.* | 5.2 | ND | ND | ND | *F.o.*, *F.p.*, *F.s.* | 1.5 | 1.9 | 130 | 190 |
| | | *F.o.* | 1.4 | ND | ND | TR | *F.o.*, *F.p.* | ND | 0.6 | ND | 60 |
| Marcelin | Eposs | *F.p.*, *F.o.* | ND | ND | ND | 1350 | *F.o.*, *F.p.* | ND | ND | ND | ND |
| | | *F.o.* | ND | ND | ND | ND | *F.p.*, *F.s.* | ND | TR[c] | ND | 90 |
| | | *F.o.* | 3.7 | ND | ND | ND | *F.o.*, *F.p.* | 0.7 | 1.4 | ND | 110 |
| | | *F.o.* | ND | ND | ND | TR[c] | *F.o.* | 0.6 | 0.8 | ND | ND |
| | | *F.o.* | 3.1 | ND | ND | 180 | *F.o.* | ND | ND | ND | ND |
| | Gijnlim | *F.o.* | 1.7 | ND | ND | TR | *F.o.* | ND | ND | ND | 40 |
| | | *F.o.* | 5.6 | ND | ND | ND | *F.s.*, *F.o.* | 1.6 | 2.4 | 140 | 320 |
| | | *F.o.* | ND | ND | ND | ND | *F.s.* | 2.2 | 2.0 | ND | ND |
| | | *F.o.* | 3.0 | ND | ND | ND | *F.o.* | 0.8 | 0.5 | ND | ND |
| | | *F.o.* | ND | ND | ND | ND | *F.s.* | 1.6 | ND | 120 | ND |
| LSD[d] | Cultivar (C) | | 1.75 | 0.42 | 0.26 | 298.5 | | 0.93 | 1.15 | 65.6 | 113.8 |
| | Locality (L) | | 1.75 | 0.42 | 0.26 | 298.5 | | 0.93 | 1.15 | 65.6 | 113.8 |
| | C × L | | 2.47 | 0.60 | 0.37 | 422.1 | | 1.32 | 1.63 | 92.8 | 160.9 |

[a] *F.o.*, *Fusarium oxysporum*; *F.p.*, *Fusarium proliferatum*; *F.s.*, *F. solani*.
[b] ND, not detectable.
[c] TR, trace amount below 0.5 μg/kg for $FB_1$ and 20 μg/kg for MON.
[d] LSD, least significant difference

cycle, with *F. proliferatum* dominating under specific conditions.

In contrast, $FB_1$ was not detected in any of the samples from 13 *F. proliferatum*-infected asparagus spear samples collected in this study during the main harvest in June 2002 from the German crop, even though all the 18 *F. proliferatum* isolates, recovered from the epidermal, pericycle and vascular tissues, were capable of $FB_1$ production *in vitro* (Table 5). When these isolates were grown on maize kernels the $FB_1$ concentrations ranged from 101.4 to 205.8 μg/g (dry weight).

Both asparagus cultivars, Eposs and Gijnlim, at the Polish locations were contaminated with $FB_1$ (Table 4). The percentage of spears with no detectable amounts of the toxin was similar for both cultivars and both locations. Samples contaminated with $FB_1$ at concentrations of ≤1 μg/kg did not depend on cultivars and locations. The percentage of samples with $FB_1$ concentrations above 1 μg/kg was similar at Marcelin and Swidwowiec with the highest $FB_1$ concentration (5.6 μg/kg) for Gijnlim at the former location. Gijnlim exhibited also a significantly (at $\alpha = 0.05$) higher percentage of samples with $FB_1$ concentrations $> 1$ μg/kg compared with Eposs.

