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Fusarium species complex on maize in Switzerland: occurrence, prevalence, impact and mycotoxins in commercial hybrids under natural infection

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Abstract The *Fusarium* species complex of maize kernels and stem pieces as well as mycotoxin contamination of commercial grain maize hybrids for animal feed were evaluated in Switzerland. Throughout 2 years, natural *Fusarium* infection varied significantly between the years and the locations and it ranged from 0.4% to 49.7% for kernels and from 24.2% to 83.8% for stem pieces. Using the agar plate method, 16 different *Fusarium* species were isolated from kernels and 15 from stem pieces. The *Fusarium* species composition, prevalence and impact differed between the north and the south and between kernel and stem piece samples. The dominant species on kernels in the north were *F. verticillioides* (32.9%), *F. graminearum* (31.3%), *F. proliferatum* (7.3%) and *F. crookwellense* (7.1%), in the south *F. verticillioides* (57.1%), *F. subglutinans* (24.6%), *F. proliferatum* (14.8%) and *F. graminearum* (1.5%) and on stem pieces *F. equiseti* (36.0%), *F. verticillioides* (20.1%), *F. graminearum* (9.5%), *F. crookwellense* (6.2%) and *F. subglutinans* (6.2%). In the south, fumonisin concentration of most hybrids exceeded guidance values for animal feed. Other *Fusarium* species isolated were *F. avenaceum*, *F. culmorum*, *F. oxysporum*, *F. poae*, *F. sambucinum*, *F. semitectum*, *F. sporotrichioides*, *F. solani*, *F. tricinctum* and *F. venenatum*. Maize hybrids varied in their susceptibility to *Fusarium* infection. Because of the high diversity of *Fusarium* species encountered in Switzerland representing a high toxigenic potential, we propose to screen maize hybrids for resistance against various *Fusarium* species and examine maize produce for several mycotoxins in order to ensure feed safety.

Keywords Crop residue · Ear and stem rot · Monitoring · Seed health test · Trichothecenes · Zearalenone

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Introduction

Diseases of maize plants caused by *Fusarium* species occur worldwide, causing lodging (Ares et al. 2004), significant yield losses (Logrieco et al. 2002) and qualitative loss of the harvested produce through contamination with mycotoxins (Wu 2007). Of all agricultural commodities, maize is the one showing the greatest contamination with *Fusarium* mycotoxins (Munkvold 2003a) and therefore, its products are of major concern for animal (Wu 2007) and human health (CAST 2003). The best-characterised mycotoxins produced by *Fusarium* species are deoxynivalenol, zearalenone and fumonisins, which occur worldwide on maize (Munkvold 2003a). Further

Fusarium mycotoxins may be of increasing concern, such as fusaproliferin, beauvericin, enniatins and moniliformin (Jestoi 2008). All of these have been detected in maize ears in Europe (Logrieco et al. 2002). *Fusarium graminearum*, *F. verticillioides*, *F. proliferatum* and *F. subglutinans* are considered as major pathogens of maize (Leslie and Summerell 2006) and *F. verticillioides* is probably the most common pathogen on maize cobs throughout the world (Leslie and Summerell 2006). Other *Fusarium* species, while not considered as pathogens, colonise maize plants and both pathogenic and non-pathogenic *Fusarium* species may produce a wide range of mycotoxins (Leslie and Summerell 2006; Desjardins 2006). *Fusarium* species distribution and prevalence varies depending on geographical region, environmental conditions and the plant parts from which they are isolated. Overall, in Europe, *F. graminearum* and *F. subglutinans* seem to be more dominant in the northern, wetter areas and *F. verticillioides* in the drier, hotter areas (Botallico 1998).

Fusarium fungi infect all parts of maize plants (di Menna et al. 1997; Munkvold et al. 1997). They have various points of entry into the plant and can infect during the whole cropping season (Munkvold et al. 1997). Furthermore, *Fusarium* fungi survive on maize crop residues (Naef and Défago 2006; Cotton and Munkvold 1998). These residues were shown to be the primary source of inoculum for kernels of the subsequent maize crop (reviewed in Munkvold 2003b).

