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Solvolysis Procedures for the Determination of Bound Residues of the Mycotoxin Deoxynivalenol in *Fusarium* Species Infected Grain of Two Winter Wheat Cultivars Preinfected with Barley Yellow Dwarf Virus

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A trichloroacetic acid treatment at 140 °C for 40 min was successfully established as a suitable solvolysis procedure for the recovery of bound deoxynivalenol or its derivatives in *Fusarium*-infected plants. Deoxynivalenol itself was not decomposed in the procedure. The derivative 15-acetyl-deoxynivalenol was chosen as a model compound for setting free deoxynivalenol in an acid-catalyzed deesterification reaction, developing the method. This is the first report using a trichloroacetic acid solvolysis procedure as a sample incubation step to free bound deoxynivalenol and determine free from bound deoxynivalenol in the sample. Between 13 and 63% of the total deoxynivalenol consisted of nonextractable deoxynivalenol. Deoxynivalenol contents in grain of the susceptible cultivar "Agent" infected with *Fusarium* spp. were 12−24 times higher when compared to those for the corresponding moderately resistant cultivar "Petrus". The highest deoxynivalenol amounts were determined in grain infected with *Fusarium* spp. as well as simultaneously infected with BYDV. This solvolysis procedure may be of importance for distinguishing between resistant and susceptible plants and their ability to immobilize (bound) mycotoxins as a plant defense mechanism.

KEYWORDS: Nonextractable deoxynivalenol; *Fusarium*; barley yellow dwarf virus (BYDV); wheat grain.

INTRODUCTION

Fusarium species causing Fusarium head blight (FHB) in wheat may secrete a variety of mycotoxins into the infected plant tissue. Mycotoxins are secondary metabolites, and in general they are not essential for normal growth or survival of the *Fusarium* species, but they are harmful to animals and humans (*1*, *2*). Trichothecenes, such as deoxynivalenol and nivalenol, are economically the most important mycotoxins produced by *Fusarium* species.

Until now, analytical methods were only available for analyzing nonconjugated, extractable mycotoxins (3−*12*). On the basis of the identification of a deoxynivalenol conjugated compound (*13*), further conjugates could be assumed to be present in contaminated samples. The occurrence of bound residues in fruits and plants is a well-known problem in analyzing pesticide residues (*14*−*20*). Thus, the aim of this study was to develop solvolysis procedures to free the nonextractable deoxynivalenol and thereby gain more information about the presence of extractable and nonextractable amounts of deoxynivalenol conjugates in inoculated grain samples. This ability to deactivate mycotoxins by binding them could be correlated to Fusarium head blight susceptibility in wheat cultivars.

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EXPERIMENTAL PROCEDURES

Chemicals and Reagents. Acetonitrile, pro analysis and gradient grade (Mallinckrodt-Baker Griesheim, Germany), isooctane for residue analysis (Fluka-Chemie, Buchs, Switzerland), ethyl acetate (Merck, Darmstadt, Germany), and toluene p.a. (Carl Roth, Karlsruhe, Germany) were used as solvents. The extraction solution used acetonitrile/water (84:16, v/v), sodium chloride (Merck), 4-(dimethylamino)pyridine (Fluka), sodium hydrogen carbonate (Merck), potassium hydroxide (Merck), and heptafluorobutyric anhydride (Fluka). The DMAP/toluene mixture used 2 mg of 4-(dimethylamino)pyridine (DMAP)/mL of toluene, 3% aqueous sodium hydrogen carbonate solution. The mycotoxin standards, nivalenol, deoxynivalenol, 3-acetyl-deoxynivalenol, and 15-acetyl-deoxynivalenol certified trichothecene standards, were from Romer Labs, Inc. (Union, USA). Their purity was verified by comparison with compounds previously received from a different manufacturer or production lot. The stock standard solutions contained 333 μg of each mycotoxin/mL.

