JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 3043–3049 3043
DOI:10.1021/jf903545j

Effects of *Fusarium* Infection on the Phenolics in Emmer and Naked Barley

Kai Eggert,*,† Jürgen Hollmann,‡ Beate Hiller,‡ Hans-Peter Kruse,§ Hashadrai M. Rawel,§ and Elke Pawelzik†

†Quality of Plant Products, Department of Crop Sciences, Faculty of Agricultural Sciences, Georg-August University Göttingen, 37075 Göttingen, Germany, ‡Max Rubner Institute, Federal Research Institute of Nutrition and Food, Schützenberg 12, 32756 Detmold, Germany, and §Department of Food Chemistry, Institute of Nutritional Science, University of Potsdam, 14558 Nuthetal, Germany

Inoculated or non-inoculated naked barley and emmer cultivars were investigated with regard to their influence on phenolic acid profiles and their arabinoxylan content. Two groups of phenolic compounds were differentiated—methanol-soluble and hydrolyzable covalent-bound phenolic compounds. Chromatographic methods were applied for their analysis. The results showed ferulic acid as the predominant phenol in both total and covalent-bound fractions. The inoculation significantly reduced the ferulic acid content within a range of 5.6−6.6% in the two cereals and all their cultivars. Naked barley cultivars additionally contained the flavonoid catechin in the soluble fraction. The innoculation led here to a significant increase in the catechin content of about 4.5%. These results document an induction of the synthesis of catechin in naked barley after artificial *Fusarium* infection, whereas the ferulic acid content declined.

KEYWORDS: Fusarium infection; phenolic acids; arabinoxylan; trichothecenes

INTRODUCTION

Fusarium graminearum and *Fusarium culmorum* infection of cereal grains leads to pathogenic effects on the plant and spike in wheat, barley, and emmer (*1*−*4*). These effects can result in yield loss and quality reduction (*1*, *5*). Fusarium head blight (FHB) is the visible effect of this fungal infection, first recorded by Worthington G. Smith in 1884, and represents a problem known worldwide (*1*−*4*).

The *Fusarium* species *F. graminearum* and *F. culmorum* are producers of various trichothecene mycotoxins, including deoxynivalenol (DON), its precursors 3- and 15-acetyldeoxynivalenol (3-Ac-DON and 15-Ac-DON), and other compounds (*1*). DON is a potential inhibitor of protein biosynthesis. In mammals, DON leads to unspecific effects in the intestines, causing diarrhea with vomiting, a reduced food intake, and raised bleeding tendency in the intestines. Its specific effects are a reduced leukocyte content connected with a loss of immune function and a rise in free radicals in the liver (*6*−*9*). These negative effects underline the necessity of reducing the infection-derived effects of *Fusarium spp*. on food and feed. This problem has been addressed effectively by limiting the maximum amount of DON by a threshold value in the European Union for products destined for human (*10*) and animal nutrition ((*EG*) *No. 576*/*2006*, 2006).

The main phenol in cereal grains is the cinnamic acid derivate ferulic acid, whereas other phenolic acids such as caffeic acid and *p*-coumaric acid are also found in lower concentrations (*10*−*13*). In monocotyledons, ferulic acid is incorporated into plant cell wall structures. Ferulic acid provides cross-linkages via ester bonds between arabinoxylans (AX) and other cell wall components such as cellulose, lignin, and proteins (*14*−*18*).

For artificially inoculated wheat grains existing data showed a significant reduction of ferulic and *p*-cumaric acid. Concentrations of ferulic acid measured during grain development attained similar values in both resistant and susceptible cultivars (*19*). In a further study different wheat cultivars were evaluated on basis of the distinction in FHB-resistant and FHB-susceptible cultivars derived from FHB disease incidence and severity data (*13*). However, no differences in the content of free and bound phenolic acids in FHB-resistant and FHB-susceptible cultivars were found (*13*). Previous studies with artificially inoculated maize grains showed a negative correlation between the extent of exposure to *F. graminearum* and the ferulic acid content in the pith tissue, but the different genotypes did not show any correlation with regard to their resistance and/or susceptibility to *F. graminearum* and the corresponding content of phenolic compounds (*12*).

Barley grains contain, in addition to cinnamic acid derivates, phenols of the flavonoid group, especially those belonging to the proanthocyanidin group (*20*). Additionally, some colored cultivars contain anthocyanidins (*21*). Catechin is the most commonly described flavonoid present in barley. All these soluble phenolic compounds are known to have protective effects against pathogen infection, UV irradiation, and oxidative stress and these effects indirectly reflect the resistance of the plant against stress (*22*). Flavan derivates have been described as inducible antimicrobial metabolites in rice as phytoalexins (*23*). The proanthocyanidins found in barley are oligomers of epicatechin and gallocatechin. They have antioxidant and radical scavenging

*To whom correspondence should be addressed.

Published on Web 02/09/2010

activities and are capable of metal complexation, of exhibiting antimicrobial properties, and of having an affinity for proteins, resulting in enzyme inhibition and/or protein precipitation (*24*). The combination of these properties of phenolic compounds and their participation in the strengthening of the cell wall material therefore illustrates their role in the defense response of the plants against pathogenic infection.

Emmer (*Triticum dicoccum*) and naked barley (hull-less barley, *Hordum vulgare* var. nudum) cultivars are usually grown in organic farming systems, and to date, no data exist about their behavior under conventional farming conditions. In addition, not much data are available on the potential effects of *Fusarium* infections on the phenolic compound profile in the grains of these cereals. More knowledge about the interaction between the fungus and grain phenolics production could contribute to a better understanding of the mechanisms involved in the reaction of the plant hosts and thereby provide criteria for determination of their susceptibility to pathogenic infection.

