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Beauvericin and enniatins A, A1, B and B1 in Norwegian grain: a survey

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Abstract

Norwegian grain samples (73 oats, 75 barley, 80 wheat) from the 2000 to 2002 growing seasons were examined for contamination with five different enniatins and the association between the found concentrations and the prevalence or infection level with several common *Fusarium* species investigated. Enniatin B was the fungal metabolite with the highest prevalence (100%) and the highest maximum concentration (5800 μg/kg, wheat). The maximum concentration of all five enniatins together in a single sample was 7400 μg/kg (wheat). Enniatin concentrations were correlated with several independent variables, among them grain species. Beauvericin was only sporadically detected in barley and wheat and at concentrations just above the limit of detection of 3 μg/kg, while amounts up to 120 μg/kg were found in oats. The likelihood of detecting enniatin A1 as well as the concentrations of enniatins B and B1 could be mainly related to infection with *Fusarium avenaceum/arthrosporioides*, and the likelihood of detecting beauvericin could be related to infection with *Fusarium poae*. This survey indicates that the prevalence of enniatins A1, B and B1 in Norwegian grain is high, and that enniatin B concentrations of above 1000 μg/kg are common in barley and wheat.
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Keywords: Depsipeptides; Food analysis; *Fusarium*; Grain; Mycotoxin

1. Introduction

The enniatins are a group of cyclic hexadepsipeptides that have been known as secondary fungal metabolites for several decades. They were discovered as early as 1947 in a strain of *Fusarium oxysporum* (Gäumann, Roth, Ettlinger, Plattner, & Nager, 1947). Today, also species in the fungal genera *Beauveria*, *Halosarpheia*, *Paecilomyces*, *Polyporus* and *Verticillium* are known to produce different enniatins (Bernardini, Carillia, Pacioni, & Santurbano, 1975; Deol, Ridley, & Singh, 1978; Hamill, Higgens, Boaz, & Gorman, 1969; Lin et al., 2002; Nilanonta et al., 2003). Naturally occurring enniatins commonly consist of three D-2-hydroxycarboxylic acid residues linked alternatively to three L-*N*-methyl-amino acid residues (Fig. 1).

The ionophoric properties of the enniatins have been extensively studied (Ivanov et al., 1973; Levy, Bluzat, Seigneuret, & Rigaud, 1995), but beauvericin has so far received most attention from a toxicological point of view. The compound was found to be toxic against several mammalian cell lines (Macchia et al., 1995) and affected the electromechanical and physiological properties of isolated smooth and heart muscle preparations (Lemmens-Gruber et al., 2000). Beauvericin is also known to induce a type of cell death similar to apoptosis, which is accompanied by DNA fragmentation (Macchia et al., 2002; Ojcius, Zychlinsky, Zheng, & Young, 1991). The mitochondriotoxic properties of enniatins other than beauvericin have recently been demonstrated (Hoornstra, Andersson, Mikkola, & Salkinoja-Salonen, 2003) as well as their effect on the

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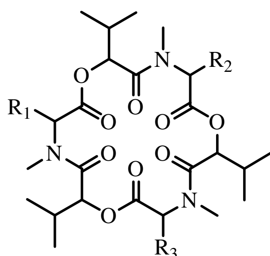

Compound	
Beauvericin	R_1=R_2=R_3= -$CH_2C_6H_5$
Enniatin A	R_1=R_2=R_3= -$CH(CH_3)CH_2CH_3$
Enniatin A1	R_1=R_2= -$CH(CH_3)CH_2CH_3$, R_3= -$CH(CH_3)_2$
Enniatin B	R_1=R_2=R_3= -$CH(CH_3)_2$
Enniatin B1	R_1=R_2= -$CH(CH_3)_2$, R_3= -$CH(CH_3)CH_2CH_3$

Fig. 1. Chemical structure of the enniatins.

electrophysical properties of guinea pig heart muscle preparations and ventricular myocytes (Kamyar, Studenik, & Lemmens-Gruber, 2004). Moreover, the herbicidal, antibiotic and insecticidal properties of the enniatins are well documented (Gäumann, Naef-Roth, & Kern, 1960; Herrmann, Zocher, & Haese, 1996; Nilanonta et al., 2003; Strongman, Strunz, Giguere, Yu, & Calhoun, 1988).

