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Toxigenicity and pathogenicity of *Fusarium poae* and *Fusarium avenaceum* on wheat

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Abstract In a field experiment between 2004 and 2006, 14 winter wheat varieties were inoculated with either a mixture of three isolates of *F. poae* or a mixture of three isolates of *F. avenaceum*. In a subsequent climate chamber experiment, the wheat variety Apogee was inoculated with individual single conidium isolates derived from the original poly conidium isolates used in the field. Disease symptoms on wheat heads were visually assessed, and the yield as well as the fungal incidence on harvested grains (field only) was determined. Furthermore, grains were analysed using LC-MS/MS to determine the content of *Fusarium* mycotoxins. In samples from field and climate chamber experiments, 60 to 4,860 μg kg^{-1} nivalenol and 2,400 to 17,000 μg kg^{-1} moniliformin were detected in grains infected with *F. poae* and *F. avenaceum*, respectively. Overall, isolate mixtures and individual isolates of *F. avenaceum* proved to be more pathogenic than those of *F. poae*, leading to a higher disease level, yield reductions up to 25%, and greater toxin contamination. For *F. poae*, all variables except for yield were strongly influenced by variety (field) and by isolate (climate chamber). For *F. avenaceum*, variety had a strong effect on all variables, but isolate effects on visual disease were not reflected in toxin production. Correlations between visual symptoms, fungal incidence, and toxin accumulation in grains are discussed.

Keywords *Fusarium* head blight · Isolate · Moniliformin · Monoacetoxyscirpenol · Nivalenol · Virulence

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Introduction

The fungal cereal disease complex *Fusarium* head blight (FHB), also called *Fusarium* scab of small grains, has been extensively studied over the last decades (e.g. Goswami and Kistler 2004). Epidemics caused by FHB pathogens result in severe yield losses and a decline in cereal quality. Furthermore, infections by these pathogens lead to contamination of grain and straw by a wide array of mycotoxins. These fungal metabolites pose serious threats to human and animal health (Bennett and Klich 2003).

In many cases, FHB is caused by several *Fusarium* species. In Europe, the predominant FHB pathogens are *F. graminearum* (teleomorph *Gibberella zeae*), *F. culmorum* (no teleomorph known), *F. avenaceum*

(teleomorph *G. avenaceae*), *F. poae* (no teleomorph known), as well as the non-toxigenic species *Microdochium nivale* (teleomorph *Monographella nivalis* var. *nivalis*) and *M. majus* (teleomorph *Monographella* (Glynn et al. 2005)) (Nicholson et al. 1997). Although *F. graminearum* is globally the most prevalent FHB-causing species, *F. poae* and *F. avenaceum* are repeatedly found in contaminated samples. In cereal surveys from Finland, Germany, Hungary, Ireland, Italy, and the UK, *F. avenaceum* and *F. poae* were among the most frequently isolated FHB pathogens (Birzele et al. 2002; Jestoi et al. 2004; Xu et al. 2005; Anonymous 2007). Furthermore, in a long-term trial conducted in Switzerland (Therwil, BL) comparing different agricultural production systems, *F. poae* was the most prevalent *Fusarium* species in wheat in 2003 (Gunst et al. 2005).

Fusarium poae has been reported to produce a number of trichothecene compounds, which are potent inhibitors of eukaryotic protein synthesis (Bennett and Klich 2003). These include the type A trichothecenes diacetoxyscirpenol (DAS), monoacetoxyscirpenol (MAS), scirpentriol (STO), HT-2 toxin, T-2 toxin, and neosolaniol (NEO), as well as the type B trichothecenes nivalenol (NIV) and fusarenone-X (FX) (Liu et al. 1998; Grabarkiewicz-Szczesna et al. 1999). In addition, beauvericin (BEA) and enniatins (ENNs) (Uhlig et al. 2006b), possessing insecticidal properties and toxicity to human cell lines (Calo et al. 2004; Ivanova et al. 2006), have been found.

Fusarium avenaceum has been associated with the production of moniliformin (MON) (Abramson et al. 2001), which causes muscular weakness and acute cardiotoxicity (Nagaraj et al. 1996), as well as BEA and ENNs (Abramson et al. 2001).

Compared with the trichothecene deoxynivalenol (DON) produced by *F. graminearum* and *F. culmorum*, NIV and the group A trichothecenes by *F. poae* as well as the non-trichothecene toxins by *F. avenaceum* display a far greater toxicity (Ueno et al. 1973; Burmeister et al. 1980). In Europe, the toxins produced by *F. poae* and *F. avenaceum* have been frequently found in various cereal samples (e.g. Uhlig et al. 2004; Schollenberger et al. 2006; Uhlig et al. 2006b).

Strategies to prevent FHB attack and mycotoxin contamination in cereals caused by *F. graminearum* and *F. culmorum* include inoculum reduction through crop rotation, optimised tillage and straw management, and the use of varieties with low susceptibility (Edwards 2004). A great deal of effort has been spent on developing and evaluating wheat and barley lines that are resistant to FHB and subsequent DON accumulation (e.g. Legge et al. 2004; Gosman et al. 2007). Highly significant correlations were observed between FHB severity, *Fusarium*-damaged kernels, yield loss, and DON accumulation (Haidukowski et al. 2005). In most cases, the resistance screenings are restricted to *F. graminearum* and *F. culmorum* and the results from these assays are not representative for the entire FHB complex. Few reports are available about particular resistance to attack by *F. poae* and *F. avenaceum* (Mesterhazy et al. 2005) or toxin accumulation by these two species in different wheat varieties (e.g. Brennan et al. 2007).