The present study focuses on the profiling of phenolics in emmer and naked barley grains after infection by *Fusarium* spp. In an earlier study the total content of phenolics was determined (*19*), but in the present investigation it was intended to fractionate the phenolic compounds into two groups: those that are free or soluble and those that are AX-bound. Further, our objective was, in comparison to previous studies, to study fully developed and harvested grains, reflecting a status after the complete *Fusarium* infection period (*10*). Moreover, we were interested in characterizing probable modifications of the phenolic compound composition after *Fusarium* infection as a stress-induced response of the plant. This response is provoked by fungal metabolites, including effects on the plant cell wall structures, where phenolic compounds are incorporated. These data explain the role of phenolic compounds as a response to the *Fusarium* infection and as stress-influenced molecules. We want to demonstrate the possible defense mechanisms of grains, showing differences in these defense strategies between the species. Additionally we will discuss different phenol fractions, free and cell wall bound, in connection with the fungal infection.

MATERIALS AND METHODS

Chemicals. For toxin analysis all standards were purchased from Biopure Co. Ltd. (Austria) as certified analytical standards. Reference compounds used for phenol quantification in the experiment are gallic acid (Sigma Aldrich), protocatechuic acid (Roth), (+) catechin (Roth); vanillic, caffeic, ferulic, *p*-cumaric, and salicylic acids (Roth), phloroglucinol (Fluka), pyrogallol (Fluka), and quercitin (Riedel-de-Häen). External standards for arabinose were D-(−)-arabinose and D-(+)-xylose (highest purity available; Sigma-Aldridge, Taufkirchen, Germany).

Experimental Design and Sample Preparation. Three emmer cultivars and seven naked barley cultivars grown in 2007 with eight replications were randomized in two field trials (Reinshof (RH) and Sattenhausen (SH)) in the center of Germany near the city of Göttingen and were chosen for the analysis. The location conditions at Reinshof are 152 m above sea level, wind sheltered, and dale area near a river border. The N_{min} content was 145 kg ha^{-1} recorded at a 90 cm depth of the soil. At Sattenhausen, the conditions are 260 m above sea level, hilly, and windy. The N_{min} content was 95 kg ha^{-1} at a 90 cm soil depth with an additional fertilization of 40 kg ha^{-1} 2 months after sowing.

In each block, the plants in the second row were artificially inoculated with a mixed DON-producing *Fusarium culmorum* and *Fusarium graminearum* spore suspension (50 mL m^{-2}; 1×10^5 spores/mL) three to five times during flowering by spray inoculation. Three strains of both *F. culmorum* (FC34, FC35, FC36) and *F. graminearum* (FG142, FG143, FG144) were used for conidiospore production. The DON-producing strains were isolated from wheat spike in Bavaria and are reference stocks from the Division of Plant Pathology and Crop Protection at the Department of Crop Science of the Georg-August-University Göttingen.

After the harvest, the grains without inoculation (later termed as natural infection) and those with inoculation (later termed as artificial infection) from the respective plots (each four replications) in both field trials were mixed. The grains from the different plots were mixed to create conditions which are also found under normal harvesting conditions. Whole grain flour was milled with a Retsch Rotormill ZM 100 to a particle size of 0.5 mm. These mixed-grain flours were then used three times for sampling from different regions of the complete sample of each cultivar and growing region.

HPLC-MS/MS of DON and 3-Ac-DON. *Sample Preparation.* Five grams of whole grain flour was extracted with 40 mL of an acetonitrile−water mixture (80:20) overnight on a reciprocal shaker. The extracts were centrifuged for 12 min at 5000*g*, and 4 mL of the supernatant was used for solid-phase extraction according to the manufacturer's instructions (Bond-Elut Mycotoxin, Varian GmbH, Darmstadt, Germany). Two milliliters of the cleaned extract was evaporated to dryness under vacuum, and the residue was redissolved in 200 μL of methanol−water (50:50) containing 0.2 mM ammonium acetate and applied for the analysis.

Analysis. For HPLC-MS/MS, a Varian 1200 L MS/MS system (Varian Inc., Palo Alto, CA) equipped with a triple-quadrupole mass spectrometer, two ProStar 210 liquid chromatographic pumps, a 410 autosampler, and a 500 MS Ion Trap mass spectrometer with ESI interface was used. Ten microliters of the solution prepared as described above was injected onto a C18 column (100 × 2 mm, 3 μm particle size) filled with polar modified material (Polaris Ether, Varian GmbH, Darmstadt, Germany), and the analytes were eluted with a methanol−water gradient (15−70% during 20 min) containing 0.2 mM ammonium acetate at a flow rate of 0.2 mL/min. DON and 3-Ac-DON were detected by tandem mass spectrometry as described previously (*25*). The other DON precursor, 15-Ac-DON, was not found in our samples. The peak intensity was used for the quantitative and qualitative analysis applying external standards (see Chemicals).

RP-HPLC of Grain Phenols. The analysis of the phenolic compounds was conducted according to a modified method of Kim et al. and Yu et al. (*21*, *26*).

Sample preparation. The aqueous methanol soluble phenolic acids were extracted consecutively three times (15, 15, and 10 mL for 12, 3, and 3 h) in a 50 mL falcon tube from 2 g of whole grain flour with 80% aqueous methanol containing 1% acetic acid at 20 °C. The sample solutions were centrifuged three times for 5 min at 4000 rpm. The supernatants were pooled and freeze-dried. The dried phenolic acids were resuspended in 4 mL of 80% aqueous methanol containing 1% acetic acid and were stored at −20 °C until analysis. Just before the HPLC was performed, the cold samples were centrifuged for 3 min at 6000 rpm and the supernatant was transferred into a vial for injection (**Figure 1**).

To compare the extraction using two different methods, this procedure was altered and the supernatant was divided into two parts: a 20 mL aliquot of the extract was freeze-dried, and in the case of the other 20 mL aliquot the methanol was evaporated at 100 °C and 5 mL of 0.2 M H_2SO_4, was added, followed by 1 h of hydrolysis at 100 °C. The rest of the procedure followed the extraction of insoluble phenolic acids as described below (**Figure 1**).

