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FUNGI OF THE *FUSARIUM* GENUS IN THE GRAINS OF CONVENTIONAL HYBRIDS AND TRANSGENIC BT-HYBRIDS OF MAIZE (*ZEA MAYS* L.) IN THE CZECH REPUBLIC

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Abstract

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Fungi of the *Fusarium* genus, the agent of ear rot in maize, not only causes decrease in yields but also negatively affects grain quality and, in relation to mycotoxins production, the health of humans and animals. This study focuses on determining the species range of *Fusarium* fungi in naturally infected stands of conventional hybrids and transgenic Bt-hybrids of maize in the Czech Republic during 2008 and 2009. Individual species of the *Fusarium* genus were determined on the basis of morphological characteristics and using polymerase chain reaction. Ten mycotoxigenic species were identified in hybrid maize grains: *F. subglutinans* (40.4%), *F. graminearum* (19.8%), *F. verticillioides* (18.2%), *F. poae* (9.3%), *F. proliferatum* (4.0%), *F. avenaceum* (3.8%), *F. oxysporum* (1.7%), *F. sporotrichioides* (1.3%), *F. sambucinum* (1.3%) and *F. culmorum* (0.2%). The species *F. subglutinans*, *F. graminearum* and *F. verticillioides* were dominant in both years. The frequency of individual *Fusarium* species did not significantly differ between conventional and transgenic Bt-hybrids. Differences in species representation were determined between individual years and sites. The hypothesized markedly lower infection of individual Bt-hybrids with fungi of the *Fusarium* genus was not statistically significant, although most Bt-hybrids did demonstrate lower infection without that being statistically significant. The average level of infection by the *Fusarium* genus in 2008 was 13.2% for grains of conventional hybrids and 6.6% for Bt-hybrids (50% lower). In 2009, the average infection level was 13.6% for conventional hybrids and 12.6% for Bt-hybrids (7.4% lower). The average infection level for grains of Bt-hybrids by the species *F. subglutinans*, *F. graminearum* and *F. proliferatum* was lower than that for grains of conventional hybrids in both years.

maize, *Fusarium* spp., microbiological method, PCR

Maize (*Zea mays* L.) is an agricultural crop of worldwide importance grown not only for the food industry but for other purposes, as well. In the Czech Republic, it is mainly grown to produce animal feed, although interest in maize as a raw material for the production of biogas and bioethanol has increased in recent years. In 2011, maize for grain was planted on ca 109,600 ha. By comparison, 10 years ago grain maize was only planted on ca 54,300 ha. Mostly conventional hybrids are grown, although in recent years so-called transgenic Bt-hybrids (MON810) resistant to infestation by caterpillars of the European corn borer (*Ostrinia nubilalis* Hübner 1796) have also been introduced. In all European maize-growing areas, considerable losses are caused by infection of *Fusarium* fungi, resulting in red and pink rot (Bottalico, 1998; Logrieco *et al.*, 2002). Red rot is caused by *F. graminearum* Schwabe 1839 (teleomorph *Gibberella zeae* Schwein [Petch] 1936), *F. culmorum* (W.G. Sm.) Sacc. 1982 and *F. avenaceum* (Fr.) Sacc. 1886 (t. *G. avenacea* Cook, 1967), while pink rot is caused by the species *F. verticillioides* (Sacc.) Nirenberg 1976 (t. *G. moniliformis* [Sawada] Wollenw. 1931), *F. proliferatum* (Matsuch.) Nirenberg ex Gerlach &

Nirenberg 1976 (t. *G. intermedia* [Kuhlman] Samuels) and *F. subglutinans* (Wollenw. & Reinking) P. E. Nelson, Toussoun & Marasas 1983 (t. *G. subglutinans* [E.T. Edwards] P.E. Nelson, Toussoun & Marasas). Species causing both types of rot include *F. equiseti* (Corda) Sacc. 1886 (t. *G. intricans* Wollenw. 1930), *F. poae* (Peck) Wollenw. 1913, *F. sporotrichioides* Sherb., 1915, *F. solani* (Mart.) Sacc. 1881 (t. *Nectria haematococca* [Berk. & Broome] Samuels & Rossman 1999), and *F. oxysporum* Schltdl. 1824 (Logrieco *et al.*, 2002). Apart from decreasing yields (Qiang *et al.*, 2008), species of the *Fusarium* genus produce the secondary metabolites and mycotoxins trichothecene, zearalenone and fumonisin (Logrieco, 2003), which may cause serious acute and chronic diseases in humans and farm animals (D'Mello *et al.*, 1999). Predisposing factors for infection of maize by fungi of the *Fusarium* genus include stress (drought, waterlogging, etc.) and infestation by the European corn borer. Damage to maize by caterpillars of the European corn borer is a frequent cause of infection by fungi of the *Fusarium* genus (Munkvold *et al.*, 1997; Munkvold & Hellmich, 1999). One option for protection is to grow resistant Bt-hybrids, whose genome contains a gene from the soil bacteria *Bacillus thuringiensis* (Bt) which induces in the plant the production of a delta endotoxin that is lethal to certain insect pests (Magg *et al.*, 2002).