Fungi from the genus *Fusarium* frequently colonise small-grain cereals throughout Europe and are associated with grain diseases such as *Fusarium* head blight and foot rot as well as the accumulation of potentially toxic metabolites in the kernels (Bottalico & Perrone, 2002). In Norway, *Fusarium avenaceum* is the most frequently isolated *Fusarium* species from grain (Kosiak, Torp, Skjerve, & Thrane, 2003) – a species that has been known to produce enniatins for a long time (Gäumann, Naef-Roth, & Ettlinger, 1949). *Fusarium* spp. in Norwegian grain that have been shown to produce enniatins under laboratory conditions include *Fusarium tricinctum*, *Fusarium poae*, *Fusarium sporotrichioides* and *Fusarium langsethiae* (Burmeister & Plattner, 1987; Thrane et al., 2004). *Fusarium culmorum* and *Fusarium graminearum* are frequently isolated species in Norwegian grain, which to date have not been reported to produce enniatins. This situation, together with the recent report of high levels of beauvericin, enniatins A1, B and B1 in *F. avenaceum* contaminated Finnish grain (Logrieco, Rizzo, Ferracane, & Ritieni, 2002), prompted us to explore the natural contamination of Norwegian grain with these fungal metabolites. The objectives of our study were: to determine the extent of contamination of Norwegian oats, barley and wheat with the enniatins beauvericin, enniatin A, A1, B and B1, and to investigate the association between enniatin contamination and the following independent variables: infection of the grain with *Fusarium* species, grain species, region and year.

2. Materials and methods

2.1. Sampling

In 2000 and 2001, the samples were collected at the grain delivery sites from a continuously sampled flowstream in connection with the delivery of the grain by the farmers in autumn. The sampling in 2000 and 2001 was carried out by the Norwegian Agricultural Inspection Service. The 2002 sampling was carried out by repeated manual probe sampling on the farm at harvest, following the regulations enacted by the Norwegian Agricultural Inspection Service. In each case, one bulk sample was collected, from which two sub-samples of 500 g were taken, one for chemical analysis and the other for mycological examination. Since the sub-samples were taken from the same well-mixed bulk sample, they were assumed to be comparable. The sample lot was composed as follows: 52 samples from the year 2000 (21 oats, 19 barley, 12 wheat), 83 samples from 2001 (26 oats, 23 barley, 34 wheat) and 93 samples from 2002 (26 oats, 33 barley, 34 wheat). Samples were obtained from most of the grain-producing areas of Norway. On arrival at the National Veterinary Institute, Oslo, samples destined for enniatin analysis were stored at −21 °C, while samples destined for mycological analysis were stored at 4 °C. Mycological analysis was carried out within six weeks after arrival of the grain samples, whereas chemical analysis was carried out in the period March 2003–August 2003.

2.2. Chemical analysis

The method for the analysis of the five enniatins in grain and its validation has been described by Uhlig and Ivanova (2004). Briefly, grain samples were extracted with acetonitrile:water (84:16, v/v) and analysed without further purification using liquid chromatography with mass spectrometric detection. Mean recoveries (n = 5–12) of enniatins from spiked grain samples over a period of 6 months were 99–115%, 86–131%, 97–113%, 73–100% and 78–114% for beauvericin, enniatin A, A1, B and B1, respectively. The limits of detection (LOD) were 3.0 μg/kg for beauvericin, enniatin A, B and B1 and 4.0 μg/kg for enniatin A1, while the limits of quantification (LOQ) were 10 μg/kg for beauvericin, enniatin A, B and B1 and 13 μg/kg for enniatin A1.

2.3. Fusarium *analysis*

For enumeration and isolation of *Fusarium* spp., 98 kernels from each of the 2001 samples were plated out on Czapek-Dox Iprodion Dichloran agar (CzID) (Abildgren, Lund, Thrane, & Elmholt, 1987), without prior treatment, with seven kernels per 90 mm dish. In 2002, half of the kernels (49) from each sample were sur-

face disinfected for 2 min with a sodium hypochlorite solution (0.40% active chlorine) before outplating. The agar plates were incubated at 25 °C for seven days in combined black light (Philips TLD 36W/08) and cold daylight (Philips TLD 36W/33) alternating with darkness, using 12 h photoperiods. All colonies resembling *Fusarium* spp. were distinguished into groups based on morphological criteria, counted, and representative colonies from the groups were transferred by one-point inoculations onto Spezieller nährstoffarmer Agar (SNA) with filter paper (Nirenberg, 1981) and potato sucrose agar (PSA) (Booth, 1971) for identification. Conditions for SNA incubation were as described above, while PSA plates were incubated in darkness at 25 °C. *Fusarium* spp. were identified according to Nelson, Toussoun and Marasas (1983); Nirenberg (1995); Samson, Hoekstra, Frisvad and Filtenborg (2002).

