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ORIGINAL PAPER

Screening survey of co-production of fusaric acid, fusarin C, and fumonisins B_1, B_2 and B_3 by *Fusarium* strains grown in maize grains

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Abstract *Fusarium* species isolated from Belgian maize were screened for their ability to produce fusarin C, fusaric acid, fumonisins B_1 (FB_1), FB_2 and FB_3 in maize grains. First, cultivation of *Fusarium* species in Myro liquid medium allowed overcoming the shortage of the standard of fusarin C on the market. All *Fusarium verticillioides* produced much higher contents of mycotoxins in Myro compared to *Fusarium graminearum* or *Fusarium venenatum*. The optimization of the LC-MS/MS method resulted in low limits of detection and quantification for fusarin C, fusaric acid, FB_1, FB_2 and FB_3 determination in maize grains. Its application for screening the potential toxin production ability evidenced that the concentrations of the analytes were significantly increased at various levels when *F. verticillioides* strains were cultivated in maize grains and reached 441 mg kg^{-1} for fusaric acid, 74 mg kg^{-1} for fusarin C, 1,301 mg kg^{-1} for FB_1, 367 mg kg^{-1} for FB_2 and 753 mg kg^{-1} for FB_3.

Keywords Mycotoxin · Fumonisin · Fusaric acid · Fusarin C · *Fusarium* · Maize

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Introduction

Many *Fusarium* species are worldwide occurring fungi that can infect and colonize maize (Thrane et al. 2004; Kokkonen et al. 2010). Recently, a new species of *Fusarium temperatum*, morphologically similar and phylogenetically close to *Fusarium subglutinans* was isolated from maize in Belgium (Scauflaire et al. 2011). Over the past years, it was demonstrated that *Fusarium* species can produce mycotoxins such as fusarin C, fusaric acid, fumonisins B_1 (FB_1), FB_2 and FB_3 (Porter et al. 1995; Cantalejo et al. 1999; Song et al. 2004; Arino et al. 2007). Figure 1 presents the chemical structures of these mycotoxins which exhibit various toxic effects to plants and animals and may pose a potential health risk for humans. Fusarin C has demonstrated a mutagenic activity and several immunosuppressive effects comparable to those of aflatoxin B_1 and sterigmatocystin intoxications (Cantalejo et al. 1999). This toxin stimulates growth of the breast cancer cell line MCF-7 and can act as an oestrogenic agonist (Sondergaard et al. 2011). Fusaric acid has a broad spectrum and is considered to be directly related to the severity of damping off, vascular wilt and root rot diseases of numerous vegetable crops (Bacon and Hinton 1996; Stipanovic et al. 2011). In addition to the critical roles in plant pathogenesis, fusaric acid can be mildly toxic to some animals such as rat (Porter et al. 1995). Fusaric acid can increase the overall toxicity of other mycotoxins by synergistically interacting on the toxicity of other naturally co-occurring mycotoxins (Chamberlain et al. 1993; Bacon et al. 1996). Consumption of FB-contaminated food and feedstuffs has been associated with severe disorders in animals and humans, i.e. leukoencephalomalacia in horses, pulmonary oedema in swine and hepatocarcinoma in rats, inhibition of sphingolipid synthesis and increased risk of oesophageal cancer in humans (Marasas 1996). Fusarin C and FB_1 have been classified as potential carcinogens for humans (group 2B) by the International Agency for Research