The epidemiology of *Fusarium* diseases is complex and multi-faceted (Munkvold 2003b; Schaafsma and Hooker 2007). Hence, it is not surprising that as of yet, no direct control measures for Gibberella ear rot, Fusarium kernel rot and stem rot are available. Furthermore, climatic factors (Stewart et al. 2002), physiological stress (Dodd 1980) and infestations by herbivores (Dowd et al. 2005) strongly influence the health status of maize plants. Consequently, prevention by cultural methods of *Fusarium* infection and subsequent mycotoxin contamination of maize is of limited success (Munkvold 2003a), although sources of resistance against *F. graminearum* (Vigier et al. 2001; Presello et al. 2006) and *F. verticillioides* (Afolabi et al. 2007; Presello et al. 2006) seem to be available.

Because of increasing health concerns, the European Union (2006/576/EC) and subsequently Switzerland (FIV 817.021.23) adjusted maximum limits for food and guidance values for feed with respect to *Fusarium* mycotoxins in maize and small-grain cereals. To fulfill these requirements in maize production, information on the occurrence and prevalence of the different *Fusarium* species in a given geographical area is crucial for predicting contamination risks. In addition, such knowledge is important for selecting appropriate *Fusarium* species in resistance screening and to improve the cropping system.

As of yet, no such information is available for Switzerland and other countries of central Europe. Therefore, the objectives of this study were (1) to monitor systematically at several sites the occurrence and prevalence of *Fusarium* species on maize kernels and stem pieces from commercial maize grown for animal feed in different areas of Switzerland under natural infection and (2) to determine the relationship between the incidence of *Fusarium* species and the mycotoxin content in maize kernels.

Materials and methods

Location of field sites

In 2005 and 2006, sampling was conducted at various locations in Switzerland representing different environmental conditions. Two field sites were located in the north-east at Hüntwangen (47°35′47″ N, 8°30′14″ E; 391 m above sea level (m.a.s.l.)) and Reckenholz (47°25′24″ N, 8°44′47″ E; 441 m.a.s.l.), one in the northwest at Goumoëns (46°39′17″ N, 6°36′93″ E; 628 m.a.s.l.) (sampling conducted in 2006 only) and one in the south of Switzerland at Cadenazzo (46°9′56″ N, 8°56′58″ E; 202 m.a.s.l.) (Fig. 1).

Weather data and climatic norm values

During both years, daily air temperature and precipitation from the site Reckenholz and Cadenazzo as well as the climatic norm values for mean temperature and amount of precipitation for both sites were recorded for the north and south of Switzerland by weather stations of the Federal Office of Meteorology and Climatology MeteoSwiss. Based on these data, daily mean temperature from seeding to harvest and from silking to harvest as well as the daily amount of

Fig. 1 Location of the field sites in Switzerland sampled for kernels (all sites) and stem pieces (Hüntwangen and Reckenholz only).

precipitation were calculated and the days with precipitation determined.

Maize hybrids sampled and field site management

The most commonly grown commercial maize hybrids were chosen from the Swiss National List (Menzi et al. 2006). The samples were collected in maize variety evaluation trials set up by the Agroscope research stations ART and ACW. At the field sites in the north (Hüntwangen, Reckenholz and Goumoëns), 14 maize hybrids were selected, including Anjou 209, Axxur, Birko, Goldenso, Dolmen, LG 22.22, LG 32.25, LG 32.45, PR39G12, Anjou 249, DCK 3420, LG 22.75, Eurostar and Benicia. In the south, the eight hybrids sown were PR35P12, PR38A24, PR36B08, PR38H20, PR38V12, PR37F73, Maxxis and Benicia. Maize was sown at a density of 6.5 to ten plants m^{-2}, depending on the maturation category of the hybrid. Experimental design was identical at each field site. Plots were arranged in a randomised complete block design with three replicates for each maize hybrid tested. Each experimental plot contained four rows. Rows were 4 m long and the space between rows was 0.75 m. The sowing date for Hüntwangen was 13 May 2005 and 16 May 2006, for Reckenholz 19 May 2005 and 22 May 2006, for Goumoëns 2 May 2006 and for Cadenazzo 6 May 2005 and 5 May 2006. The harvesting dates for Hüntwangen were between 19 October 2005 and 27 October 2005 and between 23 October 2006 and 31 October 2006; for Reckenholz between 17 October 2005 and 2 November 2005 and between 1 November 2006 and 6 November 2006; for Goumoëns on 2 November 2006; for Cadenazzo between 17 October 2005 and 18 October 2005 and on 11 October 2006. Soil management, fertilisation as well as pest and weed control were conducted according to agricultural practice for integrated production in Switzerland. Maize hybrids were exposed to natural *Fusarium* infection only.

