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Formation of fumonisins and other secondary metabolites by *Fusarium oxysporum* and *F. proliferatum* - a comparative study

A. Waskiewicz^{1*}, P. Golinski¹, Z. Karolewski², L. Irzykowska², J. Bocianowski³, M. Kostecki¹, Z. Weber²

¹ Department of Chemistry, Poznan University of Life Sciences, Wojska Polskiego 75, 60-625 Poznan, Poland

² Department of Phytopathology, Poznan University of Life Sciences, Dabrowskiego 159, 60-594 Poznan, Poland

³ Department of Mathematical and Statistical Methods, Poznan University of Life Sciences, ul. Wojska Polskiego 28, 60-637 Poznan, Poland

Abstract

The principal aim of this study was to estimate fumonisins (FB₁ and FB₂), moniliformin (MON) and ergosterol (ERG) formation by *Fusarium oxysporum* and *Fusarium proliferatum* while beauvericin (BEA) by the latter one only. Moreover, the effect of temperature on mycotoxins biosynthesis was also evaluated. Fumonisins were formed by *F. proliferatum* with the highest yield at 18°C (720.0-1976.6 µg g⁻¹ - FB₁, 74.2-670.8 µg g⁻¹ - FB₂) and only by three of four *F. oxysporum* strains at a very low level (0.02-4.77 µg g⁻¹ - FB₁, 0.02-2.15 µg g⁻¹ - FB₂). The amount of MON formed by *F. proliferatum* was the highest (p< 0.001) at 32°C (3056.87 µg g⁻¹), while MON biosynthesis by *F. oxysporum* was lower 227.54 µg g⁻¹ (p<0.001). BEA was produced by *F. proliferatum* with the highest level at 25°C (p<0.001). ERG - recognized as an indicator of fungal biomass development and as a consequence of mycotoxin formation - was found at the highest concentration at biosynthesis temperature 25°C for *F. proliferatum* and *F. oxysporum* (p<0.001).

Keywords: *Fusarium proliferatum*, *Fusarium oxysporum*, biosynthesis, fumonisins, moniliformin, beauvericin, ergosterol, HPLC, PCR

Introduction

Fungi of the *Fusarium* genus belong to a group of the most severe pathogens of asparagus (Elmer et al. 1996), commonly producing mycotoxins – secondary metabolites with well-documented health hazard, significantly affecting food safety. Both *F. proliferatum* and *F. oxysporum* are known as the *Fusarium* species causing stem and crown rot of asparagus (Weber et al. 2006, Waskiewicz et al. 2008). *Fusarium proliferatum* can form a variety of mycotoxins including fumonisins, beauvericin and moniliformin (Shephard et al. 1990), while *F. oxysporum* mainly is recognized as moniliformin producer (Logrieco et al. 2003).

Fumonisin (FB₁, FB₂) are structurally similar to sphinganine and they disrupt sphingolipid metabolism by the inhibition of ceramide synthase – a process underlying the mechanism of toxicity and pathogenesis of fumonisin-related diseases (Brown et al. 2007). They cause different toxicological effects in both humans and animals (Soriano and Dragacci 2004). The feeds contaminated with fumonisins has been shown to cause a number of mycotoxicoses, including leukoencephalomalacia in horses, pulmonary oedema in swine, altered hepatic and immune function in cattle as well as liver cancer and neural tube defects in experimental rodents (Logrieco et al. 2003, Desjadrins et al. 2007). FB₁ is toxic to the liver and kidneys in many species, causing apoptosis followed by mitosis in the affected tissues and it is also toxic to the cardiovascular system in pigs and horses (Voss et al. 2007). Epidemiological studies also suggest that fumonisins could be associated with human esophageal cancer in some regions, where maize contaminated with fumonisins is used as food (Torres et al. 2003).

Beauvericin (BEA) is a bioactive cyclohexadepsipeptide containing an alternating sequence of three N-methyl L-phenylalanyl and three D- α -hydroxyisovaleryl residues. This toxin is a specific cholesterol acyltransferase inhibitor and it is toxic to several human cell lines by the induction of apoptosis and DNA fragmentation (Logrieco et al. 2002).