Apparatus. A hemacytometer (Thoma cell counter, Marienfeld, Germany), Ultraturrax homogenizer (Janke & Kunkel, Staufen, Germany), and a Mycosep No. 227 cleanup column (Romer Labs, Inc., Union, MO) were used.

GC/EI-MS Trichothecene Analysis. Gas chromatography (GC)/electron impact (EI)-mass spectra were recorded by a Varian 3800 gas chromatograph, with the use of an 8200 CX autosampler coupled to a Saturn 2000 ion trap mass spectrometer operating in EI mode. A volume of 1 μL of the trichothecenecontaining sample in isooctane was injected at a continuous flow of 1.4 mL/min on a Varian-Chrompack SIL-24 CP MS column (30 m × 0.25 mm i.d.). The column temperature ranged

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from 80 to 290 °C (with a temperature ramp of 10 °C/min), while the injector and detector (ion trap) temperature were maintained at 250 and 225 °C, respectively. Full-scan EI-mass spectra (*m*/*z* 100−600) were recorded for peak identification. The retention times (min) of the trichothecenes were as follows: nivalenol, 10.84; deoxynivalenol, 12.52; 3-acetyl-deoxynivalenol, 15.87; 15-acetyl-deoxynivalenol, 15.47.

Plant Material. Two winter wheat (*Triticum aestivum* L.) cultivars, "Agent" and "Petrus", that differed in their susceptibility to FHB according to the "Beschreibende Sortenliste" (*21*) were used for our experiments. Cv "Agent" is classified as 7 and cv "Petrus" as 2 within a rating scale varying from 1 (highly resistant) to 9 (highly susceptible). The seeds were sown on December 19 and 20 in 2000, 2001, and 2002 in 10 L pots filled with loamy organic soil under semifield conditions (outdoor pot experiments). After growth stage BBCH 25 (*22*, *23*) plants were fertilized weekly with 100 mL of 1% (v/v) solution of the fertilizer "Wuxal" (N−P−K: 8−8−6) (Aglukon, Germany). At BBCH 31 (first node detectable) all side tillers were eliminated, allowing only the main tillers to grow, and the plants were thinned out to 15 plants per pot. After harvesting, the wheat spikes were threshed by hand. All the grain samples were dried to 13% humidity and stored at −20 °C until used.

BYDV Vector and Virus Inoculation. Oat seedlings (*Avena sativa* L.) of cv "Jumbo" were infected with BYDV−PAV-strain (barley yellow dwarf virus transmitted by *Rhopalosiphum padi*) which was kindly provided by Dr. W. Huth (BBA, Braunschweig, Germany). The aphid species *R. padi* L. was used as the vector for transmission of the virus from infected to healthy oat seedlings. The BYDV−PAV bearing aphids and virus-free aphids were fed separately in screened cages in different cabins under greenhouse conditions (13 h of daylight at 23 °C; 11 h of dark at 20 °C). At BBCH 51 (early head stage, beginning of heading; 16 and 14 days before inoculation of wheat spikes with *F. culmorum* in 2001 and with *F. graminearum* in 2003, respectively) and at BBCH 39 (flag leaf stage, flag leaf fully unrolled; 22 days before inoculation of wheat spikes with *F. graminearum* in 2002), approximately 10 viruliferous aphids were placed in a small triangular paper bag which was attached to the *ab* axial surface of the flag leaf. Simultaneously, the same number of virus-free aphids were transmitted to the control wheat plants. After a feeding period of 7 days, the aphids were killed by spraying the plants with the insecticide Tamaron (methamidophos, 0.15% a.i., Bayer AG).

Seven days after transmission of the virus vectors, the infection of the wheat plants by BYDV was determined with ELISA (*24*) using a small section of the tip of the flag leaf of each inoculated plant.

