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Full Length Research Paper

Genetic, pathogenic and toxigenic variability by *F. proliferatum* isolated from maize kernels

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Genetic (mating population and vegetative compatibility), pathogenic and toxigenic variability of *F. proliferatum* isolated from maize kernels originating from different localities in Serbia were studied. Investigated isolates of *F. proliferatum* were fertile and most of them (8/13) belonged to the mating type *MATD-2*. Based on the complementary test isolates, *F. proliferatum* were grouped into 11 vegetative compatibility groups (VCGs) and two isolates were self-incompatible. All tested field isolates of *F. proliferatum* and *nit* mutants were pathogenic and most expressed medium pathogenicity. Field isolates of *F. proliferatum* showed a higher potential for biosynthesis of FB_1 (up to 1,246.00 µg g^{-1}) in relation to their *nit1* (up to 53.41 µg g^{-1}) and *NitM* mutants (up to 56.17 µg g^{-1}). Based on genetic, pathogenic and toxigenic properties of 13 isolates of *F. proliferatum*, relatively high variability of this fungus originated from maize grain was found.

Key words: *Fusarium proliferatum*, mating type, vegetative compatibility, pathogenicity, fumonisin B1.

INTRODUCTION

The fungus *Fusarium proliferatum* (Matsushima) Nirenberg occurs worldwide on a variety of plant hosts and along with *F. graminearum* Schw., *F. verticillioides* (Sacc.) Nirenberg (syn. *F. moniliforme* Sheld.) and *F. subglutinans* (Wollen. and Rein.) Nelson, Toussoun and Marasas, it is one of the most important fungus associated with maizecausing kernel rot, an important maize disease in temperate climates (Nelson et al., 1983). Maize samples were found to be infected by *F. proliferatum* from 1% in Meksiko (Gallardo-Reyes et al., 2006) to 70.0% in India (Hossain et al., 2002). In Croatia, the most frequent fusaria found in maize were *F. verticillioides*, *F. subglutinans* and *F. proliferatum*. *F. proliferatum* was found in almost all the analysed maize samples and *F. verticillioides* in 8 out of 15 samples, which is in agreement with the high frequency of FB_1-positive samples. Depending on the sample, the incidence of *F. proliferatum* varied from 2 to 40% (Domijan et al., 2005). In Serbia also, after *F. verticillioides*, *F. subglutinans* and *F. graminearum*, *F. proliferatum* is the most important pathogen of maize (Lević et al., 1997).

F. proliferatum belongs to *Liseola* section of the *Fusarium* genus (Nelson et al., 1983) and its teleomorph, *Gibberella intermedia* (Kuhlman) Samuels, Nirenberg and Seifert, belongs to the *G. fujikuroi* (Sawada) S. Ito complex, composed of at least nine reproductively isolated biological species (mating populations [MPs]), denoted by letters A to I (Leslie et al., 2004). Although *F.*

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Abbreviations: ***MATD-1*,** Mating population D**,** mating type 1; *MATD-2*, mating population D**,** mating type 2; **VCGs**, vegetative compatibility groups; **FBs,** fumonisins; **FB_1,** fumonisin B1; **PDA,** potato dextrose agar; **CLA,** carnation leaf agar; **ELISA,** enzyme-linked immunosorbent assay.

proliferatum has a teleomorph, *G. fujikuroi,* the perithecia are rarely found in nature. The perithecia can readily be produced in the laboratory by pairing with female fertile tester strains that belong to the "D" mating group of *G. fujikuroi* (Leslie, 1995). Genetic variations within each mating population have been investigated using several mycological characters such as a distribution of a mating type (Leslie, 1991), frequency of hermaphrodite (Leslie and Klein, 1996), and vegetative compatibility (Leslie, 1993).

F. proliferatum is a toxigenic species, producing a broad range of toxins, such as fumonisin B1, moniliformin, beauvericin, fusaproliferin, fusarin and others (Logrieco et al., 1995; Miller et al., 1995; Ritieni et al., 1995; Fandohan et al., 2003, Lević et al., 2004). Maize grains infected by *F. proliferatum* are primarily contaminated with fumonisins (FBs) that may have cancer-promoting activities in humans. Oesophageal cancer (Marasas et al., 1981), leukoencephalomalacia in horses, as well as pulmonary edema in swine are fatal diseases of humans and animals caused by FBs-contaminated food and feed (Thiel et al., 1991). The elevated fumonisin level in maize has become an area of concern since ingestions of fumonisin-contaminated maize associated with livestock loss and human health risks have occured (Wang et al., 2008). The relatively high intake of raw materials with the diet of livestock can lead to nutrient losses and have adverse effects on animal health and on productivity (Biagi, 2009).