Moniliformin (MON), naturally occurring as the sodium or potassium salt of 3-hydroxycyclobut-3-ene-1,2-dione, is toxic to plant and animal species (Uhlig et al. 2007). MON is also suspected of causing the Keshan's disease, a human myocardial impairment reported in rural areas of China and South Africa (Bottalico 1998, Pineda-Valdes and Bullerman 2000). The mechanism of the acute toxicity of MON is believed to be the inhibition of pyruvate dehydrogenase, i.e. blockage of entry of pyruvate into Krebs's cycle and a decrease in mitochondrial respiration (Pirrung et al. 1996). The occurrence of

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3 mycotoxins in plant tissue is affected by several factors, including fungus strain, genetic
4 background of the host and environmental conditions.
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7 Next to *Fusarium* toxins, fungal biomass is also important and ergosterol (ERG) was
8 suggested as a factor indicating fungal development (Abramson et al. 1998).
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11 In an earlier report we indicated that *F. oxysporum* was frequently found in asparagus
12 spears contaminated with fumonisins (Weber et al. 2006); however, according to available
13 literature and taking into consideration low concentration levels and sporadic occurrence of
14 the toxins, we concluded that *F. oxysporum* is not the fumonisins producer. Those results
15 were confirmed by later studies (Waskiewicz et al. 2009), indicating very low (close to the
16 detection limit) levels of fumonisin in the fungus culture. In recent literature sources (Sewram
17 et al. 2005) there are suggestions indicating a possible fumonisins (FB) biosynthesis by some
18 *F. oxysporum* isolates. The present study was undertaken to investigate mycotoxin potential
19 of the fungus isolates with an indication on such a possibility. The principal aim of this study
20 was to estimate FUM, MON and ERG biosynthesis yield by selected *F. oxysporum* and *F.*
21 *proliferatum* strains and BEA by the latter strains only. Moreover, the effect of temperature
22 on mycotoxins biosynthesis was evaluated.
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33 **Materials and methods**

34 *Fungal isolates*

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37 Four single spore isolates of both *Fusarium oxysporum* and *F. proliferatum* in our
38 culture collection originating from asparagus spears (Table 1) were used in the experiment.
39 All fungal isolates were identified based on morphological features (Booth 1971, Gerlach and
40 Nirenberg 1982, Kwasna et al. 1991). Morphological features of our identified isolates were
41 compared with standard isolates DSM-62287 (Deutsche Sammlung von Mikroorganismen und
42 Zellkulturen, Germany) of *F. oxysporum* and DSM-62261 as well as CBS-115.97
43 (Centraalbureau voor Schimmelcultures, Netherlands) of *F. proliferatum*.
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51 *DNA preparations and species-specific PCR assays*

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53 Species-specific PCRs were used to confirm identification of *Fusarium* species based
54 on morphological features. Mycelia from 9-day-old single spore cultures of *F. proliferatum*
55 and *F. oxysporum* grown on liquid medium (5 g L⁻¹ of glucose, 1 g L⁻¹ of yeast extract), were
56 collected by vacuum filtration using a Büchner funnel. DNA was extracted and purified using
57 a DNeasy Mini Kit (QIAGEN Inc., Hilden, Germany) according to the manufacturer's
58 recommendations. In the polymerase chain reaction a forward primer: 5'-
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3 TGCATCAGACCACTCAAATCCT-3' and a reverse primer: 5'
4 TGTCAGTAACTCGACGTTGTTGTT-3' were used to detect *F. proliferatum*, while a
5 forward primer: 5'-CAGCAAAGCATCAGACCACTATAACTC-3' and a reverse primer: 5'-
6 CTTGTCAGTAACTGGACGTTGGTACT-3' were used for *F. oxysporum* (Mulè et al. 2004
7 a, b) (Sigma-Genosys, Pampisford, UK). The species-specific primers were designed based on
8 a partial sequence of the calmodulin gene. The amplification reactions were carried out using
9 a *Taq* PCR Core Kit (QIAGEN, Inc., Valencia, USA). The reaction mixture was described in
10 a previous study (Irzykowska and Bocianowski 2008). Amplification was carried out in a
11 Biometra *Tpersonal* 48 thermocycler (Whatman Biometra, Goettingen, Germany) using the
12 following programme: an initial denaturation for 3 min at 94°C, followed by 35 cycles of
13 denaturation at 94°C for 40 s, primer annealing at 60°C for 40 s and extension at 72°C for 1
14 min. The amplification was completed with an additional extension at 72°C for 3 min. The
15 PCR products were separated by electrophoresis in 1.5% agarose gels with 1× TBE buffer (89
16 mM Tris-borate and 2 mM EDTA, pH 8.0) and visualised under UV light following ethidium
17 bromide staining. A Gene Ruler™ 100 bp DNA Ladder Plus (Fermentas GMBH, St. Leon-
18 Rot, Germany) was used as a molecular size standard for PCR products.