Inoculum Production of *Fusarium culmorum* and *F. graminearum* and Inoculation of Wheat Heads. The fungal species *F. culmorum* (isolate Fc 46) and *F. graminearum* (isolate Fg 18.7) were grown on oat kernels as described below (by our own laboratory method). These *Fusarium* isolates were used for inoculation processes because they produce only the mycotoxin deoxynivalenol in infected grain. One-liter bottles were each filled with 400 g of oat kernels (presoaked in tap water for 12 h), plugged with cotton wool, and autoclaved three times (each at 121 °C and 1.2 bar for 1 h). For inoculation of oat kernels, conidia of *F. culmorum* or *F.* graminearum were produced on SNA-liquid medium (special low nutrient agar) (*25*) by stirring at 21 °C and, after an incubation period of 4 days, conidia were allowed to settle, the supernatant was decanted, and the spores were suspended in sterilized tap water. The autoclaved oat kernels were inoculated with 5 mL of conidial suspension of *F. culmorum* or *F. graminearum* (each 1×10^5 conidia/mL). The flasks were incubated for 3 weeks at 21 °C with a 16/8 h day/night cycle. To obtain uniform colonization of the kernels with the fungal species, bottles were shaken every other day. Conidial production of the fungal isolates was stimulated after spreading flat layers of the oat kernels in plastic trays (40 × 60 cm) and exposing them to near UV-light (Osram tubes, L36 W/73) for 3 weeks. During this incubation period, the oat kernel layers were moistened and mixed every other day. Afterward, the oat kernels with *Fusarium* growth were stored in plastic bags at 4 °C until used.

To prepare conidial suspensions for inoculation of the wheat plants, conidia of *F. culmorum* or *F. graminearum* were washed from the oat kernels with tap water and the suspensions were filtered through two layers of muslin. The resulting conidia concentrations were counted using a hemacytometer, and the spore concentrations were adjusted with tap water to 1×10^5 conidia/mL for *F. culmorum* and *F. graminearum*.

At midflowering (BBCH 65), 10 μL of conidial suspension of *F. culmorum* in 2001 and of *F. graminearum* in 2002 and 2003 was pipetted into the cavity between the lemma and palea of a floret in the middle of a spike. The heads were covered with a plastic bag tied to the stalk to maintain high relative humidity for 24 h.

Analysis of Extractable and Nonextractable Trichothecene Mycotoxins in Wheat Grain. Trichothecenes in wheat grain of both cultivars of the three *Fusarium*-infected variants (single-*Fusarium*-infected, BYDV-infected, and aphid-infested plants) were determined using gas chromatography/mass spectrometry (GC/MS) for verification analysis. At least three samples of each variant of each cv were analyzed, and only the average values with standard deviation are reported here.

Standard Operating Procedure (SOP). Five grams of the ground grain was homogenized with 35 mL of acetonitrile/water (84:16, v/v) using an Ultraturrax for 3 min. The volume of the mixture was adjusted to 50 mL using acetonitrile/water (84:16, v/v), the mixture was filtered, and 6 mL of extract was passed through a Mycosep column. Then, 1 mL of the purified extract was transferred to graduated tubes for further cleanup and analyzed.

Solvolysis Procedure I (SPI). Five grams of the ground grain was homogenized with 35 mL of acetonitrile/water (84:16, v/v) for 3 min. The volume of the mixture was adjusted to 50 mL using acetonitrile/water (84:16, v/v). While stirring the whole mixture, 5 mL of the homogenate was pipetted and transferred to a screw-capped tube and 2 mL of water and 1 mL of 1 M aqueous CCl_3COOH solution was added. Then, the sealed tube was subjected to solvolysis at 140 °C for 40 min in a drying furnace. After the mixture had cooled, 0.5 mL of 1 M KOH solution was added to the extract, and the volume was adjusted to 10 mL with acetonitrile. Then the extract was cleaned up by passing it through a Mycosep column, and it was analyzed. Because of the inhomogeneous distribution of particles in the unfiltered extract (SPI), this sample setup was modified in the year 2003 by separating the deoxynivalenol-containing particles through filtration. Then, both the filtrate and the residue could be solvolyzed individually, freeing deoxynivalenol from extractable and nonextractable conjugates with the use of a single ground grain sample according to the above-mentioned techniques, resulting in solvolysis procedures IIa and IIb.