Collection of maize ears and stalks

In both years, ears were sampled at physiological maturity of the hybrids. For each maize hybrid and replicate, ears of 30 plants in the two middle rows of each plot were handpicked. For a first assessment of *Fusarium* species growing in maize stalks, stalk samples of the maize hybrids Dolmen, LG 22.22, PR39G12, DCK 3420, LG 22.25 and Benicia were taken in 2006 at the sites Hüntwangen and Reckenholz. Ten stalks in the two middle rows of each plot were selected with every fifth of sixth stalk in a row and stalks were cut off at the base.

Kernels - establishment of working samples

For each maize hybrid and repetition, the 30 ears were threshed at the day of harvest (Geringhoff, Typ MRF 6609, Ahlen, Germany). A subsample of approximately 800 g was collected during the threshing process and dried to 14.5% moisture. With the aid of a riffle divider

(18 channels, Schieritz & Hauenstein AG, Arlsheim, Switzerland), each kernel sample was reduced to the working sample consisting of two replications of approximately 120 to 150 kernels for the agar plate method and 50 g for mycotoxin analysis.

Nodal and internodal pieces - establishment of working samples

Stalk samples were divided into nodal and internodal samples. For nodal samples, a cross-section of approximately 1.5 cm containing the first node counted from the aerial roots was cut at the day of harvest. The cross-section was cut into quarters and the two non-adjacent sections were dried. From the adjoining internode, a piece of approximately 1.5 cm from the middle of the internode was cut as described above.

Determination of the incidence of *Fusarium* species on maize kernels

Two samples each with 100 kernels per maize hybrid and repetition were plated at two different dates. At each date, 100 kernels were immersed for 10 min in a Chloramin T solution (1%) (Riedel-de Haën, Germany), sieved and then plated on a modified Nash-Snyder medium (NS) (Papavizas 1967), with five kernels per 90-mm Petri plate. The procedure was developed and validated in preliminary tests at ART (data not shown). The plates were incubated for 12 days at 19°C in the dark. Since it is not possible to distinguish different *Fusarium* species on the modified NS medium, all colonies resembling *Fusarium* species were transferred onto potato dextrose agar (PDA; Oxoid, Hampshire, Germany) as well as on 'Spezieller nährstoffarmer Agar' (SNA) containing a filter paper, prepared as described by Singh et al. (1991). Plates were incubated for at least 10 days at 19°C with alternating dark and NUV-light using a 12 h photoperiod. *Fusarium* species were identified using morphological characteristics according to Nelson et al. (1983) and Leslie and Summerell (2006).

Determination of the incidence of *Fusarium* species on nodal and internodal pieces

From each sample and repetition, 21 nodal and internodal pieces were surface-sterilised and plated on modified NS medium as described above for the kernel samples with three pieces per 90-mm Petri plate. The plates were incubated for 7 days at 19°C in the dark. All colonies resembling *Fusarium* species were transferred again onto modified NS medium and incubated for 5 days before transferring them onto PDA and SNA for identification as described above.

Analysis of mycotoxins

Deoxynivalenol, zearalenone and fumonisins from maize kernel samples from Cadenazzo were quantified with commercial ELISA kits (RIDASCREEN® DON, RIDASCREEN® Zearalenone and RIDASCREEN® Fumonisin (total of B1, B2, B3); R-Biopharm AG, Darmstadt, Germany). For this, the 50 g subsample was ground to a fine powder with a sample mill (Cyclotec, 1093, IG AG, Zurich, Switzerland). For each mycotoxin, a 5 g milled subsample was extracted according to the manufacturer's instructions. ELISA test kits were validated for maize with a standardised maize meal (Biopure, Tulln, Austria; Coring System Diagnostix GmbH, Gernsheim a. Rhein, Germany) with known mycotoxin content for each toxin. Detection limits of deoxynivalenol were 0.2 mg kg^{-1}, of zearalenone 0.017 mg kg^{-1} and of fumonisin 0.222 mg kg^{-1}, respectively. Mycotoxin analysis was conducted only for the samples in the south, as *Fusarium* infection levels in the north were substantially lower.

Data analysis

The relative occurrence of *Fusarium* species on maize kernels and stem pieces per site and year was calculated as a percentage of the total number of *Fusarium* isolates per site.

The total number of *Fusarium* species at each site was compared between the two years, using a one-way analysis of variance (ANOVA) for normally distributed data or a Kruskal–Wallis (KW) one-way ANOVA on ranks for not normally distributed data ($P<0.05$) (Zar 1996). The total number of *Fusarium* species in 2006 among the sites Hüntwangen, Reckenholz and Goumoëns was compared using a one-way ANOVA or a KW one-way ANOVA as described above.