33 *Medium and incubation of fungal cultures*

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35 Long-kernel, white rice (RANI) tested as mycotoxin-free was used as a medium for
36 the formation of mycotoxins and ergosterol by experimental cultures of *F. oxysporum* and *F.*
37 *proliferatum*. Rice samples of 65 g were soaked in 500 ml Erlenmeyer flasks for 16 hours
38 with 81 ml of distilled water, then sterilized for 15 minutes at 120°C. For each isolate a set of
39 nine such samples was inoculated with 5 mm disks of PDA medium overgrown with a
40 mycelium of a particular fungal isolate. Cultures were incubated for 3 weeks at 3 different
41 temperatures of 18, 25, and 32°C, triplicate at each temperature (3 Erlenmeyer flasks with the
42 prepared medium).

51 *Chemicals and reagents*

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53 Standards of pure fumonisin B₁ and B₂, moniliformin, beauvericin and ergosterol were
54 purchased from Sigma (St. Louis, MO, USA).

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56 Acetonitrile, methanol (HPLC grade), disodium tetraborate, 2-mercaptoethanol and *t*-
57 butyl-ammonium hydroxide were purchased from Sigma-Aldrich. Potassium hydroxide,
58 sodium dihydrogen phosphate, acetic acid, *n*-hexane, *o*-phosphoric acid, dichloromethane,
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3 hydrochloric acid and pentane were purchased from POCh (Poland). Water for the HPLC
4 mobile phase was purified using a Milli-Q system (Millipore, Bedford, MA, USA).
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8 *Mycotoxin analyses*

9 *Fumonisin (B₁ and B₂)*

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12 Samples (5 g) of dry 3-week-old cultures were homogenized for 3 min in 10 ml of
13 methanol-water (3:1, v/v) and filtered through Whatman no. 4 filter paper according to the
14 method described by Sydenham et al. (1990). The extract was adjusted to a pH = 5.8 - 6.3
15 using 0.1 M KOH. A SAX cartridge was attached to the SPE manifold unit (Supelco,
16 Bellefonte, PA, USA) and conditioned at a flow rate of 2 ml min⁻¹ successively with 5 ml of
17 methanol, followed by 5 ml of methanol-water (3:1, v/v). An aliquot (5 ml) of the filtered
18 subsample extract was applied at the top of the conditioned cartridge at a flow rate of 2 ml
19 min⁻¹, washed with 8 ml methanol-water (3:1, v/v), immediately followed by 3 ml of
20 methanol. Fumonisin were eluted from the column to a glass collection vial, with 10 ml of
21 1% acetic acid in methanol, at a flow rate of 1 ml min⁻¹. The elute was evaporated to dryness
22 at 40°C under a stream of nitrogen. Dry residue was stored at -20°C until HPLC analyses.
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32 The OPA reagent (20 mg per 0.5 ml methanol) was prepared and diluted with 2.5 ml
33 0.1 M disodium tetraborate (Na₂B₄O₇ x 10 H₂O), then combined with 25 µl 2-
34 mercaptoethanol added to the solution. The mixture was stored for up to 1 week at room
35 temperature in a dark, capped, amber vial. The fumonisin B₁ and B₂ standard (5 µl) or extracts
36 (20 µl) were derivatized with 20 or 80 µl of the OPA reagent. After 3 min. the reaction
37 mixture (10 µl) was injected onto an HPLC column. Methanol – sodium dihydrogen
38 phosphate (0.1 M in water) solution (77:23, v/v) adjusted to pH 3.35 with o-phosphoric acid,
39 after filtration through a 0.45 µm Waters HV membrane was used as the mobile phase with a
40 flow rate of 0.6 ml min⁻¹. A Waters 2695 apparatus (Waters Division of Millipore, Milford,
41 MA, USA), with a C-18 Nova Pak column (3.9 x 150 mm) and a Waters 2475 fluorescence
42 detector ($\lambda_{\text{Ex}} = 335$ nm and $\lambda_{\text{Em}} = 440$ nm) were used in the metabolite quantitative
43 determination. The detection limits were 10 ng g⁻¹ for FB₁ and FB₂. Positive results (on the
44 basis of retention time) were confirmed by HPLC analysis and compared with the relevant
45 calibration curve (correlation coefficient for FB₁ and FB₂ were 0.9967 and 0.9966,
46 respectively). Recoveries for FB₁ and FB₂ were: 93 and 87% respectively, which were
47 measured in triplicates by extracting the mycotoxins from blank samples spiked with 10–100
48 ng g⁻¹ of the compound. The relative standard deviations were less than 8%.
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Beauvericin and moniliformin