Solvolysis Procedure IIa (SPIIa). For this, 2 g of the ground grain was extracted with 15 mL of acetonitrile/water (84:16, v/v) using an Ultraturrax for 3 min. After adjusting the volume to 25 mL and filtering the mixture, 5 mL of the extract, 2 mL of water, and 1 mL of 1 M aqueous CCl_3COOH solution were added, and the mixture was transferred to a 15 mL tube, which was then capped. Then, the sealed tube was subjected to solvolysis at 140 °C for 40 min in a drying furnace. After the mixture had cooled, 0.5 mL of 1 M KOH solution was added to the extract, and the volume was adjusted to 10 mL with acetonitrile. Then the extract was cleaned up using the Mycosep column and analyzed.

Solvolysis Procedure IIb (SPIIb). The whole residue remaining after filtration (see SPIIa) then was transferred into a 15 mL tube; 4 mL of acetonitrile/water (84:16, v/v), 2 mL of water, and 1 mL of 1 M aqueous CCl_3COOH solution were added and the mixture was solvolyzed according to solvolysis procedure IIa. After the mixture had cooled, 0.5 mL of 1 M KOH was added, and the volume was adjusted to 10 mL with acetonitrile. A total of 6 mL of the sample extract was passed through a Mycosep column and subjected to further sample clean up as described below.

Cleanup. Aliquots of 1 mL of each of the purified extracts from the SOP and solvolysis procedures I, IIa, and IIb were transferred to graduated tubes, 0.5 mL of saturated sodium chloride and 3 mL of ethyl acetate solution were added, and the components were mixed for 1 min using a Vibrax shaker (IKA-Vibrax, Janke & Kunkel; Staufen, Germany) at 900 rpm. After settling for 2 min, the mixture formed two layers. The top organic layer was removed and collected. The lower aqueous phase was extracted again using 2 mL of ethyl acetate. Then the aqueous phase was discarded. The combined organic fraction was dried by passing it through anhydrous sodium sulfate, and then it was evaporated to dryness under a gentle stream of nitrogen at 50 °C using

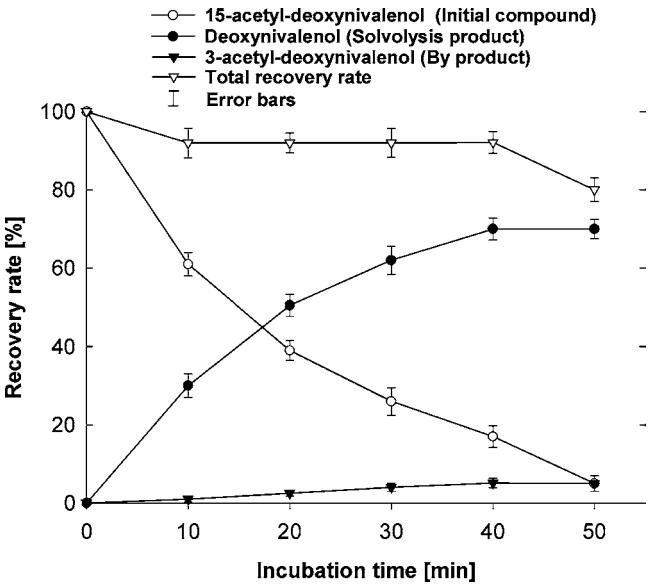


Figure 1. Recovery rates of all compounds (deoxynivalenol, 3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol) involved in our developed solvolysis processes as a function of the incubation time at 140 °C.