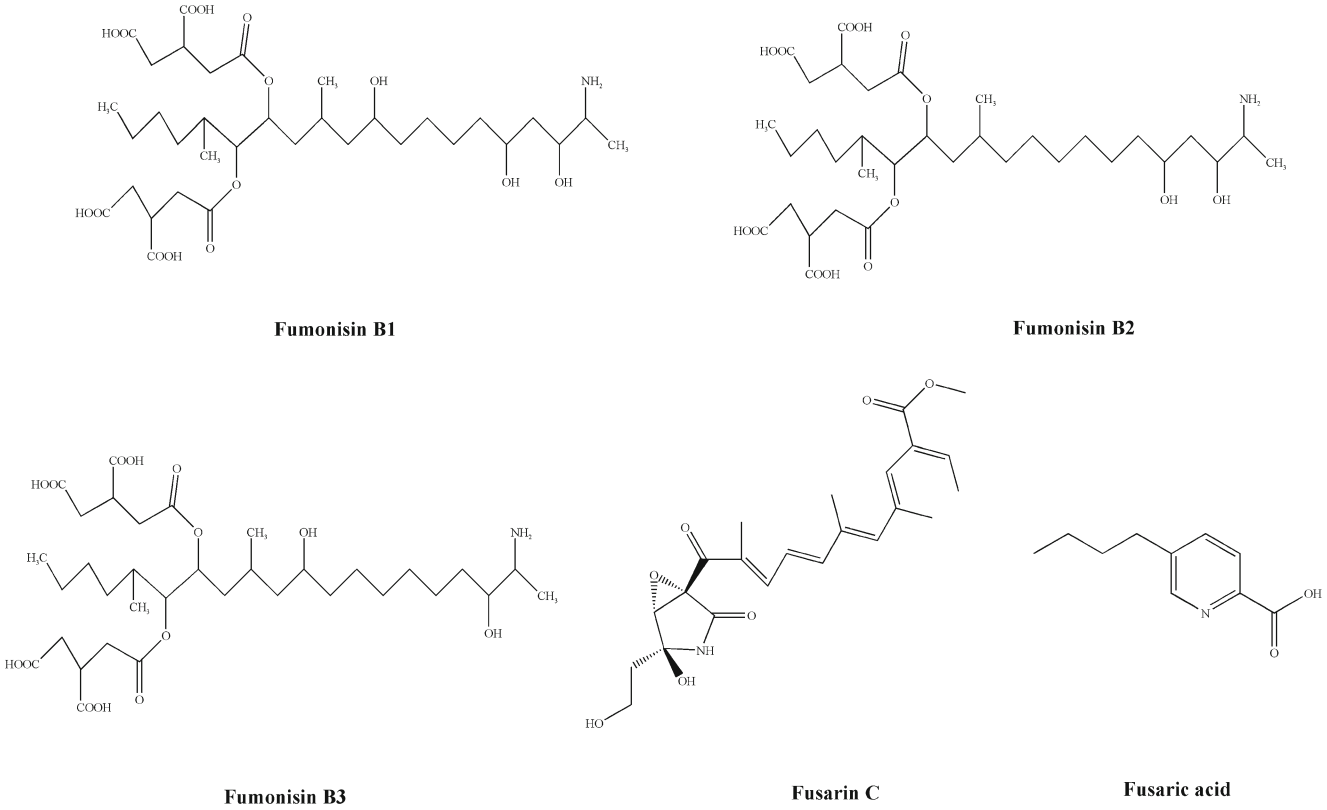

Fig. 1 Chemical structures of fusarin C, fusaric acid, fumonisin B_1 (FB_1), fumonisin B_2 (FB_2) and fumonisin B_3 (FB_3)

on Cancer (IARC 1993). Maximum acceptable levels were set at 1,000 μg kg^{-1} for FB_1 and FB_2 in maize and maize-based food intended for direct human consumption, 800 μg kg^{-1} for maize-based breakfast cereals and snacks and 200 μg kg^{-1} for processed corn-based foods and baby foods for infants and young children (European Commission 2007). It is noticeable that some health risks might be left behind since fusaric acid, fusarin C and FB_3 may co-occur and interact with FB_1 and FB_2. Since fungal strain specificity and growing media were diversely observed (Mogensen et al. 2009; Schmidt-Heydt et al. 2011; Medina et al. 2013; Nazari et al. 2014), the present study was designed to screen the ability of fungal strains isolated from maize in Belgium to produce fusarin C, fusaric acid, FB_1, FB_2 and FB_3. It also reports the isolation and characterisation works for fusarin C standard solution.

Materials and methods

Chemicals and reagents

Chemicals including $(NH_4)_2HPO_4$; KH_2PO_4; $MgSO_4{\cdot}7H_2O$; NaCl; sucrose; glycerol; potato dextrose agar anhydrous magnesium chloride ($MgCl_2$); pure crystalline form of FB_1, FB_2 and FB_3; and fusaric acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Trifluoroacetic acid (TFA) was obtained from Acros Organics (NJ, USA). Methanol, 2-propanol, acetonitrile and acetic acid were obtained from Biosolve (Valkenswaard, The Netherlands). Water used during the whole analysis was purified using the Milli-Q System (Millipore, Brussels, Belgium).

Medium preparation

Myro medium (glucose 30.0 g, $(NH_4)_2HPO_4$ 1.0 g, KH_2PO_4 3.0 g, $MgSO_4{\cdot}7H_2O$ 2.0 g, NaCl 5.0 g per L (pH 6)) was prepared according to Jackson et al. (1989), whereas the modified Myro (the second medium) was performed according to Farber and Sanders (1986) and constituted of sucrose 40.0 g, $(NH_4)_2HPO_4$ 1.0 g, KH_2PO_4 3.0 g, $MgSO_4{\cdot}7H_2O$ 2.0 g, NaCl 5.0 g and glycerol 10.0 g per L (pH 5.9).

Maize grains (40 g) were autoclaved twice after adjusting the moisture content (to 50 %) in 100-mL flasks (Scauflaire et al. 2012).

Test microorganisms and culture conditions

The cultural characteristics of *Fusarium* species were macroscopically assessed as described by Leslie and Summerell (2006), and the identification of strains at species level was achieved by sequencing of the elongation factor 1 alpha gene (O'Donnell et al. 1998).

