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Effects of *Fusarium* infection on the amino acid composition of winter wheat grain

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ABSTRACT

Winter wheat (susceptible cultivar Ritmo) was grown in 2006 near Kiel and in 2007 near Heide in northern Germany. Plants were inoculated at anthesis using a *Fusarium graminearum* macroconidial suspension. The percentage of *Fusarium*-damaged kernels (FDK) ranged from 0 ± 2% to 28 ± 2%. The contents of the *Fusarium* mycotoxin deoxynivalenol (DON) and wheat amino acids were determined in the grain. Levels of the amino acids alanine, lysine, and tyrosine increased with the percentage of FDK or DON contents whereas glutamic acid contents decreased. Aspartic acid and threonine were not related to the percentage of FDK or DON contents. Effects of *Fusarium* infection on other amino acids were significant only at the sampling site with the higher degree of *Fusarium*-damage. Interestingly, those amino acids that increased consistently and significantly with the degree of *Fusarium*-damage are derived from phosphoenolpyruvate or pyruvate, suggesting that pathogen-induced changes in the glycolytic input for amino acid biosynthesis play a significant role for the amino acid composition of *Fusarium*-damaged winter wheat grain. On average, amino acid contents decreased by 0.13% compared to the amino acid content of sound kernels upon an increase of 1% of FDK.

1. Introduction

The genus *Fusarium* includes fungi with saprophytic as well as plant-pathogenic capabilities. Wheat plants are infected at anthesis, via the open flowers, when humidity is sufficiently high to allow fungal growth (Beyer, Röding, Ludewig, & Verreet, 2004; Beyer, Verreet, & Ragab, 2005; Beyer, Klix, Klink, & Verreet, 2006). *Fusarium* species produce mycotoxins with adverse effects on the physiology of other organisms, including humans (Bondy & Pestka, 2000; Speijers & Speijers, 2004). *Fusarium* mycotoxins belonging to the group of trichothecenes inhibit eukaryotic protein biosynthesis (Rotter, Prelusky, & Pestka, 1996). Amino acids are the basic structural building units of peptides and proteins. The total storage protein content was lower in *Fusarium*-damaged kernels than in sound kernels but no significant qualitative differences in protein were detected by various analytical methods (Nightingale, Marchylo, Clear, Dexter, & Preston, 1999). The amino acid composition of *Fusarium*-damaged soybean did not vary between infected and sound seed lots (Meriles, Lamarque, Labuckas, & Maestri, 2004). Matthäus et al. (2004) found increased protein and amino acid contents in winter wheat inoculated with *F. culmorum* compared to an uninoculated control. The reasons for the contradictory results are unknown. Hermann, Aufhammer, Kübler, and Kaul (1999) found no effect of *Fusarium* infection on protein content or other quality parameters in winter wheat, rye, or triticale. However, disease severity was rather low in the latter study and thus some differences between infected and sound grain could have been too small to be detected. Rudgard and Wheeler (1985) reported that the amounts of proline and alanine were positively associated with infection of Brussels sprouts by *Erysiphe cruciferarum* and those of glutamic acid and leucine were negatively associated with infection, indicating that fungal infections can cause changes in the amino acid composition of plants. Hamzehzarghani et al. (2005) observed decreased levels of proline and glycine as a result of *F. graminearum* inoculation in susceptible wheat cultivar Roblin and resistant cultivar Sumai 3. Plant pathogens produce enzymes that cleave proteins to amino acids (i) to inactivate defence response compounds of plants (e.g. cutinases; Murphy, Cameron, Huang, & Vinopal, 1999), (ii) to obtain amino acids for nutrition (Dobinson, Lecomte, & Lazarovits, 1997), or (iii) to degrade host cell walls to facilitate penetration into the host tissue (Dow, Davies, & Daniels, 1998). It was the purpose of this study to test how far *Fusarium* infections can alter the contents of amino acids in wheat grain and to derive a hypothesis about which metabolic processes in the host plants could have been affected by *Fusarium* infection.