The extraction of the covalently bound phenolic acids was performed for the pellet remaining from the aqueous methanol extraction (**Figure 1**) or by using 2 g of the whole grain flour directly. The first step involved the addition of 20 mL of 0.1 M H_2SO_4 to the samples and 1 h of hydrolysis at 100 °C. After the extract was cooled to room temperature, 5 mL 1 M Na acetate solution (pH 5.5) containing 3900 units of α-amylase (product code 10065-10G, 30 units/mg dry matter; Sigma Aldrich, Switzerland) was added, and the mixture was incubated for 2 h at 30 °C. This treatment was followed by addition of 4 mL of 0.1 M Na acetate solution (pH 5.5) containing 94.4 units of cellulase (product code 22178-25G, 1.0 unit/mg dry matter; Sigma Aldrich, Switzerland) and incubation at 30 °C for 18 h. Finally, 6 mL of 25% NaCl solution was added and the liberated phenols were extracted three times, each with 10 mL of ethyl acetate. The ethyl acetate fraction was pooled and dried under the extractor hood. The dried extract was resuspended in 4 mL of 80% aqueous methanol containing 1% acetic acid and kept at −20 °C until analysis (**Figure 1**). Prior to HPLC, the cold samples were centrifuged for 3 min at 6000 rpm and the supernatant was transferred to a vial, ready for injection. All samples for phenol analysis were extracted three times for each location (n = 6 extract replication) so that 18 replications for emmer and 42 replications for naked barley were possible.

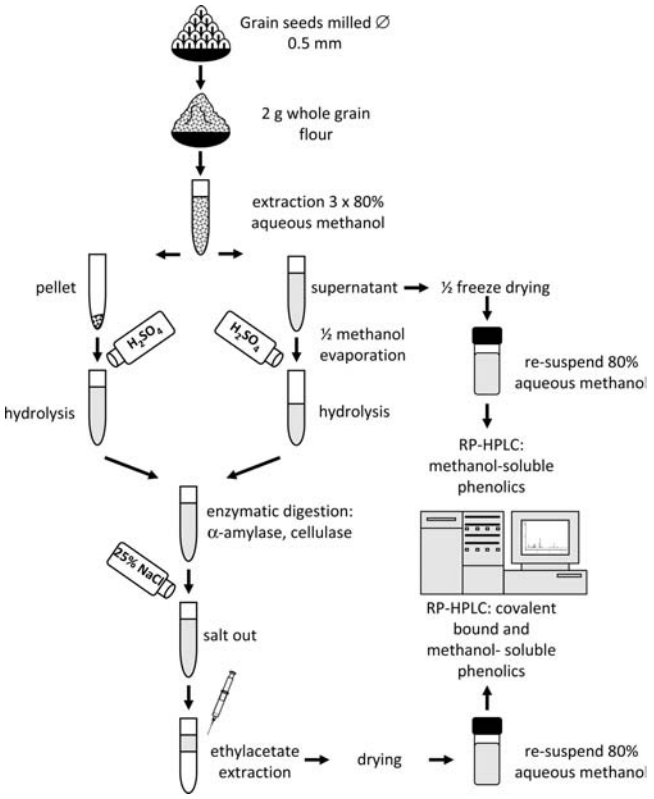

Figure 1. Extraction procedure of grain phenolics and separation procedures for the extraction of methanol-soluble phenolics and covalent bound phenolics for quantitative RP-HPLC. Aqueous methanol was made of 80% methanol with 20% distilled H_2O (v/v) containing 1% acetic acid, 20 mL of 0.1 M H_2SO_4 was used for hydrolysis of covalent bound phenolics, 5 mL of 0.2 M H_2SO_4 was used for hydrolysis of methanol-soluble phenolics, and the concentration after resuspension for analysis was 0.5 g of flour mL^{-1}.

Analysis. For the HPLC, a dual pump mode Shimadzu 10A system (Duisburg, Germany) with an LC18 column (precolumn, K2-02, 20 × 2.0 mm, Prontosil 120-5-C18 ace-EPS, 5 μm; separation column, Prontosil 120-3-C18 ace-EPS COL SC-150; Bischoff Analysentechnik and -Geräte GmbH, Leonberg, Germany) was used. The flow rate was 0.8 mL/min, and detection was performed by UV detection at 280 and 325 nm with a column temperature of 40 °C. The eluents were (A) 2% acetic acid, pH 1.9, and (B) methanol. The gradient was applied under the following conditions: 100% eluent A, 0 min; 90% eluent A, 2 min; 70% eluent A, 35 min; 10% eluent A, 50 min; 0% eluent A, 52 min; 0% eluent A, 56 min; 100% eluent A, 60−75 min (regeneration/equilibration). The injection volume of the samples was 20 μL. The quantification was performed using an external calibration with diluted standard solutions (range 12.5−200 μg/mL in 80% aqueous methanol).

HPAEC-PAD of Arabinoxylans (AX). *Sample Preparation*. For the analysis of total AX, 25 mg of flour sample and 2 mL of 1 M H_2SO_4 were incubated for 2 h in a 10 mL screw-capped glass tube in a laboratory sand bath at 110 °C in a drying oven. The samples were then cooled to room temperature in a water bath. In a following step, 2 mL of 2 M NaOH was added up to pH 7 and the pH value was checked by a pH test paper (to confirm neutral conditions). The sample solution was than centrifuged for 5 min at 3500 rpm. The supernatant (3 mL) was removed, mixed with 3 mL of yeast suspension (*Saccharomyces cervisiae*, Type II; Sigma-Aldrich, Taufkirchen, Germany, at 25 mg/mL, pH 7), and incubated for 2 h at 37 °C, while being gently shaken in a water bath. Yeast was used to digest as well as to consume the glucose liberated to avoid any interference of xylose and arabinose detection. This was followed by the addition of 1.5 mL of 2 M barium acetate ($Ba(CH_3COO)_2$). The sample was vortexed and centrifuged for 5 min at 3500 rpm. An aliquot of the supernatant was diluted 1:100, filtered through a 0.45 μm nylon filter, and analyzed.

