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Kinetics of DON, NIV and ERG Formation in Grain of Two Wheat Cultivars after Inoculation with Three Isolates of *Fusarium culmorum* (W. G. Smith) Sacc.

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ABSTRACT

During two vegetative periods – the end of September to the end of July – the grain of two wheat cultivars ('Hana' and 'Samanta') was inoculated with three isolates of *F. culmorum* (W. G. Smith) Sacc. Isolate I1 (KF 350) was the nivalenol (NIV)-producing chemotype while I2 (Haniska) and I3 (Vranov) were deoxynivalenol (DON)-producing chemotypes. In all experiments as well as in controls analysis of group B-trichothecenes (NIV, DON) and ergosterol (ERG) concentration was performed. Both wheat cultivars possessed resistance to invasion and to toxin accumulation and degradation (mainly 'Hana'). This component of resistance can be seen by kinetics curves where increased toxin production appears mainly after 14 and 21 days after inoculation. In spite of this, toxin production had a linear kinetics and small concentrations could be observed. However, a slight deviation from linearity was observed in 1998 at the 2nd and 3rd week of vegetation while in 1999 at the 3rd and 4th week. The kinetics of ERG biosynthesis can be described as linear but the amount of fungal biomass production depends on the environmental condition. *F. culmorum* isolates could be differentiated according to their chemotype; mycotoxigenicity was also confirmed. This is the first report of trichothecene contamination and their distribution during vegetation harvest in Slovakian wheat.

Keywords: resistance, three isolates of *F. culmorum*, trichothecenes group B
Abbreviations: DAI, days after inoculation; **DON**, deoxynivalenol; **ERG**, ergosterol; **FHB**, *Fusarium* head blight; **I1**, **I2**, **I3**, isolates; **NIV**, nivalenol

INTRODUCTION

The most common species of *Fusarium* responsible for scab are *F. graminearum* Schwabe and *F. culmorum* (W.G. Smith) Sacc. In Europe's climate, including Slovakia, *F. culmorum* is one of the most destructive pathogens of wheat, *Triticum aestivum* L. (Miedaner *et al*. 2001; Šrobárová 2001). A recent study we carried out in 2000 and 2001 demonstrated a drift in the populations from *F. culmorum* (W. G. Smith) Sacc to *F. graminearum* Schwabe (Šrobárová and Prokop 2007). These two pathogens are closely related. Resistance in wheat plants to *F. culmorum* is correlated with resistance to *F. graminearum* (Mesterhazy 2002). The infected grains are of low quality with damaged starch granules, storage protein and accumulation of mycotoxins (Perkowski and Wiwart 2002; Prange *et al*. 2005). At the same time ergosterol (ERG) is produced in grain. ERG, the predominant sterol in most fungi, is used as an indicator of fungal growth in cereal grain (Seitz *et al*. 1977). It has subsequently been used to estimate fungal biomass in decomposing plant material (Gessner and Schmitt 1966).

The abundant growth of *F. culmorum* can be responsible for biosynthesis of deoxynivalenol (DON) (Snijders and Perkowski 1990), but nivalenol (NIV) was also found in small-grain cereals infected by *F. culmorum* (Mirocha *et al*. 1997; Perkowski *et al*. 1997; Gang 1998). These group B trichothecenes not only contaminate cereal grain but are also an important factor in pathogenesis (Van Eeuwijk *et al*. 1995; Miedaner and Perkowski 1996). The deployment of resistant cultivars is one of the best means for combating Fusarium head blight (FHB) in wheat (Šrobárová and Pavlová 2002).

The aim of our experiments was to evaluate two different wheat cultivars for resistance to trichothecenes group B after inoculation with three isolates of *F. culmorum* (W.G. Smith) Sacc. during two vegetative periods. Isolate I1 was the NIV-producing chemotype while I2 and I3 were DON-producing chemotypes. Within these experiments kinetics and analysis of group B trichothecenes and fungal biosynthesis was measured as ERG concentration.

MATERIALS AND METHODS

Inoculum preparation

The strain of *F. culmorum* (I1) is originally from the Culture Collections of the Institute of Plant Genetics, Polish Academy of Sciences, Poland (KF 350), while I2 was isolated during 1992 from the wheat heads of 'Hana' at Haniska, Slovakia, and I3 was isolated from new lines of POR 1522 in Vranov nad Topl'ou, Slovakia. For multiplication of these fungi, strains were grown on the potato-dextrose agar (PDA; Difco Laboratories, Detroit): 40 grams was suspended into 1 L distilled H_2O, pH was adjusted to 6 (± 0.2), then medium was poured into 100 mm diameter Petri dishes. Cultures were incubated for 21 days in a 12-h photoperiod at 25°C. Conidia and air mycelia were rasped off 50 Petri dishes and homogenized by a magnetic mixer with 2500 mL of distilled water. The average number of conidia counted in a Burker chamber was $5x10^5$ per mL^{-1}.

Experimental design

Two cultivars of winter wheat 'Hana', resistant to FHB and 'Samanta', with moderate resistance to FHB (Šrobárová and Pavlová

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2002), were sown on experimental fields at the Slovak Academy of Science in 4 treatments (control and inoculated separately with isolates I1, I2, or I3) in 3 replicates each. In 1998 samples were collected every 4 weeks beginning from the first week after inoculation. In the following year (1999) the evaluation of trichothecenes (DON, NIV) and ERG concentration was prolonged to 6 week harvest terms. Both winter cultivars were sown on September 28 and 30th, 1997 and 1998, respectively and grown separately on 6 m^2 (2x3 m^2) experimental plots bordered with rows and paths.

Inoculation

Each wheat head, one by one (500 per replicate) was inoculated 4 days after anthesis (stage 10.5 on the Feekes scale) (Zadoks *et al*. 1974) with 1 mL of mycelium and spore suspension mixture. 'Hana' was inoculated on June 16th, 1998, while 'Samanta' on June 18th, 1998, and in 1999 two days later on June 20th. The inoculation was done early in the morning with a hand sprayer (100 mL).

Control variants were grown the same way but instead of 1 mL of inoculation suspension the same amount of distilled water was applied. The inoculated spikes were covered for 24 hours with plastic bags, to maintain humidity.