Knowledge about the genetic variation of important fitness traits (like fertility and aggressiveness) of *Fusarium* spp. in maize is important for resistance, estimating the evolutionary risk of the pathogen, and for planning the agricultural management practices (Leslie, 1991). In order to determine the variation of *F. proliferatum* the following was studied: (i) the fertility of isolates of *F. proliferatum* and assigning their classify-cation into a specific mating types; (ii) distribution of isolates over different VCGs; (iii) pathogenicity to maize seed under laboratory conditions; and (iv) the toxicological profile of the isolates.

MATERIALS AND METHODS

Fungal isolates

Isolates of *F. proliferatum* were obtained from maize kernels from different localities in Serbia. One hundred maize kernels from each sample (ten ears), were collected during harvest in 2006 and 2007 and after disinfection in 1% sodium hypochlorite (NaOCl) for 5 min were directly placed on Petri dishes (five kernels per plate) containing potato dextrose agar (PDA) and incubated in the dark at 25ºC for one week. The identification of the *Fusarium* species was made according to the taxonomic system developed by Burgess et al. (1994) and Leslie and Summerell (2006). Single conidium cultures on PDA and on carnation leaf agar (CLA), according to Burgess et al. (1994) and Leslie and Summerell (2006), were used for further morphological observations, vegetative compatibility and pathogenicity studies. For examining mating populations, vegetative compatibility groups, pathogenicity and production of fumonisin B_1, 13 field isolates of *F. proliferatum* were selected . The pathogenicity test and analysis of fumonisin included *nit* mutants, *nit1* and *NitM* of field isolates.

Crosses

Sexual crosses were made on carrot agar as previously described by Klittich and Leslie (1988). Briefly, testers (3628 *MATD-1* and 3627 *MATD-2*) or the male strains were grown on sloping CLA in glass test tubes for seven days at 25°C under alternating 12 h of combined light (fluorescent and near ultraviolet light) and dark, while the field isolates or the female strain were grown in Petri dishes on a carrot medium at 20°C in the dark. After crossing, 1 ml of the tester suspension was carefully displaced through the culture of field isolates. The plates were incubated for 4-6 weeks under the same growing conditions, used for the growth of testers. The presence of perithecia and mature perithecia with ascospore mass extruded from the ostioles were examined weekly. In all of the successful crosses with the female strain standard tester the unknown field isolates were classified as the male strain. Tests for female fertility of the unknown isolates were made by reversing the roles of the two strains in the cross. A cross was scored as infertile if mature perithecia were not formed after two attempts. The effective population size [$N_{e(mt)}$], the inbreeding effective population size and [$N_{e(f)}$] were calculated by using the equations of Leslie and Klein (1996).

Vegetative compatibility tests

Nitrate non-utilizing mutants (*nit* mutants) were generated on minimal agar with chlorate according to the method of Correll et al. (1987). The physiological phenotypes of *nit* mutants recovered from the representative isolates were interpreted on the basis of their growth on media containing different nitrogen sources. Complementation tests between phenotypically distinct *nit* mutants were made on the minimal medium (MM). *Nit* mutants were assigned to the same VCG based on the ability of the derived *nit1* mutants to form a heterokaryon with complementary NitM mutants.

Pathogenicity

A total of 39 isolates of *F. proliferatum*, 13 isolates each of field isolates, *nit1* and NitM mutants(13 isolates each), were used for the pathogenicity test. A simple laboratory method described by Mesterházy (1984), but with minor modifications, was used to determine the effect of selected isolates of *F. proliferatum* on maize seed germination. Twenty five maize seeds surface-sterilised with 1% NaOCl solution for 5 min and washed three times with distilled water were arranged in 200-mm Petri dishes with two layers of filter paper. A total of 40 ml of inoculum prepared in the liquid Czapek-Dox medium or 40 ml of distilled water in control were poured over each Petri dish. All treatments were repeated four times. After seven days, the number of germinated seeds in treatments with inoculum and water (control) were estimated. Pathogenicity of *F. proliferatum* was expressed as the percentage of germination of inoculated maize seeds calculated in relation to seed germination in the control and expressed as a percentage. Obtained data were

Table 1. Female fertility and the mating type of *F. proliferatum* isolates from maize kernels (*Zea mays* L.)