The objective of the study was to determine the incidence of fungi of the *Fusarium* genus in naturally infected stands of maize in the Czech Republic and the influence of conventional hybrids versus transgenic Bt-hybrids on the infection level of grains by *Fusarium* fungi in 2008 and 2009. Considering that transgenic Bt-hybrids are not damaged by the European corn borer, we hypothesized a significantly lower infection level for grains of Bt-hybrids versus conventional maize hybrids.

MATERIALS AND METHODS

In 2008 and 2009, we monitored the incidence of fungi of the *Fusarium* genus in the maize kernels of conventional and transgenic Bt-hybrids among naturally infected stands from locations with regular presence of the European corn borer. The sites (Čejč – district Hodonín, 183 m above sea; Hodonín – district Hodonín, 167 m above sea; Jiřice u Miroslavi – district Znojmo, 198 m above sea; Loštice – district Šumperk, 258 m above sea; Medlov – district Olomouc, 286 above sea; Otrokovice – district Zlín, 190 m above sea and Rostěnice – district Vyškov, 260 m above sea) were representative of the main grain maize growing area in the Czech Republic (Fig. 1). Both conventional hybrids and related transgenic Bt-hybrids were represented at each site. Sample collections (random 5 × 10 ears of each hybrid, regardless of symptoms) were made in the fully ripe growth stage (89 BBCH). Grains were disinfected on the surface (5% sodium hypochlorite for 60 s), washed twice with sterile distilled water, and placed into Petri dishes on potato-dextrose agar (PDA), always 10 pieces/Petri dish in five repetitions. Incubation was at laboratory temperature (20–24 °C). Isolation of pathogens from grains was performed between the fourth and seventh days, and pure cultures were grown from monosporic isolates for identification purposes on PDA or SNA (Spezieller nährstoffarmer Agar) for 10–25 d, at 20–24 °C in darkness. To differentiate individual *Fusarium* species, both the standard microbiological method and the molecular biological polymerase chain reaction (PCR) method were used.

Morphological characteristics of macro- and microconidia and characteristics of the fungal colony were primarily used for microbiological diagnosis of *Fusarium* spp. An optical microscope (Olympus BX41, magnification 200–400×) and stereomicroscope (Olympus SZX12) were used for identifying individual species. Determination was according to the identification keys of Leslie *et al.* (2006) and Samson *et al.* (1996). Accuracy of identification according to morphological characteristics of the five most prevalent species of the *Fusarium* genus was confirmed in a collection of selected isolates using PCR. DNA isolation was performed from 100 mg of fungus mycelia using a DNeasy Plant Minikit (Qiagene, Germany). The quality and quantity of DNA were determined using a spectrophotometer (NanoDrop 2000c). DNA purity was evaluated by comparing the 260/280 absorbance ratio. For DNA amplification, selected pairs of primers were used: JIAf/r for *F. avenaceum* (Turner *et al.*, 1998), Fg16F/R for *F. graminearum* (Nicholson *et al.*, 1998), Fp82F/R for *F. poae* (Parry & Nicholson, 1996), SUB1/2 for *F. subglutinans*, and VER1/2 for *F. verticillioides* (Mulè *et al.*, 2004). Sequences of the specific primers used are presented in Tab. I. A *Taq* PCR Core Kit (Qiagene, Germany) was used for PCR reactions. The reactions were run in a volume of 25 µl. For individual PCR reactions, reaction conditions for the thermal cycler (Apollo™ ATC401) and concentrations of reagents (buffers, nucleotides, primers, *Taq*-polymerase) were selected according to the aforementioned citations. Positive (standard) and negative (without template) controls were included into the reactions. The resulting DNA fragments were separated by horizontal electrophoresis (Consort EV265 power source, Cleaver MSMAXI 10 tank) on 1–1.5% tris-acetate-EDTA-agarose gel. Visualization of amplified DNA after colouring by 1% ethidium bromide was done using a UV-transilluminator (Photo/UV®21). A 100 bp ladder (Sigma) was used as the standard size for molecule fragments. In the case of positive reactions, gels were photo documented (Olympus C-765 UltraZoom).

Statistical evaluation of the differences in infection levels between conventional hybrids and Bt-hybrids was performed using analysis of variance and multiple comparison (Tukey's HSD; α = 0.05) in the UNISTAT 5.1 program (Unistat Ltd., London, UK). In the tables of results, each letter represents a different level of statistical significance where $p = 0.05$.