Evaluation

Both control and infected heads were harvested in 4 periods: 'Hana' on June 23rd (I = 7 days) followed by June 30th (II = 14 days), July 7th (III = 21 days) and July 14th (IV = 28 days), while 'Samanta' on June 25th (I), followed by July 2nd (II), 9th (III) and 16th (IV). In 1999 harvesting occurred two days later, periods V and VI (35 and 42 days).

Traits were evaluated by analysis of variance. Dependence among the observed parameters was checked by correlation analysis. Software STARTGRAPHICS Version 2.1, Statistical Graphics Corp., was used.

Chemical analysis of toxins

Wheat kernels were analyzed for the presence of group B-trichothecenes, DON and NIV according to Perkowski *et al*. (2003).

Briefly, samples (10 g) were extracted with acetonitrile/water (82:18) and cleaned-up on a charcoal column [Celite 545/charcoal Draco G/60/activated alumina neutral 3:9:5 (w/w/w)].

Trichothecene toxins (DON, 3-AcDON, 15-AcDON, FUS X, NIV; Sigma, St. Louis, USA) after derivatization with mixture of trimethylsilylimidazole:trichlorosilane (100:1 v/v) were analyzed using the HP 6890 gas chromatograph coupled with the HP 5972 A mass spectrometer equipped with the HP-5MS (0.25 mm × 30 m) capillary column. Samples were injected into the injection port (at 250°C), the transfer line temperature was 280°C and the analyses were performed with the programmed temperature: initial temperature 80°C, held 1 min, from 80°C to 200°C at 15°C/min held for 6 min and from 200°C to 280°C at 10°C/min, the final temperature being maintained for 3 min. The helium flow rate was constant at 0.7 cm^3/min. Quantitative analysis was performed in single ion monitored mode (SIM) using following ions for detection of DON: 103 and 512; 3-AcDON: 117 and 482; 15-AcDON: 193 and 482; FUS X: 103 and 570; NIV: 191 and 600. Qualitative analysis was performed in SCAN mode (100-700 amu). Recoveries for analyzed toxins were as follows: DON 84 ± 3.8%; 3AcDON 78 ± 4.8%; 15 AcDON 74 ± 2.2%; FUS X 87% ± 5.9% NIV 81 ± 3.8%. Limit of detection was 0.01 mg/kg.

Chemical analysis of ergosterol

Samples containing 10 g of grounded grain were placed into 17 mL culture tubes, suspended in 1 mL of methanol, treated with 0.1 mL of 2 M aqueous NaOH, and sealed tightly. Then the culture tubes were placed within 250 mL plastic bottles, sealed tightly, and placed inside a microwave oven (Whirpool model AVM 401/WH) operating at 2450 MHz and 900 W maximum output. Samples were irradiated (370 W) for 20 s, after *c*. 5 min, for an additional 20 s and extracted with pentane (HPLC grade, Sigma-Aldrich, Steinheim, Germany) (3 x 4 mL) within the culture tubes. The combined pentane extracts were evaporated to dryness in a gentle stream of a high purity nitrogen. Prior to analysis samples were dissolved in 4 mL of methanol, filtered through 13 mm syringe filters with 0.5 μm pore diameter (Fluoropore Membrane Filters), evaporated to dryness by a nitrogen stream and dissolved in 1 mL of methanol. Prepared samples were analyzed by HPLC. Separation was achieved on a 150 mm length x 3.9 mm diameter Nova Pak C-18, 4 μm particle size column and eluted with methanol/acetonitrile (90:10) at a flow rate of 0.6 mL/min. ERG was detected with a Waters 486 Tunable Absorbance Detector detector set at 282 nm. Estimation of ERG was performed by a comparison of peak areas with those of an external standard (>95%, Aldrich, Milwaukee, USA) or by co-injection with a standard.

RESULTS AND DISCUSSION

Cultivars

The objective of our experiments were to test evaluation of resistance cultivars to Fusarium Head Blight (FHB) in two wheat cultivars 'Samanta' (ancestor: 'Hana' × 'Viginta') and 'Hana' (ancestor: 'NS-984-1' × ('Mironovská 808' × 'Moisson'). 'Hana' was characterized as resistant to FHB, during previous field experiments whereas Samanta is moderately resistant (Šrobárová and Pavlová 2002). In our experiment we proved that 'Hana' became resistant to the accumulation of β-trichothecenes over two years (**Fig. 1**).

Metabolites analysed in 1998 and 1999

Toxin (DON, NIV) concentrations in 1998 were low (**Figs. 2, 3**). In 1998, when the degree of infection was low, the fungal microflora content was also very low (close to naturally contaminated kernels) and ERG was below 3 mg/kg. This indicates only slightly *F. culmorum* colonization (Schnürer and Jonsson 1992). The DON and NIV concentrations were 6.27 and 2.90 mg/kg, respectively, which is very low for inoculated samples (Perkowski *et al*. 1995). In 1998 there were no significant differences between cultivars in toxin and ERG concentrations.

In 1999 (**Figs. 4, 5**), however fungal colonization was stronger than in 1998 followed by a concomitant increase in ERG concentration in the kernels. In the case of 'Sa-manta' the difference between years was more significant (α = 0.001) than for 'Hana' (α = 0.01). In 1999 'Samanta' accumulated significantly more DON than 'Hana' (α = 0.01) while there were no significant differences in NIV or ERG concentration, although a higher concentration was detected for 'Samanta'. In 1999 toxin accumulation was higher than in 1998 but lower than that reported by others who carried out similar experiments (Savard *et al*. 2000; Reinbrecht *et al*. 2002).

The kernels of 'Samanta' accumulated significantly more DON and NIV in 1999 while in 'Hana' (**Fig. 5**) there were no significant differences between both years of the experiment. Additionally, ratios between DON/NIV, DON/ERG, and NIV/ERG presented in **Figs. 6** and **7** are indirect proof of higher resistance of 'Hana' to toxin accumulation as well as to possible toxin degradation (Parry *et al* 1995; Mesterhazy 2002; Champeil *et al*. 2004; Brennan *et al*. 2007). Disease severity depends strongly on the inoculation date and environmental conditions. The most susceptible growth stage for FHB expression in ERG is during anthesis (Parry *et al*. 1995). In both cases, however, the least susceptible genotypes had a significantly lower DON content than the highly susceptible ones ERG/DON. These results are in agreement with correlations found between FHB rated by ERG and DON content in wheat materials (Mesterházy 2002). The DON/ERG ratio showed the variation among isolates and cultivars and indicates different disease severity and mycotoxin production levels in both cultivars. We found no concentrations of DON in all samples colonized by NIV-producing isolates, unlike Champeil *et al*. (2004), who did.