Table 5
Fumonisin $B_1$ levels (μg/g) in *Fusarium proliferatum*[a] cultures on maize kernels after 19 days of incubation

| Isolate[a] no. | Asparagus cultivar | $FB_1$ concentrations[b] (μg/g) |
|---|---|---|
| 1 | Ravel | 165.4 |
| 2 | | 151.6 |
| 3 | Eposs | 170.4 |
| 4 | Ramos | 181.4 |
| 5 | | 175.8 |
| 6 | | 175.8 |
| 7 | Backlim | 169.4 |
| 8 | Thielim | 146.0 |
| 9 | | 136.0 |
| 10 | | 119.2 |
| 11 | | 146.0 |
| 12 | Grolim | 124.4 |
| 13 | | 101.4 |
| 14 | | 178.8 |
| 15 | | 205.8 |
| 16 | | 199.8 |
| 17 | | 161.0 |
| 18 | | 169.4 |

[a] *F. proliferatum* isolates were received from an asparagus field in Germany in 2002.
[b] $FB_1$ concentrations in cultures on maize kernels (based on dry weight).

## Discussion

Occurrence of *Fusarium* spp. in asparagus spears was similar to the results of Elmer (2000), who isolated *Fusarium* spp. most often from the base of spears. In California, USA, with a warm climate and the range of average temperature during summer of 35–40°C, *F. proliferatum* was the dominant species on older asparagus plantings, while in colder states (the range of average temperature during summer of 20–30°C), *F. oxysporum* was predominant, similarly to our results. In the base and centre parts of spears *F. proliferatum* prevalence was correlated with MON concentration ($r = 0.546$ significant at $\alpha = 0.05$ and $r = 0.679$ at $\alpha = 0.01$, respectively). The individual *Fusarium* species detected may also depend on the susceptibility of a given asparagus cultivar to different *Fusarium* spp. (Sadowski and Knaflewski, 1990; Elmer et al., 1996).

Gossmann et al. (2001) collected roots, crowns and spears from plants from older asparagus plantings between July and October 2000 from different fields in Germany and Austria and investigated the occurrence of endophytic fungi. Seventeen *Fusarium* species were distinguished in that study, of which nine are recognized to be of pathogenic relevance to asparagus. In their study, different *Fusarium* species – *F. acuminatum* in the range of incidence of 0–2%, *F. avenaceum* (0–14%), *F. culmorum* (0–1%), *F. oxysporum* (37–82%), *F. proliferatum* (1–30%), *F. redolens* (0–25%), *F. sambucinum* (0–30%), *F. solani* (0–2%) and *F. subglutinans* (in six of eight Austrian samples only) – were detected at different sampling sites. Apart from the effect of location, the preceding crop rotations had a major influence on the *Fusarium* spp. detected on asparagus plants. Furthermore, *F. proliferatum* was detected in perennial asparagus production sites in Germany and Austria for the first time (Gossmann et al., 2001). This species, together with *F. oxysporum*, is one of the most important fungal pathogens worldwide causing crown and root rot on asparagus (Elmer et al., 1996).

The chemical analyses showed that $FB_1$ was present in both locations in Poland. Similar results for $FB_1$ contamination of asparagus spears were observed in Germany (Gossmann et al., 2001). After the first report on the detection of $FB_1$ in *F. proliferatum*-infected asparagus in Italy (Logrieco et al., 1998), Seefelder et al. (2002) showed that this mycotoxin was found in nine of 10 samples of *F. proliferatum*-infected asparagus spears from Germany. Samples were collected from perennial asparagus production sites after the main harvest period at the end of July 2000 from plants exhibiting severe stunting. Spears infected with *F. proliferatum* as well as *F. sambucinum* and/or *F. oxysporum*, typically had $FB_1$ concentrations between 36.4 and 4513.7 $\mu$g/kg (dry weight). The inability to detect $FB_1$ in some asparagus spears infected with *F. proliferatum* might be due to the time of sampling, which took place approximately 2 months earlier in the season in Germany in our work than in the studies by Gossmann et al. (2001) and Seefelder et al. (2002). Nevertheless, the results do not exclude a potential health risk for consumers of asparagus spears infected with *F. proliferatum*. Further investigations are needed in this respect.

The different levels of mycotoxins detected in this study compared with those reported by Seefelder et al. (2002) and Logrieco et al. (1998) could be influenced by differences in the climate and the fact that samples collected in the Italian studies were heavily infected with *F. proliferatum*, with visible symptoms of fusarium crown and root rot.