Infection level and species representation were calculated according to the following formulas:

$$\textit{Infection level} = \frac{\Sigma \textit{ grains infected by the given species}}{\Sigma \textit{ grains tested}} \times 100\ [\%]$$

$$\textit{Species representation} = \frac{\Sigma \textit{ isolates of the given species}}{\Sigma \textit{ isolates of the genus}} \times 100\ [\%]$$

RESULTS AND DISCUSSION

In 2008 and 2009, ten species of the *Fusarium* genus were isolated and subsequently identified: *F. graminearum*, *F. avenaceum*, *F. verticillioides*, *F. proliferatum*, *F. subglutinans*, *F. poae*, *F. sporotrichioides*, *F. sambucinum*, *F. oxysporum* and *F. culmorum* (Fig. 2).

In 2008, *F. subglutinans* (39.4%), *F. verticillioides* (22.8%), *F. graminearum* (20.5%) and *F. avenaceum* (10.5%) were isolated and determined from grains of conventional maize hybrids. Representation of other species ranged between 0.8% and 3.0%: *F. sporotrichioides* (3.0%), *F. proliferatum* (1.5%), *F. poae* (0.8%), *F. oxysporum* (0.8%) and *F. culmorum* (0.8%) (Fig. 3).

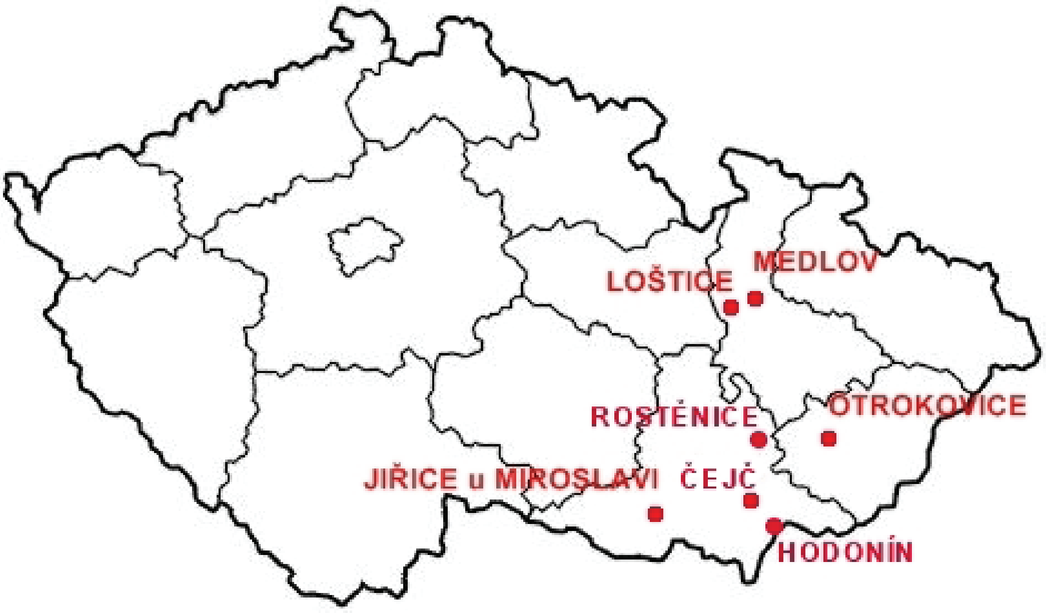
In the grains of transgenic hybrids in 2008, *F. subglutinans* (39.4%), *F. graminearum* (22.8%) and *F. verticillioides* (16.3%) were identified. Representation of other species was 1.5–4.7%: *F. poae* (4.6%), *F. avenaceum* (4.7%), *F. sambucinum* (4.7%), *F. oxysporum* (4.6%), *F. sporotrichioides* (1.5%) and *F. proliferatum* (1.5%) (Fig. 4).

In 2009, *F. subglutinans* (45.0%), *F. graminearum* (21.7%) and *F. verticillioides* (13.9%) were identified in the grains of conventional hybrids of maize. Also represented were *F. proliferatum* (7.5%), *F. poae* (6.4%), *F. avenaceum* (3.2%), *F. oxysporum* (2.2%), *F. sporotrichioides* (1.5%) and *F. sambucinum* (0.7%) (Fig. 5).

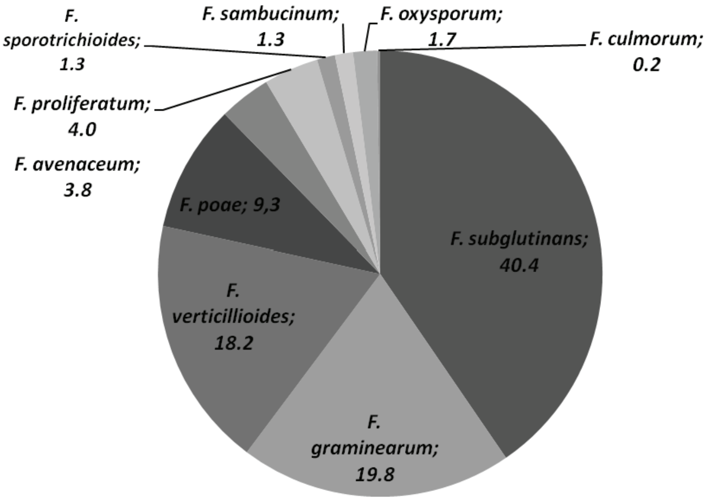
In the grains of transgenic hybrids in 2009, *F. subglutinans* (38.8%), *F. verticillioides* (19.6%), *F. graminearum* (16.2%) and *F. poae* (13.9%) were