It could, however, be experimentally verified that a

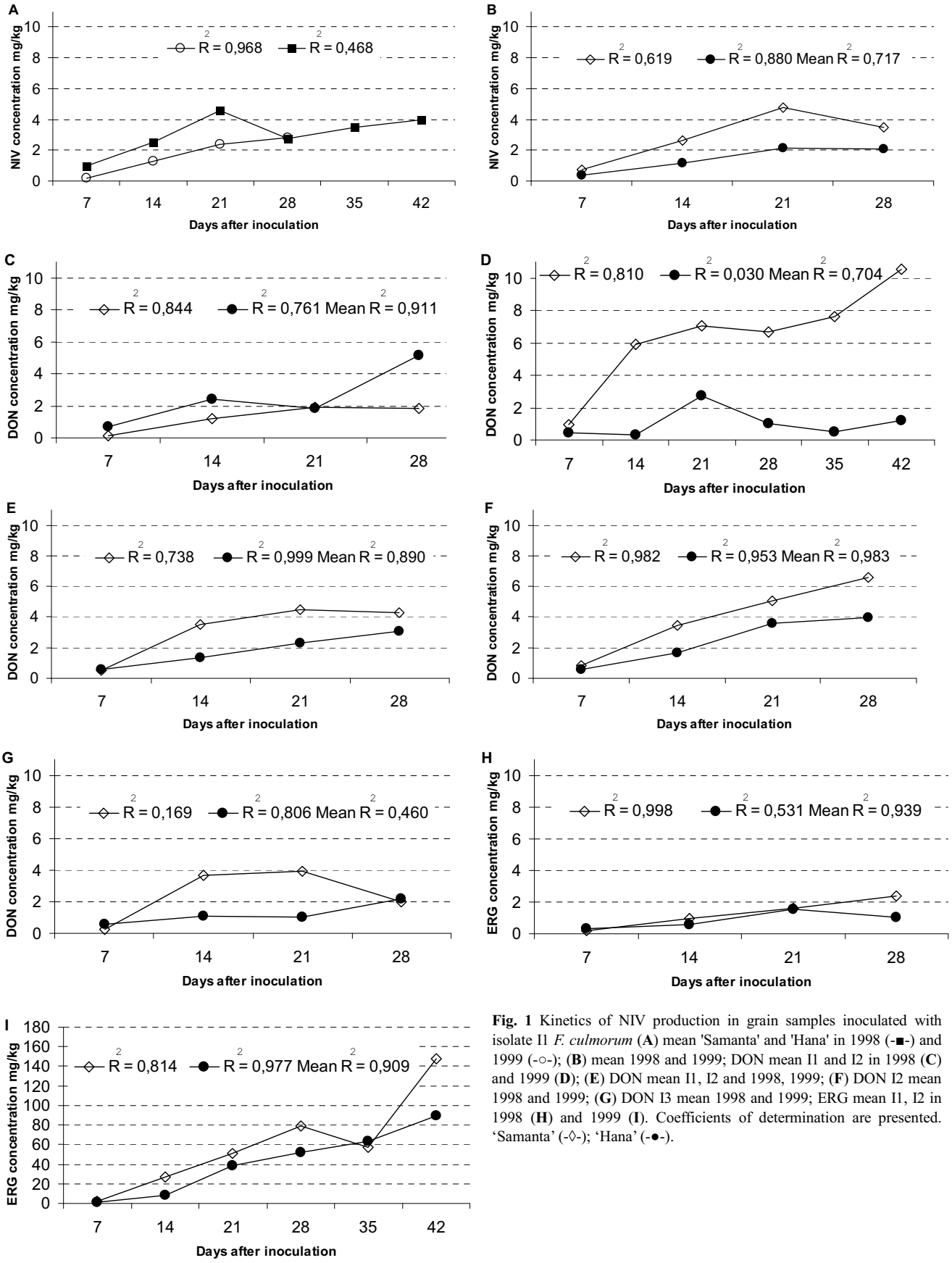

Fig. 1 Kinetics of NIV production in grain samples inoculated with isolate I1 *F. culmorum* (**A**) mean 'Samanta' and 'Hana' in 1998 (-■-) and 1999 (-○-); (**B**) mean 1998 and 1999; DON mean I1 and I2 in 1998 (**C**) and 1999 (**D**); (**E**) DON mean I1, I2 and 1998, 1999; (**F**) DON I2 mean 1998 and 1999; (**G**) DON I3 mean 1998 and 1999; ERG mean I1, I2 in 1998 (**H**) and 1999 (**I**). Coefficients of determination are presented. 'Samanta' (-◊-); 'Hana' (-●-).

correlated selection response for low DON content can be achieved by selecting for low FHB rating as ERG among winter wheat populations as was achieved by Brennan *et al*. (2007).

In 1999 those ratios decreased with harvest time and this process was faster for 'Hana' than for 'Samanta'. Additionally in 1998, when the fungal colonization and ERG concentration were low, the mean values of these ratios – presented in **Fig. 6** – were greater than one. On the other hand, in 1999, when fungal colonization was higher, the estimated ratio after 28-35 days after inoculation (DAI) was 0.03 for 'Hana' and 0.11 for 'Samanta'.

Czech researchers Šip *et al*. (2002) showed related results; infection with *F. culmorum* of 'Hana' had a large effect on grain yield but a relatively lower effect on kernel size and DON content (25 mg/kg), compared to 'Samanta'