To compare the total *Fusarium* infection of maize hybrids in the north over all sites and both years, data were pooled for each hybrid and thereafter ranked

according to infection level (one-way ANOVA on ranks).

For the samples from the south, effect of year, maize hybrid and repetition on the occurrence of total *Fusarium* species, *F. verticillioides*, *F. proliferatum*, as well as on the sum of the fumonisin producers *F. verticillioides* and *F. proliferatum* or fumonisin content was analysed with a three-way ANOVA ($P<0.05$). Thereafter, the data from the individual year were analysed separately with a two-way ANOVA ($P<0.05$). An all-pairwise multiple comparison procedure using the Holm-Sidak method ($P<0.05$) was performed to determine maize hybrids that differed significantly from each other during each year with respect to those parameters measured (Holm 1979). To determine whether the total incidence of the two fumonisin-producing species *F. verticillioides* and *F. proliferatum* would explain the level of total *Fusarium* species present or fumonisin content, the Spearman rank correlation coefficient was calculated. All statistical analyses were performed using SigmaStat 3.5 (Statcon 2006).

Results

A total of 60,000 maize kernels was analysed to determine the range of *Fusarium* species as well as their prevalence and impact. Overall, 9% of the grains sampled were infected with *Fusarium* species and a total of 5,620 *Fusarium* isolates was recovered. In the 1,523 stem pieces examined, a total of 846 *Fusarium* isolates was found. Sixteen different *Fusarium* species were isolated from maize kernels (Table 1) and 15 different *Fusarium* species from stem pieces (Table 2).

The most frequent *Fusarium* species on maize kernels in Switzerland was *F. verticillioides*. When the *Fusarium* populations from kernels were separated according to geographical areas of Switzerland, the predominant *Fusarium* species in the north were *F. verticillioides* (32.9%), followed by *F. graminearum* (31.3%), *F. proliferatum* (7.3%) and *F. crookwellense* (7.1%). In the south, *F. verticillioides* (57.1%) was dominant on maize kernels followed by *F. subglutinans* (24.6%), *F. proliferatum* (14.8%) and *F. graminearum* (1.5%) (Table 1). Throughout the two sampling years, *Fusarium* species prevalence from kernels varied at the different sites in the north (Table 1). At Hüntwangen in 2005, *F. graminearum* was the species most often observed, while in 2006, it was *F. verticillioides.* In contrast, at Reckenholz, the opposite was found. In the south, *F. verticillioides* clearly dominated over the other two species throughout both years. The mean infection level over all field sites and years ranged from as low as 0.4% up to 49.7%. In 2006, kernel infection was significantly higher than in 2005, both in the north and in the south of Switzerland ($P<0.05$). In 2006 in the north, *Fusarium* infection was highest at Goumoëns (25.6%) followed by lower infection levels at Hüntwangen (4.5%) and Reckenholz (2.6%) (all significantly different, $P<0.05$) (Table 1).

On stem pieces, the most frequent *Fusarium* species isolated were *F. equiseti* (36.0%), followed by *F. verticillioides* (20.1%), *F. graminearum* (9.5%), *F. crookwellense* (6.2%) and *F. subglutinans* (6.2%) (Table 2). Overall, infection of stem pieces was substantially higher than that of kernels. In Hüntwangen and in Reckenholz, 83.8% and 67.8% of the nodal pieces were infected by *Fusarium* species and in internodal pieces by 54.0% and 24.2%, respectively. In the nodal pieces, more *Fusarium* isolates were found and species diversity was higher compared with the internodal pieces (Table 2).

The less prevalent *Fusarium* species, both on kernels (Table 1) and stem pieces (Table 2) throughout all sites, were *F. avenaceum*, *F. culmorum, F. oxysporum, F. poae, F. sporotrichioides, F. sambucinum, F. semitectum, F. solani, F. tricinctum* and *F. venenatum*.

All maize hybrids in the north were infected with *Fusarium* species. Relative level of incidence ranged from 2.6% to 14.0% for the maize hybrids LG 22.75 and LG 32.25, respectively, as determined with the agar plate method. However, these infection limits were not significantly different from each other (data not shown).