Cultures samples (15 g) of each strain were homogenized with 75 ml of acetonitrile:methanol:water (16:3:1, v/v/v) and filtered (Whatman no. 4 filter paper). The filtrate was defatted twice with 25 ml of heptane and the bottom layer was evaporated to dryness, then the residue was dissolved in 50 ml of methanol:water (55:45, v/v) and extracted twice with 25 ml of dichloromethane. The aqueous, bottom phase (containing MON) was concentrated to 1 ml, while the CH₂Cl₂ phase (containing BEA) was evaporated to dryness and purified according to the method described by Kostecki et al. (1999).

Moniliformin was quantified using a Waters 501 apparatus (Waters Division of Millipore, Milford, MA, USA) with a C 18 Nova Pak column (3.9 x 3000 mm) and a Waters 486 UV detector ($\lambda_{\max} = 229$ nm). Acetonitrile:water (15:85, v/v) buffered with 10 ml 0.1 M K₂HPO₄ in 40% *t*-butyl-ammonium hydroxide in 1 L of solvent (Sharman et al. 1991) was used as the mobile phase (flow rate 0.6 ml min⁻¹). The MON detection limit was 25 ng g⁻¹.

Beauvericin was quantified using a Waters 2695 apparatus with a C-18 Nova Pak column (150 × 3.9 mm) and a Waters 2996 Photodiode Array Detector (Waters, Milford, USA) set at 205 nm. HPLC conditions included a constant flow rate of 0.6 ml min⁻¹ and acetonitrile:water (85:15, v/v) was used as the mobile phase. Detection limit for BEA was 10 ng g⁻¹.

Positive results (on the basis of retention time) were confirmed by HPLC analysis and comparison with the relevant calibration curve (correlation coefficient for MON and BEA were 0.9990 and 0.9997 respectively). Recoveries for MON and BEA were: 90 and 92% respectively, which were measured in triplicates by extracting the mycotoxins from blank samples spiked with 10–100 ng g⁻¹ of the compounds. The relative standard deviation (R.S.D.) value was below 7% for MON and below 5% for BEA.

Ergosterol analysis

Samples containing 100 mg of ground grains were placed into 20-ml culture tubes, suspended in 2 ml methanol, treated with 0.5 ml of 2M aqueous sodium hydroxide, and tightly sealed. Samples were irradiated (370 W) twice for 20 s. After 15 min contents of cultures tubes were neutralized with 1 M aqueous hydrochloric acid, 2 ml MeOH were added and extraction with pentane (3 × 4 ml) was carried out within the culture tubes. The combined pentane extracts were evaporated to dryness in a stream of nitrogen. Before analysis the sample extract was dissolved in 1 ml of MeOH and 50 μ l were analyzed by HPLC. Separation

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3 was performed on a 150 × 3.9 mm Nova Pak C-18, 4 μm column and eluted with
4 methanol:acetonitrile (90:10, v/v) at a flow rate of 0.6 ml min⁻¹. Ergosterol was detected with
5 a Waters 2996 Photodiode Array Detector (Waters, Milford, USA) set at 282 nm.
6 Quantification of ergosterol was made by a comparison of peak areas with those of external
7 standards of ERG. The presence of ergosterol was confirmed by a comparison of retention
8 times with that of the external standard and by co-injection of every tenth sample with an
9 ERG standard. All analyses were run in triplicate and mean values were reported. The
10 detection limit was 10 ng g⁻¹. Standard deviation was always < 5%.
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21 *Statistical analysis*

