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Natural Occurrence of *Fusarium* Toxins in Barley Grown in a Southwestern Area of Germany

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The occurrence of toxins produced by Fusarium species in cereals and other agricultural commodities has increasingly raised interest over the last two decades (Gareis et al. 1989; Müller and Schwadorf 1993). Efforts were mainly directed to the occurrence of zearalenone, an estrogenic Fusarium metabolite and to toxic trichothecenes (deoxynivalenol, nivalenol, T-2 toxin, HT-2 toxin, diacetoxyscirpenol). The occurrence of Fusarium toxins in barley grown in southwest Germany has been monitored by thin layer chromatography (TLC) and gas chromatography (GC) with FID or ECD detection (Thalmann et al. 1985). However, TLC is not sensitive enough to detect low levels of Fusarium toxins and confirmation is difficult, whereas GC method with FID and/or ECD can yield false positive results in extracts obtained from agricultural commodities for trichothecenes (Schwadorf and Müller 1991). In the present study, we used a gas chromatograph equipped with a mass selective detector, which is a sensitive and confirmatory method to determine Fusarium toxins in cereals (Schwadorf and Müller 1991, 1992). Barley samples were collected soon after harvest because preharvest contamination is of main interest for agricultural practice. The kernels were microbiologically examined for invasion by Fusarium, as well as by Penicillium and Aspergillus species, since these latter two have been described as storage fungi (Christensen and Sauer 1982).

MATERIALS AND METHODS

A total of 44 barley samples destined for feed use were randomly selected within 1-4 weeks after harvest from 43 farms located in the northeastern part of Baden-Wuerttemberg (Stuttgart governmental district). During the summer months of the crop year (1987) heavy rainfall occurred. Periodically occurring wet summers are

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characteristic for the area. Samples (approximately 1 kg) were withdrawn from a granary (bulk depth: 30 cm) or from a silo. The samples were combine harvested and most preserved by heated-air drying (7 samples),warm-air drying (2 samples), ambient air drying (11 samples), refrigeration (1 sample), and addition of propionic acid (1 sample). The other samples were not dried or otherwise preserved before analysis. All samples were examined for internal mycoflora and mycotoxins. Subsamples to be examined for internal mycoflora were stored at 4°C prior to examination and subsamples to be examined for mycotoxins were stored at -18°C.

The percentage of kernels with fungal growth was determined as follows. From each sample approximately 200 kernels were randomly collected and shaken for 2 min in 5% NaOCl (0.6% chlorine), followed by a double-rinse with sterile distilled water, and 90 kernels (15 kernels per plate) were placed on malt extract agar. After incubation for 5-8 days at 28°C the percentage of kernels with Fusaria and Penicillia and/or Aspergilli was determined.

T-2 toxin (T-2), HT-2 toxin (HT-2), diacetoxyscirpenol (DAS), deoxynivalenol (DON), 3-acetyl-deoxynivalenol (3-AcDON), nivalenol (NIV) were extracted from finely ground samples (particle size approximately 0.3 mm) as described by Tanaka et al. (1985). After trifluoracylation, quantification and confirmation was by a gas chromatograph equipped with a mass selective detector (Schwadorf and Müller 1991). Detection limits were 1 µg/kg for DON and 1-3 µg/kg for 3-AcDON, NIV, T-2, HT-2, and DAS. Zearalenone (ZON), α- and ß-zearalenol (α-, ß-ZOL) were extracted from the ground barley samples with ethylacetate, cleanup involved a base treatment, followed by partitioning with water. Confirmation and quantitation were by conversion to trimethylsilyl derivatives and measuring on a gas chromatograph equipped with a mass selective detector (Schwadorf and Müller 1992). Detection limit was 1 µg/kg.

RESULTS AND DISCUSSION

Barley samples were found to contain a high degree of Fusaria. Kernels with Fusaria were found in all samples, and the percentage of infested kernels was between 40 and 100% in 89% of samples. Penicillia and/or Aspergilli were not detected in 36% of barley samples, with the percentage of infected kernels mostly below 10% in the positive samples (Table 1). This indicates that moisture content of barley was unfavourable for the growth of Penicillium and Aspergillus species after harvest. It also strongly suggests that growth of Fusaria occurred exclusively before harvest because Fusaria and other

field fungi have higher humidity requirements than storage fungi (Christensen and Sauer 1982).

Table 1. Occurrence of Fusaria and of Penicillia and/or Aspergilli in barley samples from farms in Baden-Wuerttemberg, Germany.

FUSARIA						
Percentage[a]	0-20	20-40	40-60	60-80	80-90	90-100
Number of samples	3	2	13	16	8	2
PENICILLIA and/or ASPERGILLI						
Percentage[a]	0	0-10	10-40	40-60	60-90	90-100
Number of samples	16	18	3	3	1	3

[a] Percentage of surface-sterilized kernels that yielded the indicated fungi

The frequencies and levels of Fusarium toxins in 44 barley samples are summarized in Table 2. DON was the dominant toxin with an incidence of 98% and mean and maximum contents of 0.4 and 4.76 mg/kg, respectively. DON was followed by ZON and 3-AcDON which were found with incidences of 68 and 48% of the barley samples, respectively, but low mean and maximum levels. NIV, T-2 and HT-2 were detected in less than 11% of the barley samples with low levels. DAS and α- and ß-ZOL were not detected in the barley samples in excess of the 3 and 1 µg/kg detection limits, respectively. All samples contained at least one Fusarium toxin (Table 3) which is consistent with the high degree of Fusaria infestation (Table 1).