At Cadenazzo, maize hybrids and sampling year significantly influenced total *Fusarium* infection (hybrid: $df=7$, $F=18.4$, $P<0.001$; year: $df=1$, $F=73.6$, $P<0.001$), infection by *F. verticillioides* (hybrid: $df=7$, $F=357.1$, $P<0.001$; year: $df=7$, $F=2402.4$, $P<0.001$), *F. proliferatum* (hybrid: $df=7$, $F=16.3$, $P<0.001$; year: $df=7$, $F=133.3$, $P<0.001$), sum of *F. verticillioides* and *F. proliferatum* (hybrid: $df=7$, $F=244.8$, $P<0.001$; year: $df=7$, $F=1871.5$, $P<0.001$), whereas the fumonisin content was only influenced by the sampling year and not the maize hybrid examined (hybrid: $df=7$,

Table 1 Relative incidence (%) and number of *Fusarium* species detected from maize kernels at four sites in Switzerland in 2005 and 2006

	Relative incidence (%) of *Fusarium* species						
	2005			2006			
	Hüntwangen[a]	Reckenholz[a]	Cadenazzo[b]	Hüntwangen[a]	Reckenholz[a]	Goumoëns[a,c]	Cadenazzo[a]
Species isolated							
F. avenaceum	0.9	6.3	0.1	5.0	1.0	0.9	0.1
F. crookwellense	3.5	0.0	0.8	7.4	11.0	13.6	0.2
F. culmorum	0.0	0.0	0.0	3.5	3.7	8.4	0.0
F. equiseti	7.8	0.0	1.3	9.6	1.4	2.8	0.6
F. graminearum	53.5	21.9	1.2	24.7	36.2	20.2	1.8
F. oxysporum	0.0	0.0	0.1	0.5	0.5	3.8	0.0
F. poae	5.2	0.0	0.1	0.5	0.5	6.4	0.4
F. proliferatum	7.8	12.5	15.6	3.5	8.3	4.2	14.0
F. sambucinum	0.0	0.0	0.0	0.8	0.0	0.2	0.0
F. semitectum	0.0	0.0	0.1	0.0	0.0	0.0	0.0
F. sporotrichioides	0.0	0.0	0.3	2.7	0.5	0.4	0.1
F. solani	0.9	0.0	0.1	0.5	0.5	0.0	0.0
F. subglutinans	0.0	9.4	36.0	9.3	1.4	9.5	13.2
F. tricinctum	0.0	0.0	0.0	0.3	0.0	0.2	0.0
F. venenatum	0.9	0.0	0.0	0.5	1.4	0.1	0.0
F. verticillioides	19.9	50.0	44.5	31.3	33.9	29.3	69.7
Mean incidence (%)	1.4	0.4	31.7	4.5	2.6	25.6	49.7
Number of isolates	116.0	32.0	1520.0	377.0	218.0	972.0	2385.0
Number of species	9.0	5.0	12.0	15.0	13.0	14.0	10.0

Incidence was assessed using the agar plate technique. The four *Fusarium* species dominating on maize kernel samples are highlighted.

[a] North of Switzerland

[b] South of Switzerland

[c] Goumoëns was sampled in 2006 only.

F=2.4, P<0.05; year: df=7, F=5.2, P<0.05) (data not shown). The effect of repetition was never significant.

When the data from Cadenazzo were analysed separately for each year, maize hybrids significantly differed in their total *Fusarium* infection (2005: df=7, F=12.3, P<0.001; 2006: df=7, F=10.7, P<0.001) (data not shown), infection by *F. verticillioides* (2005: df=7, F=57.6, P<0.001; 2006: df=7, F=445.8, P<0.001), *F. proliferatum* (2005: df=7, F=66.5, P<0.001; 2006: df=7, F=25.1, P<0.001), sum of *F. verticillioides* and *F. proliferatum* (2005: df=7, F=73.3, P<0.001; 2006: df=7, F=268.8, P<0.001), but not in fumonisin content (2005: df=7, F=1.3, P>0.05; 2006: df=7, F=0.8, P>0.05) (for 2005: Fig. 2a; for 2006: Fig. 2b). The two species *F. verticillioides* and *F. proliferatum* significantly determined total *Fusarium* infection (r=0.88; P<0.001) and fumonisin concentration (r=0.53; P<0.001) over both sampling years, however, this correlation was not significant when analysed for each year separately. The effect of repetition was never significant.