22 The two-way analysis of variance was carried out to determine the effects of isolates,
23 temperatures and isolate × temperature interactions on the variability in concentrations of FB₁,
24 FB₂, MON, BEA and ERG. These analyses were conducted independently for *F. oxysporum*
25 and *F. proliferatum*. Least significant differences for each trait were calculated.
26 Homogeneous groups for the analysed traits were determined on the basis of least significant
27 differences. The relationship between concentrations of FB₁, FB₂, MON, BEA and ERG was
28 estimated using correlation coefficients.
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35 **Results and discussion**

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37 Molecular analyses confirmed morphological identification of *Fusarium* isolates used
38 in the experiment. PCR products were 526 bp and 534 bp in length from *F. proliferatum* and
39 *F. oxysporum*, respectively.
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42 Three temperatures as a biosynthesis culture condition were introduced, since 24°C is
43 generally recognized as optimal for mycotoxin formation, 18°C for fumonisins biosynthesis
44 and 32°C for moniliformin.
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48 The analysis of variance for mycotoxins and ergosterol contents for *F. oxysporum*
49 indicates a significant effect of temperature and isolate on all analyzed traits (p<0.001).
50 Moreover, the temperature × isolate (T×I) interaction determined the incidence of mycotoxins
51 and ergosterol at 0.001 level (Table 2). Analogously, the analysis of variance for contents of
52 mycotoxins and ergosterol for *F. proliferatum* shows a significant effect of temperature on
53 contents of FB₁, FB₂ and MON as well as a dependence between isolate and the level of
54 toxins and ergosterol (significant at 0.001 level). The temperature × isolate interaction has a
55 significant effect on yield of FB₁, MON, ERG (p<0.001) and FB₂ (p<0.05) (Table 3).
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3 Fumonisin were formed by all *F. proliferatum* isolates with the highest yield at 18°C
4 (720.0-1976.6 $\mu\text{g g}^{-1}$ - FB₁, 74.2-670.8 $\mu\text{g g}^{-1}$ - FB₂), while the lowest level of the compound
5 was found for 32°C (1.1-49.8 $\mu\text{g g}^{-1}$ - FB₁, 0.2-11.0 $\mu\text{g g}^{-1}$ - FB₂). In contrast, Nelson et al.
6 (1992) reported that only 50% of the *F. proliferatum* isolates from corn-based feed produced
7 FB₁. Biosynthesis of fumonisins by three of four *F. oxysporum* strains was at very low
8 concentration level (0.02-4.77 $\mu\text{g g}^{-1}$ - FB₁, 0.02-2.15 $\mu\text{g g}^{-1}$ - FB₂) also with the highest yield
9 at 18°C (Tables 4 and 5). FB₁ formation yield was always higher than of FB₂, what confirms
10 other findings in corn, wheat or rice cultures of *F. proliferatum* or *F. verticillioides* (Logrieco
11 et al. 1998, Hinojo et al 2006, Liu et al. 2007).

12
13 Moreover, statistically significant differences were also shown between mean
14 concentrations of fumonisins B₁ and B₂ for individual *F. oxysporum* isolates as well as
15 between isolate „06-51sa” and the other three analysed for *F. proliferatum* (Tables 4 and 5).
16 The amount of moniliformin (MON) formed by *F. proliferatum* was very high ($p < 0.001$) at
17 32°C (3056.87 $\mu\text{g g}^{-1}$), while in case of *F. oxysporum* was considerably lower (227.54 $\mu\text{g g}^{-1}$)
18 ($p < 0.001$) (Table 6). Beauvericin (BEA) was produced by *F. proliferatum* at the highest level
19 at 25°C ($p < 0.001$) (Table 7). Ergosterol - recognized as an indicator of fungal biomass
20 development and as a consequence mycotoxin formation - was found at the significantly
21 highest concentration at biosynthesis temperature of 25°C for *F. proliferatum* and *F.*
22 *oxysporum* ($p < 0.001$) (Table 8).