a temperature-controlled evaporation system (Barkey, Germany). The residue of the sample extract was redissolved in 1 mL of 4-(dimethylamino)-pyridine (DMAP)/toluene mixture (2 mg/mL in toluene) and 50 μL of heptafluorobutyric anhydride and derivatized in a 60 °C water bath for 20 min. After the mixture had cooled to room temperature, 1 mL of aqueous sodium hydrogen carbonate solution (0.03 g/mL in water) was added, the mixture was shaken for 10 s on a Vibrax shaker, and the layers were allowed to separate for 2 min. The lower phase was removed, and the top organic layer was washed with 1 mL of water. The top (toluene) layer was transferred to GC vials and analyzed by GC/MS. For identification and quantification purposes, a mixed standard containing nivalenol, deoxynivalenol, 3-acetyl-deoxynivalenol, and 15-acetyl-deoxynivalenol was prepared. The deoxynivalenol concentration was adjusted to 20 μg/L with isooctane, while the concentrations of the other components were adjusted to 30 μg/L. Quantification was done by external standard mode. The limit of quantification for all of the trichothecene derivatives was 10 μg/kg of flour for GC/EI-MS analysis.

RESULTS AND DISCUSSION

The total deoxynivalenol contents in grain of the susceptible cv "Agent" of the three *Fusarium*-infected variants (single *Fusarium*-infected (F), BYDV-infected (VF), and aphid-infested plants (AF)) were 12 to 24 times higher compared to the total deoxynivalenol contents in the grain of all the tested variants of the moderately resistant cv "Petrus" in the years 2001 and 2002. Deoxynivalenol contents in grain of variants VF were the highest in the three variants of both cultivars in both years. From the same grain samples higher amounts of deoxynivalenol were detected by the analytical SPI method (extractable and nonextractable deoxynivalenol) than by the SOP method (extractable deoxynivalenol) (**Figure 2**).

In 2001, deoxynivalenol amounts in grain resulting from *F. culmorum*-inoculated variants F, VF, and AF of the susceptible cv "Agent" were 14.6, 57.1, and 34.7 μg/g, respectively, determined by the SPI method. The corresponding extractable deoxynivalenol amounts determined by the SOP were 71, 79, and 72% of the deoxynivalenol contents analyzed by SPI, respectively (**Figure 2A**). Grain from the moderately resistant cv "Petrus" contained markedly lower amounts of deoxynivalenol: 1.0 μg/g (variant F), 4.7 μg/g (variant VF), and 1.5 μg/g (variant AF), respectively, determined by the SPI method. The