PR35P12 was the least susceptible maize hybrid with respect to *F. verticillioides* infection, whereas Maxxis was the most susceptible maize hybrid determined with the agar plate method. High concentrations of fumonisins were found in all maize kernels and both years in all maize hybrids, ranging from 2.5 to 22.8 mg kg^{-1} in 2005 and from 9.9 to 28.6 mg kg^{-1} in 2006 (Fig. 2a + b). However, in both years, fumonisin concentrations did not significantly differ among the hybrids. Levels of infection by the main producers of deoxynivalenol and zearalenone, namely *F. graminearum* and *F. crookwellense*, were low in both years (Table 1). Accordingly, these two mycotoxins were only detected in very low concentrations or were below detection level, except for PR38H20 in 2005 where deoxynivalenol and zearalenone concentrations were 1.4 and 0.2 mg kg^{-1}, respectively, and for PR35P12 in 2006 where deoxynivalenol

Table 2 Relative incidence (%) and number of *Fusarium* species detected from nodal and internodal stem pieces at two sites in Switzerland in 2006

	Relative incidence (%) of *Fusarium* species			
	Hüntwangen		Reckenholz	
	Nodes	Internodes	Nodes	Internodes
Species isolated				
F. avenaceum	10.7	0.0	11.2	1.7
F. crookwellense	4.5	0.5	14.8	5.0
F. culmorum	0.7	0.0	2.0	1.7
F. equiseti	34.4	25.5	32.8	51.3
F. graminearum	9.3	4.7	9.6	14.3
F. oxysporum	2.4	2.1	2.0	0.0
F. poae	0.3	1.0	0.4	0.0
F. proliferatum	1.4	10.9	2.8	0.8
F. semitectum	0.0	0.0	0.8	0.8
F. sporotrichioides	9.3	2.6	3.6	1.7
F. solani	0.0	0.5	0.0	0.0
F. subglutinans	4.5	16.7	2.0	1.7
F. tricinctum	3.8	0.0	0.4	0.0
F. venenatum	1.0	0.0	3.2	0.0
F. verticillioides	17.5	27.6	14.4	21.0
Mean incidence (%)	83.8	54.0	67.8	24.2
Number of isolates	290.0	187.0	250.0	119.0
Number of species	13.0	10.0	14.0	10.0

Incidence was assessed using the agar plate technique. The four *Fusarium* species dominating on stem pieces are highlighted.

and zearalenone were detected at 3.4 mg kg^{-1} and 0.4 mg kg^{-1}, respectively.

From flowering to harvest of the maize hybrids, mean daily temperature at Cadenazzo was approximately 3°C higher than at Reckenholz and the sum of precipitation was slightly lower in the north for both years (data not shown). These weather data are in accordance to the long-term norm values for the climate at the two experimental sites which were on average approximately 3°C warmer and received twice as much rain in the south compared with the north (data not shown).

Discussion

Maize was found to be the host of an extremely wide range of *Fusarium* species under natural infection at the sample sites examined in Switzerland. The occurrence, prevalence, diversity and impact of *Fusarium* species varied substantially between years, between field sites, between kernel and stem piece samples and between samples from the north and the south of the country. *Fusarium* species considered to be pathogenic to maize, namely *F. verticillioides*, *F. proliferatum*, *F. subglutinans* and *F. graminearum* all occurred in Switzerland, both on kernels and stem pieces.

Fusarium verticillioides was found at all sites investigated in Switzerland. In fact, it was the most prevalent species in the north and occurred at particularly high levels in the south of the country. This is in line with observations of Munkvold (2003b) and the statement of Leslie and Summerell (2006) that this *Fusarium* species is probably the most common pathogen on maize cobs. *Fusarium proliferatum* and *F. subglutinans* co-occurred with *F. verticillioides*, which is in line with observations by Botallico (1998) in Italy. However, *F. proliferatum* was also found in the north of Switzerland, which would support the earlier observation by Botallico (1998) that this species has spread northwards in Europe. *Fusarium graminearum* seems increasingly distributed from central to northern European areas (Botallico 1998), which corresponds to our study, as this species was the second most important species in the north and was present only at very low levels in the south. *Fusarium graminearum* could be displaced in maize