Fusarium graminearum (strains II F030, III R048, III S002 and III T010), *Fusarium venenatum* (strains II Q002, III A048, IV A034 and IV B035) and *Fusarium verticillioides* (strains II R078, III K048, IV B011 and IV B133) obtained from maize grown in Belgium (Tables 1 and 3) were the first test microorganisms cultivated in Myro liquid medium (100 mL) at 27 °C for 8 days in the dark under regular shaking at 100 rpm. *F. verticillioides* IV B011 was selected as super producer among the tested strains. Moreover, ten other *F. verticillioides* strains (Tables 1 and 4) including two mating-type tester strains were chosen and cultivated both in modified Myro medium (100 mL) and in maize grains. Non-inoculated media (broth or maize cultures) were used as controls for checking any incurred mycotoxin production during the trials. The liquid cultures were incubated for 2 weeks at 27 °C under regular shaking at 100 rpm. Incubation was stopped by centrifugation to isolate the supernatant in a flask wrapped with aluminium foil and storage at −20 °C until analysis. As for maize grains, inoculation was performed with four plugs of 25 mm^2 of 7-day-old mycelium grown on potato dextrose agar (PDA; Sharlau, Spain). Cultures were incubated at 25 °C for 3 weeks in the dark. For maize, incubation was stopped by autoclaving at 121 °C for 20 min and samples were thereafter dried at 40–50 °C for 18 h, ground and sieved (<800 μm) and stored at −20 °C until analysis.

Isolation and identification of fusarin C

The supernatant (1 mL) of liquid medium was diluted with methanol/water solution (50:50, *v*/*v*) to 10 mL. The dilution was vortexed for 1 min, passed through a 0.22-μm filter and injected into HPLC and LC-MS/MS. All experiments are performed in a dark room due to the instability of fusarin C towards the light. The concentration of the fusarin C was calculated using the extinction coefficient $\varepsilon = 33{,}000$ (350 nm) reported by Barrero et al. (1991).

Sample pretreatment

Maize flour (4.00±0.02 g) of each culture was macerated with 20 mL acetonitrile/2-propanol/water solution (50:30:20, *v*/*v*/*v*) for 10 min, followed by 1 h shaking on a Heidolph Reax 2. Thereafter, 6 g of anhydrous $MgSO_4$ and 1.5 g of NaCl were added to the slurry and shaken again for 5 min. The mixture

Table 1 List of the entire investigated *Fusarium* strains

Original country	Species	Strain number	Substratum	References
Belgium	*F. graminearum*	II F030	*Zea mays*	This study
		III R048 (MUCL 53453)	*Zea mays*	Scauflaire et al. 2012
		III S002 (MUCL 53454)	*Zea mays*	Scauflaire et al. 2012
		III T010 (MUCL 53455)	*Zea mays*	Scauflaire et al. 2012
	F. venenatum	II Q002	*Zea mays*	This study
		III A048	*Zea mays*	This study
		IV A034	*Zea mays*	This study
		IV B035	*Zea mays*	This study
	F. verticillioides	I E061	*Zea mays*	This study
		I M102	*Zea mays*	This study
		I S054	*Zea mays*	This study
		II K020	*Zea mays*	This study
		II L084	*Zea mays*	This study
		II M015	*Zea mays*	This study
		II R078	*Zea mays*	This study
		III K048 (MUCL 53471)	*Zea mays*	Scauflaire et al. 2012
		IV B011 (MUCL 53472)	*Zea mays*	Scauflaire et al. 2012
		IV B022	*Zea mays*	This study
		IV B133	*Zea mays*	This study
USA		X085 (FGSC 7600[a]-MUCL 43478)	*Zea mays*	Gerlach and Nirenberg 1982
		X086 (FGSC 7603[b]-MUCL 43479)	*Zea mays*	Gerlach and Nirenberg 1982
Philippines		MUCL 51655	*Zea mays*	This study

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[a] Mating type tester strain of *F. verticillioides*, MAT1-1

[b] Mating type tester strain of *F. verticillioides*, MAT1-2

was subsequently centrifuged at 10,000*g* for 15 min. The supernatant (5 mL) was diluted with methanol/water solution (50:50, *v*/*v*) to 50 mL and mixed. This mixture (1 mL) was taken and passed through a 0.22-μm filter for injection.

HPLC-diode array detector conditions

The HPLC system consisted of a Waters LC system (Waters, Milford, MA, USA), an Atlantis T3 column (150 mm× 4.6 mm, 5 μm) maintained at 30 °C and a diode array UV detector set at 363 nm with simultaneous monitoring wavelengths from 200 to 450 nm. A computer using a Millennium Empower software was used to process the signals of the detector and for data management.

The mobile phase was made up of solvent A (0.05 % TFA in water) and solvent B (0.05 % TFA in acetonitrile) eluted at a flow rate of 1 mL min^{-1}. Isocratic elution during 20 min was used with 40 % solvent B. The injection volume was 10 μL.