I: *Sequences of specific primers used*

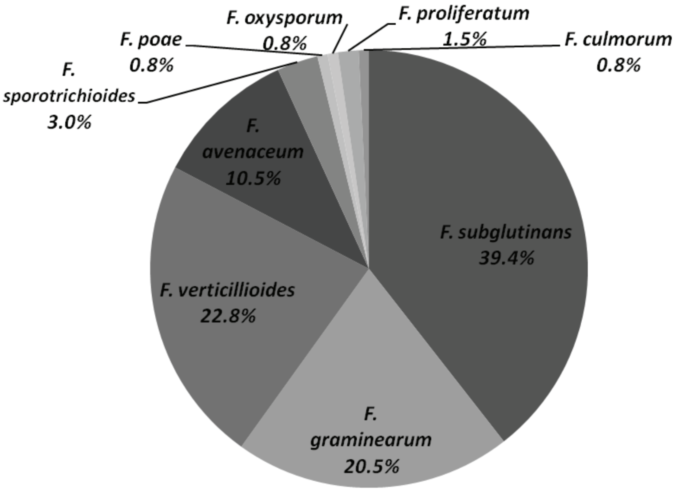
Type of pathogen	Primer name	Sequences of primers	Amplicon size (bp)
Fusarium subglutinans	SUB1 SUB2	5´-CTGTCGCTAACCTCTTTATCCA-3´ 5´-CAGTATGGACGTTGGTATTATATCTAA-3´	631
Fusarium verticillioides	VER1 VER2	5´-CTTCCTGCGATGTTTCTCC-3´ 5´-AATTGGCCATTGGTATTATATATCTA-3´	578
Fusarium graminearum	Fg16F Fg16R	5´-CTCCGGATATGTTGCGTCAA-3´ 5´-GGTAGGTATCCGACATGGCAA-3´	400–500 (variable)
Fusarium avenaceum	JIAf JIAr	5´-GCTAATTCTTAACTTACTAGGGGCC-3´ 5´-CTGTAATAGGTTATTTACATGGGCG-3´	220
Fusarium poae	Fp82F Fp82R	5´-CAAGCAAACAGGCTCTTCACC-3´ 5´-TGTTCCACCTCAGTGACAGGTT-3´	220



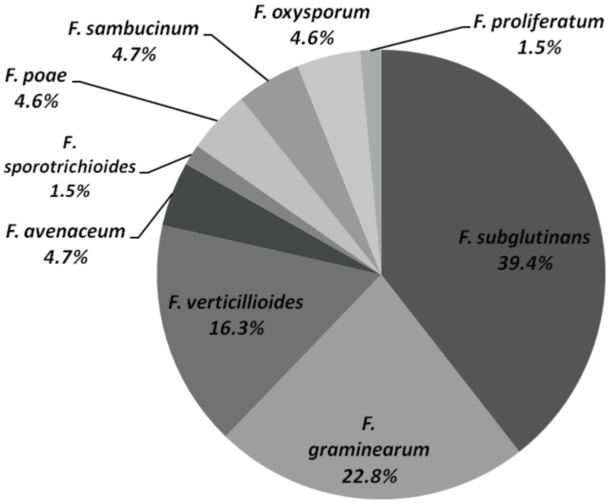
1: *Map of the localities of interest*



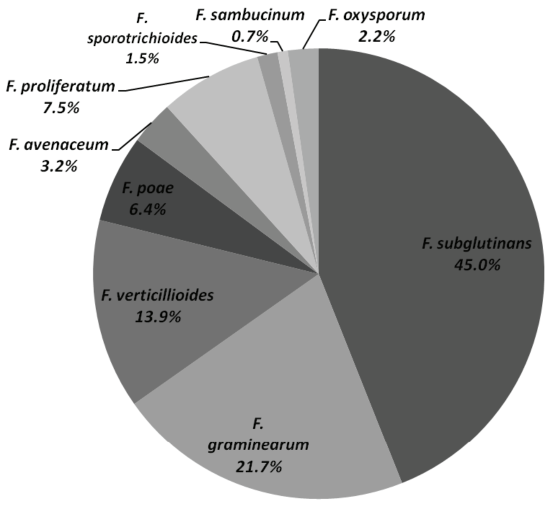
2: *Representation of species of the Fusarium genus isolated from grains of conventional and transgenic maize hybrids in 2008 and 2009*