2.4. Statistical analysis

Arithmetic means and medians of analyte concentrations and fungal prevalence (percentage of infected samples) and infection level (percentage of infected kernels per sample) were calculated using Microsoft Excel 2000 or JMP 5.0.1a (SAS Institute Inc., Cary, NC, 2002). Multiple regression analyses were performed with SAS-PC 8.2 for Windows with Enterprise Guide 2.0 (SAS Institute Inc., 2002). The samples from 2000, as well as seven samples from the 2001 season, lacked mycological data and were excluded from the multiple regression analyses.

The assumption of normality of the analyte concentrations was assessed using stem-and-leaf plots and normal probability plots, revealing that the distribution of all the five outcomes was skewed to the left. A logarithmic transformation of the outcome of enniatins B and B1 was applied to two different general linear models. In the case of non-detectable concentrations, $\text{LOD}/\sqrt{2}$ was used. This approach is expected to give good estimates of both the geometric mean and the standard deviation as long as the distribution is not highly skewed (Hornung & Reed, 1999).

The transformations of the outcomes of beauvericin, enniatin A and A1 did not improve the normality of the data, probably because of the high amount of negative samples (see Section 3). Therefore the LOD was used as the cut-off value for categorisation in positive and negative samples. These binary variables were applied to three different logistic regression models using PROC GENMOD. Three possible risk factors were included as categorical variables: grain species (three levels), year (two levels) and geographical region (see Tables 3 and 4 below). Also the fungal prevalences of *F. culmorum*, *F. graminearum*, *F. tricinctum*, *F. poae*, *F. langsethiae* and *F. sporotrichoides* in the samples were handled as categorical variables (positive and negative) because no linear relationship was found between them and the outcome variables. The log-transformed concentrations of enniatin B and B1 and the sum of the infection levels of *F. avenaceum* and *F. arthrosporioides*, were handled as continuous variables. The elimination criterion was the type-III *F*-test, a *P*-value of 0.05 being used as the level for exclusion from the model. The modelling was manually conducted, starting with all independent variables. Separate models were assessed using the results from *Fusarium* analysis with or without surface disinfection. The least square means were estimated for all levels of the significant independent variables in the final general linear models and the odds ratios in the logistic models. The two-way interaction terms of the variables in the final models were also tested for significance.

The correlation between the concentrations of individual enniatins, as well as the moniliformin concentrations from an earlier study of the same samples (Uhlig et al., 2004) was investigated using Spearman correlation coefficients.

3. Results

3.1. Chemical analysis

Beauvericin, enniatins A, A1, B and B1 were found in 73 (32%), 58 (25%), 153 (67%), 228 (100%) and 214 (94%) of the 228 analysed grain samples, respectively (Table 1 and Figs. 2–4). The concentrations of the fungal metabolites varied both with regard to year and grain species. The prevalence of beauvericin as well as enniatins A1 and B1 was highest in the samples from

Table 1
Means and medians of enniatin concentrations (μg/kg) from the analysis of 73 oats, 75 barley and 80 wheat samples from 2000 to 2002

	Oats		Barley		Wheat	
	Arithmetic mean	Median	Arithmetic mean	Median	Arithmetic mean	Median
Beauvericin	16	7.6	<3.0	<3.0	<3.0	<3.0
Enniatin A	<3.0	<3.0	4.5	<3.0	5.8	<3.0
Enniatin A1	6.0	<4.0	35	14	22	11
Enniatin B	47	36	490	270	790	360
Enniatin B1	20	11	170	95	180	71