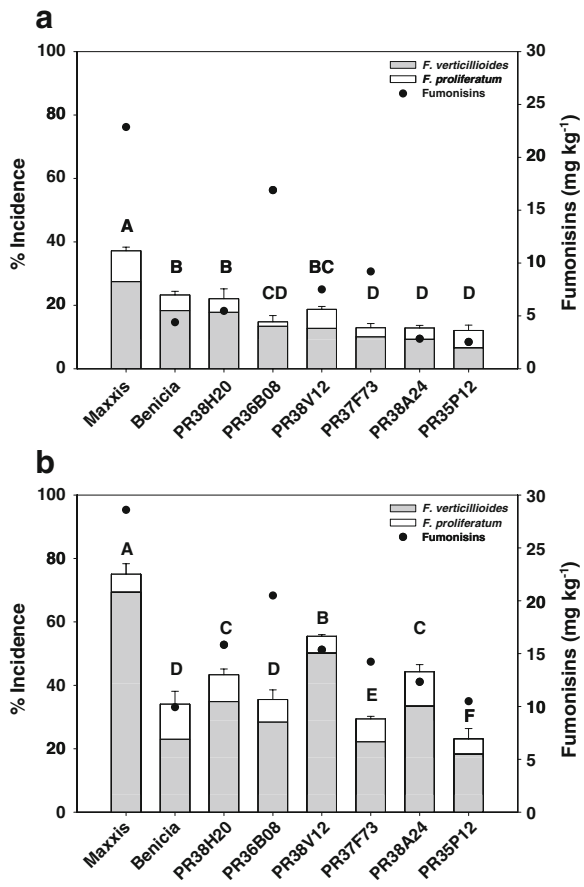

Fig. 2 Infection of maize hybrids in Cadenazzo in 2005 (**a**) and 2006 (**b**) with *Fusarium verticillioides* (*grey columns*) and *F. proliferatum* (*white columns*) and fumonisin concentration (mg kg^{-1}) (*black circles*). *Letters* indicate significant differences of incidence levels of the hybrids (two-way ANOVA; $P<0.001$). *Error bars* indicate the standard error of means for the sum of both species.

ears when *F. verticillioides* is present (Reid et al. 1999); however, this relationship was probably not the major component shaping the *Fusarium* species complex in the south in Switzerland. Most likely, the occurrence of *Fusarium* species in Switzerland seems to be influenced by climatic factors, as previously investigated by various researchers for other regions of the world. Generally, *F. verticillioides* is favoured by dry and hot conditions, whereas *F. graminearum* and *F. subglutinans* require cooler temperatures for optimal growth (Vigier et al. 1997). Additionally, *F. graminearum* is favoured by wetter conditions (Stewart et al. 2002). These observations, however, only partly fit with the results of our study.

In contrast to the observations that suggested that *F. verticillioides* occurrence in maize kernels is favoured by dry conditions or drought stress during grain-filling (reviewed by Munkvold 2003b), the amount and the number of days with precipitation from silking to harvest was similar in the northern and southern part of Switzerland. However, the mean temperature in the south was about 3°C higher than in the north. Since the temperature alone does not explain the different predominance of species in the north and the south, and after silking, wet weather followed and no drought stress occurred, we suppose that parameters other than weather parameters also have a strong influence on the prevalence of *Fusarium* species.

In our study, the diversity of *Fusarium* species and their incidence on freshly harvested maize stalks was high. *Fusarium equiseti* was the dominant species. More *Fusarium* species and higher *Fusarium* incidence were found in nodes compared with internodes on both trial sites. For maize, no adequate publication on *Fusarium* distribution on maize stalks with respect to node or internodes could be found; however, on wheat, recovery of *F. graminearum* was higher on the first node than on subcrown internodes (Salas and Dill-Macky 2004).

In Switzerland, a large number of additional *Fusarium* species to the ones mentioned above was detected from maize. All these species were already found to occur occasionally on maize in other parts of the world (Leslie and Summerell 2006). Most of these additional species are considered as saprophytes or opportunistic invaders of maize plants; however, they still cause yield losses and contaminate the harvested produce with mycotoxins. Therefore, with respect to the high number of different *Fusarium* species on maize, it is not surprising that of all agricultural commodities, maize is the crop showing the highest contamination with *Fusarium* mycotoxins (Chelkowski 1998; Munkvold 2003a). Subsequently, the number of different *Fusarium* metabolites occurring in maize is expected to be significantly greater than that encountered in wheat and other small-grain cereals. Furthermore, total amounts of *Fusarium* metabolites accumulating in maize kernels are about ten times higher than amounts present in wheat kernels when intensity of infection is similar and the same *Fusarium* species are involved (Chelkowski 1998). Based on information about *Fusarium* species and associated mycotoxins from Leslie and Summerell (2006) and

Desjardins (2006), the 16 *Fusarium* species identified in Switzerland are capable of producing 37 different mycotoxins or other fungal metabolites that could cause a great danger for human and animal health. Additionally, reports indicate that leaves and stalks of maize are even more contaminated with *Fusarium* mycotoxins than kernels (di Menna et al. 1997). This suggests that *Fusarium* infection is also of great concern for ensiled maize (Wilkinson 1999). For example, *F. equiseti* and *F. avenaceum* are capable of producing critical mycotoxins, such as the acute cardiotoxic moniliformin. Furthermore, deoxynivalenol (Mansfield et al. 2005), zearalenone (reviewed by Wilkinson 1999) and fumonisins (Mansfield et al. 2007) persisted in silage, although the *Fusarium* fungi themselves would not survive the ensiling process (Mansfield and Kuldau 2007).