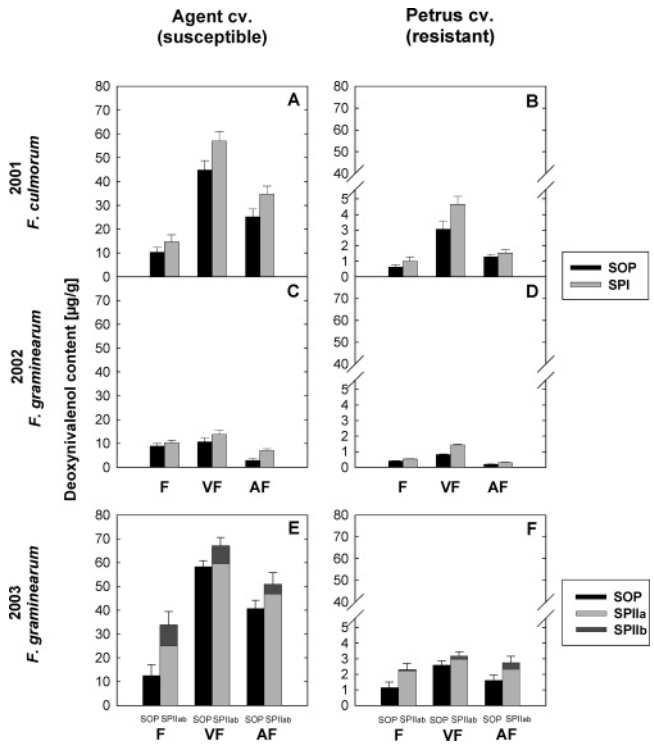


Figure 2. Deoxynivalenol content [μg/g of ground grain] of the winter wheat cvs "Agent" (susceptible) and "Petrus" (resistant) in different experimental variants: F = *Fusarium culmorum* infection in 2001, *F. graminearum* infection in 2002 and 2003; AF = *Rhopalosiphum padi* infestation plus *Fusarium* spp. infection; VF = BYDV infection plus *Fusarium* spp. infection. (Error bars are one standard error.)

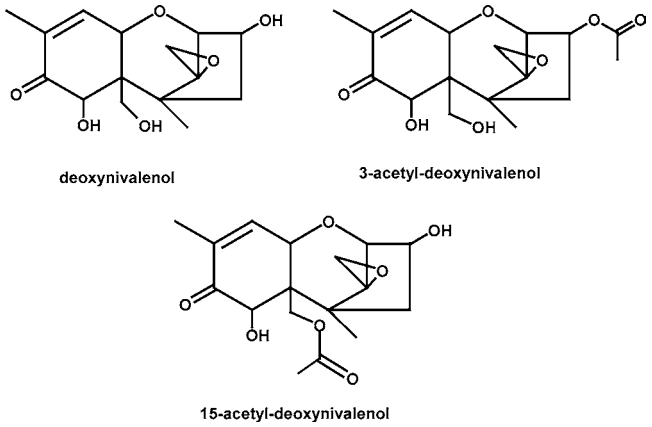
corresponding extractable deoxynivalenol amounts determined by the SOP were 61, 66, and 83% of the deoxynivalenol contents analyzed by SPI, respectively (**Figure 2B**).

In 2002, deoxynivalenol contents of all *F. graminearum*-infected variants were lower than those determined in 2001 of both cvs. In cv "Agent" the deoxynivalenol contents of the variants F, VF, and AF were 10.4, 13.9, and 7.1 μg/g, respectively, determined by the SPI method. The corresponding extractable deoxynivalenol amounts determined by the SOP were 87, 77, and 41% of the deoxynivalenol contents analyzed by SPI, respectively (**Figure 2C**). In cv "Petrus" the deoxynivalenol contents of the variants F, VF, and AF were 0.5, 1.4, and 0.3 μg/g, respectively, determined by the SPI method. The corresponding extractable deoxynivalenol amounts determined by SOP were 76, 56, and 67% of the deoxynivalenol amounts analyzed by the SPI method, respectively (**Figure 2D**).

When the deoxynivalenol amounts in grain of the susceptible and moderately resistant cvs in 2003 are considered, a similar contamination profile could be observed as in 2001 and 2002, respectively. Deoxynivalenol amounts in the grain of cv "Agent" of the three variants F, VF, and AF were 15 to 21 times higher compared to the amounts in the grain of cv "Petrus". When both solvolysis procedures SPIIa and SPIIb are applied to the variants F, VF, and AF, the deoxynivalenol amounts analyzed were 33.8, 67.0, and 50.9 μg/g in the case of the susceptible cv "Agent" and 2.3, 3.2, and 2.8 μg/g in the case of the moderately resistant cv "Petrus", respectively (**Figure 2** parts **E** and **F**).