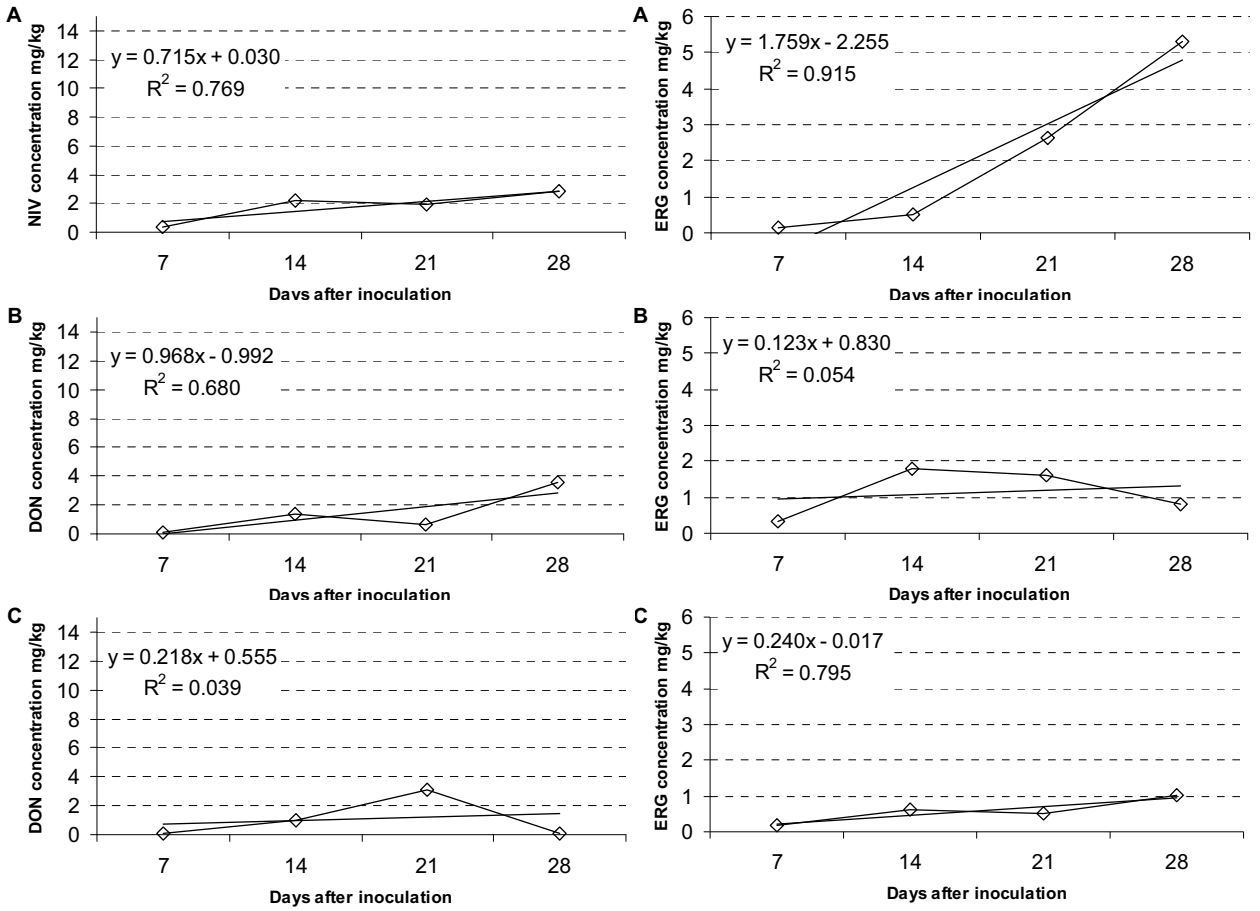

Fig. 2 Kinetics of NIV, DON and ERG production in grain samples inoculated with I1 (**A**), I2 (**B**), I3 (**C**) *F. culmorum* isolates in 'Samanta' in 1998. Linear regression curves, their equations and coefficients of determination are presented.

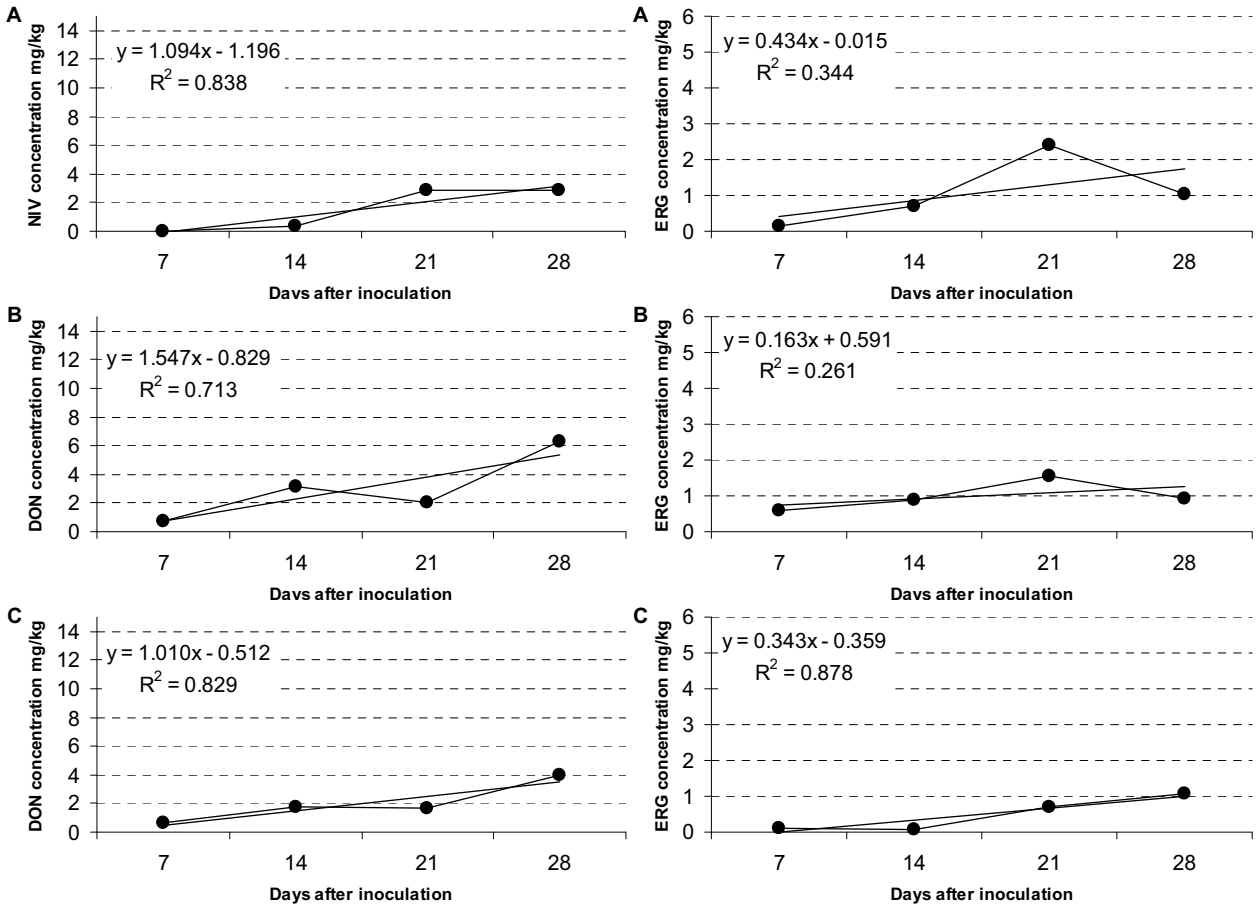

Fig. 3 Kinetics of NIV, DON and ERG production in grain samples inoculated with I1 (**A**), I2 (**B**), I3 (**C**) *F. culmorum* isolates in 'Hana' in 1998. Linear regression curves, their equations and coefficients of determination are presented.