Collection number[a]	Year	Testers[b]				*MAT* type
		3628 *MATD-1*		3627 *MATD-2*		
		N	R	N	R	
1275	2006	-	-	+	-	D-1
1598	2007	+	-	-	-	D-2
1574	2007	+	+	-	-	D-2
1345	2006	-	-	+	-	D-1
1555	2007	+	-	-	-	D-2
1584	2007	+	-	-	-	D-2
1616	2007	+	-	-	-	D-2
1374	2006	+	-	-	-	D-2
1609	2007	-	-	+	+	D-1
1248	2006	+	-	-	-	D-2
1641	2007	-	-	+	-	D-1
1653	2007	-	-	+	-	D-1
1322	2006	+	-	-	-	D-2

[a]All cultures are deposited in the Culture Collection of the Maize Research Institute, Zemun Polje (MRIZP), Belgrade-Zemun, Republic of Serbia. [b]N, Crosses of tester (♀) to the field isolate (♂); R, crosses of field isolate (♀) to the tester (♂).

transformed into arcsin using Microsoft Office Excel 2007 according to the equation = ASIN (SQRT (X/100)) * 180/PI (), where X = average value of germination expressed as a percentage, and PI () = 3.14159. Seeds used in these studies were obtained from a seed lot of maize hybrids whose germination amounted to 97%.

Potential for fumonisin B_1 production

Thirteen isolates each of field isolates, *nit1* and NitM mutants, or a total of 39 isolates of *F. proliferatum* were used for the analysis of fumonisin B_1 (FB_1) by ELISA (Enzyme linked immunosorbent assay). Investigated isolates of *F. proliferatum* were grown on 50 g maize kernels in 250 ml Erlenmeyer flasks. Maize kernels, with previously increased humidity to about 40%, were autoclaved. Kernels were inoculated with three fragments (5 × 5 mm) of the *F. proliferatum* colony, previously developed on PDA. Cultures were incubated at 27°C for three weeks. Harvested cultures were dried in a fan oven at 50°C for 72 h, finely ground, and stored at 4°C. Control kernels were treated the same way, except that they were not inoculated.

Furthermore, 5 g of ground sample were mixed with 25 ml of methanol and 1 g NaCl. This sample was mixed in a blender for 3 min and then was strained through a Whatman filter paper No. 1, and the filtrate was collected. The dilution of the filtrate was carried out based on the concentration of toxin in the sample and and the ELISA procedure was performed by following the manufacturer's recommendations (Tecna S. r. l., Trieste, Italy). Absorbance was determined using the spectrophotometer Elisa reader BioTek EL × 800™ (Absorbance Microplate Reader) at 450 nm.

RESULTS AND DISCUSSION

According to the Republic Hydrometeorological Service of Serbia in 2006 and 2007, the moderate warm (daily temperatures of around 25°C) and mostly dry weather with infrequent precipitations during the reproductive period of maize were recorded. These climatic conditions were convenient for developing *Fusarium* species on maize. The optimal conditions for *F. verticillioides* and *F. proliferatum* maize ear rot tend to be hot and dry (Doohan et al., 2003). According to Miller (2001), the incidence of *Fusarium* kernel rot (*F. verticillioides* and *F. proliferatum*) is higher in warmer climates under dry conditions. In such environments, insect damage is well recognised as a collateral factor.

Crosses

Both *MATD-1* and *MATD-2* individuals were identified among 13 isolates of *F. proliferatum* collected from maize kernels from different locations. Five, that is eight isolates out of the isolates selected for the pathogenicity test, were *MATD-1* and *MATD-2*, respectively (ratio 1: 2.46), according to the ratio found in the whole population tested (ratio 1:2.60) (Table 1). This ratio reduced the effective population number $N_{e(mt)}$ to 94.69%. Moreover, among 13 fertile isolates, two isolates (1574 and 1609) were hermaphrodites and 11 isolates were female sterile, giving an $N_{e(f)}$ of 2.67% of the count (total population). One out of the two hermaphrodites belonged to the mating type *MATD-1* and the other to *MATD-2*.

Also, the report of Stanković et al. (2007) showed that *MATD-2* was predominant over the *MATD-1* in this

Table 2. The effects of field isolates and *nit* mutants of *F. proliferatum* on seed germination and fumonisin B_1 production.