In breeding programmes and field studies where several varieties are tested for their reaction to a given pathogen, a mixture of isolates from the same species is often used. In the case of toxigenic *Fusarium* species, such a mixture might mask differences between isolates not only in their ability to cause disease but also in their potential to produce mycotoxins. A number of studies have shown that different isolates of the same *Fusarium* species can vary substantially in the concentration and type of toxin production both *in vitro* and *in planta* (e.g. Toth et al. 2004). Differences in the type of toxin production have been used to group *F. graminearum* in two different chemotypes producing primarily either DON and acetylated forms of DON or primarily NIV and/or FX (Miller et al. 1991). Furthermore, it was shown that DON represents a virulence factor for *F. graminearum* (Desjardins and Plattner 2003). Virulence is a component of pathogenicity and can be defined as the relative ability of a pathogen to cause damage on a host (Shaner et al. 1992). As of yet, no chemotypes of *F. poae* or *F. avenaceum* have been reported and the role of their toxins in pathogenesis has not been studied. We assume, however, that populations of these two species consist of individuals with variable toxigenicity.

The objectives of this study were (1) to examine in the field the responses of several Swiss wheat varieties to inoculation by a mixture of *F. poae* or *F. avenaceum* isolates and (2) to investigate in the climate chamber the effect of individual isolates on disease and mycotoxin variables on a single wheat variety.

Materials and methods

Fungal cultures and inoculum production

Stock cultures of poly and single conidium isolates of *F. poae* and *F. avenaceum* were maintained in 50% glycerol at −80°C. For both species, three isolates were used in this study. Their geographic origin and the hosts from which they were derived are shown in Table 1. All single conidium isolates were deposited in the public culture collection of the Fungal Biodiversity Centre, Centraalbureau voor Schimmelcultures (CBS), NL. Fungal inoculum for field and climate chamber experiments was produced by placing individual agar plugs with mycelium and conidia from stock cultures onto potato dextrose agar (PDA; CM0139, Oxoid Ltd., Basingstoke, UK) in Petri dishes (∅ 9 cm). After incubation for 6 days at 19±1°C with a photoperiod of 12 h dark/12 h near-UV light, the inoculum was multiplied by rinsing fungal cultures in each dish with 10 ml sterile deionised water and by distributing 0.2 ml of the resulting conidial suspensions with a glass rod onto plates containing substrate S agar (Messiaen et al. 1991). Plates were subsequently incubated for 5 to 7 days as described above. Conidial suspensions for inoculation of plants were obtained by washing each plate with tap water containing Tween 20 (0.0125%) using an air pressure (2 bars) device, filtering the resulting suspension through gauze, and adjusting to the desired conidial concentration.

Field experiment with mixtures of poly conidium isolates on several varieties

In 2003/2004, 2004/2005, and 2005/2006, a field experiment was conducted using 14 winter wheat (*Triticum aestivum*) varieties to assess the susceptibility to *F. poae* and *F. avenaceum* poly conidium isolates. The choice of varieties was according to the Swiss national catalogue of recommended varieties (classes Top, I, II, III, and biscuit) for the harvest year 2004, including Lona, Runal, Tamaro, Titlis, Segor, Zinal, Arolla, Arina, Levis, Galaxie, Asketis, Pegassos, Habicht, and Arbola. The experiments were carried out on the experimental farm of the Research Station Agroscope ART in Zurich-Reckenholz. The soil type was a loamy cambisol with 2.8% organic matter. Plot size was 1.3×5.5 m and wheat was drilled at 400 seeds per square metre. The sowing dates for the individual years were October 15, 2003; October 25, 2004; and October 12, 2005. Husbandry operations were standard for the farm except that no fungicides were applied. For inoculations of the wheat plants, mixtures of three poly conidium isolates from the respective *Fusarium* species were used. Treatments consisted of inoculations with *F. poae*, inoculations with *F. avenaceum*, as well as applications of water with Tween 20 (0.0125%), that served as the control treatment. The final suspensions contained 2.5×10^5 conidia per millilitre with equal amounts of each isolate within a mixture and were applied at a total volume of 730 l ha^{-1} using a back-pack sprayer (width 1.5 m, 3 bar, Birchmeier M125, Birchmeier Sprühtechnik AG, Stetten, Switzerland) covering the entire plot surface. Due to differing growth stages between wheat varieties at a given date and in order to ensure that for each variety at least one application was conducted during mid-anthesis (DC 65), inoculations were carried out three times for each variety. Weather data were obtained from the MeteoSwiss–operated weather station located at Zurich-Reckenholz approximately 0.5 to 1 km from the experimental site.

Table 1 Source and origin of fungal isolates

Fusarium species	Isolate	Host/variety	Geographic origin (community/canton)
F. poae	Fp 0335	Spring wheat, var. Greina	Solothurn/SO
F. poae	Fp 0338	Winter wheat, var. Arina	Belpberg/BE
F. poae	Fp 0378	Winter wheat, var. Pegassos	Seedorf/BE
F. avenaceum	Fa 0376	Winter wheat, var. Runal	Zurich/ZH
F. avenaceum	Fa 0379	Winter wheat, var. Arina	Montricher/VD
F. avenaceum	Fa 0380	Spring wheat, var. Greina	Zurich/ZH

All isolates originated from wheat grains, were collected in 2003, and have been deposited as single conidium isolates in the public culture collection of the Fungal Biodiversity Centre, Centraalbureau voor Schimmelcultures (CBS), NL: Fp 0335=CBS 121298, Fp 0338=CBS 121297, Fp 0378=CBS 121299, Fa 0376=CBS 121289, Fa 0379=CBS 121294, Fa 0380=CBS 121290.