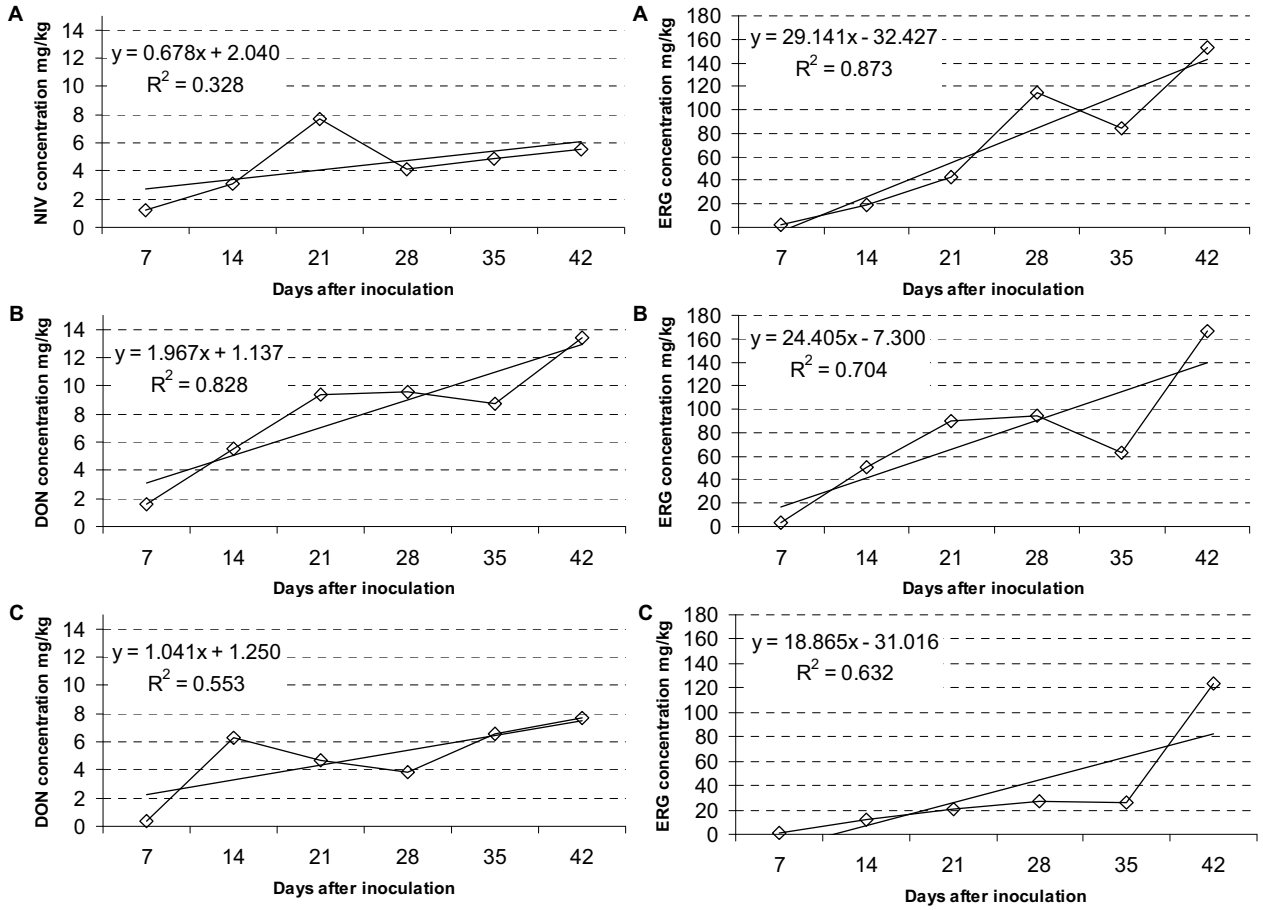

Fig. 4 Kinetics of NIV, DON and ERG production in grain samples inoculated with I1 (**A**), I2 (**B**), I3 (**C**) *F. culmorum* isolates in 'Samanta' in 1999. Linear regression curves, their equations and coefficients of determination are presented.