Table 2. Fusarium toxins in barley samples from farms in Baden-Wuerttemberg.

Mycotoxin	No and % of samples	µg/kg Range	Mean
ZON	30 (68)	1 - 310	35
α-ZOL	0		
ß-ZOL	0		
DON	43 (98)	4 - 4764	399
3-AcDON	21 (48)	3 - 17	5
NIV	5 (11)	3 - 10	5
T-2	4 (9)	3 - 9	5
HT-2	2 (5)	10 - 32	21
DAS	0		

The dominance of DON observed in the present study is in accordance with previous results. Monitoring of barley collected from several European countries revealed that

DON occured with higher frequency and mostly at higher levels than ZON, 3-AcDON, NIV and T-2, regardless of the barley crop year (Gareis et al. 1989). In Norwegian barley the incidence of DON (68%) was less than NIV (100%), but maximum and mean levels of DON were higher than NIV (Sundheim et al. 1988). There is meager information on the presence of T-2, HT-2 and DAS in barley samples, and no information on the occurrence of α- and ß-ZOL in barley is available. DON is the major Fusarium toxin detected in other cereals and agricultural commodities in Europe and other countries (Gareis et al. 1989).

Thirty-four of the 44 barley samples analysed contained two or more toxins. The simultaneous occurrence of two, three or four toxins were detected in 23, 48 and 7% of the barley samples, respectively (Table 3). The presence of ZON/DON/3-AcDON occurred with highest frequency, followed by ZON/DON in 34% and 18% of barley samples, respectively. The frequency of combinations of ZON, DON, 3-AcDON and NIV with type A-trichothecenes (T-2, HT-2) was rare (Table 3).

Table 3. Co-occurrence of Fusarium toxins in barley samples from farms in Baden-Wuerttemberg, Germany

Toxins detected	Number of samples
ZON	1
DON	9
ZON / DON	8
ZON / 3-AcDON	2
ZON / DON/ 3-AcDON	15
ZON / DON / NIV	2
ZON / DON / T-2	2
DON / 3-AcDON / NIV	1
DON / 3-AcDON / HT-2	1
ZON / DON / 3-AcDON / NIV	1
ZON / DON / T-2 / HT-2	1
DON / 3-AcDON / NIV / T-2	1

The co-occurrence of either ZON/DON/3-AcDON or ZON/DON in the barley samples suggests the presence of F.culmorum and F.graminearum. Strains of each species of European origin were found to produce several Fusarium toxins in pure culture, with ZON/DON and ZON/DON/3-AcDON among the combinations detected (Bottallico et al. 1989). Both of these species have been isolated from barley in southern Germany and northern Switzerland (Bauer et al. 1980; Häni 1980; Rintelen 1985; Schmidt 1975). The single occurrence of ZON in only one barley sample may be due to the occurrence of Fusarium strains

producing only this toxin. Further, the single occurrence of DON may be due to the suppression of ZON formation by environmental conditions. The absence of 3-AcDON from some of the positive DON samples may have been caused by its conversion into DON by Fusarium mycelia, plant tissue, yeasts or bacteria. Likewise, the failure to detect α- and ß-ZOL may be due to its transformation into ZON. The co-occurrence of NIV, ZON, and DON may be due to the simultaneous presence of Fusarium strains producing either NIV and ZON or DON and ZON. (For literature see Müller and Schwadorf 1993).

Fusarium poae and Fusarium sporotrichioides are important producers of type A-trichothecenes (T-2, HT-2, DAS) and toxigenic strains of both species have been found in barley of German origin (Bauer et al. 1980; Rintelen 1985). Thus the presence of T-2 and HT-2 together with ZON, DON, 3-AcDON and NIV (Table 3) suggests the occurrence of F.poae and/or F.sporotrichioides together with F.culmorum in the same samples.

The low incidence of T-2 and HT-2 and the absence of DAS in the present study may be due to the low incidence of toxigenic Fusarium strains in barley in the crop year studied, or else environmental conditions may have been unfavourable for toxin formation.

The ZON and DON content of most barley samples were below 100 and 500 µg/kg, respectively (Table 4). The other toxins also were detected at low levels (Table 2). A toxicological evaluation of these levels may be warranted for future research since there is little information on the effects of low levels of Fusarium toxins, especially with regard to pigs. The co-occurrence of Fusarium toxins which may produce additive or synergistic effects also must be considered (for literature see Müller and Schwadorf 1993). Our results stress the need for regular screening for Fusarium toxins in barley grown under adverse weather conditions.

Table 4. Frequency distribution of DON and ZON in barley samples from farms in Baden-Wuerttemberg

Toxin level	Number of samples with indicated level	
	ZON	DON
1 - 100	28	18
100 - 500	2	18
500 - 1000	-	3
1000 - 2000	-	1
> 2000	-	3

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