Depending on the year and the variety, visual disease assessment was conducted in the field during two occasions after inoculation (15 to 25 days and 20 to 30 days post-inoculation – dpi, respectively) by counting all kernels or glumes with typical symptoms from 4×10 randomly selected wheat heads per plot (*F. poae*: distinct lesions or bleaching of individual kernels or glumes, often with a dark margin; *F. avenaceum*: bleached lesions, mostly covering entire spikelets, sometimes with adjacent salmon-coloured spore masses). For each variety, the average number of spikelets with three kernels was determined and disease was expressed as percent kernels with symptoms. Plots were combine-harvested on August 2, 2004, July 28, 2005, and July 21, 2006. Grains were passed through a grain cleaning machine (aspiration cleaner KF12, Kongskilde, Sorø, Denmark) to remove harvest by-products. Grain yield (t ha^{-1}) was determined at 15% moisture content and samples of 600 g were taken. To ensure a random distribution of different grain fractions, samples were further processed using a grain divider (sample splitter RT6.5, Retsch GmbH, Haan, Germany). Five grams were taken to determine the incidence (% infection) by *F. poae* and *F. avenaceum* using a seed health test (SHT; Hecker et al. 2004). To account also for infection by naturally present FHB species, incidence of the two locally most common species, *F. graminearum* and *M. nivale*, was determined as well.

For the SHT, grains were surface-sterilised (10 min, 1% chloramine T) and 100 grains were placed on PDA (10 grains $plate^{-1}$) and incubated for 6 days by 19±1°C with a photoperiod of 12 h dark/12 h near-UV light. Colonies of *Fusarium* spp. and *M. nivale* were identified according to Leslie and Summerell (2006). For assessment of the mycotoxin content, a sub-sample of 50 g was taken and finely ground with a sample mill (Model 1093, Tecator, Cyclotec). Fungal incidence in samples from control plots indicated very low levels of FHB-causing species, and only samples from inoculated plots were analysed for mycotoxin content.

Climate chamber experiment with individual single conidium isolates on Apogee spring wheat

Seeds of Apogee, a rapid maturing full-dwarf hard red spring wheat variety with high susceptibility to *F. graminearum* (Mackintosh et al. 2006), were pre-germinated on moist filter paper in plastic containers for 2 days at 10°C and subsequently for 3 days at 19°C in the dark. Three germinated seeds with emerged radicles between 3 and 4 cm in length were sown at a depth of 2 cm in pots (16 cm ∅, 13 cm height) containing a potting mix for herbaceous perennials ('Staudenerde', white peat 41%; bark humus 36%; expanded clay 20%, clay 3%, N/P=20:20; Obi-Ter, Märwil, Switzerland). Pots were placed in a growth chamber (Conviron, model PGV36, Controlled Environments Ltd., Winnipeg, Canada) at 21/16±1°C day/night temperature with a 14 h photoperiod (max 350 μmol m^{-2} sec^{-1}) and 70/85% RH during day/night, respectively. Before inoculation, plants were fertilised weekly with 5 ml Vegesan Standard per litre water and 100 ml per pot (N/P/K=80:70:80, Hauert, Grossaffoltern, Switzerland). After inoculation, plants were fertilised one more time. At DC 61–63, three heads per pot were assigned for inoculation and the selected heads were numbered and marked with threads. For inoculation, each pot was placed on a rotary disk to ensure even coverage of inoculum. For each pot, a 40 ml suspension containing 1×10^5 conidia ml^{-1} of each isolate was sprayed onto the three heads in one pot using a gravity spray gun (ITW DeVilbiss®). The appropriate conidial concentration for inoculation of Apogee was determined in preliminary trials to ensure that the disease expression was great enough to be measured and low enough to obtain enough grain material for mycotoxin analysis. Tap water with Tween 20 served as the control treatment. To ensure high humidity immediately after inoculation, pots were placed in saucers filled with water and covered for 48 h with polyethylene bags placed over metal frames and bags were tucked under the pots. During coverage, light was reduced to approximately 70 μmol m^{-2} sec^{-1} to avoid an increase in temperature under the bags. After removing the plastic bags and the saucers, light intensity was set back to the original conditions.

Visual disease assessment was conducted at 7, 11, and 14 dpi by counting the number of symptomatic kernels from each inoculated head. Using the average number of spikelets with three kernels for Apogee, disease was expressed as percent kernels with symptoms. For harvest, inoculated heads were cut at 10 weeks after sowing and weighed. Heads were individually threshed (single head threshing machine, Woods of Colchester LTD A.C. Fan, UK) and the

number of grains per head, the weight of the grains per head as well as the thousand kernel weight (tkw) were determined. Assessment of fungal incidence on grains with a SHT was not possible since all grain material was needed for mycotoxin analyses. For the latter, grains from the three heads per pot (2–5 g), including those from the control treatments, were finely ground with a coffee grinder (Mio*Star Beany, Migros, Switzerland).