Collection number	Seed germination[a]			Fumonisin B_1 (µg g^{-1})[b]		
	Field isolates	*nit1*	NitM	Field isolates	*nit1*	NitM
1275	47.1^{h}	59.5^{ab}	56.9^{b}	111.20	52.14	46.54
1598	65.3^{f}	62.3^{bc}	64.5^{b}	109.90	41.76	14.33
1574	70.3^{e}	68.6^{bc}	58.8^{bc}	109.90	26.99	3.75
1345	84.2^{b}	67.7^{c}	60.9^{bcd}	1,246.00	51.13	56.17
1555	70.3^{e}	62.3^{bc}	60.9^{bc}	141.99	4.33	54.13
1584	70.3^{e}	57.5^{ab}	57.5^{d}	205.80	2.77	12.11
1616	74.4^{d}	60.2^{c}	56.9^{bcd}	138.50	10.53	15.15
1609	84.2^{b}	59.5^{bc}	58.8^{b}	76.80	12.28	47.91
1374	50.7^{g}	66.1^{a}	63.7^{cd}	128.50	53.41	11.55
1641	84.2^{b}	61.6^{bc}	55.6^{b}	128.10	12.69	12.05
1248	65.3^{f}	62.3^{a}	57.5^{a}	219.50	42.55	37.15
1322	78.3^{c}	53.7^{c}	61.6^{d}	2.37	27.01	48.27
1653	84.2^{b}	60.9^{ab}	51.9^{d}	67.80	16.97	15.39
Average	72.2	61.7	58.9	206.64	27.27	28.01

[a]Data transformed into arcsin. Mean values followed by the same letters are not significantly different (P > 0.05) according to Duncan's multiple rang test. b Isolates grown on autoclaved maize kernels in the dark at 25°C for four weeks.

population (ratio 2.6:1) with the similar estimated ratio in population of *F. proliferatum* isolated from onion in Serbia. However, this ratio reduced the effective population number and could be a consequence of the lack of female fertility that was detected among the analysed isolates. These results suggest that the mating type *MATD-2* dominates on maize kernels in Serbia. On the other hand in Iran, isolates from maize mainly belonged to *MATD-1*, while the isolates from rice roots belonged to *MATD-2* (Alizadeh et al., 2010). According to these authors, isolates originating from the roots of sugarcane were equally represented with *MATD-1* and *MATD-2*, while all isolates from the roots of onion belonged to *MATD-1*.

The lowest $N_{e(f)}$ values were recorded in isolates belonging to the mating population D, suggesting that sexual reproduction was relatively infrequent, but that there was potential for sexual reproduction within the Serbian population D of *G. fujikuroi* and thus an expansion of the genetic diversity by recombination. Sexual recombination probably occurs often enough to ensure that most populations usually will appear to be randomly mating. The effective population size is affected much more severely by a decrease in the number of female-fertile strains than it is by any known inequalities in the relative numbers of strains carrying the different mating-type idiomorphs (Leslie and Klein, 1996).

Vegetative compatibility

Thirteen isolates of *F. proliferatum* were examined for their ability to sector spontaneously on the toxic chlorate medium. Moreover, 69% of over 155 colonies examined formed chlorate-resistant mutant sectors. After the growth of isolates on media that contained one of three nitrogen sources, the *nit* mutants were divided into three phenotypic classes. The *nit* mutants occurred in seven loci: a structural gene for nitrate reductase (*nit1*), a regulatory gene specific for the nitrate reduction pathway (*nit3*), and five genes (*nit2*, *nit4*, *nit5*, *nit6* and *nit7*) controlling the production of a molybdenum-containing cofactor that is necessary for nitrate reductase activity (NitM). Mutations of *nit1* were recovered most frequently (11.1 to 90%, depending on the strain) followed by NitM (10 to 50%) and *nit3* mutations (0 to 13.3%). Based on the inability of pairings between *nit1* and NitM, mutants of isolates 1345 and 1598 on the minimum medium revealed that these isolates were self-incompatible. These isolates belonged to the mating types *MATD-1* (isolate 1345) and *MATD-2* (isolate 1598). Among the 13 isolates, 11 VCGs were identified on the basis of positive complementation reactions of *nit1* and NitM mutants.