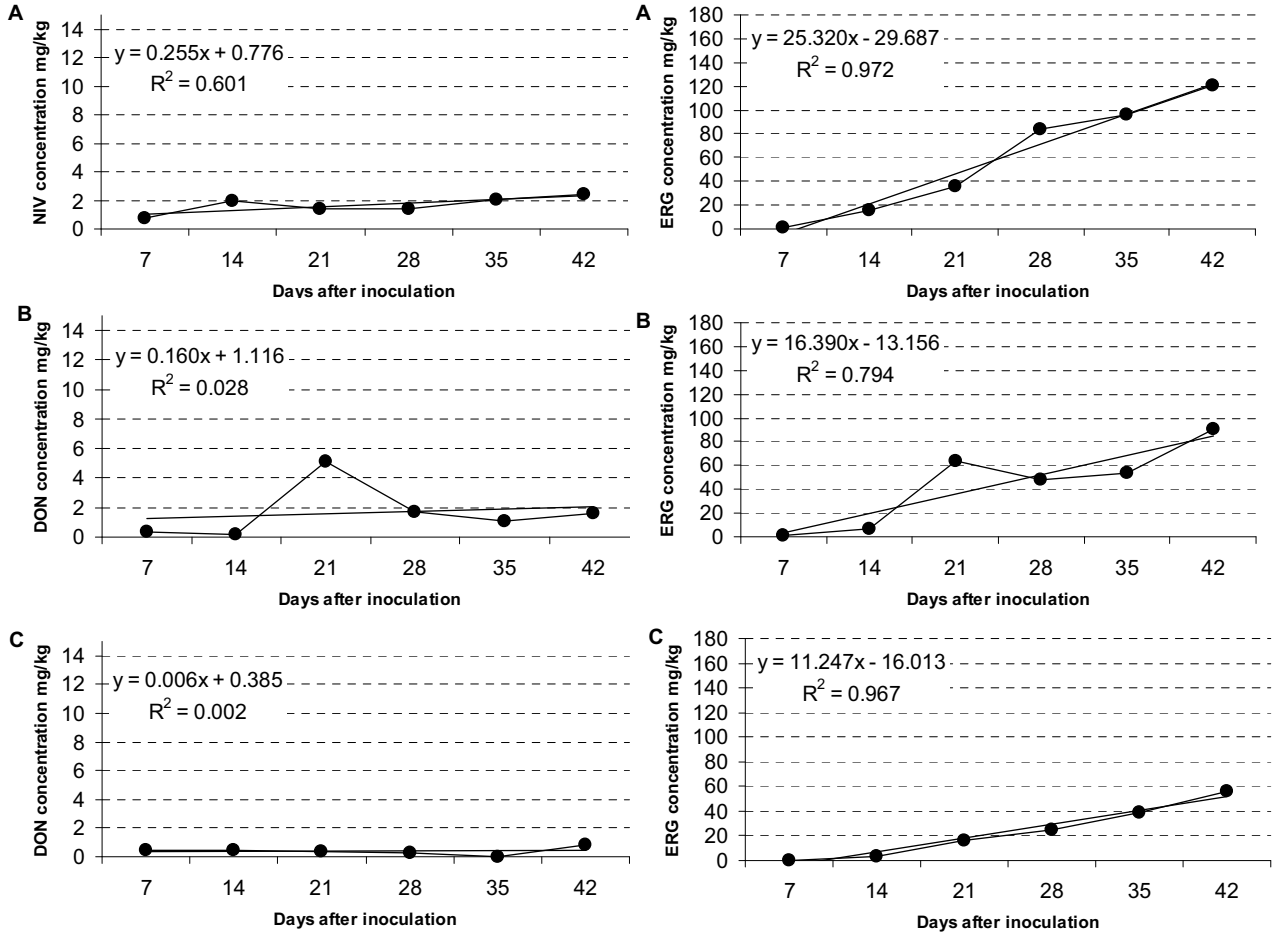

Fig. 5 Kinetics of NIV, DON and ERG production in grain samples inoculated with I1 (**A**), I2 (**B**), I3 (**C**) *F. culmorum* isolates in 'Hana' in 1999. Linear regression curves, their equations and coefficients of determination are presented.

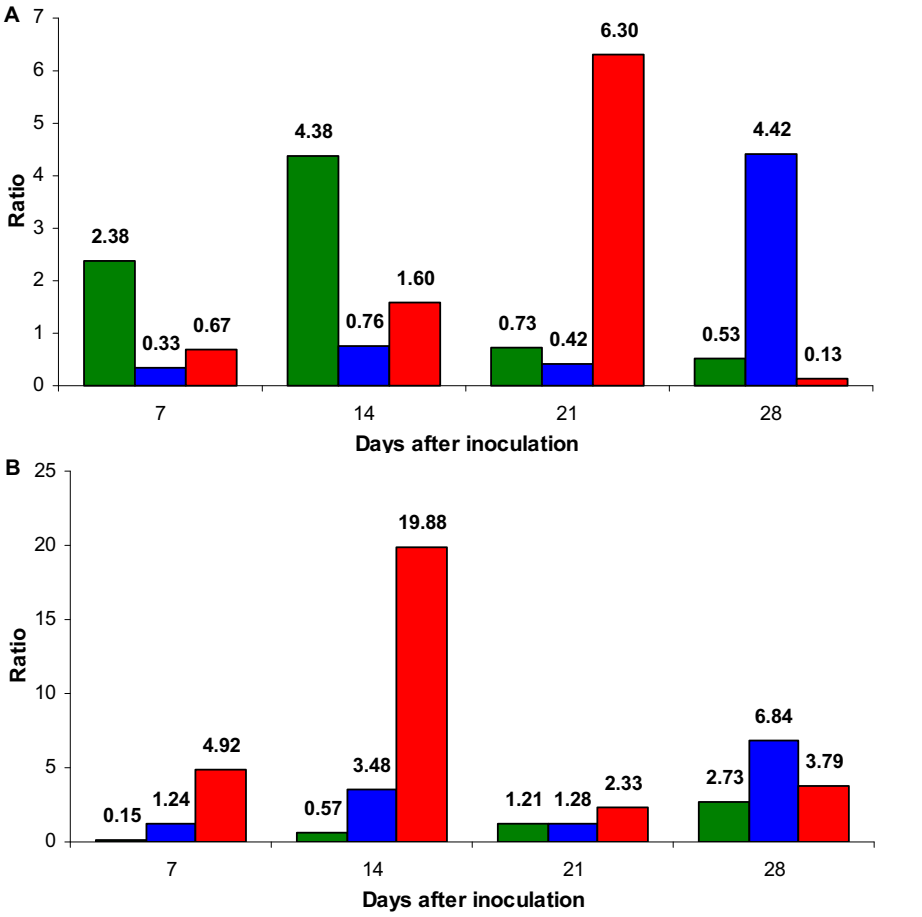

Fig. 6 Ratios NIV/ERG (I1) and DON/ERG (I2; I3) for grain samples of wheat cultivar 'Samanta' (**A**) and 'Hana' (**B**) after inoculation with isolates I1, I2, I3 *F. culmorum* in 1998.