Mycotoxin analysis

Ground grain samples were analysed at the Centre for Analytical Chemistry, Department for Agrobiotechnolgy, IFA-Tulln for *Fusarium* mycotoxins using an LC-MS/MS-based method for wheat, which has been described in detail (Sulyok et al. 2006). For the field experiment, samples inoculated with *F. poae* were analysed for NIV, T-2, HT-2, and DAS, whereas samples inoculated with *F. avenaceum* were analysed for MON. For the climate chamber experiment, all samples were additionally analysed for MAS, BEA, and ENNs A, A1, B, and B1. In brief, 0.5 g of sample was extracted for 90 min with a mixture of acetonitrile/water/acetic acid (79+20+1, *v*+*v*+*v*) on a rotary shaker. After centrifugation, the raw extract was diluted using acetonitrile/water/acetic acid (20+79+1, *v*+*v*+*v*) and directly injected into the LC-MS/MS instrument. Due to the wide range of concentrations of the target toxins, three different dilutions had to be prepared and analysed per sample: 1+1, 1+99 and 1+7,499 (*v*+*v*). Chromatographic separation was performed using an 1100 Series HPLC System (Agilent, Waldbronn, Germany) equipped with a Gemini® C_{18}-column, 150×4.6 mm i.d., 5 μm particle size, and a C_{18} 4×3 mm i.d. security guard cartridge (all from Phenomenex, Torrance, CA, USA) in gradient elution mode. Detection and quantification of the mycotoxins was performed with a QTrap 4000 LC-MS/MS System (Applied Biosystems, Foster City, CA, USA) equipped with a Turbo V Ion source for electrospray ionisation (ESI).

Quantification was performed using external calibration. Limits of detection were estimated in each sequence from signal-to-noise ratios (S/N) of calibration standard peaks at low concentration levels and corresponded to S/N=3:1. To verify that the analytical process was under control during the analysis of the inoculated cereal samples, blank matrix samples derived from the control treatments of the respective experiments were spiked with a defined mixture of the target toxins and analysed together with the inoculated samples of the respective test series. The recoveries for the spiked samples ranged between 80% and 112%. These findings were in good agreement with the validation results of the used analytical method (Sulyok et al. 2006). Results were not corrected for recoveries.

The toxin measurements were conducted over a period of several years; hence, due to differences in the sensitivity of the LC-MS/MS instrument, detection limits varied between the experiments and replicate trials. For toxins occasionally showing concentrations below detection limits, the ranges were as follows (in μg kg^{-1}): NIV, 10–80; T-2/HT-2, 4–10; MAS/DAS, 1–10; BEA, 60–240.

Experimental design and analyses

The field experiment investigating the response of various wheat varieties was performed throughout three consecutive years and was set up in a strip-plot design with fungal applications as the vertical treatments, varieties as the horizontal treatments and four replications. Varieties were arranged in strips across each replication. The climate chamber experiment examining the effect of different isolates was performed twice and set up in a completely randomised design with six replications (pots), with pooled results from three inoculated heads per pot. For each experiment, results from the replicate trials were pooled, since differences between the trials were not significant for all variables. Percentage data (disease symptoms, fungal incidence) were *arcsin (square root)* transformed, whereas yield and toxin content were *ln* transformed before analysis of variance (ANOVA). When the overall effect of the relevant factor was significant in ANOVA, an all-pairwise multiple comparison procedure according to Holm-Sidak (α= 0.05; Holm 1979) was performed in order to evaluate differences between treatment means.

In treatments where toxin concentrations were below the detection limit, analyses were first performed with the toxin concentration set to zero, half of the detection limit, and the full detection limit. Since the use of these diverse detection limit values did not lead to different results with respect to effect of variety or isolate, half the detection limit was used

for samples in which no toxin was detected. For the field experiment, linear regression was used to describe the relationship between disease symptoms, fungal incidence on grains, and different toxins. Disease symptoms were regressed on fungal incidence and on toxins, and fungal incidence was regressed on toxins. Regressions were computed with mean values of transformed data. For plotting of graphs, non-transformed data were used. All statistical analyses were performed using SigmaStat® 3.5 (Systat Software Inc., San Jose, CA, USA).

Results

Field experiment with mixtures of poly conidium isolates on several varieties

In 2004 and 2006, more precipitation occurred during wheat anthesis and thus conditions were presumably more favourable for fungal infection compared with 2005 (data not shown). We observed a strong year effect for *F. poae* as disease, fungal incidence, and toxin accumulation in grains were substantially greater in 2004 and 2006 compared with 2005 (Fig. 1). However, overall trends for the inoculations with respect to variety responses were the same. The

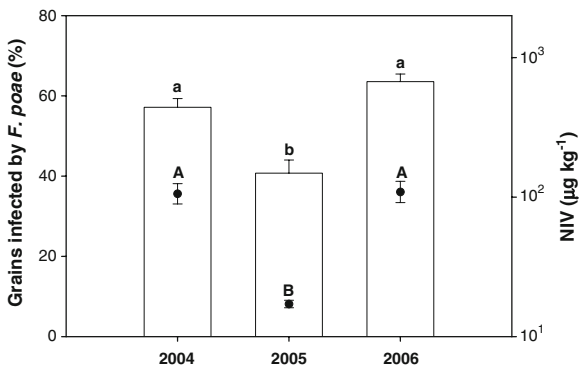

Fig. 1 Fungal incidence (*circles*) and production of NIV in wheat grains (*bars*) following inoculation with *Fusarium poae* during three consecutive years in a field experiment at Zurich-Reckenholz. Data represent means over all 14 winter wheat varieties. *Vertical bars* adjacent to the means indicate the standard error of the mean (SEM). For plotting of means and SEMs, non-transformed data were used; for significance letters, *arcsin*(*square root*)-(percentage data) and *ln*-(toxins) transformed data were used. Means with the *same letters* (*capital*, fungal incidence; *lower-case*, toxins) are not significantly different at α=0.05; *n.s.* not significant

14 varieties tested in this study varied strongly in terms of disease symptoms, fungal incidence on harvested grains, and toxin concentrations. Treatments with *F. poae* isolates resulted in fewer symptoms and a lower incidence on grains compared with those obtained with *F. avenaceum*.