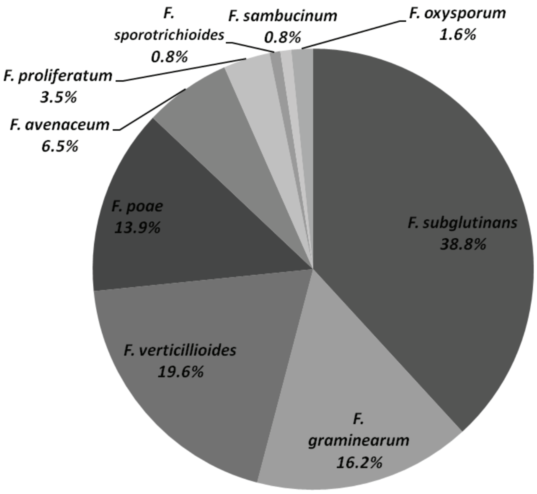
3: *Representation of species of the Fusarium genus isolated from grains of conventional maize hybrids in 2008*



4: *Representation of species of the Fusarium genus isolated from grains of transgenic Bt-hybrids in 2008*



5: *Representation of species of the Fusarium genus isolated from grains of conventional hybrids in 2009*



6: *Representation of species of the Fusarium genus isolated from grains of transgenic hybrids in 2009*

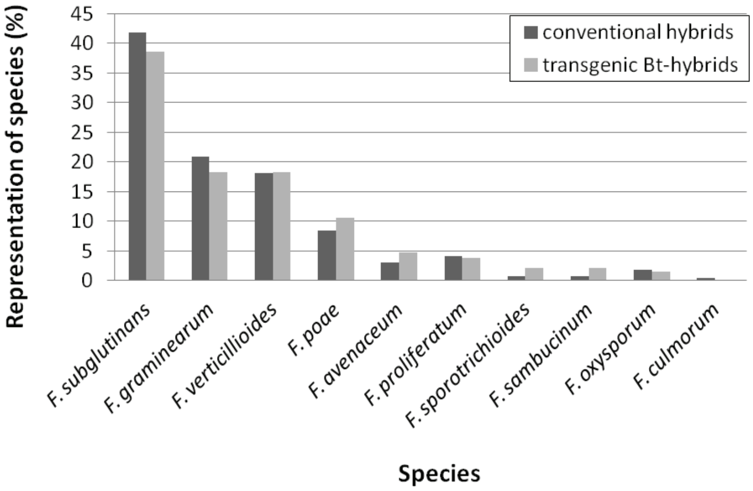
determined. Representation of other species was in the range 0.8–6.5%: *F. avenaceum* (6.5%), *F. proliferatum* (3.5%), *F. oxysporum* (1.6%), *F. sporotrichioides* (0.8%) and *F. sambucinum* (0.8%) (Fig. 6).

In both study years, *F. subglutinans*, *F. graminearum* and *F. verticillioides* were dominant species in grains of maize hybrids. Lew *et al.* (2001) and Logrieco *et al.* (2002) report that *F. subglutinans*, *F. verticillioides* and *F. graminearum* are among the most frequently isolated species on maize in Europe. Görtz *et al.* (2008) related a similar spectrum in their study as to the biological diversity of *Fusarium* spp. on maize grains in Germany. *F. culmorum*, which was determined only in 2008 in the grains of conventional hybrids (0.8%) is, according to Logrieco *et al.* (2002), one of the dominant species in Europe. In contrast, they report that *F. sambucinum*, which was identified only in the grains of Bt-hybrids in 2008 (4.7%), is a sporadically isolated species.

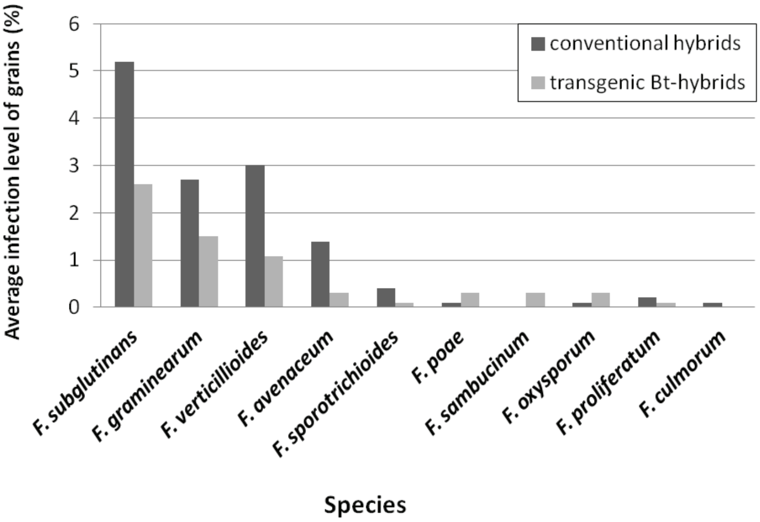
Representation of the individual *Fusarium* species in maize grains did not significantly differ between conventional and transgenic Bt-hybrids (Fig. 7).