LC-MS/MS analysis

The UHPLC system consisted of a Waters Acquity LC system (Waters, Milford, MA, USA), an Acquity UPLC T3 column (100 mm×2.1 mm, 1.8 μm) maintained at 35 °C and Waters Xevo TQ-S MS (Waters, Milford, MA, USA) with an electrospray source in positive electrospray ionization (ESI^+) mode. The mobile phase eluted at 0.5 mL min^{-1} consisted of (A) 1 % acetic acid in water, (B) methanol and (C) water. A linear gradient elution programme was applied as follows: 0 min 5 % A, 24 % B; 3 min 5 % A, 69 % B; 5 min 5 % A, 69 % B; 5.2 min 5 % A, 95 % B; 5.7 min 5 % A, 95 % B; and 5.8 min 5 % A, 24 % B, and the rest was always C (water). The composition was held at 5 % A and 24 % B for 2.2 min for equilibration, giving a total run time of 8 min. The injection volume was 2.0 μL (partial loop with needle overfill).

The ionization source conditions were set as follows: capillary voltage of 0.5 kV, cone voltage of 60 V, source offset voltage of 50 V, source temperature of 150 °C, desolvation temperature of 450 °C and nebulizer of 7.0 bar. The cone and desolvation gas flows were 150 and 1,000 L h^{-1}, respectively. Data acquisition and processing were performed using MassLynx v4.1 (Waters).

Results and discussion

Isolation and identification of fusarin C

Out of 12 strains cultivated in Myro medium, LC-diode array detector (DAD) analyses revealed two different peaks for *F. verticillioides* (IV B011) against the control (Supporting information Fig. S1). The spectra of these two peaks were very similar with the maximum absorption at 363 nm, reflecting the presence of pentaene chain. A minor difference was seen in the range of 200–250 nm (Fig. 2a), which was crucial because this absorption range reflected the pyrrolidone ring (Kleigrewe et al. 2012). The fractions corresponding to both peaks were isolated using LC-DAD, pooled for each peak and dried by nitrogen gas, avoiding the light. The residues were re-dissolved in a 50 % methanol (2 mL). They were noted as peaks 1 and 2 according to the different retention times and then analyzed by LC-MS/MS for further identification.

For LC-MS/MS analysis, first, the full-scan mode was used and multiple injections indicated that peak 1 only showed the precursor ion *m*/*z* 432 ($[M+H]^+$) whilst peak 2 showed different precursor ions : *m*/*z* 432 ($[M+H]^+$), 454 ($[M+Na]^+$) and 470 ($[M+K]^+$). Kleigrewe et al. (2012) have previously indicated that the response of $[M+Na]^+$ should be higher than that of $[M+H]^+$ for fusarin C. This was exactly observed for the precursor ions of peak 2 (454 for $[M+Na]^+$, the highest one). Therefore, peak 2 was identified as fusarin C. After investigation of the fragments of peak 2, the main product ions, i.e. *m*/*z* 436, 419, 406, 396, 368, 352, 335, 290 and 267 (Fig. 2b), correspond with the data of Kleigrewe et al. (2012).

The UV spectrum of peak 1 was similar to that of fusarin C showing the same *m*/*z*. This compound might be identified as the *open-chain fusarin C* or one of its stereoisomers (*epi*-fusarin C) which was also obtained upon storing the standard solution of fusarin C (peak 2) in methanol at room temperature for 2 days. Fusarin C is then shown to be instable in methanol at room temperature and was transformed to another compound as stated by Gelderblom et al. (1983) and Kleigrewe et al. (2012).

Optimization of LC-MS/MS conditions

The mobile phase allowed the improvement of separation and ionization efficiency with high selectivity and sensitivity of analysis. Mixture of methanol and water was initially used as the mobile phase for separation of the five targeted analytes, whilst the peak shape of fusaric acid was unsatisfactory (Fig. 3a). Three mobile phases: (1) methanol–water containing 5 mM ammonium acetate, (2) methanol–water containing 5 mM ammonium and 0.05 % acetate acid and (3) methanol–water containing 0.05 % acetate acid were compared (Fig. 3). Mobile phase 2 or 3 allowed all mycotoxins to be efficiently separated with good peak shapes. Alternatively, methanol–water containing 0.05 % acetate acid was selected due to the relatively higher sensitivity. Under such situation, fusarin C, fusaric acid, FB_1, FB_2 and FB_3 were completely separated from each other in less than 8 min, providing narrow peaks with good peak symmetry. Compared to the use of TFA as an additive in the mobile phase (Brown et al. 2012), the