2. Materials and methods

2.1. Plant material and fungal isolates

Winter wheat (*Triticum aestivum* L., susceptible cultivar Ritmo) was grown in northern Germany in 2006 near Kiel (latitude 54° 21′ N, longitude 10° 28′ E) and in 2007 near Heide (latitude 54° 12′ N,

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longitude 9° 6′ E). The field experiments were originally carried out to assess different fungicide regimes. For the present study, only grain obtained from control plots (no fungicide use) was utilised. The experimental design was a completely randomised block design with three replicates at Kiel and two replicates at Heide. Plants were inoculated at anthesis during light rainfall by overhead application of a *F. graminearum* macroconidial suspension containing spores of six isolates, as described by Beyer, Klix, and Verreet (2007). Briefly, fungal material was propagated in sterile containers in PDB medium and applied using a plot sprayer (Baumann Saatzucht, Waldenburg, Germany) equipped with standard nozzles (Agrotop, Obertraubling, Germany) such that whole plots were inoculated. Plot size was 10 m^2 (2 × 5 m). Grain was harvested using a combine harvester for experimental plots. Approximately 8 kg of grain were obtained per plot.

Small and light kernels (including high levels of FDKs) were separated from heavy and large kernels (largely excluding FDKs) using a sample cleaner (model SLN, Zuther GmbH, Karwitz, Germany). Furthermore, sand, weed seed and other contaminants were removed during the cleaning process. Four levels of *Fusarium*-damage were generated by mixing varying amounts of light and heavy kernels. Each subsample was mixed well and approximately 200 g of grain were transferred into the laboratory for following analyses. The percentage of FDK was determined based on kernel colour and the degree of shrivelling (Jackowiak, Packa, Wiwart, & Perkowski, 2005; Sinha & Savard, 1997) for each lot of grain, using subsamples of at least 40 kernels. Disease severity levels were 0 ± 2%, 17 ± 2%, 19 ± 2%, and 28 ± 2% FDK for sampling site Kiel and 0 ± 1%, 9 ± 1%, 13 ± 0%, and 25 ± 4% FDK for sampling site Heide. Subsamples were mixed well and approximately 20 g were milled for mycotoxin analyses and 180 g were milled for amino acid analyses.

2.2. Deoxynivalenol (DON) analyses

DON contents of freeze-dried (model Gamma 1-20, Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany) grain were determined as described by Beyer et al. (2007). Briefly, dry samples were milled (laboratory mill model MF 10 basic, IKA-Werke, Stauffen, Germany) and sample material (10 g) was extracted using 50 ml MeOH + H_2O (84 + 16 [v/v]) in Erlenmeyer flasks and shaken for 1.5 h at ≈20 °C. Extracts (10 ml) were filtered and 8 ml of filtered extract were cleaned up using MycoSep 227 Trich+ columns (Coring System Diagnostix GmbH, Gernsheim/Rhein, Germany). Cleaned extract (4 ml) was transferred to round-bottom flasks and evaporated to dryness (Rotavapor model R-210, Büchi Labortechnik AG, Flawil, Switzerland). Residues were re-dissolved in 500 μl of aqueous 12.5% MeOH, transferred to glass vials and stored at −70 °C prior to use. DON contents of the extracts were determined by comparison with a certified type B trichothecene mix standard (biopure Referenzsubstanzen GmbH, Tulln, Austria) using an HPLC system consisting of two L-2130 pumps, a diode array detector (model L-2450) and an EZChrom Elite data analysis unit (all devices from VWR International, Darmstadt, Germany). Five μl of extract were injected into a Chromolith RP-18e column adjusted to 20 °C. Elutions were carried out with MeOH and H_2O. Flow rates were 1.9 ml min^{-1} (0–11 min, 18 min) or 3.7 ml min^{-1} (12–17 min). The gradient programme was as follows. 0–10 min: 12.5% MeOH, 10–12 min: linear gradient from 12.5% to 19% MeOH, 12–17 min: 19% MeOH, 17–18 min: linear gradient from 19% to 12.5% MeOH. Chromatograms were analyzed at 220 nm.

2.3. Determination of amino acids

Contents of amino acids within dry grain samples were determined, following the official protocol given in directive 98/64/EC of the European Commission (Anonymous 1998) in detail.