Representation of the individual species in the genus did differ between years and locations. In 2008, the grains of transgenic hybrids had lower representation of the species *F. verticillioides*, *F. avenaceum* and *F. sporotrichioides*. In 2009, by contrast, *F. subglutinans*, *F. graminearum*, *F. proliferatum*, *F. oxysporum* and *F. sporotrichioides* were less represented. Meanwhile, *F. poae* was in both years more highly represented in the grains of transgenic hybrids. In 2008, *F. subglutinans* was dominant at the sites Čejč, Medlov, Otrokovice and Jiřice while *F. graminearum* was the dominant species at the Loštice site. In 2009, *F. subglutinans* was the dominant species at the sites Otrokovice, Hodonín and Čejč while *F. graminearum* was dominant at Rostěnice.

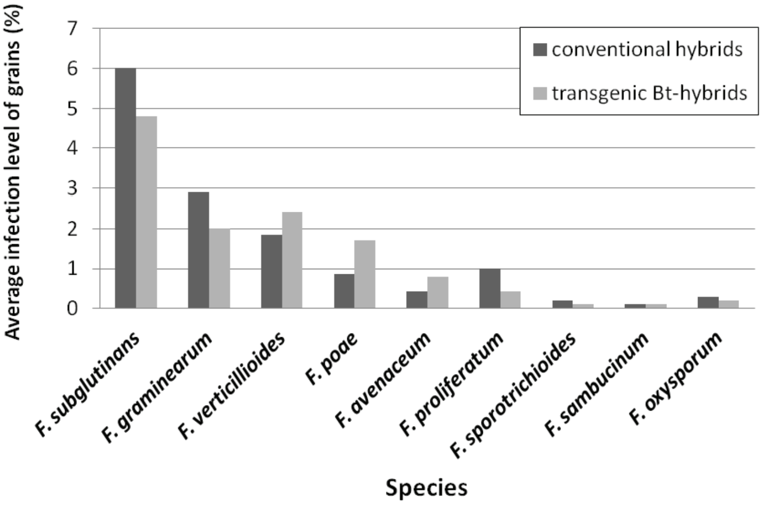
In 2008, the highest infection level of maize grains was recorded for the species *F. subglutinans*, which



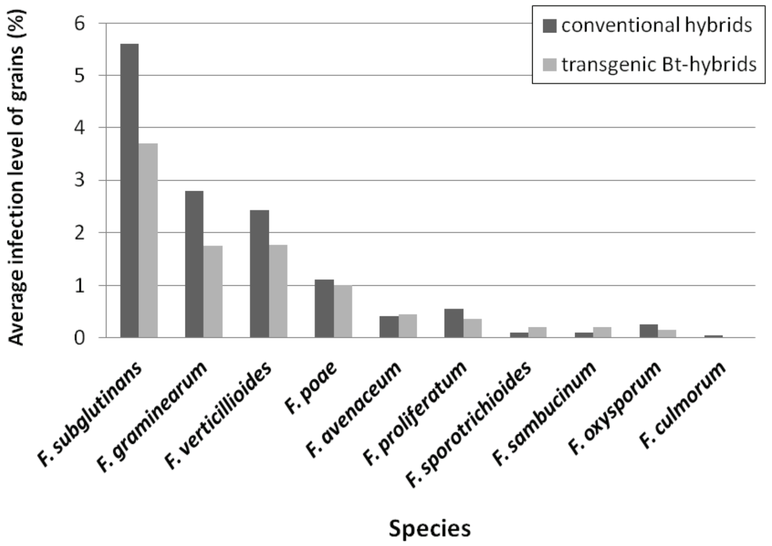
7: *Representation of species of the Fusarium genus isolated from grains of conventional and transgenic maize hybrids in 2008 and 2009*



8: *Average infection level of grains of maize hybrids by the Fusarium genus in 2008*



9: *Average infection level of grains of maize hybrids by the Fusarium genus in 2009*



10: *Average infection level of grains of maize hybrids by the Fusarium genus in 2008 and 2009*

II: *Infection level (averages) of grains of conventional (C) and transgenic (Bt) maize hybrids by fungi species of the Fusarium genus in 2008*

Hybrids/localities		Čejč	Medlov	Otrokovice	Loštice	Jiřice u Miroslavi
DKC 3511	C	6.0^{a}	10.0^{a}	28.0^{b}	18.0^{b}	14.0^{a}
DKC 3512 YG	Bt	6.0^{a}	6,0^{a}	10.0^{ab}	2.0^{ab}	6.0^{a}
DK 440	C	6.0^{a}	NT	NT	NT	20.0^{a}
DKC 4442 YG	Bt	2.0^{a}	NT	NT	NT	20.0^{a}
DK 315	C	NT	14.0^{a}	6.0^{a}	10.0^{ab}	NT
DKC 3946 YG	Bt	NT	4.0^{a}	2.0^{a}	2.0^{ab}	NT
DKC 3420	C	NT	18.0^{a}	14.0^{ab}	NT	NT
DKC 3421 YG	Bt	NT	2.0^{a}	2.0^{a}	NT	NT
PR38A24	C	2.0^{a}	NT	NT	NT	NT
PR38A25 YG	Bt	10.0^{a}	NT	NT	NT	NT