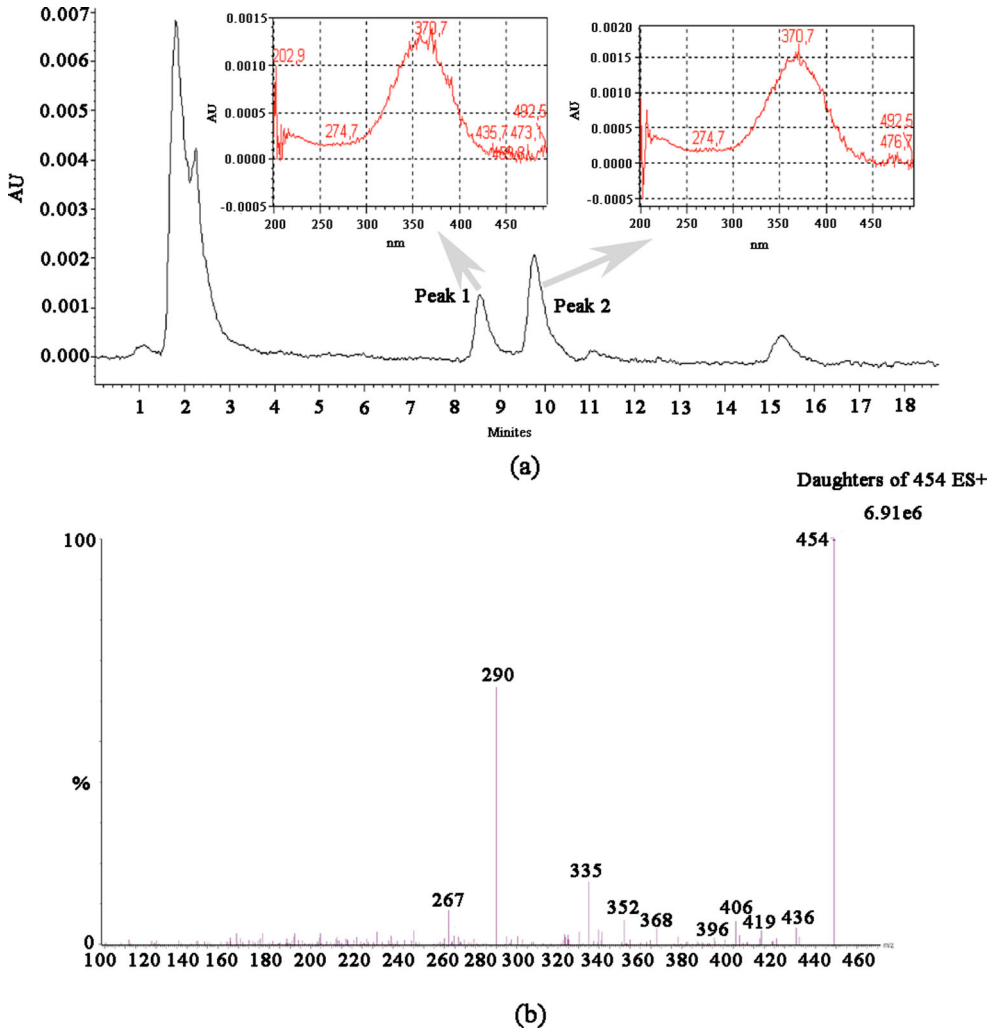

Fig. 2 Identification of fusarin C by UV spectra using HPLC with diode array detector (DAD) (**a**) and by fragmentation upon LC-MS/MS analysis (**b**)

developed method is simpler, more quick and economic with nice peak shapes.

The MS/MS conditions were optimized for each mycotoxin by direct injection of the standard solution. Identification of precursor ions was performed under the full-scan mode by recording from *m/z* 100 to 800, both in ESI^+ and ESI^- modes. The results showed that fusarin C generated the precursor ion of $[M+Na]^+$ with much higher abundance than that of $[M+H]^+$, whilst all the other compounds formed $[M+H]^+$ precursor ions with the highest intensity under ESI^+ mode. On the basis of the confirmation of precursor ions, two product ions for each precursor ion were selected according to the highest sensitivity and optimal selectivity for the targets. Cone voltages were selected according to the sensitivity of the precursor ions, and collision energies were chosen to give the maximum intensity of the fragment ions. The final selections of precursor ions, product ions, cone energies and collision energies are reported in Table 2.

Sample preparation and preliminary method characterisation

Based on the quick, easy, cheap, effective, rugged and safe (QuEChERS) protocol adapted for the analysis of aflatoxins, ochratoxin A, enniatin A, citrinin, fusarin C and FB1 (Frenich et al. 2011; Arroyo-Manzanares et al. 2013; Zhang et al. 2013), the present study did not use the primary and secondary amine (PSA) as the base sorbent for removing the impurities of the sample solutions (for sample purification) since it can remove FB_1, FB_2 and FB_3 from extracts due to ion exchange.

A matrix-matched calibration was used for the compensation of the matrix effect during the ionization process. The matrix-matched calibration was performed with freshly prepared mycotoxin calibration solutions in ranges of 1–100 ng mL^{-1} for fusarin C and 1–1,000 ng mL^{-1} for fusaric acid, FB_1, FB_2 and FB_3. The sample solutions with high concentration levels of the analytes were diluted with the blank matrices for fitting the linear range of the calibration curve. The sensitivity was evaluated by determining the limit of detection (LOD) and limit of quantitation (LOQ) defined as