Analysis. The subsequent HPLC was performed with a DIONEX BioLC 500 chromatography system (DIONEX GmbH, Germany); consisting of an AS 50 autosampler, an ED 50 amperometrical detector with PAD cell and two GS 50 gradient pumps. The BioLC system was operated by Chromeleon 6.50 SP 7 chromatography software (DIONEX GmbH, Germany). A CarboPac PA-1 precolumn, 50 mm × 2 mm (DIONEX GmbH, Germany) and, for separation, a CarboPac PA-1 column 250 mm × 2 mm, 10 μm (DIONEX GmbH, Germany) were used and operated at 25 °C in a column oven. The mobile phases were H_2O (solvent A) and 0.1 M NaOH (solvent B). A 0.2 mL/min isocratic flow (A:B 80:20) under helium was applied for 50 min, and 25 μL per sample was injected. A reference Ag/AgCl electrode was used in combination with a working gold electrode for detection using the following pulse potential sequence and durations: +0.1 V for 0.40 s, −2 V for 0.01 s, +0.6 V for 0.01 s, and −0.1 V for 0.06 s. The detector response for the external standards D-(−)-arabinose and D-(+)-xylose (highest available purity; Sigma-Aldridge, Taufkirchen, Germany) was linear in the concentration range of 0.5−20.0 mg/L ($R^2 > 0.95$) and was used to determine the concentration in the samples using the following equation: $C_{\text{arabinoxylans}} = 0.88[C_{\text{arabinose}} + C_{\text{xylose}}]$. To exclude arabinose and xylose from sources other than from AX, the factor of 0.88 given in the equation was introduced according to Hollmann et al. (*27*). For testing the influence of the AX content from each species, one emmer (Klein) and one naked barley (ZFS) cultivar were used as representatives for investigation.

Table 1. Total *Fusarium* Toxin Content Based on DON + 3-Ac-DON Concentration in Naturally and Artificially Infected Emmer (Three Cultivars) and Naked Barley (Seven Cultivars) Grains

	Fusarium toxin content (mg kg^{-1})		
species	natural infection	srtificial infection	p^a
emmer	1.1 ± 0.75	4.9 ± 3.5	0.0245
naked barley	0.2 ± 0.22	2.8 ± 1.9	0.0002

[a] Based on paired Student *t* test (emmer, $n = 18$; naked barley, $n = 42$). Data are presented as mean value ± standard deviation; p = significance.

Data Analyses. Data analyses were performed using Microsoft Excel 2003 for mean values and standard deviation. SigmaPlot 10.0 was used for the correlation (r) and statistical significance (p).

RESULTS AND DISCUSSION

Effects of *Fusarium* Infection on the Grain Phenolics. The content of the *Fusarium* toxins DON and 3-Ac-DON in emmer and naked barley showed a significant increase in the grains of artificially infected plants in comparison to the grains of naturally infected plants (**Table 1**). The accumulation of these mycotoxins indicates that both types of cereal and all the tested cultivars are liable to a certain degree of infection, underlining their susceptibility to *Fusarium* spp. (*4*, *5*, *28*). The obtained data showed in part high standard deviations for both toxins and phenolics (**Tables 1** and **2**) due to the field trials at two separate locations and the different cultivars investigated within one species.

Phenolic compounds were detected in small amounts in the hydrolyzed methanol-soluble fraction. They mainly represented the cinnamic acid derivates caffeic and ferulic acid (**Table 3**). These results confirm the literature data, where such hydroxycinnamates were also identified in the methanol-soluble fraction (*21*, *29*).

The flavonoid catechin was the predominant phenol in the methanol-soluble fraction in naked barley, but it was completely missing in emmer (**Tables 2** and **3**). Some flavonoids reported in other studies as being present in barley besides catechin and proanthocyanidins (e.g., hesperidin, kaempferol, myricetin, naringenin, quercetin, and rutin) (*20*, *21*, *30*) could not be detected in the investigated naked barley cultivars. However, to the best of our knowledge, this is the first study documenting that artificial *Fusarium* infection led to a significant increase of about 4.5% in the catechin content in the grains in comparison to the content for natural infection (**Tables 2** and **3**). We postulate, therefore, that in naked barley the biosynthesis of catechin can be induced as a

Table 2. Phenol Content in Grains of Naturally and Artificially *Fusarium* Infected Emmer (Three Cultivars) and Naked Barley (Seven Cultivars) Samples: Fraction 1, Methanol Soluble (80% MeOH + 1% Acetic Acid); Fraction 2, Acid−Enzyme Hydrolyzable (H_2SO_4/Enzyme)

		phenol content (mg kg^{-1})					
		emmer			naked barley		
fraction	phenol	natural infection	artificial infection	p^b	natural infection	artificial infection	p^b
1	catechin	n.d.[c]	n.d.		94.7 ± 34.1	99.3 ± 33.2	0.046
2	caffeic acid	n.d. (3.2)[a]	n.d. (2.6)[a]		19.6 ± 2.4	18.6 ± 2.7	<0.001
	ferulic acid	310.4 ± 12.7	292.9 ± 13.9	<0.001	283.6 ± 32.7	264.9 ± 32.7	<0.001
	p-coumaric acid	10.5 ± 3.6	9.6 ± 2.7	0.093	11.1 ± 5.5	11.1 ± 6.3	0.719

[a] Data of cultivar Klein. [b] Based on paired Student *t* test (emmer, $n = 18$; naked barley, $n = 42$); p = significance. [c] n.d. = not detectable.