In general, disease symptoms increased from the first to the second assessment date and only results from the second date are presented here. The mean percentage of kernels with symptoms in the non-inoculated control plots ranged from 0.1% to 0.4% (data not shown), whereas in the plots inoculated with *F. poae* and *F. avenaceum*, 0.3% to 4.3% and 3.6% to 15.7% of the kernels were visibly affected, respectively (Fig. 2a). For both *Fusarium* species, Arina showed the lowest and Pegassos the highest percentage of symptomatic kernels. The differences between varieties were highly significant for both species ($P<$ 0.001). The SHT revealed that the *Fusarium* spp. incidence on grains from non-inoculated control plots was very low, ranging from 0.1% to 4.3% for *F. poae*, 0.7% to 2.6% for *F. avenaceum*, and 0.3% to 1.9% for *F. graminearum*. In contrast, the non-toxigenic FHB species *M. nivale* showed higher prevalence between 2.5% and 14.3% (data not shown). Treatments with *F. poae* and *F. avenaceum* resulted in 5.5% to 49.1% and 68.7% to 91.8% infected grains, respectively (Fig. 2b). As with disease symptoms, differences between varieties in fungal incidence from inoculated plots were highly significant ($P<0.001$). For *F. poae*, the relationship of fungal incidence (fi) with visual disease assessment (vd) was best described by the following linear model: $\mathrm{fi} = 0.15 + 2.96\mathrm{vd}$, accounting for 57% of the total variation in fungal incidence (P=0.002). The standard error (SE) for the intercept (i) and the slope (s) were 0.09 and 0.74, respectively. The corresponding model for *F. avenaceum* was $\mathrm{fi} = 0.87 + 1.01\mathrm{vd}$, accounting for 51% of the total variation (P=0.004), with SE(i)=0.08 and SE(s)= 0.29. For *F. poae*, Galaxie was the variety with the lowest percentage of infected grains, whereas Arolla was highest. For *F. avenaceum*, Titlis and Arbola were the varieties with the lowest and the highest levels of fungal incidence, respectively.

Pooled over all varieties, the effect of *F. avenaceum* inoculations on yield was highly significant ($P<$ 0.001), whereas *F. poae* applications had no effect ($P=$ 0.66). The mean yield (t ha^{-1}) was 6.15 (ranging from 5.37 to 7.55) from control plots, and 6.20 (5.36 to

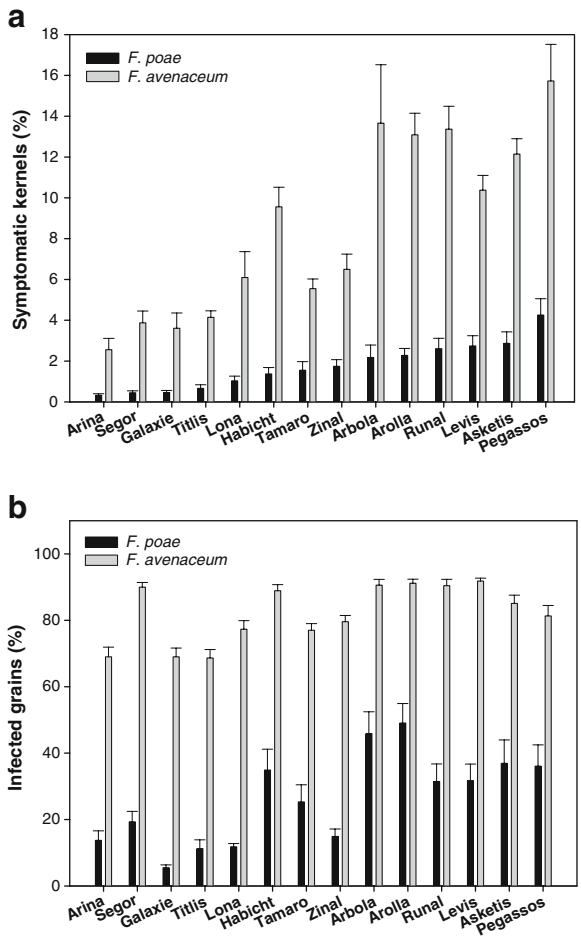

Fig. 2 Susceptibility of 14 winter wheat varieties to *Fusarium poae* or *Fusarium avenaceum* in a field experiment at Zurich-Reckenholz pooled over data from 2004 to 2006. **a** Disease symptoms based on a visual assessment. **b** Incidence of infected grains based on a seed health test. *Columns* represent means from individual varieties. Depiction and statistics as in Fig. 1

7.52) for those treated with *F. poae*. For *F. avenaceum*, mean yield was 5.44 (4.29 to 6.27), corresponding to an average yield reduction of 11.3%, ranging from 3.8% for the var. Galaxie to 25.3% for Arolla.

Nivalenol was detected from almost all plots inoculated with *F. poae* and differences between the varieties were highly significant ($P<0.001$). Mean values for individual varieties were highly variable with concentrations between 58.4 and 1,560 μg kg^{-1}. Detection of HT-2 was inconsistent and concentrations were low, ranging from below the detection limit to 19.7 μg kg^{-1}. T-2 was detected in only three samples throughout the experimental years. No DAS was detected in any of the samples. Samples from plots that were inoculated with *F. avenaceum* isolates contained substantial concentrations of MON from 2,350 to 14,700 μg kg^{-1} and differences between varieties in toxin production were highly significant ($P<0.001$).