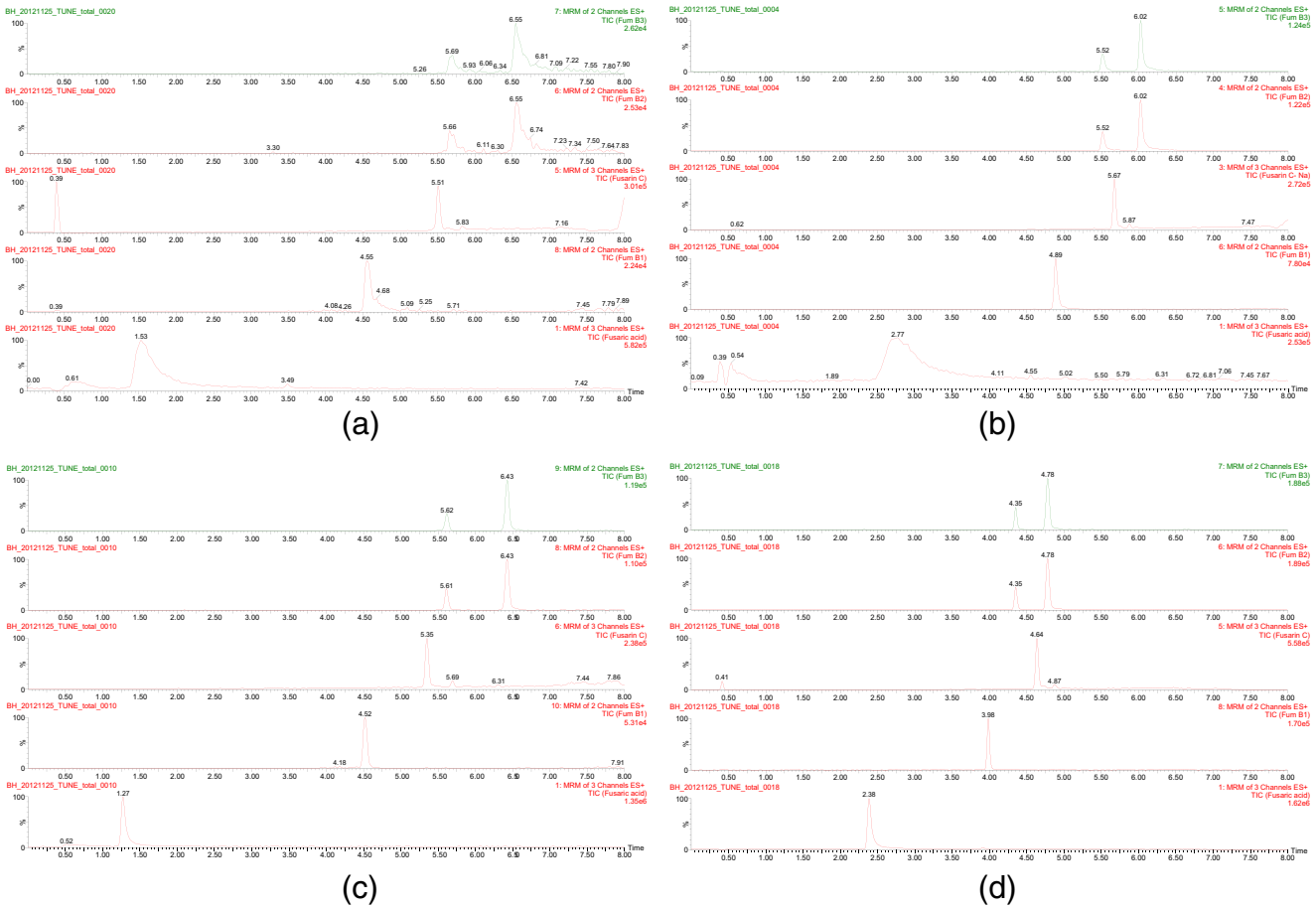

Fig. 3 Comparison of separation and ionization efficiencies of the five mycotoxins using four candidate mobile phases. **a** Methanol–water, **b** methanol–water containing 5 mM ammonium acetate, **c** methanol–water containing 5 mM ammonium and 0.05 % acetate acid, **d** methanol–water containing 0.05 % acetate acid

the concentrations of the mycotoxins that yielded a signal-to-noise ratio (S/N) of 3 and 10. They were both determined by decreasing the spike concentrations in matrix. Similar LOD and LOQ values obtained for fusarin C, fusaric acid, FB_1, FB_2 and FB_3 were 0.1 and 0.3 ng mL^{-1}, respectively. These limits were lower than those obtained by Kleigrewe et al. (2011) and Zhang et al. (2013), indicating that the sensitivity of the present method is high.

Toxin-producing ability by fungal strains

In Myro medium, inoculation of *F. graminearum*, *F. venenatum* and *F. verticillioides* showed that four strains were fusaric acid producers whilst eight strains were fusarin C producers. Both FB_1 and fusaric acid were co-produced by *F. verticillioides* strains (Table 3). *F. verticillioides* IV B011 strain was able to produce relatively high amounts of toxins

Table 2 The MS/MS parameters for fusaric acid, fusarin C and fumonisins B_1, B_2 and B_3

Names	Precursor ion (*m*/*z*)	Cone energy (eV)	Primary product ion (*m*/*z*)	Collision energy (eV)	Secondary product ion (*m*/*z*)	Collision energy (eV)
Fusaric acid	180	15	92	24	65	30
Fusarin C	454	20	290	18	335	20
FB_1	722	50	334	38	352	34
FB_2	706	50	336	37	318	39
FB_3	706	50	336	37	318	39