Table 3. Phenolic Compounds in Grains of One Emmer and One Naked Barley Cultivar after Natural and Artificial *Fusarium* Infection As Determined by Different Extraction Procedures: Fraction 1, methanol soluble (80% MeOH + 1% acetic acid); Fraction 2, Acid−Enzyme Hydrolyzable (H_2SO_4−Enzyme)

		phenol content (mg kg^{-1})			
		emmer cv. Klein		naked barley cv. ZFS	
fraction	phenol	natural infection	artificial infection	natural infection	artificial infection
1	catechin	n.d.[a]	n.d.	178.3	183.6
	caffeic acid	4.7	4.8	7.5	7.3
	ferulic acid	13.2	11.1	9.0	7.8
	p-coumaric acid	n.d.	n.d.	n.d.	n.d.
2	catechin	n.d.	n.d.	n.d.	n.d.
	caffeic acid	1.7	1.8	18.4	14.2
	ferulic acid	345.5	321.4	336.0	270.5
	p-coumaric acid	5.5	5.7	1.6	n.d.
content in fraction 1 + 2	catechin	n.d.	n.d.	178.3	183.6
	caffeic acid	6.4	6.5	25.9	21.5
	ferulic acid	345.5	332.6	345.1	278.3
	p-coumaric acid	5.5	5.7	1.6	n.d.
Total phenol content		357.4	344.8	550.9	483.4

[a] n.d. = not detectable.

reaction to *F. graminearum* and *F. culmorum* infection (**Figure 2**). We further postulate that catechin may represent a valuable marker to explain the lower susceptibility of barley to *Fusarium* spp. in comparison to emmer (**Tables 1** and **2**), because the degree of infection based on the *Fusarium* toxin content was much lower in naked barley than in emmer. However, the naked barley cultivars with the highest catechin content did not show the lowest susceptibility in all cases (data not shown). Therefore, we could conclude that catechin could be just one of the factors in naked barley characterizing its natural plant disease defense response as described in the literature (*23*). The induction of specific enzymes involved in the phenylpropanoid pathway after *Fusarium* infection may explain the observed change in catechin content (*31*).

In comparison, no catechin was found in the extracted cell-wall-bound fraction (H_2SO_4/enzyme hydrolyses) (**Tables 2** and **3**). Here, ferulic acid represented the predominant hydroxycinnamate derivate (*13*), followed by caffeic acid and *p*-coumaric acid. A statistically significant lower content of both ferulic and caffeic acids was found after artificial infection of naked barley, in comparison to the naturally infected samples (**Table 2**). In emmer only ferulic acid was affected. In general, all the investigated cultivars of both emmer and naked barley responded with a reduction in their hydroxycinnamic acid contents (data not shown). These results are in agreement with those obtained for wheat cultivars also showing a reduced total phenol content (*19*). A reasonable tentative conclusion can thus be postulated that during *Fusarium* infection the synthesis of these substances may be reduced or disturbed. One of the reasons could be the inhibition of peptidyl transferase as shown in rats, representing an integral part of the 60S ribosomal subunit, which is ubiquitous in eukaryotes, as well as the inhibition of DNA and RNA biosynthesis through the trichothecenes (*6*, *9*). However, other investigations have shown that the organelle which is mostly affected by *F. graminearum* in the plant cell is the chloroplast, in which the synthesis of the aromatic amino acids, the precursors of phenolics, occurs (*32*).

In the present study, an increase of ferulic and caffeic acid contents in the methanol-soluble fraction was not detected either in artificial infected or in naturally infected samples. Therefore, an enzymatic degradation, e.g. by fungal α-*N*-arabinofuranosidase and feruloyl esterase (*33*), can be excluded. In naked barley, a reduced production of these substances was observed, probably due to the catechin synthesis being favored (**Figure 1**). In grain, the derivates *p*-coumaric and caffeic acid are produced in 30 and 20 times lower concentrations than ferulic acid, respectively. These substances can be considered as being precursors of ferulic acid (*34*). It seems that the synthesis of *p*-coumaric and caffeic acid in the studied species is based on the amino acid tyrosine, because we did not find cinnamic acid as a precursor of *p*-coumaric acid. Therefore, the synthesis over the precursor phenylalanine and cinnamic acid may not have taken place in the grains of emmer and naked barley (**Figure 1**) (*34*).

The catechin concentration shown in **Table 3** was determined by a different extraction procedure, and we found its content doubled in comparison to the extraction without H_2SO_4/enzyme hydrolysis after methanol extraction (**Table 3**). The reason for this doubling could have been the release of catechin out of precursors after hydrolysis and a more certain signal in the chromatogram (*20*). In the investigated naked barley cultivar ZFS, the soluble phenol proportion of the total phenol content rose to 35%, whereas the emmer cultivar Klein showed only 5% soluble phenols. These differences in soluble phenol content between