The distribution of *F. proliferatum* isolates obtained from maize from various locations into different VCGs is reported for the first time in Serbia. We identified 11

VCGs among 13 isolates with a genetic diversity number of VCGs (number of VCGs/number of isolates) of 0.86. Alizadehl et al. (2010) identified 10 VCGs of 20 *F. proliferatum* isolates collected from maize in different regions of Iran. Nine isolates belonged to VCG 1, two isolates in each to VCG 3 and VCG 4, while seven isolates belonged to different VCGs (VCG 2, VCG 8, VCG 9, VCG 10, VCG 11, VCG 12 and VCG13). Some isolates of the fungus, which were isolated from the roots of rice and sugar cane, were classified into VCG 1 and VCG 2, while all isolated originated from onion were classified into VCG 1. The same authors concluded that natural populations of *F. proliferatum* in Iran were probably genetically divergent and included isolates representing a potential risk for disease development. Elmer (1991) and Elmer et al. (1999) collected 110 and 77 isolates of *F. proliferatum* from asparagus originating from different locations in the USA and seven regions in Australia, respectively, and they grouped them into 20 VCGs. Lim et al. (2001) also paired the six isolates from the mating population D originating from sorghum in Korea and received three different VCGs.

Pathogenicity

The study of *F. proliferatum* pathogenicity, both for field isolates and *nit* mutants, revealed that all isolates affected seed germination (Table 2), but effects of some of field isolates were stronger than effects of their *nit* mutants. On the average, field isolates had a lower effect on seed germination (72.2%) compared to *nit1* (61.7%) and NitM mutants (58.9%). Germination of inoculated seeds, with a spore suspension of field isolates, *nit1* and NitM mutants ranged from 47.1 to 84.3%, 53.7 to 68.6% and from 51.9 to 64.5%, respectively. Only two out of 13 field isolates (1275 and 1374) significantly affected seed germination (47.1 and 50.7%) compared to the other field isolates or their *nit* mutants. The greatest effect on seed germination was expressed by *nit1* mutants isolates 1322 and 1584 (53.7 and 57.5%, respectively) and by NitM isolates 1653 (51.9%) and 1584 and 1248 (57.5%).

All 13 field isolates of *F. proliferatum* exhibited lower pathogenicity under laboratory conditions compared to *nit* mutants (13 *nit1* and 13 NitM). The results achieved by Pascale et al. (2002) showed *F. proliferatum* to be highly pathogenic to maize under field conditions. On the basis of the effect on seed germination of maize field isolates, they can be classified into six groups, while 11 VCGs among 13 isolates were identified by the comple-mentation test. Discrepancies between the results of pathogenicity and the complementation test are likely a result of the methodology applied in studying pathogenicity. Elmer (1991) also found no correlation between VCG and virulence of isolates of *F. proliferatum*.

Toxigenic potential for fumonisin B_1

The analysis of FB_1 showed that field isolates of *F. proliferatum,* except the isolate 1322, had showed a higher production of this mycotoxin than their isolates of *nit* mutants. Field isolates biosynthesised FB_1 at a concentration ranging from 2.37 to 1,246 µg g^{-1} with an average of 206.64 µg g^{-1}, while their *nit1* and NitM mutants produced 2.77 to 53.41 (average 27.27 µg g^{-1}) and 3.75 to 56.17 µg g^{-1} (average 28.08 µg g^{-1}), respectively. On the average, the field isolates biosynthesised 7.6 times more FB_1 than their *nit* mutants. Typically, isolates belonging to the mating type *MATD-1* produced higher concentrations of the mycotoxin FB_1 than isolates of the mating type *MATD-2*. In relation to this, there were no differences between field isolates and their *nit* mutants. Only one field isolate (1345) produced 1,246.00 µg g^{-1} FB1, while the remaining field isolates, *nit1* and NitM mutants, produced FB_1 to 219.50 µg g^{-1}, 53.41 µg g^{-1} and 56.17 µg g^{-1}, respectively. Ross et al. (1990) identified isolates of *F. proliferatum* that biosynthesised FB_1 in the range from 1.670 to 2.790 µg g^{-1}, while Nelson et al. (1992) found that strains of *F. proliferatum* (19 of 31.61%) produced this mycotoxin in amounts ranging from 155 to 2.936 µg g^{-1}, similar to our results for field isolates. On the average, isolates of the mating type *MATD-1* produced a higher concentration of FB_1 in relation to the isolates of the mating type *MATD-2*. Similar results were also obtained from Leslie et al. (2004).