Table 3 Concentrations (mg L^{-1}) of the five mycotoxins in Myro liquid medium inoculated with *Fusarium graminearum*, *F. venenatum* and *F. verticillioides*

Species	Samples	Fusaric acid	Fusarin C	FB_1	FB_2	FB_3
F. graminearum	II F030	–[a]	–	–	–	–
	III R048	–	0.1	–	–	–
	III S002	–	–	–	–	–
	III T010	–	–	–	–	–
F. venenatum	II Q002	–	0.1	–	–	–
	III A048	–	0.1	–	–	–
	IV A034	–	–	–	–	–
	IV B035	–	0.1	–	–	–
F. verticillioides	II R078	128	0.3	0.2	–	–
	III K048	55	0.7	0.3	–	–
	IV B011	137	0.5	1.8	0.7	0.3
	IV B133	1	0.3	0.1	–	–

[a] Not detected

(137 mg L^{-1} of fusaric acid and 1.8 mg L^{-1} of FB_1) together with 0.5 mg L^{-1} of fusarin C, 0.7 mg L^{-1} (FB_2) and 0.3 mg L^{-1} (FB_3). A similar trend was also observed by Arino et al. (2007), Brown et al. (2012) and Butchko et al. (2012). The highest concentration of fusarin C (0.7 mg L^{-1}) was produced by the strain of *F. verticillioides* III K048. These results demonstrate that *F. verticillioides* simultaneously produced fusarin C, fusaric acid, FB_1, FB_2 and FB_3 in contrast to *F. graminearum* or *F. venenatum* under the same cultivation conditions.

MRM chromatograms of the five mycotoxins in the standard solution (a) and in a representative contaminated sample (maize inoculated with *F. verticillioides* IV B011 strain) (b) are shown in Fig. 4. For this *F. verticillioides* strain, the concentration of fusaric acid was similar in both media, whilst the concentration of fusarin C was higher in the modified Myro. Very low concentrations of FB_1, FB_2 and FB_3 were observed in both liquid media (Tables 3 and 4).

Cultivation of ten other strains in the modified Myro and on the sterilized maize grains clearly showed that the contents of fusarin C and FBs were substantially increased in maize cultures up to 441 mg kg^{-1} for fusaric acid, 74 mg kg^{-1} for fusarin C, 1,301 mg kg^{-1} for FB_1, 367 mg kg^{-1} for FB_2 and 753 mg kg^{-1} for FB_3 (Table 4). These results indicate that the components of the medium seriously affected the production of the target mycotoxins (Jackson et al. 1989; Jackson and Freer 1991). Likewise, Baird et al. (2008) have found that the concentration of FB production was obviously lower in the liquid than in rice medium. Myro medium and maize were completely different, and consequently, several factors could affect the in vitro production of FBs, including limited nitrogen, moderate water activity and pH levels. Whilst a medium of pH 5.9 was used by Baird et al. (2008), an optimum of 2.0 was reported by Miller (2001) for FB production. It could also be obviously seen that fusarin C, fusaric acid and FB_3 were easily produced at different ratios (Table 5) along with FB_1 and FB_2 when maize was infected by *F. verticillioides*. In contrast to fusarin C and FB_3, the level of fusaric acid production may exceed the sum of FB_1 and FB_2 concentrations, depending on the strain. If the same is true in maize-based food or feedstuffs, the co-occurrence of these mycotoxins should be a major matter of concern for human and animal health. We therefore emphasized in the monitoring of these five mycotoxins in the control programmes of agricultural practices, crop management and food production by focusing not only on *F. verticillioides* but also on the