In cv "Agent" (cv "Petrus") the extractable deoxynivalenol amounts of the variants F, VF, and AF determined by the SOP were 37 (50), 87 (81), and 80 (59)% of the deoxynivalenol amounts determined by the combination of SPIIa and SPIIb, respectively. Because of the individual method properties of

Chart 1. Chemical Structures of Deoxynivalenol, 3-Acetyl-deoxynivalenol, and 15-Acetyl-deoxynivalenol



SPIIa and SPIIb, we can focus deoxynivalenol analysis on either the filtered extract (SPIIa) or the residual pellet after filtration (SPIIb), and thus it is possible to compare these amounts to the corresponding SOP results. When the residual pellet after filtration of the grain extracts of variants F, VF, and AF of the cv "Agent" ("Petrus") is examined, by the application of only SPIIb analysis, the deoxynivalenol contribution was 26 (3), 11 (7), and 8 (16)% of the deoxynivalenol amounts analyzed by the combination of SPIIa and SPIIb, respectively (**Figure 2** parts **E** and **F**). On the other hand, compared to the deoxynivalenol amounts analyzed by the combination of SPIIa and SPIIb, only 74 (97), 89 (93), and 92 (84)% of the deoxynivalenol amounts were determined in cv "Agent" ("Petrus") when only the SPIIa analysis was applied to the filtered extracts of the grain of the variants F, VF, and AF. When the deoxynivalenol amounts determined by the SOP in the filtered extracts of the grain of the different variants are compared to the corresponding SPIIa data, this new analysis procedure revealed higher deoxynivalenol amounts.

To establish suitable solvolysis conditions for freeing deoxynivalenol from conjugated deoxynivalenol species, 15-acetyl-deoxynivalenol (**Chart 1**) was chosen as a model compound for testing the applied solvolysis procedures. With the use of this well-known, available trichothecene toxin as a model compound for an esterified "deoxynivalenol conjugate", we were able to prove and optimize the solvolysis efficiency in our experiments freeing deoxynivalenol up to 70% (**Figure 1**) and preventing it from decomposing. With regard to this developed solvolysis process (**Figure 1**) the optimal solvolysis conditions could be established by increasing the incubation time to 40 min at 140 °C, thereby achieving a 92% total recovery rate of all of the involved compounds (deoxynivalenol, 3-acetyl-deoxynivalenol, and 15-acetyl-deoxynivalenol). When the incubation time was increased to 50 min, then a decreased total recovery rate (<80%) could be observed, assuming decomposition processes of these compounds. On the other side, we get all the data of our solvolysis experiments at room temperature, too. As an example, the extracted and analyzed amount of deoxynivalenol of a sample extract at room temperature (0.053 μmol/L) could be increased to 0.059 μmol/L by increasing the extracting temperature to 140 °C; but the addition of acid at 140 °C (solvolysis) pushes up the analyzed amount of deoxynivalenol to 0.065 μmol/L, an improvement of about 22% extraction efficiency. Thus, the above-mentioned solvolysis procedures could be developed effectively.

It has been demonstrated that analysis by gas chromatography using MS detection together with a deoxynivalenol solvolysis procedure provides a suitable and selective trace determination of total deoxynivalenol in the sample. In 2003, the optimized solvolysis procedures SPIIa and SPIIb were developed to distinguish extractable and nonextractable deoxynivalenol using a single acetonitrile/water homogenized grain sample. With the application of this new analytical tool as a pretreatment step to all of the *Fusarium*-infected grain samples, increasing amounts of deoxynivalenol could be analyzed compared to the original SOP method. Therefore, we checked all sample extracts as a first analysis step by GC/MS for (natural) free, extractable 15-acetyl-deoxynivalenol and 3-acetyl-deoxynivalenol, because these compounds will be converted to deoxynivalenol in our applied solvolysis process, too. However, our aim was to check the freed amounts of deoxynivalenol coming only from the conjugated species. When this is taken into account, our results indicate that considerable amounts of deoxynivalenol exist in a conjugated or bound form in the inoculated grain.

Consideration of the amounts of deoxynivalenol in the grain of the moderately resistant cv "Petrus" shows an increase of up to 95% of deoxynivalenol could be identified using the extended SPIIa and SPIIb treatment procedures compared to the SOP results (variants F and AF, **Figure 2**). When the optimized solvolysis procedures SPIIa and SPIIb were applied to the grain samples from the year 2003, the nonhomogeneous distribution of particles in the unfiltered extract (SPI) was overcome, and thus we were able to determine the extent of nonextractable deoxynivalenol amounts.