2.4. Statistics

The number of replicates per disease severity level was $n = 2$ at sampling site Heide and $n = 3$ at sampling site Kiel. Data are presented as median ± standard error. Since the variability of amino acid contents was very small (average coefficient of variation = 1.57%), standard errors of amino acid data were omitted for clarity in Table 1. Regressions were carried out using all data points, not treatment medians or means, and the statistical software package SPSS (version 14, SPSS, Inc., Chicago, Illinois, USA).

3. Results

The percentages of FDK ranged from 0% to 33% at sampling site Kiel and from 0% to 28% at sampling site Heide. DON contents at Kiel were higher than at Heide, even at the same level of disease severity (Fig. 1). For instance, at 20% FDK, DON contents were estimated to be 7.28 mg kg^{-1} at Kiel and 5.01 mg kg^{-1} at Heide (Fig. 1).

The contents of proteinogenic amino acids changed with the percentage of FDK, as well as with the DON content of wheat grain (Table 1). However, significance of the effects depended on the environment. At Kiel, alanine, lysine, and tyrosine contents significantly increased with the DON content or the percentage of FDK whereas arginine, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, methionine, phenylalanine, proline, serine and valine contents decreased (Table 2). Aspartic acid and threonine were not related to the percentage of FDK or DON contents (Table 2). At Heide, alanine, arginine, lysine, and tyrosine contents significantly increased with the DON content or the percentage of FDK, whereas the glutamic acid level decreased (Table 2). Other amino acids were not significantly affected by *Fusarium* infection at Heide.

Table 1
Sampling sites, percentages of *Fusarium*-damaged kernels (FDK), deoxynivalenol (DON) content, and contents of selected amino acids of winter wheat cultivar Ritmo

Sampling site	FDK (%)	DON (mg kg^{-1})	Amino acid content (% [w/w])[a]															
			ALA	ARG	ASP	CYS	GLU	GLY	HIS	ILE	LEU	LYS	MET	PHE	PRO	SER	THR	TYR	VAL
Kiel	0 ± 2	1.49 ± 0.42	0.45	0.61	0.70	0.29	3.69	0.51	0.33	0.43	0.85	0.35	0.20	0.59	1.24	0.59	0.37	0.33	0.53
Kiel	17 ± 2	3.54 ± 0.62	0.46	0.59	0.70	0.28	3.52	0.50	0.28	0.41	0.82	0.35	0.19	0.57	1.17	0.57	0.37	0.31	0.52
Kiel	19 ± 2	7.49 ± 1.13	0.46	0.58	0.71	0.28	3.42	0.49	0.28	0.41	0.82	0.36	0.19	0.56	1.13	0.56	0.37	0.33	0.52
Kiel	28 ± 2	20.2 ± 4.33	0.46	0.57	0.71	0.27	3.22	0.48	0.27	0.40	0.79	0.36	0.19	0.56	1.09	0.54	0.37	0.35	0.50
Heide	0 ± 1	0.52 ± 0.02	0.44	0.60	0.73	0.29	3.65	0.50	0.29	0.42	0.84	0.36	0.20	0.57	1.18	0.58	0.37	0.31	0.42
Heide	9 ± 1	1.87 ± 1.31	0.44	0.59	0.71	0.29	3.63	0.49	0.28	0.41	0.83	0.35	0.19	0.57	1.20	0.57	0.36	0.32	0.52
Heide	13 ± 0	1.90 ± 0.39	0.44	0.60	0.72	0.29	3.68	0.50	0.29	0.42	0.83	0.36	0.19	0.57	1.17	0.58	0.37	0.31	0.53
Heide	25 ± 4	8.36 ± 3.15	0.45	0.61	0.72	0.29	3.59	0.50	0.29	0.42	0.83	0.37	0.20	0.57	1.17	0.58	0.37	0.34	0.54

The number of replicates was $n = 3$ at sampling site Kiel and $n = 2$ at sampling site Heide. Data represent medians.
[a] ALA = alanine, ARG = arginine, ASP = aspartic acid, CYS = cysteine, GLU = glutamic acid, GLY = glycine, HIS = histidine, ILE = isoleucine, LEU = leucine, LYS = lysine, MET = methionine, PHE = phenylalanine, PRO = proline, SER = serine, THR = threonine, TYR = tyrosine, VAL = valine.