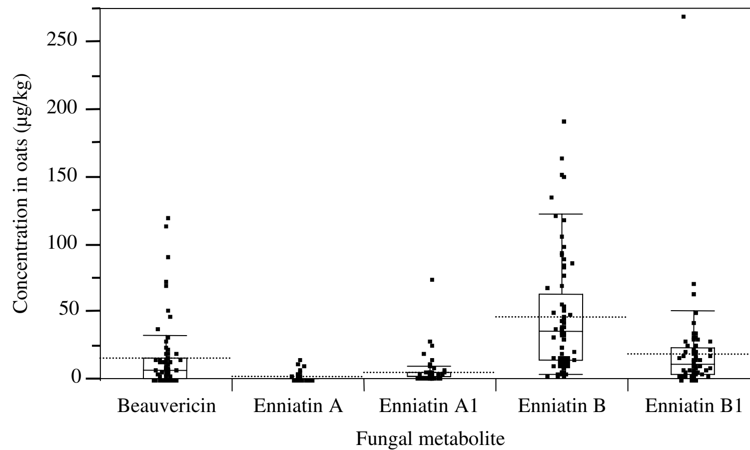

Fig. 2. One-way plot of the results from the analysis of five different enniatins in 73 Norwegian oats samples from the 2000 to 2002 growing seasons. Box plots represent median, quartiles and 90% whiskers; the dotted line represents the arithmetic mean.

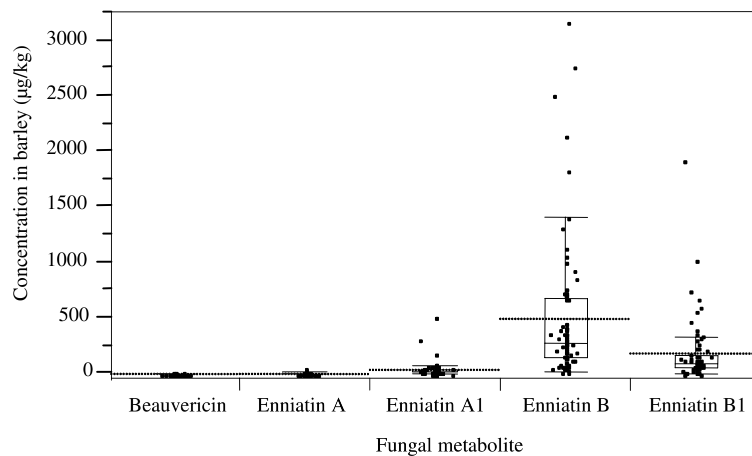

Fig. 3. One-way plot of the results from the analysis of five different enniatins in 75 Norwegian barley samples from the 2000 to 2002 growing seasons. Box plots represent median, quartiles and 90% whiskers; the dotted line represents the arithmetic mean.

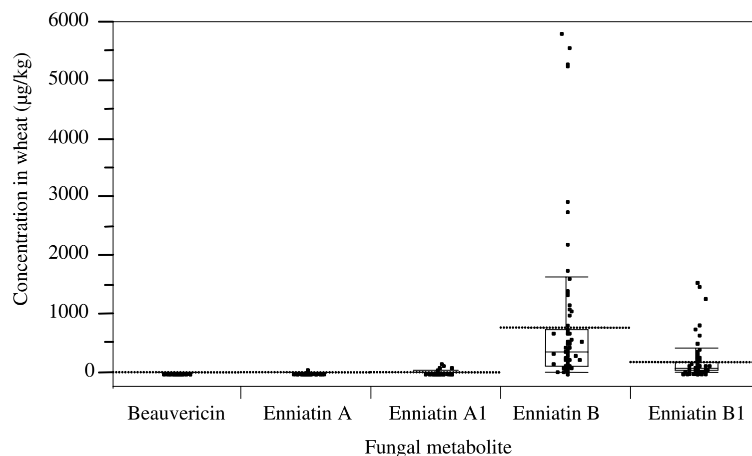

Fig. 4. One-way plot of the results from the analysis of five different enniatins in 80 Norwegian wheat samples from the 2000 to 2002 growing seasons. Box plots represent median, quartiles and 90% whiskers; the dotted line represents the arithmetic mean.