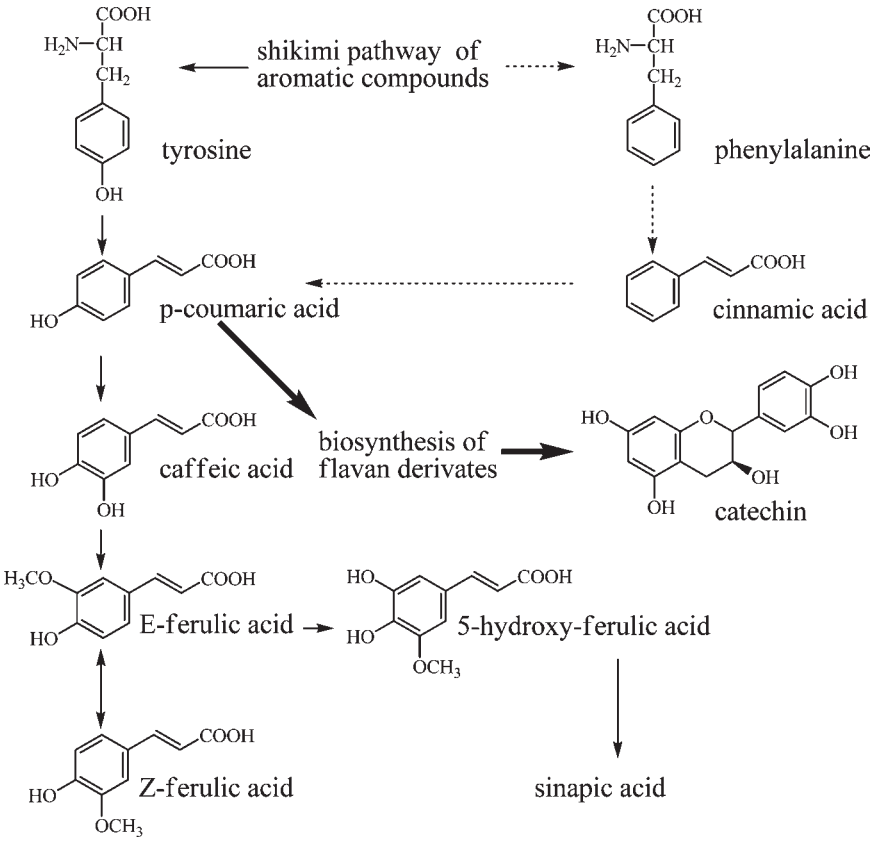

Figure 2. Supposed biosynthesis of caffeic, ferulic, and *p*-coumaric acid as well as catechin in the grains. Preferred ways of synthesis in grain and those postulated after *Fusarium* infection in barley are shown with thick arrows. Normal arrows show the steps of synthesis of ferulic acid, the main phenol in grain; dotted arrows show synthesis of phenolic acids postulated by the literature.

Table 4. Content of Total Arabinoxylan Monosaccharides in Grains of One Emmer and One Naked Barley Cultivar after Natural and Artificial *Fusarium* Infection[a]

	monosaccharide content (g kg^{-1})			
	emmer cv. Klein		naked barley cv. ZFS	
monosaccharide	natural infection	artificial infection	natural infection	artificial infection
xylose	23.4 ± 0.3	23.9 ± 0.4	25.7 ± 0.3	25.5 ± 0.5
arabinose	20.9 ± 0.2	20.2 ± 0.2	29.3 ± 0.1	29.4 ± 0.2

[a] Data are presented as mean value ± standard deviation.

both the two cereals may confirm the higher susceptibility of emmer to *Fusarium* infection.

Total AX Content. After artificial *Fusarium* infection, the investigated samples showed only marginal changes in their content of AX sugars, xylose and arabinose, in comparison to the content in the naturally infected samples (**Table 4**). There was no recognizable tendency that *Fusarium* infection changed the AX content (**Table 4**). The variations in emmer and naked barley were very low and so were not due to the presence of infection. Overall, AX was much less influenced by *Fusarium* infection than the ferulic acid content (**Table 2**). Ferulic acids acting as cross-linking molecules between AX chains showed a reduced content (**Table 2**). This reduction in cross-linking can diminish the function of AX as a barrier against biodegradability by microorganisms and reflects a reduced cell wall rigidity (*35*). Also, cross-links with other cell wall components bound to AX, such as lignin (ether bound), protein, cellulose, or other polysaccharides (ester bound) may also be influenced by the *Fusarium*-induced ferulic acid reduction (*15*, *17*, *18*, *36*). This process thus illustrates how *Fusarium* infection may succeed. However, these alterations in grain cell wall structure and cross-linking may also contribute to a lower and modified baking quality (*37*) as well as provoking a fragmentation of AX.

Looking at the differences in the content of arabinose, xylose, and AX between the two cereals, a lower content of AX in emmer of about 20% in comparison to that in naked barley was found. The literature data showed a wide range for AX in the different cereal species from 21 to 171 g kg^{-1}, for arabinose from 12 to 55 g kg^{-1}, and for xylose from 7 to 148 g kg^{-1} (*38*). The AX sugars in our investigation were not reduced in equal quantities: the xylose content in emmer was about 10% lower than in naked barley, and the arabinose concentration was reduced about 30%. The lower arabinose content in emmer in comparison to that in naked barley emphasizes a reduced cross-linking potential in the cell walls of the former species. This result thus provides a further reason for the lower susceptibility of naked barley to *Fusarium* infection, since a more complex network in the cell wall leads to higher cell wall rigidity and a reduced enzymatic degradation by *Fusarium*.

The higher caffeic acid concentration in the grains of naked barley compared to that in emmer may also be a further relevant factor in reducing its susceptibility to *Fusarium* in addition to its high ss-glucan content of about 5% (*39*), the latter being 10−15% higher than in emmer. The participation of these compounds in a stronger interaction with other cell wall compounds may contribute to barley's resistance against the mycelia growing from the fungus and thus reduce the degree of infection.

The presented results document that phenolic acid contents in the grains of both emmer and naked barley were influenced by

Fusarium infection, leading to a significant reduction in the total content of ferulic acid as the predominant phenol in the grains. The total AX did not change as a result of the *Fusarium* infection. However, comparing the two types of cereals, we found higher AX contents in naked barley than in emmer and also more arabinose, providing a possible site for phenol-involved cross-linking. In addition, naked barley grains contained the flavonoid catechin, which was significantly more highly concentrated after artificial *Fusarium* infection in comparison to naturally infected samples. Catechin is lacking in emmer, and this might be one of the reasons for the higher susceptibility of this species to the fungus. Further investigations are necessary to clarify the putative degradation of AX and to show the influence of fungal enzymes on structures containing AX. Furthermore, studies with colored barley cultivars, such as blue, purple, and black cultivars, should be performed to clarify the influence of flavonoids, proanthocyanidins, and anthocyans on the extent of *Fusarium* infection. Additionally, it is important to clarify and review the postulated and preferred mechanism of the phenol synthesis in infected grains described here and to elucidate the factors determining this modulation.