23
24 The correlation matrix for the traits studied for *F. oxysporum* and *F. proliferatum*
25 indicates that the concentration of FB₂ is significantly correlated with the level of FB₁
26 ($p < 0.001$), while in case of moniliformin an opposite dependence was shown, i.e. the higher
27 FB₁ concentration, the lower MON level ($p < 0.05$). For *F. proliferatum* isolates it was also
28 shown that beauvericin concentration is directly proportional to the concentration of
29 fumonisins (FB₁ and FB₂), significant at 0.01 level (Table 9).

30
31 The effect of temperature and water activity on the fumonisins formation by *F.*
32 *proliferatum* and *F. verticillioides* was previously reported (Liu et al. 2007) showing that all
33 tested strains isolated from asparagus crowns could form FB₁ and FB₂ on maize within a wide
34 range of 10-11499 $\mu\text{g g}^{-1}$ and 2-965 $\mu\text{g g}^{-1}$, respectively. There was a high variability in the
35 amount of fumonisins produced by different strains of *F. proliferatum*. In similar studies, on
36 autoclaved maize kernels *F. proliferatum* isolates produced high levels of FB₁ (up to 2500 mg
37 kg^{-1}) (Logrieco et al. 2003). In addition, FB₁ and FB₂ (up to 0.5 mg kg^{-1} and 0.06 mg kg^{-1} ,
38 respectively) were detected in samples from asparagus crowns (Logrieco et al. 1998).

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3 Stankovic et al. (2007) investigated pathogenicity and mycotoxin biosynthesis ability of *F.*
4 *proliferatum* originated from onion and garlic. Tested isolates, grown on autoclaved maize
5 kernels, produced MON in amounts ranging from 50 to 420 mg kg⁻¹, FB₁ (25-3000 mg kg⁻¹),
6 and BEA from 400 to 550 mg kg⁻¹. Moretti et al. (2007) showed that all tested strains of *F.*
7 *proliferatum* produced beauvericin (at 25°C) in a range of concentrations of 5-1300 mg kg⁻¹.
8 The effect of temperature on the formation of beauvericin and moniliformin by *F.*
9 *subglutinans* on rice has been reported by KostECKI et al. (1999). They achieved results
10 similar to our findings, since the highest BEA levels were recorded at 20-25°C (mean 704 mg
11 kg⁻¹), while MON production was the highest at 30°C (mean 219 mg kg⁻¹). For *F.*
12 *proliferatum* the optimum temperature for fumonisin biosynthesis, as it was shown in our
13 study, was 18°C, which corresponds with the results reported by other authors (Hinojo et al.
14 2006). However, it was shown by Marin et al. (1999a) that fumonisins were formed with the
15 highest yield by *F. proliferatum* and *F. verticillioides* at 30°C and 0.98 water activity (a_w) for
16 all examined isolates (0.78-143.95 µg g⁻¹). Furthermore, there was an increase in fumonisins
17 concentration level at reduced a_w levels (0.92-0.95) at lower temperatures, particularly 15°C,
18 when compared to concentrations obtained at higher temperatures and even at a higher a_w. In
19 experiment with a wider range of temperatures (7-37°C) conducted by the same author (Marin
20 et al. 1999b), the optimal temperature for fumonisins production by *F. proliferatum* at 15°C
21 was confirmed. According to Hinojo et al. (2006), the effect of temperature on fumonisins
22 biosynthesis by *F. verticillioides* is more relevant than the effect of a_w. Investigations were
23 conducted at four assayed a_w values (0.97-1.00) and it did not affect significantly fumonisins
24 accumulation in rice cultures. Accumulation of these mycotoxins was the highest at 20°C
25 (5.61-3550 mg kg⁻¹ - FB₁, 0.66-326.20 mg kg⁻¹ - FB₂) and the lowest at 37°C (5.41-40.72 mg
26 kg⁻¹ - FB₁, 0.35-5.30 mg kg⁻¹ - FB₂). These data also show clearly that environmental factors
27 have a significant effect on fumonisins concentration.
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48 It was shown that mycelium of *F. oxysporum* grows optimally at 26°C (Omer et al.
49 1999; Hibar et al. 2006). However, present study indicates that the most efficient biosynthesis
50 of FB₁ by *F. oxysporum* isolates occurs under temperature stress conditions (at 18°C), what
51 was not described so far. More extensive study are necessary to assess the importance of this
52 findings for human and animal health protection. In further investigation more strains of *F.*
53 *oxysporum* will be tested to determine whether the potential for toxin production was
54 widespread within this species.
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Conclusions