However, contrary to the results of pathogenicity tests, field isolates produced higher concentrations of FB_1 (in average 206.64 µg g^{-1}) in relation to *nit1* (27.27 µg g^{-1}) and NitM mutants (28.01 µg g^{-1}). A positive correlation between production of FB_1 and the effect on seed germination was found only for *nit1* mutants ($r = 0.41$ significant at $P_{0.10}$). Desjardins and Hohn (1997) working on the preliminary genetic analysis of *G. fujikuroi* mating type A (*F. verticillioides*) indicated a possible link between the ability of a fumonisin production and virulence expressed in kernels of maize.

Conclusion

F. proliferatum showed relatively high variability based on comparison of results of mating types, vegetative compatibility groups, pathogenicity and the production of FB_1. This can be explained by isolations of the pathogen from different locations in Serbia, as well as high genetic diversity within *F. proliferatum* species and its teleomorph *G. intermedia*. Genetic diversity of a fungal population is an important factor when developing a biocontrol strategy against that fungus. This is also necessary in order to develop management strategies in the prevention of

mycotoxin contamination, as well as the selection of maize genotypes resistant to Fusarium kernel rot.

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REFERENCES

Alizadeh A, Nikkhah MJ, Fotouhifar Kh, Rabiee Motlagh E, Rahjoo V (2010). Genetic Diversity of *Fusarium proliferatum* populations from maize, onion, rice and sugarcane in Iran based on vegetative compatibility grouping. Plant Pathol. J. 26: 216-222.

Biagi G (2009). Dietary supplements for the reduction of mycotoxin intestinal absorption in pigs. Biotechnol. Anim. Husb. 25(5-6): 539-546.

Burgess WL, Summerell AB, Bullock S, Gott PK, Backhouse D (1994). Laboratory Manual for Fusarium Research. 3rd Edition. Fusarium Research Laboratory, Department of Crop Sciences, University of Sydney and Royal Botanic Gardens, Sydney.

Doohan FM, Brennan J, Cooke BM (2003). Influence of climatic factors on *Fusarium* species pathogenic to cereals. Eur. J. Plant Pathol. 109: 755-768.

Correll JC, Klittich CJ, Leslie JF (1987). Nitrate non-utilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. Phytopathology, 77: 1640-1646.

Desjardins AE, Hohn MT (1997). Mycotoxins in plant pathogenesis. Mol. Plant Microbe Interact. 10: 147-152.

Domijan A-M, Peraica M, Cvjetković B, Turčin S, Jurjević Ž, Ivić D (2005). Mould contamination and co-occurrence of mycotoxins in maize grain in Croatia. Acta Pharm. 55: 349-356.

Elmer WH (1991). Vegetative compatibility groups of *Fusarium proliferatum* from asparagus and comparisons of virulence, growth rates, and colonization of asparagus residues among groups. Phytopathology, 81: 852-857.

Elmer WH, Summerell BA, Burgess LW, Backhouse D, Nigh EL (1999). Vegetative compatibility groups in *Fusarium proliferatum* from asparagus in Australia. Mycologia, 91: 650-654.

Fandohan P, Hell K, Marasas WFO, Wingfield MJ (2003). Infection of maize by Fusarium species and contamination with fumonisin in Africa. Afr. J. Biotechnol. 2(12): 570-579.

Gallardo-Reyes ED, Ibarra-Moreno GM, Sanchez-Mariner RI, Cuamea-Cruz G, Molina-Gil D, Parra-Vergava NV, Rosas-Burgos EC, Cortezocha MO (2006). Micobiota de maiz (*Zea mays* L.) recien cosechado y produccion de fumonisina B_1 por cepas de *Fusarium verticillioides* (Sacc.) Nirenb. Rev. Mexicana Fitopat. 24: 27-34.

Hossain SMJ, Khan M, Rahman MA, Hossain MA, Haque SS, Janardham GR (2002). Biodiversity study of *Fusarium* spp. on stored cereal grains in Karnataka State, India. Pak. J. Biol. Sci. 5: 446-448.

Klittich CRJ, Leslie JF (1988). Nitrate reduction mutants of *Fusarium moniliforme (Gibberella fujikuroi).* Genetics, 118: 417-423.

Leslie JF (1991). Mating populations in *Gibberella fujikuroi* (*Fusarium* section *Liseola*). Phytopathology, 81: 1058-1060.

Leslie JF (1993). Fungal vegetative compatibility. Annu. Rev. Phytopathology, 31: 127-150.