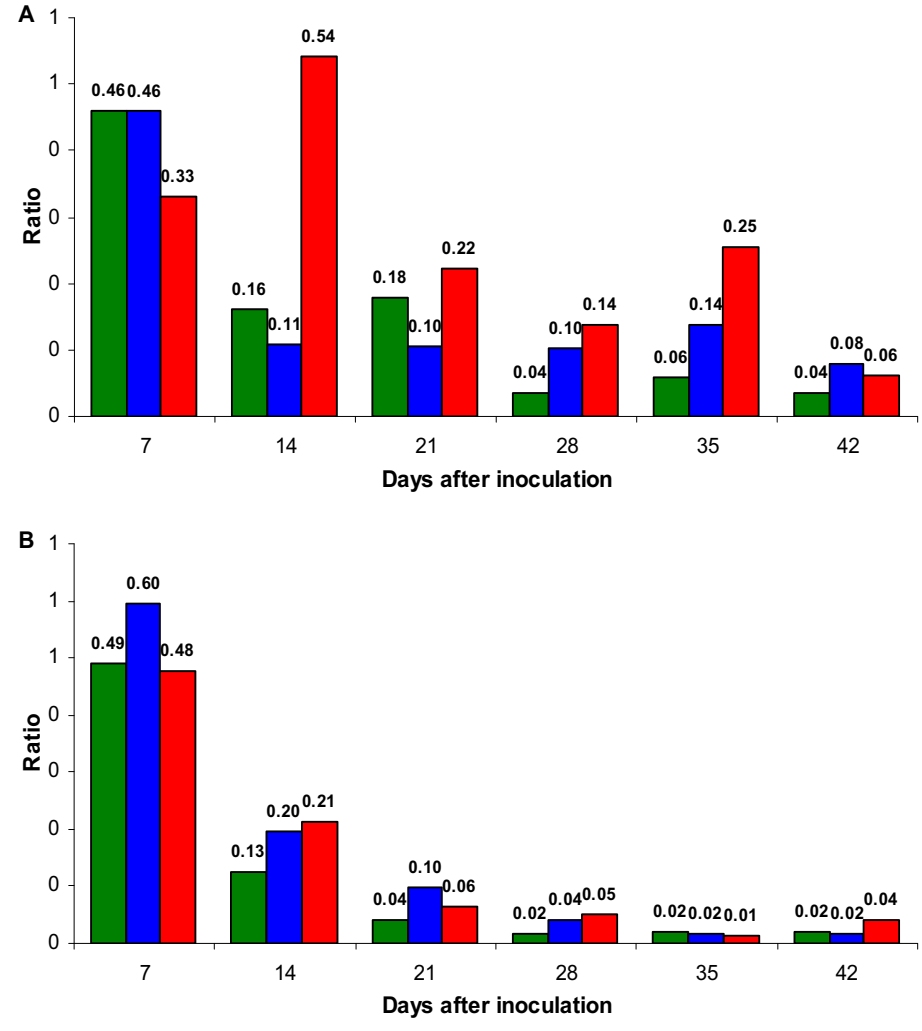

Fig. 7 Ratios NIV/ERG (I1) and DON/ERG (I2; I3) for grain samples of wheat cultivar 'Samanta' (**A**) and 'Hana' (**B**) after inoculation with isolates I1, I2, I3 *F. culmorum* in 1999.

with higher DON content (28 mg/kg), but the same effect as 'Hana' on grain yield.

Harvest term

In our study the first harvest term was at 7 days after inoculation (DAI). In all experiments in this harvest term *Fusarium* toxins (NIV, DON) as well as ERG were already present in grain **Figs. 2-5**. The mean DON concentration for the first harvest term after inoculation was from 0.08 ('Samanta' 1998) to 0.52 mg/kg ('Samanta' 1999); NIV from 0.08 ('Hana' 1998) to 0.84 mg/kg ('Hana' 1999); ERG from 0.22 ('Hana' 1998) to 1.84 mg/kg ('Samanta' 1999). Similar results were obtained by Savard *et al*. (2000) and Reinbrecht *et al*. (2002). Savard *et al*. (2000), after inoculation of the highly susceptible cultivar 'Roblin' with *F. graminearum* DAOM 178148 found 383 mg/kg of DON in inoculated spiklets at 9 DAI. Reinbrecht *et al*. (2002) reported that for susceptible winter-wheat cultivar 'Kontrast' over 110 mg/kg of DON and NIV was observed at 7 DAI due to inoculation with isolate *F. culmorum* 46. According to Bai *et al*. (2001) FHB is in an advanced stage of development at 14 DAI. In our experiments at 14 DAI the highest DON, NIV and ERG concentrations (mg/kg) were: 6.32 for I3, 3.07 for I1, and 51.03 for I2, respectively for 'Samanta' in 1999; in 1998, for 'Hana' the maximum concentrations of DON I2, NIV I1 and ERG I1 were as follows: at 28 DAI, 6.27 mg/kg, at 21 DAI, 2.90 mg/kg and at 21 DAI, 2.40 mg/kg, respectively. For 'Samanta', maximum DON I2, NIV I1, and ERG I1 concentrations were detected at 28 DAI, at 3.57, 2.83, and 5.30 mg/kg, respecttively. In 1999 when the fungal colonization was stronger maximum concentrations were as follows: for 'Hana', DON I2 at 21 DAI, 5.09 mg/kg while for NIV I1 and ERG I1 42 DAI, at 2.43 mg/kg and 120.77 mg/kg, respectively; for 'Samanta' DON I2 and ERG I2 were at 42 DAI, at 13, 38 mg/kg and 166, 47 mg/kg, respectively while for NIV I1 at 21 DAI, 7.68 mg/kg.

Our results are comparable to those of Reinbrecht *et al*. (2002) whose assessment of DON and NIV concentrations in a susceptible cultivar 'Kontrast' exceeded 400 and 100 mg/kg, respectively while for a moderately resistant cultivar 'Pegassos' 100 and 20 mg/kg, respectively indicated that low toxin concentration in spite of heavy colonization of heads in 1999 was due to significant resistance of 'Hana' and 'Samanta'. With reference to the above, in 1999 for example, for 'Samanta' the mean DON concentration for I2 was 8.02 mg/kg (**Fig. 4**), for I3 4.89 mg/kg (**Fig. 4**) while that of NIV for I1 was 3.73 mg/kg (**Fig. 4**). Similar trends were observed for different variants of this experiment.