Toxin content could be predicted well by the incidence of *F. poae*-infected grains with $r^2=0.90$ for NIV and $r^2=0.72$ for HT-2 (Fig. 3a). The linear models were as follows: NIV = 1.90 + 6.51fi, $P<0.001$, SE(i)=0.33, SE(s)=0.63; HT-2 = 0.76 + 1.89fi, $P<$

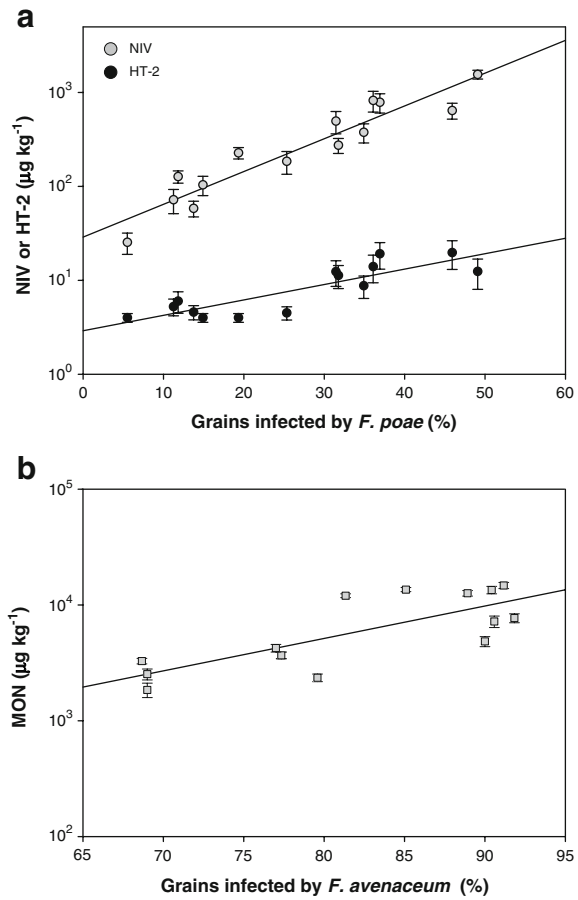

Fig. 3 Relation between the incidence of *Fusarium poae* or *Fusarium avenaceum* in grains and their toxins as observed in samples from a field experiment at Zurich-Reckenholz from 2004 to 2006 with 14 winter wheat varieties. *Circles* (NIV, HT-2) or *squares* (MON) represent means from individual varieties pooled over 3 years. **a** Relation between the incidence of *F. poae* and the NIV and the HT-2 content in grains. **b** Relation between the incidence of *F. avenaceum* and the MON content in grains. Regression equations are given in the text. Depiction and statistics as in Fig. 1

0.001, SE(i)=0.18, SE(s)=0.34. Prediction based on disease symptoms was less accurate (NIV = 2.82+ 20.10vd, r^2=0.56, P=0.002, SE(i)=0.64, SE(s)=5.19; HT-2 = 0.92 + 6.76vd, r^2=0.60, P=0.001, SE(i)= 0.20, SE(s)=1.60). For *F. avenaceum*, the trend was different with a higher correlation between disease symptoms and MON (MON = 6.43 + 7.87vd, r^2= 0.77, P<0.001, SE(i)=0.36, SE(s)=1.25) than between incidence of infected grains and MON (MON = 3.03+ 4.87fi, r^2=0.59, P=0.001, SE(i)=1.36, SE(s)=1.17; Fig. 3b).

Climate chamber experiment with individual single conidium isolates on Apogee spring wheat

The wheat var. Apogee proved to be susceptible to all isolates of *F. poae* and *F. avenaceum*. Disease symptoms increased during the assessment period from 7, 11, to 14 dpi, independently of the isolates used (data not shown). For *F. poae*, inoculation with Fp 0335 resulted, for all assessment dates, in significantly (P<0.001) less symptoms compared with Fp 0338 and Fp 0378 (Fig. 4a). Inoculations with Fp 0335 did not only lead to fewer symptomatic kernels, but this isolate also resulted in substantially smaller lesions compared with the other two isolates (Fig. 5). Between *F. avenaceum* isolates, differences were not significant during the first two assessment dates (7 and 11 dpi); however, at 14 dpi, plants treated with Fa 0376 showed significantly (P=0.007) more symptoms compared with Fa 0379 and Fa 0380 (Fig. 4b). Sizes of individual lesions or bleached areas were comparable among the isolates.

None of the *F. poae* isolates had a significant effect on any of the yield variables. In contrast, all *F. avenaceum* isolates resulted in substantial reductions in ear weight (data not shown), grain weight per ear, and tkw with no differences between the isolates (Fig. 6). None of the fungal inoculations had an effect on the number of grains per ear (data not shown).

All *F. poae* isolates produced mycotoxins in grains of Apogee. NIV was detected in concentrations greater than those observed in field samples (mean amounts of isolates ranging from 946 to 4,860 μg kg^{-1}) and MAS was also consistently found (59 to 145 μg kg^{-1}). DAS (4.8 to 6.6 μg kg^{-1}) was only produced in one out of the two trials, whereas BEA was detected in both trials but only in a single sample from isolate Fp 0338 (data not shown). Mean concentrations of NIV and MAS