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3 Achived results revealed that among asparagus pathogens not only *F. proliferatum* but
4 also *F. oxysporum* was able to form toxic metabolites, including fumonisins B₁ and B₂. It is
5 known that most enzyme-encoding genes required for fumonisins biosynthesis have been
6 identified and the order and orientation of *Fum* genes in *F. oxysporum* is the same as in *F.*
7 *proliferatum* and *F. verticillioides*. However, The differences between *F. oxysporum* and *F.*
8 *proliferatum* and also intraspecies variation of *F. oxysporum* in efficiency of FBs formation
9 can be explained by some variability in genetic background of tested strains, different
10 expression of global regulatory genes, host-pathogen interactions and environmental
11 conditions (such as medium content, temperature, and water activity).
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19 Since there is no known effective therapy of fumonisin-related diseases, and
20 prevention of intoxication is the most important for human health, both species infecting
21 asparagus *F. proliferatum* as well as *F. oxysporum* should be considered as a pathogens
22 affecting food safety.
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Table 1. Isolates of *F. oxysporum* and *F. proliferatum* used in the experiment

Species	Isolate	Source of asparagus spears	Year of isolation
<i>F. oxysporum</i>	06-39na	field, cv. Eposs, Poland	2006
	06-45nc	field, cv. Eposs, Poland	2006
	06-49sd	field, cv. Eposs, Poland	2006
	07-17n	market, Poland	2007
<i>F. proliferatum</i>	06-51sa	field, cv. Eposs, Poland	2006
	06-98wa	field, cv. Eposs, Poland	2006
	SE-3wz	field, cv. Eposs, Poland	2005
	SE-7sz5	field, cv. Eposs, Poland	2005

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Table 2. Mean squares from analysis of variance for investigated traits for *F. oxysporum*

Source of variation	Degrees of freedom	Mean squares			
		FB ₁	FB ₂	MON	ERG
Isolates (I)	3	11.82***	0.99***	214871.5***	729430***
Temperature (T)	2	14.38***	2.23***	18703.8***	3360140***
I × T	6	4.13***	0.65***	18532***	584424***
Residual	24	0.21	0.05	870.8	11292

*** - significant at 0.001 level

Table 3. Mean squares from analysis of variance for investigated traits for *F. proliferatum*

Source of variation	Degrees of freedom	Mean squares				
		FB ₁	FB ₂	MON	BEA	ERG
Isolates (I)	3	1650464***	176895***	2023783.6***	176958***	36539
Temperature (T)	2	7355710***	533002***	2885264.3***	251629***	1039334***
I × T	6	419224***	43558*	1978001.5***	32225***	204549***
Residual	24	55737	15219	711,2	1880	13317

* - significant at 0.05 level

*** - significant at 0.001 level

Deleted: biosynthesis

Table 4. Mean values for fumonisin B₁ (FB₁) formation yield ($\mu\text{g g}^{-1}$)

<i>F. oxysporum</i>					<i>F. proliferatum</i>				
Isolates	Temperature			Means	Isolates	Temperature			Means
	18°C	25°C	32°C			18°C	25°C	32°C	
06-39na	1.72	0.64	0.63	0.99B	06-51sa	710	57	1	256B
06-45nc	4.69	3.72	0.00	2.80A	06-98wa	1773	1479	40	1097A
06-49sd	0.00	0.00	0.00	0.00C	SE-3wz	1904	1613	12	1176A
07-17n	3.21	0.77	0.34	1.44D	SE-7sz5	1701	1412	11	1041A
Means	2.43a	1.29b	0.24c		Means	1522a	1140b	16c	
LSD _{0.001} : for Isolates - 0.81, for Temperature - 0.7, for I × T - 1.4					LSD _{0.001} : for Isolates - 416.8, for Temperature - 361, for I × T - 722				

Means with the same letter are not significantly different

Table 5. Mean values for fumonisin B₂ (FB₂) formation yield ($\mu\text{g g}^{-1}$)