Kinetics curves of DON, NIV and ERG biosynthesis

In most cases (**Figs. 2**-**5**) high values of determination coefficients of estimated regression curves were obtained. In the remaining cases the values of determination coefficients were definitely lower, e.g. for kinetics of DON formation in 1998 for isolate I3 or ERG I2 in grains of 'Samanta' (**Fig. 2**), and in 1999 NIV I1 for 'Samanta' (**Fig. 4**) or DON I2 for 'Hana' (**Fig. 5**). This is due to a significant deviation of the metabolite concentration results from the linear regression curve, especially from 14 to 21 DAI what is presented on **Figs. 4** and **5**. In 1999, where evaluation was prolonged, at 35 DAI there was a breakdown of the kinetics curve (**Fig. 5**). Reinbrecht *et al*. (2002) made similar conclusions, reporting that the highest differences between analysed cultivars 'Kontrast' and 'Pegassos' took place at 21 and 28 DAI, respectively. Additionally, Smith *et al*. (2004) identified in a time course experiment that this effect of cultivar (recombinant inbred lines of barley, FEG8) on DON accumulation after inoculation with *F. graminearum* (isolate Butte86ADA-ll) was observed at 10 DAI.

Taking into account the kinetic curves for particular metabolites, in the case of NIV in 1998 for 'Hana' and 'Samanta' the linear mean kinetics curve was $R^2 = 0.968$. In 1999 a similar regression was observed for 'Hana' while for 'Samanta' NIV concentration reached a significantly higher level than the other observations 21 DAI followed by a decrease in its concentration (**Fig. 1**). For both years of the experiment as well as for both cultivars the mean coefficient of determination equalled $R^2 = 0.714$ implying that 71% of results can be explained by linear regression. These results indicate that high levels of DON and NIV can be produced after 20 days even by early infection. Similar effects are presented by Yoshida *et al*. (2005) in the case of late infection and a lack of FHB symptoms.

Generally for 'Hana' and for isolates I2 and I3, in both 1998 and 1999, estimated coefficients (**Fig. 1**) of determination ($R^2 = 0.999$) definitely indicate a linear increase of DON concentration over time. For 'Samanta' the estimated coefficient was lower ($R^2 = 0.738$) whereas for cultivars, years and isolates the value equalled $R^2 = 0.890$ (**Fig. 1**). Analysing the two isolates separately, in the case of I2, DON concentration increased linearly over time ($R^2 = 0.983$ for both cultivars; $R^2 = 0.982$ for 'Samanta' and $R^2 = 0.952$ for 'Hana'). For I3, especially in the case of 'Samanta', the assessment of coefficients of determination were lower due to significant oscillations of DON concentration which depended on the harvest term whereas for 'Hana' (**Fig. 1**) this coefficient remained high ($R^2 = 0.806$).

For ERG (**Fig. 1**) an estimation of its biosynthetic kinetics could only be carried out for each year separately since ERG concentration in both years differed significantly ($\alpha = 0.001$ 'Samanta'; $\alpha = 0.01$ 'Hana').

In 1998 the coefficient of determination for both analyzed cultivars and for the three isolates was $R^2 = 0.939$ while in 1999 it was $R^2 = 0.909$. Such results indicate that the kinetics of ERG production (**Fig. 1**) is linear despite a definitely different range of concentrations of this metabolite in both years (mean <3 mg/kg in 1998 and <120 mg/kg in 1999). The disassociation between visual estimates of disease and DON contamination was noted by Paul *et al*. (2005).

A strong regression was observed for 'Samanta' in 1998 ($R^2 = 0.998$) and 'Hana' in 1999 ($R^2 = 0.977$) but in 'Hana' in 1998 and 'Samanta' in 1999 deviations from the linear regression could be detected at 21 or 35 DAI, respectively. In general the single deviations were averaged to finally estimate the coefficients of determination for linear regression; if it was >0.9 that would allow us to conclude that ERG biosynthesis in grain is linear ($\alpha = 0.01$). In conclusion, selection of cultivars for reduced FHB symptoms should lead to a correlated selection response in low fungal biomass and low DON content in grain (Miedaner *et al*. 2004a), confirmed by significant genetic variation for these traits reported for 57 isolates (Cumagun *et al*. 2004). In some cases, however, fungal biomass *in vivo* (**Fig. 1**) was not proportional to the development of disease symptoms and DON production when different field isolates were investigated. It is crucial to calculate DON production relative to the amount of fungal biomass in host tissue.

Chemotypes of *F. culmorum* and toxigenocity of isolates

Profile of trichothecenes produced by *F. culmorum* under laboratory and under field conditions were: DON (dominating chemotype), nivalenol and 3-AcDON as DON precursor (Bottalico and Perrone 2002; Quarta *et al*. 2006).

Isolate I1 was the *F. culmorum* chemotype responsible for producing NIV whereas isolates I2 and I3 (chemotypes) produced DON. The mean concentration of NIV for I1 in all combinations ranged from 1.54 to 3.73 mg/kg. Considering that DON concentration was only 10% of NIV concentration its biosynthesis has no effect on the biosynthesis of NIV, which was the main product. NIV producing isolates are recognized as less aggressive on wheat and rye than DON producers. The highest amount of NIV con-

trasted with the NIV content in grain from field trials and no correlated between field and *in vitro* assays (Gang *et al*. 1998; Cumagun *et al*. 2004).

Comparing the mycotoxigenicity of isolates I2 and I3, both being DON producing chemotypes (although they produce only a negligible amount of DON in FHB-resistant cultivars) noticeable differences between them could be observed. I2 produced in both 1998 and 1999 slightly more DON and ERG (**Fig. 1**) than I3, despite similar culture conditions. Similarly, there was a strong, positive correlation between DON and ERG contents in the infected grains, for isolates I2 and I3 it was $r = 0.73$, $P = 0.001$. It seems that ERG production is closely linked with DON and FDK, indicating that aggressiveness is proportional to the fungal biomass in the infected grains (Lamper *et al*. 2000). Miedaner *et al*. (2004b) evaluated 42 isolates of *F. culmorum*, most of which belong to more aggressive isolates producing a higher mean DON content in grain from field trials ($r = 0.69$; $P = 0.01$). *In vitro* assays reliably distinguished DON- and NIV-producing types of *F. culmorum*; however, these assays could not predict the production of DON by these isolates in the field. However, DON production rate per unit of fungal biomass, estimated as the DON/ERG ratio at harvest, changed along with environmental conditions and isolates used for inoculation but not correlates with their aggressiveness (**Figs. 6, 7**).

Our results prove that conditions in a particular year influence fungal metabolite biosynthesis differently for different cultivars, especially considering the quantitative aspect of this process described by biosynthetic yield. Differentiation of *F. culmorum* isolates based on their chemotype and mycotoxigenicity was also confirmed.

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