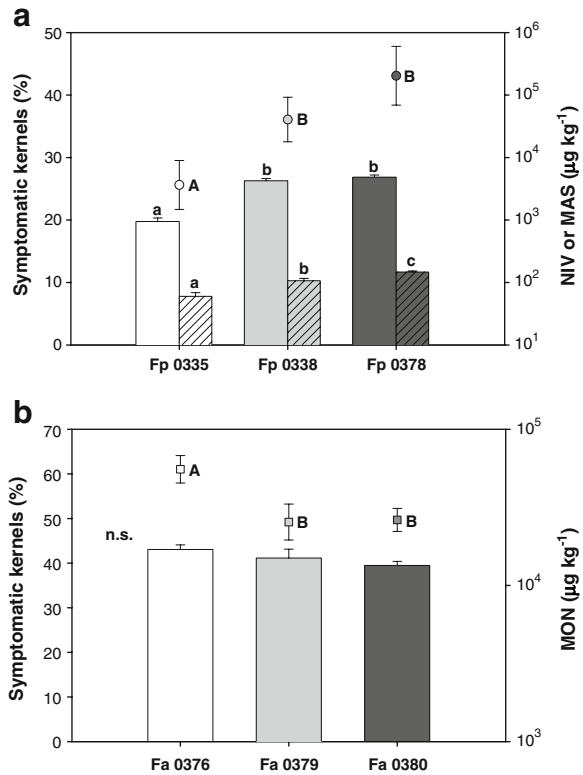

Fig. 4 Effect of different *Fusarium* isolates on disease symptoms at 14 dpi (*circles* or *squares*) and toxin production (*bars*) in grains of the wheat var. Apogee in a controlled environment. **a** Symptoms caused by *F. poae* and NIV (*plain bars*) and MAS (*hatched bars*) in grains. **b** Symptoms caused by *F. avenaceum* and MON in grains. Depiction and statistics as in Fig. 1

from the individual isolates correlated closely with observed disease symptoms: Fp 0335 proved to be the lowest-yielding isolate for both toxins and the differences with respect to Fp 0338 and Fp 0378 were highly significant (P<0.001; Fig. 4a). The production of MAS demonstrated significant differences between all three isolates and they could be ranked according to increasing concentrations with Fp 0335<Fp 0338<Fp 0378 (Fig. 4a).

All *F. avenaceum* isolates produced MON (mean concentrations ranging from 13,400 to 17,000 μg kg^{-1}) in grains of Apogee as well as ENNs A, A1, B, and B1 (sum of ENNs from 65,700 to 129,000 μg kg^{-1}). Since ENNs were also found in non-inoculated control plants in the first replicate trial, these toxin data were not further analysed. In contrast to NIV and MAS produced by *F. poae*, MON produced by *F. avenaceum* was not correlated with disease symptoms. Although Fa 0376 was the isolate resulting in significantly higher

Fig. 5 Differences in symptom development on the wheat var. Apogee after inoculation of the *Fusarium poae* isolates Fp 0335 and Fp 0338 in a controlled environment

numbers of symptomatic kernels compared with the other two isolates, no differences between isolates were observed in MON concentrations (P=0.22; Fig. 4b).

Discussion

The pathogenicity and toxigenicity of *F. poae* and *F. avenaceum* were investigated in the field with mixtures of poly conidium isolates inoculated onto 14 winter wheat varieties. From the poly conidium isolates, single conidium isolates were derived and tested individually on the spring wheat var. Apogee under a controlled environment.

In the field, we observed a strong year effect for *F. poae*, leading to different levels of disease and toxin contamination. However, results for the susceptibility of the varieties to this fungus were similar. The isolate mixture of *F. avenaceum* proved to be more pathogenic than that of *F. poae*, leading to greater disease levels and higher toxin accumulation. In particular, inoculations with *F. avenaceum* significantly reduced the yield in most varieties whereas *F. poae* infection had no effect on yield. Similar results have been found in a controlled environment study with barley, demonstrating the greater pathogenicity of *F. avenaceum* compared with that of other FHB species including *F. poae* (Xue et al. 2006).

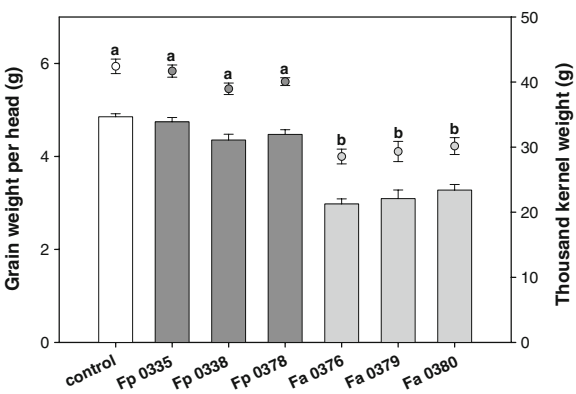

Fig. 6 Effect of different *Fusarium poae* and *Fusarium avenaceum* isolates on grain weight (*bars*) and thousand kernel weight (*circles*) of the wheat var. Apogee following inoculation in a controlled environment. Depiction and statistics as in Fig. 1

For both *Fusarium* species, the wheat varieties differed substantially in their reactions to the variables observed, which is in line with other reports where the susceptibility of cereal varieties to different *Fusarium* species was investigated (e.g. Brennan et al. 2007). In the current study, the var. Pegassos showed at least 10 times more kernels with symptoms than the var. Arina upon inoculation with *F. poae*. For the more pathogenic species *F. avenaceum*, this ratio was still five to one. With respect to *F. poae* incidence on grains, 10-fold differences between the highest and the lowest infection levels were observed. In contrast, for *F. avenaceum*, potential differences were masked by the overall high level of infection of grains in all varieties, ranging between 70% and 90%. Hence, a lower conidial concentration than the one used in this study (2.5×10^5 ml^{-1}) might be more appropriate to reveal differences in variety responses to this species.