NT – not tested

III: *Infection level (averages) of grains of conventional (C) and transgenic (Bt) maize hybrids by fungi species of the Fusarium genus in 2009*

Hybrids/ localities		Otrokovice	Rostěnice	Hodonín	Čejč
DKC 3511	C	10.0^{a}	12.0^{a}	16.0^{a}	34.0^{a}
DKC 3512 YG	Bt	16.0^{a}	10.0^{a}	8.0^{a}	18.0^{a}
DKC 4490	C	4.0^{a}	14.0^{a}	16.0^{a}	14.0^{a}
NC 4702 KDDZ	Bt	16.0^{a}	4.0^{a}	30.0^{a}	16.0^{a}
DK 440	C	6.0^{a}	4.0^{a}	16.0^{a}	NT
DKC 4442 YG	Bt	2.0^{a}	12.0^{a}	10.0^{a}	NT
DK 315	C	4.0^{a}	2.0^{a}	NT	NT
DKC 3946 YG	Bt	4.0^{a}	8.0^{a}	NT	NT
DKC 3420	C	8.0^{a}	NT	NT	NT
DKC 3421 YG	Bt	18.0^{a}	NT	NT	NT

NT – not tested

infected 5.2% of grains of conventional hybrids and 2.6% of transgenic Bt-hybrids. *F. graminearum* infected 2.7% of grains of classic hybrids and 0.7% of Bt-hybrids. For the species *F. verticillioides*, the level of infected grains in classic hybrids was 3.0% and that in Bt-hybrids was 1.1%. In 2008, the grains of conventional and transgenic hybrids of maize were further infected by the species *F. avenaceum*, *F. poae*, *F. sambucinum*, *F. sporotrichioides*, *F. oxysporum*, *F. proliferatum* and *F. culmorum*. A lower average infection level of grains of Bt-hybrids in 2008 was determined for the species *F. subglutinans*, *F. graminearum*, *F. verticillioides*, *F. avenaceum*, *F. sporotrichioides* and *F. proliferatum*. The infection level of grains in the conventional and Bt-hybrids of maize in 2008 is presented in Fig. 8.

In 2009, the highest infection of maize hybrids was recorded for the species *F. subglutinans*, which infected 6.0% of grains of classic hybrids and 4.8% of transgenic Bt-hybrids. *F. graminearum* infected 2.9% of the grains of classic hybrids and 2.0% of Bt-hybrids. For *F. verticillioides*, the infection level of classic hybrids was 1.9% and that of Bt-hybrids 2.4%. In 2009, the grains of conventional and Bt-hybrids were further infected by the species *F. poae*, *F. avenaceum*, *F. proliferatum*, *F. sporotrichioides*, *F. sambucinum* and *F. oxysporum*. Lower average infection of transgenic Bt-hybrids in 2009 was determined for the species *F. subglutinans*, *F. graminearum*, *F. proliferatum* and *F. oxysporum*. The infection levels of grains in the conventional and Bt-hybrids in 2009 are presented in Fig. 9.

Using Tukey's HSD test in both years (Tabs. II and III), for the most part, no statistically significant differences were determined in the infection levels of grains of conventional and transgenic Bt-hybrids by the *Fusarium* genus, although most Bt-hybrids manifested lower infection without evidencing statistical significance (Fig. 10). Average infection level of grains of Bt-hybrids by the species *F. subglutinans*, *F. graminearum* and *F. proliferatum* was lower in both years than the infection level of grains of conventional hybrids (with a larger difference in 2008) (Figs. 8 and 9). Gatch & Munkvold (2002) had determined in Bt-maize, which is less susceptible to European corn borer, lower infection levels of the species *F. subglutinans* and *F. verticillioides* than in hybrids without the transgene. In contrast, for the species *F. graminearum* they proved higher infection level in Bt-hybrids. In 2008, the average infection level for grains of conventional hybrids by the *Fusarium* genus was 13.2% and that of Bt-hybrids was 6.6% (50% lower). In 2009, the average infection level of grains of the conventional hybrids by the *Fusarium* genus was 13.6% and that of Bt-hybrids was 12.6% (7.4% lower). Considering that transgenic Bt-hybrids are not damaged by the European corn borer, in contrast to conventional hybrids, we were expecting statistically significantly lower infection levels of their grains. While Munkvold & Hellmich (1999), Munkvold & Desjardins (1997), Munkvold *et al.* (1997) and Clements *et al.* (2003) state that ears of Bt-maize are significantly less infected by fungi of the *Fusarium* genus versus non-transgenic maize, Nelson & De'fago (2006), who detected fungi in maize stems, determined no differences between the conventional and Bt-hybrids. The species of the *Fusarium* genus may infect the maize during germination or after the plant has been damaged by birds (Reid, 1999). Maize grains may be infected by conidia growing through stigma of pistils, which are very sensitive during the first six days after formation (Reid & Hamilton, 1996; Munkvold *et al.*, 1997b). Infection via stigmata is important in the case of *F. verticillioides* and probably also of *F. proliferatum* and *F. subglutinans* (Munkvold *et al.*, 1997).