ABBREVIATIONS USED

AX, arabinoxylan; FHB, *Fusarium* head blight; DON, deoxynivalenol; 3-Ac-DON, 3-acetyldeoxynivalenol; LC-MS/MS, liquid chromatography with coupled mass spectrometry; *p*, significance factor; RP-HPLC, reverse-phase high-pressure liquid chromatography, GC-MS/MS, gas chromatography with coupled mass spectrometry.

ACKNOWLEDGMENT

Special thanks are given to the growers who provided the seeds for this research trial, Dr. Karl-Josef Müller, of Getreidezüchtungsforschung Darzau, Eckard Irion of Verein für Pflanzenzucht Hof Grub e.V.;, Claus Einfeldt of Saatzucht Ackermann, and Hans-Werner Klein of the UGB Gesundheitsförderung, as well as for the toxin analysis by the group of Prof. Petr Karlovsky and Sasithorn Limsuwan, Molecular Phytopathology and Mycotoxin Research, Department of Crop Sciences, University of Göttingen, 37075 Göttingen, Germany.

LITERATURE CITED

(1) Bottalico, A.; Perrone, G. Toxigenic Fusarium species and mycotoxins associated with head blight in small-grain cereals in Europe. *Eur. J. Plant Pathol.* **2002**, *108* (7), 611–624.

(2) Parry, D. W.; Jenkinson, P.; McLeod, L. Fusarium Ear Blight (Scab) in Small-Grain Cereals - A review. *Plant Pathol.* **1995**, *44* (2), 207–238.

(3) Ramirez, M. L.; Reynoso, M. M.; Farnochi, M. C.; Chulze, S. Vegetative compatibility and mycotoxin chemotypes among Fusarium graminearum (Gibberella zeae) isolates from wheat in Argentina. *Eur. J. Plant Pathol.* **2006**, *115* (2), 139–148.

(4) Yang, L.; van der Lee, T.; Yang, X.; Yu, D.; Waalwijk, C. Fusarium populations on Chinese barley show a dramatic gradient in mycotoxin profiles. *Phytopathology* **2008**, *98* (6), 719–727.

(5) Buerstmayr, H.; Stierschneider, M.; Steiner, B.; Lemmens, M.; Griesser, M.; Nevo, E.; Fahima, T. Variation for resistance to head blight caused by Fusarium graminearum in wild emmer (Triticum dicoccoides) originating from Israel. *Euphytica* **2003**, *130* (1), 17–23.

(6) Eriksen, G. S.; Pettersson, H. Toxicological evaluation of trichothecenes in animal feed. *Anim. Feed Sci. Technol.* **2004**, *114* (1−4), 205–239.

(7) Thuvander, A.; Wikman, C.; Gadhasson, I. In vitro exposure of human lymphocytes to trichothecenes: individual variation in sensitivity and effects of combined exposure on lymphocyte function. *Food Chem. Toxicol.* **1999**, *37* (6), 639–648.

(8) Tsuda, S.; Kosaka, Y.; Murakami, M.; Matsuo, H.; Matsusaka, N.; Taniguchi, K.; Sasaki, Y. F. Detection of nivalenol genotoxicity in cultured cells and multiple mouse organs by the alkaline single-cell gel electrophoresis assay. *Mutat. Res.* **1998**, *415* (3), 191–200.

(9) Yabe, T.; Hashimoto, H.; Sekijima, M.; Degawa, M.; Hashimoto, Y.; Tashiro, F.; Ueno, Y. Effects of nivalenol on hepatic drug-metabolizing activity in rats. *Food Chem. Toxicol.* **1993**, *31* (8), 573–581.

(10) Siranidou, E.; Kang, Z.; Buchenauer, H. Studies on symptom development, phenolic compounds and morphological defence responses in wheat cultivars differing in resistance to Fusarium head blight. *J. Phytopathol.* **2002**, *150* (4−5), 200–208.

(11) Hernanz, D.; Nunez, V.; Sancho, A. I.; Faulds, C. B.; Williamson, G.; Bartolome, B.; Gomez-Cordoves, C. Hydroxycinnamic acids and ferulic acid dehydrodimers in barley and processed barley. *J. Agric. Food Chem.* **2001**, *49* (10), 4884–4888.

(12) Santiago, R.; Reid, L. M.; Arnason, J. T.; Zhu, X.; Martinez, N.; Malvar, R. A. Phenolics in maize genotypes differing in susceptibility to Gibberella stalk rot (Fusarium graminearum Schwabe). *J. Agric. Food Chem.* **2007**, *55* (13), 5186–5193.

(13) Zhou, K.; Hao, J.; Griffey, C.; Chung, H.; O'Keefe, S. F.; Chen, J.; Hogan, S. Antioxidant properties of Fusarium head blight-resistant and -susceptible soft red winter wheat grains grown in Virginia. *J. Agric. Food Chem.* **2007**, *55* (9), 3729–3736.

(14) Allerdings, E.; Ralph, J.; Schatz, P. F.; Gniechwitz, D.; Steinhart, H.; Bunzel, M. Isolation and structural identification of diarabinosyl 8-O-4-dehydrodiferulate from maize bran insoluble fibre. *Phytochemistry* **2005**, *66* (1), 113–124.

(15) Bunzel, M.; Ralph, J.; Marita, J. M.; Hatfield, R. D.; Steinhart, H. Diferulates as structural components in soluble and insoluble cereal dietary fibre. *J. Sci. Food Agric.* **2001**, *81* (7), 653–660.

(16) Izydorczyk, M. S.; Biliaderis, C. G. Cereal arabinoxylans: Advances in structure and physicochemical properties. *Carbohydr. Polym.* **1995**, *28* (1), 33–48.