Moniliformin was observed in spear samples as frequently as $FB_1$, but at much higher concentrations (significant at $\alpha = 0.001$) than $FB_1$ (Table 4). This is the first report on the association between the occurrence of *F. proliferatum* and the presence of $FB_1$ and MON in asparagus spears, demonstrating that asparagus spears can simultaneously be contaminated with $FB_1$ and MON and may pose a potential risk for human health (JECFA, 2000). Selection of spears without brown spots may decrease this risk.

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## References

Barnett HL, Hunter BB. *Illustrated Genera of Imperfect Fungi.* St Paul, MN, APS Press, 1998.

Bobko P. *Correlation and Regression.* Thousand Oaks, CA, Sage Publications, 2001.

Bogartz RS. *An Introduction to the Analysis of Variance.* Westport, CT, Praeger, 1994.

Booth C. *The Genus Fusarium.* Kew, Surrey, CABI, 1971.

Chelkowski J, Zawadzki M, Zajkowski P, Logrieco A, Bottalico A. (1990) Moniliformin production by *Fusarium* species. *Mycotoxin Res* **6**:41–44.

Doko MB, Visconti A. (1994) Occurrence of fumonisins B1 and B2 in corn and corn-based human foodstuffs in Italy. *Food Addit Contam* **11**:433–439.

Duncan DB. (1955) Multiple range and multiple test. *Biometrics* **11**:1–42.

Elmer WH. (2000) Incidence of infection of asparagus spears marketed in Connecticut by *Fusarium* spp. *Plant Dis* **84**:831–834.

Elmer WH, Johnson DA, Mink GI. (1996) Epidemiology and management of the diseases causing to asparagus decline. *Plant Dis* **80**:117–125.

Franceschi S, Bidoli E, Baron AE, La Vecchia C. (1990) Maize and risk of cancers of the oral cavity, pharynx, and esophagus in Northeastern Italy. *J Natl Cancer Inst* **82**:1407–1411.

Gerlach W, Nirenberg H. *The Genus Fusarium.* A Pictorial Atlas. Berlin-Dahlem, Germany, Mitt Biol Bundesanst Land Forstwirtschaft, 209, 1982.

Golinski P, Kostecki M, Lasocka I, Wisniewska H, Chelkowski J, Kaczmarek Z. (1996) Moniliformin accumulation and other effects of *Fusarium avenaceum* (Fr.) Sacc. on kernels of winter wheat cultivars. *J Phytopathol* **144**:495–499.

Golinski P, Kiecana I, Kaczmarek Z, Kostecki M, Kaptur P, Wisniewska H, Chelkowski J. (1999) Scab response of selected winter wheat cultivars after inoculation with *Fusarium avenaceum* (Fr.) Sacc. *J Phytopathol* **147**:717–723.

Golinski P, Kaczmarek Z, Kiecana I, Wisniewska H, Kaptur P, Kostecki M, Chelkowski J. (2002) *Fusarium* head blight of common Polish winter wheat cultivars – comparison of effects of *Fusarium avenaceum* and *Fusarium culmorum* on yield components. *J Phytopathol* **150**:135–141.

Gossmann M, Büttner C, Bedlan G. (2001) Untersuchungen zum Spargel (*Asparagus officinalis* L.) aus Jung- und Ertragsanlagen in

Deutschland und Österreich auf Infektionen mit *Fusarium*-Arten. *Pflanzenschutzberichte* **59**:45–54.

JECFA. *Joint FAO/WHO Expert Committee on Food Additives, 53rd Report. Safety Evaluation of Certain Food Additives*, WHO Food Additive Series 44. Geneva, WHO, 2000.

Kellermann TS, Marasas WFO, Thiel PG, Gelderblom WCA, Cawood ME, Coetzer JAW. (1990) Leukoencephalomalacia in two horses induced by oral dosing of fumonisin B1. *Onderstepoort J Vet Res* **57**:269–275.

Kiecana I, Mielniczuk E, Kaczmarek Z, Kostecki M, Golinski P. (2002) Scab response and moniliformin accumulation in kernels of oat genotypes inoculated with *Fusarium avenaceum* in Poland. *Eur J Plant Pathol* **108**:245–251.

Kostecki M, Grabarkiewicz-Szczesna J, Chelkowski J, Wisniewska H. (1995) Beauvericin and moniliformin production by Polish isolates of *Fusarium subglutinans* and natural co-occurrence of both mycotoxins in maize samples. *Microbiol Alim Nutrit* **13**:67–70.