No statistically significant differences in the infection levels of grains of the maize hybrids were determined between the sites (data not shown).

CONCLUSION

In 2008 and 2009, ten species of the *Fusarium* genus were isolated and determined in the grains of maize using microbiological and PCR methods: *F. subglutinans* (40.4%), *F. graminearum* (19.8%), *F. verticillioides* (18.2%), *F. poae* (9.3%), *F. proliferatum* (4.0%), *F. avenaceum* (3.8%), *F. oxysporum* (1.7%), *F. sporotrichioides* (1.3%), *F. sambucinum* (1.3%) and *F. culmorum* (0.2%). The identified species were classified into five sections: *Liseola*, *Sporotrichiella*, *Discolor*, *Roseum* and *Elegans*.

The species *F. subglutinans*, *F. graminearum* and *F. verticillioides* were dominant in the grains of maize hybrids in both study years. In contrast, the species *F. culmorum*, *F. sambucinum*, *F. oxysporum* and *F. sporotrichioides* were isolated only sporadically.

Representation of individual species of the *Fusarium* genus did not significantly differ between conventional and transgenic Bt-hybrids. The representation of the species in the genus differed by year and site. In 2008, lower representation of the species *F. verticillioides*, *F. avenaceum* and *F. sporotrichioides* was determined in the grains of transgenic hybrids. In 2009, the species *F. subglutinans*, *F. graminearum*, *F. proliferatum*, *F. oxysporum* and *F. sporotrichioides* were less represented.

In 2008, the average infection level of grains of conventional hybrids by the *Fusarium* genus was 13.2% while that of Bt-hybrids was 6.6% (50% lower). In 2009, the average infection level of grains of conventional hybrids by the *Fusarium* genus was 13.6% while that of Bt-hybrids was 12.6% (7.4% lower). For the most part, no statistically significant difference was determined in the infection level of grains of conventional and transgenic Bt-hybrids by fungi of the *Fusarium* genus, even though most Bt-hybrids did demonstrate lower infection without that being statistically significant. The anticipated significantly lower infection of the individual Bt-hybrids by fungi of the *Fusarium* genus was not statistically confirmed. However, in the species *F. subglutinans*, *F. graminearum* and *F. proliferatum* lower average infection level of the grains of Bt-hybrids was determined in both years. The lower infection level of transgenic hybrids (although not statistically significant) can probably be explained by lower infection rate by caterpillars of the European corn borer, as the other infection methods (via stigmata, roots, damage by birds, etc.) are identical both for conventional and transgenic hybrids of maize. No statistically significant differences in the infection level of maize hybrid grains by the species of the *Fusarium* genus were determined between the sites.

As the harvested area of maize is today being extended, the potential for infection by *Fusarium* spp. is growing, and subsequent contamination of that production by mycotoxin presents a risk to health. Monitoring of mycotoxigenic fungi of the *Fusarium* genus is necessary not only to ensure good health of the plants themselves (for selecting suitable hybrids for planting) but also to protect the health of humans and farm animals by preventing contamination of the food chain by undesirable mycotoxins.

SUMMARY

The objective of the study was to determine the fungi of the *Fusarium* genus in naturally infected stands of maize in the Czech Republic and the influence of conventional hybrids versus transgenic Bt-hybrids on the infection level in grains by fungi of the *Fusarium* genus in 2008 and 2009. The individual species of *Fusarium* were determined on the basis of morphological characteristics and using PCR.

In the hybrid maize grains, ten mycotoxigenic species were identified: *F. subglutinans* (40.4%), *F. graminearum* (19.8%), *F. verticillioides* (18.2%), *F. poae* (9.3%), *F. proliferatum* (4.0%), *F. avenaceum* (3.8%), *F. oxysporum* (1.7%), *F. sporotrichioides* (1.3%), *F. sambucinum* (1.3%) and *F. culmorum* (0.2%). The representation of the individual species of the *Fusarium* genus did not significantly differ between the conventional hybrids and transgenic Bt-hybrids. Differences in species representation were determined between years and locations. The average infection level in grains of conventional hybrids by the *Fusarium* genus in 2008 was 13.2% and for Bt-hybrids it was 6.6% (50% lower). In 2009, the average infection level in grains of conventional hybrids was 13.6% and that in Bt-hybrids was 12.6% (7.4% lower). The expected significantly lower infection of the individual Bt-hybrids by fungi of the *Fusarium* genus did not prove to be statistically significant, although most Bt-hybrids did demonstrate lower infection without those differences being statistically significant.

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