(17) Philippe, S.; Tranquet, O.; Utille, J. P.; Saulnier, L.; Guillon, F. Investigation of ferulate deposition in endosperm cell walls of mature and developing wheat grains by using a polyclonal antibody. *Planta* **2007**, *225* (5), 1287–1299.

(18) Pitkanen, L.; Tuomainen, P.; Virkki, L.; Aseyev, V.; Tenkanen, M. Structural comparison of arabinoxylans from two barley side-stream fractions. *J. Agric. Food Chem.* **2008**, *56* (13), 5069–5077.

(19) McKeehen, J. D.; Busch, R. H.; Fulcher, R. G. Evaluation of wheat (Triticum aestivum L.) phenolic acids during grain development and their contribution to Fusarium resistance. *J. Agric. Food Chem.* **1999**, *47* (4), 1476–1482.

(20) Quinde-Axtell, Z.; Baik, B. K. Phenolic compounds of barley grain and their implication in food product discoloration. *J. Agric. Food Chem.* **2006**, *54* (26), 9978–84.

(21) Kim, M. J.; Hyun, J. N.; Kim, J. A.; Park, J. C.; Kim, M. Y.; Kim, J. G.; Lee, S. J.; Chun, S. C.; Chung, I. M. Relationship between phenolic compounds, anthocyanins content and antioxidant activity in colored barley germplasm. *J. Agric. Food Chem.* **2007**, *55* (12), 4802–4809.

(22) Zagoskina, N. V.; Olenichenko, N. A.; Iun'vei, C.; Zhivukhina, E. A. [Formation of phenol compounds in various cultivars of wheat (Triticum aestivum L.)]. *Prikl. Biokhim. Mikrobiol.* **2005**, *41* (1), 113–116.

(23) Dixon, R. A. Natural products and plant disease resistance. *Nature* **2001**, *411* (6839), 843–847.

(24) Santos-Buelga, C.; Scalbert, A. Proanthocyanidins and tannin-like compounds - nature, occurrence, dietary intake and effects on nutrition and health. *J. Sci. Food Agric.* **2000**, *80* (7), 1094–1117.

(25) Adejumo, T. O.; Hettwer, U.; Karlovsky, P. Occurrence of Fusarium species and trichothecenes in Nigerian maize. *Int. J. Food Microbiol.* **2007**, *116* (3), 350–357.

(26) Yu, J.; Vasanthan, T.; Temelli, F. Analysis of phenolic acids in barley by high-performance liquid chromatography. *J. Agric. Food Chem.* **2001**, *49* (9), 4352–4358.

(27) Hollmann, J.; Lindhauer, M. G. Pilot-scale isolation of glucurono-arabinoxylans from wheat bran. *Carbohydr. Polym.* **2005**, *59* (2), 225–230.

(28) Oliver, R. E.; Stack, R. W.; Miller, J. D.; Cai, X. Reaction of wild emmer wheat accessions to Fusarium head blight. *Crop Sci*. **2007**, *47* (2), 893–899.
(29) Mattila, P.; Pihlava, J. M.; Hellstrom, J. Contents of phenolic acids, alkyl- and alkenylresorcinols, and avenanthramides in commercial grain products. *J. Agric. Food Chem*. **2005**, *53* (21), 8290–8295.
(30) Gu, L.; Kelm, M. A.; Hammerstone, J. F.; Beecher, G.; Holden, J.; Haytowitz, D.; Prior, R. L. Screening of foods containing proanthocyanidins and their structural characterization using LC-MS/MS and thiolytic degradation. *J. Agric. Food Chem*. **2003**, *51* (25), 7513–7521.
(31) Boddu, J.; Cho, S.; Kruger, W. M.; Muehlbauer, G. J. Transcriptome analysis of the barley-Fusarium graminearum interaction. *Mol. Plant-Microbe Interact*. **2006**, *19* (4), 407–417.
(32) Zhou, W.; Eudes, F.; Laroche, A. Identification of differentially regulated proteins in response to a compatible interaction between the pathogen Fusarium graminearum and its host, Triticum aestivum. *Proteomics* **2006**, *6* (16), 4599–4609.
(33) Wanjiru, W. M.; Kang, Z. S.; Buchenauer, H. Importance of cell wall degrading enzymes produced by Fusarium graminearum during infection of wheat heads. *Eur. J. Plant Pathol*. **2002**, *108* (8), 803–810.
(34) Schoch, G.; Goepfert, S.; Morant, M.; Hehn, A.; Meyer, D.; Ullmann, P.; Werck-Reichhart, D. CYP98A3 from Arabidopsis thaliana is a 3′-hydroxylase of phenolic esters, a missing link in the phenylpropanoid pathway. *J. Biol. Chem*. **2001**, *276* (39), 36566–36574.
(35) Ishii, T. Structure and functions of feruloylated polysaccharides. *Plant Sci*. **1997**, *127* (2), 111–127.
(36) Renger, A.; Steinhart, H. Ferulic acid dehydrodimers as structural elements in cereal dietary fibre. *Eur. Food Res. Technol*. **2000**, *211* (6), 422–428.
(37) Izydorczyk, M.; Biliaderis, C. G.; Bushuk, W. Comparison of the Structure and Composition of Water-Soluble Pentosans from Different Wheat-Varieties. *Cereal Chem*. **1991**, *68* (2), 139–144.
(38) Blakeney, A. B.; Flinn, P. C. Determination of non-starch polysaccharides in cereal grains with near-infared reflectance spectroscopy. *Mol. Nutr. Food Res*. **2005**, *49* (6), 546–550.
(39) Bhatty, R. S. The potential of hull-less barley. *Cereal Chem*. **1999**, *76* (5), 589–599.

Received for review October 9, 2009. Revised manuscript received January 27, 2010. Accepted January 30, 2010. This work is a part of the FAEN Joint Project 3 "Quality-related plant production under modified basic conditions: mycotoxins in the context of production, quality and processing", financed by the Ministry of Science and Culture of Lower Saxony, Germany.