Deleted: biosynthesis

<i>F. oxysporum</i>					<i>F. proliferatum</i>				
Isolates	Temperature			Means	Isolates	Temperature			Means
	18°C	25°C	32°C			18°C	25°C	32°C	
06-39na	0.29	0.22	0.13	0.21C	06-51sa	83	21	0	35B
06-45nc	1.60	0.63	0.00	0.74A	06-98wa	492	408	9	303A
06-49sd	0.00	0.00	0.00	0.00C	SE-3wz	520	466	3	329A
07-17n	1.54	0.10	0.02	0.55B	SE-7sz5	476	454	1	310A
Means	0.86a	0.24b	0.04b		Means	393a	337a	3b	
LSD _{0.001} : for Isolates - 0.4, for Temperature - 0.34, for I × T - 0.69					LSD _{0.001} : for Isolates - 217.8, for Temperature - 188.6, for I × T - 377.3				

Means with the same letter are not significantly different

Table 6. Mean values for moniliformin (MON) biosynthesis yield ($\mu\text{g g}^{-1}$)

<i>F. oxysporum</i>					<i>F. proliferatum</i>				
Isolates	Temperature			Means	Isolates	Temperature			Means
	18°C	25°C	32°C			18°C	25°C	32°C	
06-39na	0.0	0.0	0.0	0.0B	06-51sa	1.0	15.3	66.9	27.7C
06-45nc	0.0	0.2	0.5	0.2B	06-98wa	8.7	41.9	2981.7	1010.8A
06-49sd	235.4	202.5	490.0	309.3A	SE-3wz	4.2	48.5	229.8	94.2B
07-17n	0.0	0.9	1.1	0.7B	SE-7sz5	0.3	21.7	188.3	70.1BC
Means	58.9b	50.9b	122.9a		Means	3.6b	31.8b	866.7a	
LSD _{0.001} : for Isolates - 52.1, for Temperature - 45.12, for I × T - 90.24					LSD _{0.001} : for Isolates - 47.09, for Temperature - 40.78, for I × T - 81.56				

Means with the same letter are not significantly different

Table 7. Mean values for ergosterol (ERG) level of concentration ($\mu\text{g g}^{-1}$)

<i>F. oxysporum</i>					<i>F. proliferatum</i>				
Isolates	Temperature			Means	Isolates	Temperature			Means
	18°C	25°C	32°C			18°C	25°C	32°C	
06-39na	97	483	8	196C	06-51sa	303	1271	63	546A
06-45nc	108	2242	313	888A	06-98wa	362	827	513	567A
06-49sd	317	950	540	602B	SE-3wz	401	792	739	644A
07-17n	212	952	384	516B	SE-7sz5	286	690	494	490A
Means	183b	1157a	311b		Means	338b	895a	452b	
LSD _{0.001} : for Isolates - 187.6, for Temperature - 162.5, for I × T - 325					LSD _{0.001} : for Isolates - 203.7, for Temperature - 176.4, for I × T - 352.9				

Means with the same letter are not significantly different

Table 8. Mean values for beauvericin (BEA) biosynthesis yield ($\mu\text{g g}^{-1}$)

<i>F. proliferatum</i>				
Isolates	Temperature			Means
	18°C	25°C	32°C	
06-51sa	13.4	31.9	9.6	18.3B
06-98wa	214.0	481.2	236.6	310.6A
SE-3wz	280.5	506.6	159.6	315.6A
SE-7sz5	174.1	532.8	49.4	252.1A
Means	170.5b	388.1a	113.8b	

LSD_{0.001}: for Isolates - 76.55, for Temperature - 66.29, for I × T - 132.58

Means with the same letter are not significantly different

Table 9. The correlation matrix for the traits studied for *F. oxysporum* (above diagonal) and *F. proliferatum* (below diagonal)

Trait	FB ₁	FB ₂	MON	BEA	ERG
FB ₁	1	0.888***	-0.409*	-	0.185
FB ₂	0.937***	1	-0.326	-	-0.077
MON	-0.369*	-0.329	1	-	0.022
BEA	0.591**	0.596**	0.003	1	
ERG	-0.119	-0.101	-0.036	0.307	1

* - significant at 0.05 level

** - significant at 0.01 level

*** - significant at 0.001 level