Leslie JF (1995). *Gibberella fujikuroi*: Available populations and variable traits. Can. J. Bot. 73: S282-S291.

Leslie JF, Klein KK (1996). Female fertility and mating type effects on effective population size and evolution in filamentous fungi. Genetics, 144: 557-567.

Leslie JF, Summerell BA (2006). The *Fusarium* Laboratory Manual. Blackwell Publishing, Iowa, USA.

Leslie JF, Zeller KA, Logrieco A, Mulé G, Moretti A, Ritieni A (2004). Species diversity of and toxin production by Gibberella fujikuroi species complex strains isolated from native prairre grasses in Kansas. Appl. Environ. Microbiol. 70: 2254-2265.

Lević J, Tamburić-Ilinčić LJ, Petrović T (1997). Maize kernel infection by *Fusaria* in the period 1994-1996. Cereal Res. Commun. 25: 773-775.

Lević J, Stanković S, Bočarov-Stančić A, Škrinjar M, Mašić Z (2004). The overview on toxigenic fungi and mycotoxins in Serbia and Montenegro. In: Logrieco A, Visconti A (ed.). An Overview on Toxigenic Fungi and Mycotoxins in Europe, Kluwer Academic Publishers, Dordrecht, Boston, London. pp. 201-218.

Lim S-H, Yun S-H, Lee Y-W (2001). Mating behaviour, mycotoxin production, and vegetative compatibility of *Gibberella fujikuroi* species complex from sorghum in Korea. Plant Pathol. J. 17: 276-280.

Logrieco A, Moretti A, Ritieni A, Bottalico A, Corda P (1995). Occurrence and toxigenicity of *Fusarium proliferatum* from preharvest maize ear rot, and associated mycotoxins, in Italy. Plant Dis. 79: 727-731.

Marasas WFO, Wehner FC, Van Rensburg SJ, van Schalkwyk DJ (1981). Mycoflora of corn produced in human esophageal cancer areas in Transkei, southern Africa. Phytopathology, 71: 792-796.

Mesterházy A (1984). A laboratory method to predict pathogenicity of *Fusarium graminearum* in field and resistance of wheat to scab. Acta Phytopathol. Acad. Sci. Hung. 19: 205-218.

Miller JD (2001). Factors that affect the occurrence of fumonisin. Environ. Health Perspect. 109: 321-324.

Miller JD, Savard ME, Schaafsma AW, Seifert K, Reid LM (1995). Mycotoxin production by *Fusarium moniliforme* and *Fusarium proliferatum* from Ontario and occurrence of fumonisin in the 1993 corn crop. Can. J. Plant Pathol. 17: 233-239.

Nelson PE, Plattner RD, Schackelford DD, Desjardins AE (1992). Fumonisin B1 production by *Fusarium* species other than *F. moniliforme* in section *Liseola* and by some related species. Appl. Environ. Microbiol. 58: 984-989.

Nelson PE, Toussoun TA, Marasas WHO (1983). *Fusarium* Species: An Illustrated Manual for Identification, Pensylvania State University Press, University Park, PA.

Pascale M, Visconti A, Pronczuk M, Wisniewska H, Chelkowski J (2002). Acumulation of fumonisins, beauvericin and fusaproliferin in maize hybrids inoculated under field conditiond with *F. proliferatum*. Mycol. Res. 106: 1026-1030.

Ritieni A, Fogliano V, Randazzo G, Scarallo A, Logrieco A, Moretti A, Mannina L, Bottalico A (1995). Isolation and characterization of fusaproliferin, a new toxic metabolite from *Fusarium proliferatum*. Nat. Toxins. 3: 17-20.

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.

Stanković S, Lević J, Petrović T, Logrieco A, Moretti A (2007). Pathogenicity and mycotoxin production by *Fusarium proliferatum* isolated from onion and garlic in Serbia. Eur. J. Plant Pathol. 118: 165-172.

Thiel PG, Shephard GS Sydenham EW Marasas WFO Nelson PE, Wilson TM (1991). Levels of fumonisin B_1 and B_2 in feeds associated with confirmed cases of equine leukoencephalomalacia. J. Agric. Food Chem. 39: 109-111.

Wang J, Zhou Y, Liu W, Zhu X, Du L, Wang Q (2008). Fumonisin level in corn-based food and feed from Linxian County, a high-risk area for esophageal cancer in China. Food Chem. 106: 241-246.