2002, where 40%, 76% and 100% of the samples, respectively, proved positive. Enniatin A was detected most frequently in the samples from 2001, where 42% of the samples were positive. Enniatin B was detected in all samples. The highest concentration of beauvericin (120 µg/kg) was found in an oat sample from 2002. However,

Table 2
Infection level of grain samples with selected Fusarium species from the 2001 and 2002; growing season – positive samples only

Species	2001						2002					
	Oats		Barley		Wheat		Oats		Barley		Wheat	
∑*F. avenaceum/arthrosporioides*	53.3[a]	44.0[b]	67.3[a]	68.4[b]	67.0[a]	66.4[b]	61.2[a]	60.2[b]	73.9[a]	77.6[b]	75.8[a]	80.6[b]
F. tricinctum	7.5	4.6	13.0	8.2	4.8	4.1	7.8	4.1	9.1	6.1	9.9	4.1
F. culmorum	8.4	4.1	10.3	9.2	13.9	8.2	7.9	6.1	8.2	6.1	7.0	6.1
F. graminearum	2.4	2.0	2.0	2.0	1.4	1.0	13.5	8.2	13.8	14.3	8.7	5.1
F. poae	5.1	5.1	3.5	1.5	4.1	3.1	16.8	12.2	6.0	4.1	5.9	6.1
F. sporotrichioides	–	–	1.0	1.0	1.0	1.0	7.2	2.0	7.5	4.1	5.7	2.0
F. langsethiae	4.7	3.7	1.8	2.0	1.7	1.5	7.9	6.1	2.8	2.0	2.0	2.0

Numbers represent the percentage of infected kernels per sample.
[a] Arithmetic mean of positives.
[b] Median of positives.

the barley and wheat samples from 2001 contained the highest amounts of the other four enniatins. They were as high as 59, 500, 3200 and 1900 μg/kg of enniatins A, A1, B and B1, respectively, in barley and 58, 190, 5800 and 1600 μg/kg of enniatins A, A1, B and B1, respectively, in wheat. The maximum concentration of the five enniatins together was 7400 μg/kg in a wheat sample from 2001. The lowest amounts were detected in the samples from 2000 with maximum concentrations of 22, 190, 740 and 470 μg/kg of enniatins A, A1, B and B1, respectively, in barley and 4.3, 26, 1400 and 190 μg/kg of enniatins A, A1, B and B1, respectively, in wheat.

3.2. Fusarium analysis

The results from the *Fusarium* analyses were presented in a corresponding paper about the contamination of Norwegian grain with moniliformin (Uhlig et al., 2004). The present study included three samples less than the moniliformin survey. However, this did not influence the *Fusarium* results. *F. avenaceum/arthrosporioides* were the dominating species and were found in all samples. The accumulated sum of infection with *F. avenaceum/arthrosporioides* is used in this investigation rather than *F. avenaceum* alone because it is often difficult to distinguish the two species morphologically or by molecular methods (Yli-Mattila, Paavanen-Huhtala, Bulat, Alekhina, & Nirenberg, 2002). Supplementary *Fusarium* data are shown in Table 2.

3.3. Multiple regression and correlation analysis

The regression models (Tables 3 and 4) showed that the likelihood of detecting beauvericin and enniatin A1 was significantly related to grain species. The likelihood of detecting beauvericin was significantly higher in oats and the likelihood of detecting enniatin A1 was significantly higher in barley and wheat. The regression models showed that also the concentrations of enniatins B and B1 were significantly related to grain species (Table 4). The concentrations of enniatin B and B1 were significantly higher in barley and wheat than in oats.

The likelihood of detecting beauvericin was significantly related to the presence of *F. poae* (Table 3). The interaction terms in the final model indicate a stronger effect of the presence of *F. poae* in oats than in the other grain species. The likelihood of detecting enniatin A1 as well as the concentrations of enniatin B and B1 were significantly related to the infection level with *F. avenaceum/arthrosporioides* in all grain species. The presence of the enniatin-producer *F. tricinctum* was only significantly related to the likelihood of detecting enniatin A1 as was the occurrence of *F. culmorum*, which is not known to produce enniatins. The likelihood of detecting enniatin A was not significantly related to any *Fusarium* species. For the samples from 2002, separate models were assessed for the untreated and treated kernels. When the models were conducted using the *Fusarium* data from the surface-disinfected kernels, significant correlations could only be detected between the likelihood of detecting enniatin A1 and the presence of *F. culmorum*, and the likelihood of detecting beauvericin and the presence of *F. poae*.