Kwasna H, Chelkowski J, Zajkowski P. *Fungi (Mycota). XXII.* Warszawa, Krakow, Polish Academy of Sciences, 1991.

Logrieco A, Dako B, Moretti A, Frisullo S, Visconti A. (1998) Occurrence of fumonisins B1 and B2 in *Fusarium proliferatum* infected asparagus plants. *J Agric Food Chem* **46**:5201–5204.

Nirenberg H. *Untersuchungen über die morphologische Differenzierung in der Fusarium-*Sektion *Liseola*. Berlin-Dahlem, Germany, Mitt Biol Bundesanst Land Forstwirtschaft, 169, 1976.

Reams RY, Thacker HL, Harrington DD, Novilla MN, Rottinghaus GE, Bennet GA, Horn J. (1997) A sudden death syndrome induced in poults and chicks fed diets containing *Fusarium fujikuroi* with known concentrations of moniliformin. *Avian Dis* **41**:20–35.

Ross PF, Nelson PE, Richard JL, Osweiler GD, Rice LG, Plattner RD, Wilson TM. (1990) Production of fumonisins by *Fusarium moniliforme* and *Fusarium proliferatum* isolates associated with equine leukoencephalomalacia and pulmonary edema syndrome in swine. *Appl Environ Microbiol* **56**:3225–3226.

Rubatzky VE, Yamaguchi M. *World Vegetables*. New York, Chapman & Hall, 1997.

Sadowski Cz, Knaflewski M. (1990) Susceptibility of selected asparagus cultivars to *Fusarium* spp. under field conditions. *Acta Hortic* **271**:343–351.

Seefelder W, Gossmann M, Humpf HU. (2002) Analysis of fumonisin B1 in *Fusarium proliferatum*-infected asparagus spears and garlic bulbs from Germany by liquid chromatography-electrospray ionization mass spectrometry. *J Agric Food Chem* **50**:2778–2781.

Shao Y, Poobrasert O, Kennelly E, Chin CT, Ho CT, Hguang MT, Garrison SA, Cordell GA. (1999) Cytotoxic activity of steroidal saponins from *Asparagus officinalis*. *Acta Hortic* **479**:277–282.

Sharman M, Gilbert J, Chelkowski J. (1991) A survey of the occurrence of the mycotoxin moniliformin in cereal samples from sources worldwide. *Food Addit Contam* **4**:459–466.

Shepard GS, Sydenham EW, Thiel PG, Gelderblom WCA. (1990) Quantitative determination of fumonisins B1 and B2 by high-performance liquid chromatography with fluorescence detection. *J Liq Chromatogr* **13**:2077–2087.

Sydenham EW, Gelderblom WCA, Thiel PG, Marasas WFO. (1990) Evidence for the natural occurrence of fumonisin B1, a mycotoxin produced by *Fusarium moniliforme*, in corn. *J Agric Food Chem* **38**:285–290.

Thiel PG, Shephard GS, Sydenham EW, Marasas WFO, Nelson PE, Wilson TM. (1991) Levels of fumonisins B1 and B2 in feeds associated with confirmed cases of equine leukoencephalomalacia. *J Agric Food Chem* **39**:109–111.

Thiel PG, Marasas WFO, Sydenham EW, Shephard GS, Gelderblom WCA. (1992) The implications of naturally occurring levels of fumonisins in corn for human and animal health. *Mycopathologia* **117**:3–9.

Vesonder RF, Golinski P. Metabolites of *Fusarium*. In: Chelkowski J (ed.), *Fusarium*: Mycotoxins, Taxonomy and Pathogenicity, Amsterdam, The Netherlands, Elsevier, 1989, pp. 1–39.

Vesonder R, Haliburton J, Golinski P. (1989) Toxicity of field samples and *Fusarium moniliforme* from feed associated with equine-leucoencephalomalacia. *Arch Environ Contam Toxicol* **18**:422–439.

Visconti A, Doko MB. (1994) Survey of fumonisin production by *Fusarium* isolated from cereals in Europe. *J AOAC Int* **77**:546–550.