Of special concern is also the co-production of different mycotoxins (e.g. Kubena et al. 1997; Speijers and Speijers 2004). Maize samples intended for animal consumption should be analysed for the occurrence of the two to four most prevalent *Fusarium* species and their toxins. Without the determination of the toxin producers, the samples should be analysed not only for deoxynivalenol, zearalenone and fumonisin, but also for other harmful mycotoxins, such as fusaproliferin (produced by *F. proliferatum*, *F. subglutinans*, *F. oxysporum*, and *F. sporotrichioides*), beauvericin (*F. equiseti*, *F. verticillioides*, *F. proliferatum*, *F. subglutinans*, *F. poae*, *F. oxysporum* and *F. sporotrichioides*), enniatins (*F. avenaceum*, *F. proliferatum*, *F. oxysporum*, *F. poae*, *F. sporotrichioides, F. tricinctum* and *F. venenatum*) or moniliformin (*F. subglutinans*, *F. proliferatum*, *F. avenaceum*, *F. equiseti*, *F. culmorum*, *F. oxysporum*, *F. semitectum*, *F. sporotrichioides* and *F. tricinctum*).

In the north of Switzerland, a vast range of commercial maize hybrids was chosen to analyse a wide genetic resistance pool. Unfortunately, the overall natural *Fusarium* infection level was too low to allow clear conclusions with respect to the susceptibility of maize hybrids. In contrast, in the south of Switzerland, maize hybrids were heavily infected by *F. verticillioides* and *F. proliferatum*. The ranking of the least and the most susceptible hybrids with respect to *Fusarium* infection was identical in both years, but the other hybrids varied somewhat in their ranking. Still, *Fusarium* incidence in kernels of the least susceptible hybrid was 18%, which is rather high as the resistance against *Fusarium* is of high agronomic relevance. All samples showed high content of fumonisins, with 13 out of 16 exceeding the guidance values of the European Union and Switzerland for animal feed of 5 mg kg^{-1} for pigs. This is in line with other studies that demonstrated that some hybrids react similarly with respect to *Fusarium* infection or fumonisin concentrations while others do not (Afolabi et al. 2007; Camargos et al. 2001). The mycotoxins deoxynivalenol and zearalenone only occurred at low limits in the south or could not be detected at all, which is not surprising as the *Fusarium* species responsible for their production only occurred at low frequencies or not at all.

No direct control measures for *Fusarium* diseases on maize are available and effective prevention strategies for crop rotations with maize and cereals are so far not available. Based on our results, we assume that breeding of resistant maize hybrids is the most advantageous measure to reduce *Fusarium* infection and the resulting mycotoxin contamination. Therefore, these data on natural *Fusarium* infection and *Fusarium* species occurrence and prevalence in Switzerland allow not only the identification of key *Fusarium* species, but are also a means to promote both the cropping of hybrids with low susceptibility to *Fusarium* species and resistance breeding in order to reduce risks emanating from infected maize kernels.

From this study, it is apparent that a great complexity of *Fusarium* species occurs on maize in Switzerland and all maize hybrids tested supported the growth of *Fusarium* species. We have shown that species composition and prevalence is different depending on geographical region and plant part analysed, and that infection of maize kernels and stems can be high. Pooled over both years and all sites, the order of *Fusarium* species prevalence was *F. verticillioides*, *F. subglutinans*, *F. proliferatum* and *F. graminearum* on kernels and *F. equiseti*, *F. verticillioides*, *F. graminearum*, *F. crookwellense* and *F. subglutinans* on stem pieces. With this information, we can estimate the most important *Fusarium* species that should be used for resistance screening of maize hybrids within Swiss growing environments, probably in a multi-species approach.

Further research is in progress to analyse samples with respect to *Fusarium* species and a

wide range of mycotoxins from large-scale maize hybrid trials and harvest samples from growers over different years. Additionally, agronomic factors which lead to *Fusarium* infection and mycotoxin contamination of maize under Swiss growing conditions will be evaluated in order to reduce mycotoxin risks.

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