The likelihood of detecting enniatin A and enniatin B concentrations was significantly related to year – both were significantly higher in the grain samples from 2001 than from 2002. Interaction terms indicated a stronger yearly variation of the likelihood of detecting enniatin A as well as enniatins B and B1 concentrations in wheat compared to oats and barley. The likelihood of detecting beauvericin and enniatin B1 concentrations was significantly related to region. In the case of beauvericin, the likelihood of detecting the metabolite was significantly higher in samples from the areas west and north of the Oslofjord region than in samples from Trøndelag (mid-Norway) and east of the Oslofjord. Enniatin B1 concentrations were significantly higher in samples from the area north of the Oslofjord than in the areas west and east of the Oslofjord and from Trøndelag.

Table 3
The results from the final multiple logistic regression models with demonstration of beauvericin, enniatin A and A1 above or below the detection limit as the outcome in the study of Norwegian grain samples collected in 2001 and 2002; 95% confidence intervals are given in brackets

Independent variable	Category	Logistic regression models					
		Beauvericin		Enniatin A		Enniatin A1	
		Adjusted odds ratio	*P*-value	Adjusted odds ratio	*P*-value	Adjusted odds ratio	*P*-value
Grain species	Oats	1.8 (1.6–2.1)	<0.001	1.2 (1.1–1.4)	0.35	1.5 (1.3–1.7)	<0.001
	Barley	1.3 (1.1–1.4)		1.4 (1.3–1.6)		2.3 (2.1–2.6)	
	Wheat	1.1 (1.0–1.2)		1.4 (1.3–1.6)		2.1 (1.9–2.3)	
Year	2001		n.s.[a]	1.5 (1.4–2.0)	<0.001		n.s.
	2002			1.2 (1.1–1.3)			
Year*grain species	2001*oats		n.s.	1.3 (1.1–1.4)	0.02		n.s.
	2001*barley			1.5 (1.3–2.0)			
	2001*wheat			1.8 (1.5–2.1)			
	2002*oats			1.2 (1.0–1.4)			
	2002*barley			1.3 (1.1–1.5)			
	2002*wheat			1.1 (0.9–1.3)			
Region	Østfold & Akershus	1.3 (1.2–1.4)	0.006		n.s.		n.s.
	Hedmark & Oppland	1.5 (1.2–1.8)					
	Buskerud, Vestfold &Telemark	1.6 (1.4–1.7)					
	Sør & Nord Trøndelag	1.2 (1.0–1.4)					
F. avenaceum/arthrosporioides	Continuous		n.s.[a]		n.s.		0.01[b]
F. culmorum	Positive		n.s.		n.s.	2.1 (1.6–2.0)	0.01
	Negative					1.8 (2.0–2.3)	
F. tricinctum	Positive		n.s.		n.s.	2.1 (1.6–1.9)	0.02
	Negative					1.7 (1.9–2.3)	
F. poae	Positive	1.6 (1.4–1.7)	<0.001		n.s.		n.s.
	Negative	1.2 (1.1–1.3)					
*F. poae**grain species	Oats*positive	2.4 (2.2–2.7)	0.03		n.s.		n.s.
	Oats*negative	1.4 (1.1–1.8)					
	Barley*positive	1.4 (1.2–1.6)					
	Barley*negative	1.2 (1.0–1.4)					
	Wheat*positive	1.2 (0.9–1.2)					
	Wheat*negative	1.0 (1.0–1.3)					

[a] Not significant ($P > 0.05$).
[b] Regression coefficient $\beta = 0.003$.

Table 4
The results from the final general linear regression models with demonstration of enniatin B and B1 as the outcome in the study of Norwegian grain samples collected in 2001 and 2002; 95% confidence intervals are given in brackets