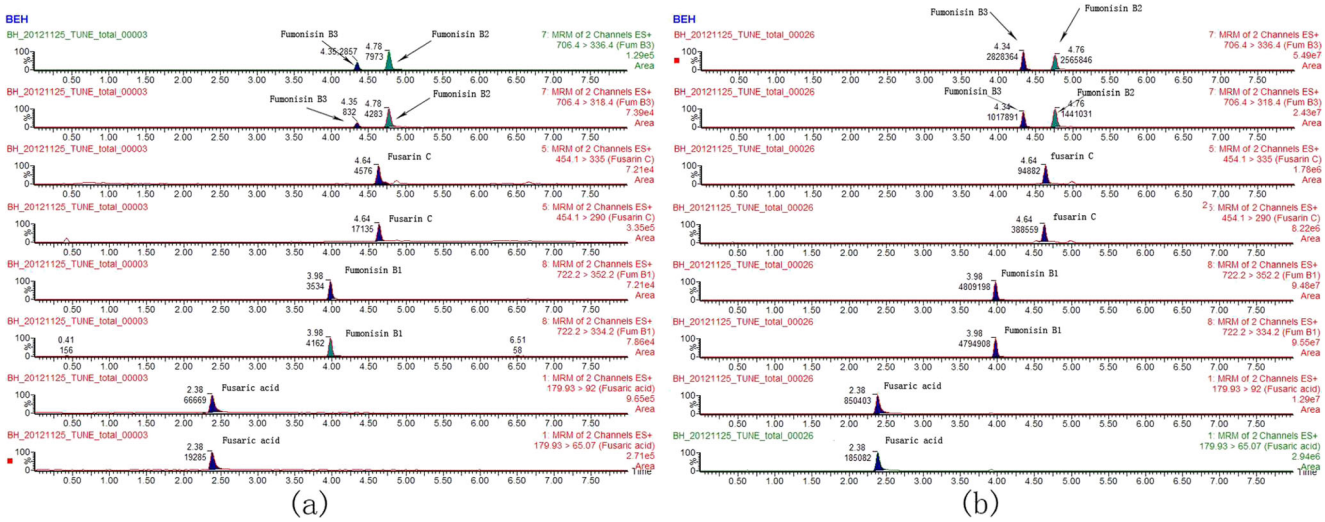

Fig. 4 Chromatographs of five mycotoxins in the standard solution (**a**) and in the medium solution (diluted ten times) of *F. verticillioides* (**b**). The concentrations of the five mycotoxins in **a** are 20 ng mL^{-1} for fusaric acid and 10 ng mL^{-1} for fusarin C, fumonisin B_1 (FB_1), fumonisin B_2 (FB_2) and fumonisin B_3 (FB_3), respectively

Table 4 Concentrations (mg L^{-1}, mg kg^{-1}) of the five mycotoxins in modified Myro liquid medium and in maize grains inoculated with *F. verticillioides* in modified Myro liquid and maize grains

Strains	Modified Myro liquid medium (mg L^{-1})					Maize grains (mg kg^{-1})				
	Fusaric acid	Fusarin C	FB_1	FB_2	FB_3	Fusaric acid	Fusarin C	FB_1	FB_2	FB_3
I E061	24	0.9	–	–	–	10	20	1,301	367	505
I M102	–[a]	–	–	–	–	0.7	13	382	72	166
I S054	91	1	–	–	–	25	33	135	13	83
II K020	369	0.2	–	–	–	132	28	50	15	24
II L084	154	0.7	–	–	–	441	43	96	18	14
II M015	118	2	–	–	–	154	18	95	22	48
IV B011	126	3	0.3	–	–	31	20	968	268	753
IV B022	79	3	–	–	–	10	11	638	122	563
MUCL 43478	31	2	–	–	–	3	11	697	106	324
MUCL 43749	80	0.9	–	–	–	77	74	310	39	174
MUCL 51655	147	4	0.2	–	–	8	50	864	120	340

MUCL Mycothèque de l'Université catholique de Louvain (Belgium)

[a] Not detected

population of the *F. temperatum* that is newly observed in Belgium by Scauflaire et al. (2012). We also suggest that early detection methods of the high-fusarin C, fusaric acid, FB_1, FB_2 and FB_3 producers (*F. verticillioides*) could be developed and used for taking corrective actions in the field or even during storage for discarding the contaminated batches from the food chain. As FB_1 is chemically modified during many food processing techniques, including nixtamalisation, heat treatment and reaction with sugars (Bullerman and Bianchini 2007; De Girolamo et al. 2011), we suggest performing further study on the stability of fusarin C and fusaric acid during cooking and food processing based on maize products such as popcorn, cornflakes, tinned maize, tortilla chips, beer and corn starch.

Conclusions

New information about the ability of Belgian *Fusarium* strains has been provided as far as fusarin C, fusaric acid, FB_1, FB_2 and FB_3 co-production is concerned either in liquid or in maize cultures. In contrast to the *F. graminearum* or *F. venenatum*, relatively higher toxin-producing ability could be observed for *F. verticillioides* under the same culture conditions. Significant increase in the production of all tested mycotoxins was found in maize grains. The multi-mycotoxin contamination according to fungal strains and culture medium is a very important issue that is able to support decision-making for managing fusarin C, fusaric acid, FB_1, FB_2 and FB_3 contamination in crops.

Table 5 Ratios of fusaric acid, fusarin C, FB_1, FB_2 and FB_3 contents obtained on sterilized maize grains inoculated with *Fusarium verticillioides* strains

Strains	FB_1+FB_2 (mg/kg)	Ratios		
		Fusaric acid/(FB_1+FB_2)	Fusarin C/(FB_1+FB_2)	FB_3/(FB_1+FB_2)
I E061	1,669	0.01	0.01	0.30
I M102	454	0.002	0.03	0.37
I S054	147	0.17	0.22	0.57
II K020	64	2.06	0.43	0.38
II L084	114	3.88	0.38	0.12
II M015	117	1.32	0.15	0.41
IV B011	1,236	0.02	0.02	0.61
IV B022	760	0.01	0.01	0.74
MUCL 43478	803	0.003	0.01	0.40
MUCL 43749	349	0.22	0.21	0.50
MUCL 51655	983	0.01	0.05	0.35

MUCL Mycothèque de l'Université catholique de Louvain (Belgium)

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Conflict of interest None

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