This dramatic increase of conjugated deoxynivalenol amounts could also be observed in the year 2001 but not in the year 2002. These fluctuations might depend on different climatic conditions that moderate plant physiology and helping more or less to prevent *Fusarium* infection. Otherwise it could be assumed that the first developed solvolysis procedure I was less effective.

Because mycotoxins, e.g. deoxynivalenol, are regarded as a virulence factor of *F. graminearum* and *F. culmorum*, causing head blight in wheat plants, our aim was to distinguish between grain produced by the moderately resistant and susceptible wheat cvs with regard to the ability to immobilize mycotoxins as a plant defense strategy by inactivating those compounds. When the data of both wheat cvs are compared, an effective plant inactivating process producing conjugated deoxynivalenol amounts could be assumed not only in the case of the moderately resistant cv "Petrus" but also in the susceptible cv "Agent". To prove this mycotoxin immobilizing process, there is a need for more data regarding this complex behavior in plants.

Miller and Young (*26*) monitored deoxynivalenol and zearalenone in wheat plots that were inoculated with a strain of *F. graminearum*. They found that deoxynivalenol levels increased for 6 weeks after inoculation, and then decreased. Reinbrecht et al. (*27*) reported on the kinetics of trichothecene accumulation in two winter wheat cvs "Pegassos" (moderate resistant) and "Kontrast" (susceptible). Heads of cv "Pegassos" exhibited lower deoxynivalenol concentrations than those of cv "Kontrast". The highest deoxynivalenol accumulations in heads were analyzed between 28 and 42 days after inoculation; afterward toxin contents declined at ripening (56 days after inoculation). Yi et al. (*28*) stated that independent of precrops and treatments, deoxynivalenol contents of winter wheat grain increased from growth stage BBCH 75 (medium milk) to BBCH 83 (early dough), and then the contents declined from 48 to 86% in harvested grain in 1998. These changes in deoxynivalenol toxin contents might be attributed to either degradation and/or incorporation of trichothecenes in grain with increasing ripening

period. Thus, bound trichothecenes could not be extracted with conventional procedures and up to now only the extractable trichothecene fraction in grain could be analyzed. When our experimental data is taken into account, the changes in toxin content mentioned above could be explained by the formation of nonextractable products (*29*).

Plants are well-known to incorporate endogenous compounds (e.g. phenolics) (*30*, *31*) and exogenous chemicals (e.g. pesticides) into bound and nonextractable residues. After radioactive-labeling methods were employed to elucidate the metabolic fate of pesticides, the formation of bound residues was detected in crop plants. In an early compilation, Baldwin (*32*) reported on 14 examples of bound pesticide residues with up to 50% incorporation rates.

Recently, Poppenberger et al. (*33*) isolated and characterized a gene from *Arabidopsis thaliana* encoding a UDP-glucosyltransferase that catalyzed the transfer of glucose from UDP-glucose to the hydroxyl group at carbon 3 of deoxynivalenol. This deoxynivalenol-glucosyltransferase was also found to detoxify the acetylated derivative 15-acetyl-deoxynivalenol. Constitutive expression in *Arabidopsis* led to enhanced tolerance against deoxynivalenol.

Because the digestive system of animals and humans uses acid hydrolysis for food treatment, a higher daily intake of mycotoxins than that calculated up to now could be assumed. Some experiments revealed that animal bioavailability rates of bound pesticide residues are above 60% (*34*, *35*). For the first time we have developed an analytical tool, the liberation of deoxynivalenol from its conjugates, that enables us to investigate bound residues of the nonlabeled mycotoxin deoxynivalenol in inoculated grain samples.

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