The fact that Arina was one of the best performing varieties in terms of low susceptibility towards these

two species parallels our previous findings with respect to the response to *F. graminearum* (Hecker et al. 2004). Moreover, Doohan et al. (2000) identified antifungal activity in wheat grain extracts from Arina against *F. culmorum*.

Toxin concentrations in grains where plants are artificially inoculated might not reflect the situation in commercial grain fields; however, the span between 60 and 1,500 μg kg^{-1} NIV or 2,400 and 15,000 μg kg^{-1} MON in grains underlies the tremendous impact of varieties on toxin contamination. Resistance to *F. culmorum* and *F. graminearum* has been grouped into three different components, including (I) resistance to penetration, (II) resistance to colonisation, and (III) mechanisms that influence the DON content in grains (Snijders 2004). Such components could also be applicable to the *Fusarium* species from the current study and might explain the rather low correlation between disease symptoms (components I and II) and final toxin content (III).

Pooled over all varieties and all years, the NIV content and *F. poae* incidence in grains were highly correlated with an r^2 of 0.90. Other publications have reported similar results for *F. graminearum* incidence and DON content (Browne 2007). The correlation between fungal incidence and toxin was lower for HT-2 (r^2=0.72). We assume that this is due to the low production of HT-2, often close to or below the detection limit.

The correlation coefficient between *F. avenaceum* incidence and MON content was rather low (r^2= 0.59). It is possible that high concentrations of MON can be produced even with a lower prevalence of the fungus in the plant. However, this finding might also be due to the overall high infection rate by *F. avenaceum* starting at 70%. Hence, similar to the variety effects discussed above, a lower inoculum dose could improve the correlation.

In the climate chamber experiment, the spring wheat var. Apogee was inoculated with individual single conidium isolates of *F. poae* or *F. avenaceum*. This approach was chosen in order to work with genetically homogenous material and to elucidate differences in toxigenicity and pathogenicity between the isolates that had been used as a mixture in the field. Apogee proved to be a suitable variety since the overall response to these two species was similar to that of the varieties used in the field. Inoculations with *F. avenaceum* resulted in greater visual disease and greater toxin accumulation compared with *F. poae*. In addition, and in accordance to the field trials, yield reduction was observed only after inoculation with *F. avenaceum*.

Despite lower inoculum doses, toxin concentrations were higher than those detected in the field. In addition, DAS was detected in grain samples from Apogee but not in field grain samples. These results could be explained by the variety. However, differing environmental conditions and the presumed absence of other competing fungal species certainly played an important role in toxin accumulation. In particular, the absence of the non-toxigenic species *M. nivale*, which was present in the field in all years and in most varieties, could have resulted in higher and more varied toxin accumulation since this species has been shown to suppress the growth of *F. culmorum in planta* and to reduce DON production *in vitro* (Simpson et al. 2004).

Marked differences were found between the three isolates of *F. poae*. Starting with disease symptoms, one isolate not only caused the lowest number of kernels with symptoms but also substantially smaller lesions. Hence, if the disease assessment method was based on total lesion surface instead of number of symptomatic kernels, the determined differences between the isolates would have been even greater. The differences in disease symptoms were reflected in distinct toxin concentrations, with the highest-yielding isolate producing approximately five times more NIV than the lowest-yielding isolate. Differences in MAS production were also substantial and significant between all three isolates. These correlations are in line with published work on disease severity by *F. graminearum* and DON accumulation (Haidukowski et al. 2005) and could be a first indication that the trichothecenes NIV or MAS might also play a role in the pathogenesis of *F. poae*.

The fact that DAS and BEA were inconsistently detected from grains inoculated with *F. poae* was at first somewhat surprising, since the same isolates produced substantial concentrations of these toxins under *in vitro* conditions after inoculation of autoclaved wheat grains (Vogelgsang et al. 2008). The results of the current study could be explained by the different wheat varieties used in these experimental systems. More importantly though is the fact that the metabolism of a toxigenic fungus in a living plant is certainly different than in an *in vitro* system.

The differences between the *F. avenaceum* isolates were less obvious. Although some variation was found in disease symptoms, these differences were not reflected in yield reduction or MON production. Moreover, the toxin results are in contrast to the above mentioned *in vitro* experiments, where Fa 0379, compared with the other two isolates, was consistently the lowest-yielding isolate on four different cereal substrates (Vogelgsang et al. 2008).

None of the three *F. avenaceum* isolates used in the current study synthesised BEA. This result contrasts with other investigations that reported BEA production by this species (Logrieco et al. 2002) but is in line with recent reports by Jestoi et al. (2006) and Uhlig et al. (2006a) that did not detect any BEA from *F. avenaceum*.

In the current study, a small number of isolates from two important FHB species showed remarkable variation in their pathogenicity displayed by the formation of disease symptoms and, in the case of *F. poae*, in toxigenicity. It is very likely that other strains of *F. poae* and *F. avenaceum* from different hosts and various geographic areas show even more variability, including quantitative and qualitative differences in the production of toxins. Hence, more research is needed including a greater number of isolates in order to further investigate the potential variability in pathogenicity, yield reduction, and to possibly reveal distinct metabolite profiles or even chemotypes. Resulting correlations, for example between pathogenicity and toxin accumulation, as well as knowledge about distinct populations in terms of qualitative and quantitative toxigenicity could contribute to an improved prediction of the expected toxin content in harvested goods and is needed to enable a risk analysis for cereals, in particular for the highly toxic compounds NIV and MON. Finally, the role of trichothecenes in the pathogenesis of *F. poae* on cereals needs to be elucidated in greater detail.

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