Independent variable	Category	General linear regression models			
		Enniatin B		Enniatin B1	
		Adjusted least square mean	*P*-value	Adjusted least square mean	*P*-value
Grain species	Oats	47 (40–67)	<0.001	15 (11–20)	<0.001
	Barley	340 (270–450)		120 (90–170)	
	Wheat	410 (330–490)		97 (72–130)	
Year	2001	220 (180–270)	0.05	57 (43–75)	0.80
	2002	170 (130–200)		55 (42–71)	
Year*grain species	2001*oats	47 (33–67)	<0.001	12 (8.1–19)	<0.001
	2001*barley	320 (200–490)		100 (60–170)	
	2001*wheat	710 (560–990)		150 (110–380)	
	2002*oats	54 (37–74)		18 (12–27)	
	2002*barley	350 (270–450)		150 (110–220)	
	2002*wheat	240 (160–330)		62 (42–91)	
Region	Østfold & Akershus		n.s.[a]	53 (45–67)	0.003
	Hedmark & Oppland			110 (67–200)	
	Buskerud, Vestfold &Telemark			62 (49–82)	
	Sør & Nord Trøndelag			26 (15–45)	
F. avenaceum/arthrosporioides	Continuous	–	<0.001[b]		<0.001[c]

[a] Not significant ($P > 0.05$).
[b] Regression coefficient $\beta = 0.02$.
[c] $\beta = 0.01$.

Apart from beauvericin, the concentrations of all enniatins were strongly correlated with each other (Spearman correlation coefficients 0.4106–0.9719, $P < 0.001$) and to moniliformin concentrations (Spearman correlation coefficients 0.6007–0.7284, $P < 0.001$).

4. Discussion

The grain samples, which were analysed in this survey, were from most of the grain producing areas of Norway. They were therefore assumed to reflect the real situation. The statistical analyses showed that different *Fusarium* species are responsible for the production of beauvericin, on the one hand and the other enniatins, on the other hand, in different grain species. Accumulation of beauvericin in maize or corn has before been related to *F. subglutinans* (Kostecki, Szczesna, Chelkowski, & Wisniewska, 1995; Krska et al., 1996) or *F. proliferatum* (Munkvold, Stahr, Logrieco, Moretti, & Ritieni, 1998; Pascale, Visconti, Pronczuk, Wisniewska, & Chelkowski, 2002), which do not commonly infect Norwegian grain (Kosiak et al., 2003). However, it has been shown that beauvericin may be produced by *F. poae* under laboratory conditions (Logrieco et al., 1998; Thrane et al., 2004), and this species which seems to be responsible for the contamination of Norwegian oats with the metabolite. *F. poae* is also one of the most prevalent species in Norwegian wheat and the question is why the species does not seem to produce beauvericin in this grain species. An explanation could be the lower infection level of *F. poae* (Table 2) or interactions between the host plant and the fungus.

The statistical analyses also show that *F. avenaceum/arthrosporioides* are likely responsible for the amounts of enniatins A1, B and B1 in Norwegian oats, barley and wheat. This is in accordance with expectations since *F. avenaceum* is known to be a strong enniatin producer (Blais, Apsimon, Blackwell, Greenhalgh, & Miller, 1992) and does commonly infect grain in Norway (Kosiak et al., 2003). The occurrence of the enniatin-producing *F. tricinctum* was only significantly related to the presence of one of the enniatins. The reasons for this may be the lower level of infection with the fungus on the one hand and/or its lower pathogenicity compared to *F. avenaceum* on the other (Bottalico & Perrone, 2002). The higher pathogenicity of the more prevalent *F. avenaceum/arthrosporioides* could result in outcompeting of *F. tricinctum* and suppresion of the production of metabolites by the latter. The presence of *F. culmorum* was significantly related to the presence of enniatin A1. This peculiarity was found before when the likelihood of detecting moniliformin in Norwegian grain was significantly related to the prevalence of *F. culmorum* (Uhlig et al., 2004). The reason for this connection is not clear but it could possibly be explained by fungal interactions in the field with the stronger pathogen *F. culmorum* influencing the metabolite production of *F. avenaceum/arthrosporioides*. However, the genetic pathways, as well as the regulation and expression of the genes involved in the biosynthesis of the enniatins, are not yet known. Another explanation might be that

both *F. culmorum* and *F. avenaceum* thrive under the same climatic conditions and that the observed correlation is therefore an artefact.

When the statistical models were studied using the *Fusarium* data from the surface disinfected kernels, the otherwise strong relationship of enniatin B and B1 concentrations, as well as the likelihood of detecting enniatin A1, to the infection level of *F. avenaceum/arthrosporioides*, disappeared, while the occurrence of beauvericin and enniatin A1 could still be related to the occurrence of *F. poae* and *F. culmorum*, respectively. This indicates that *F. avenaceum/arthrosporioides* predominantly infect the surface of the kernels while *F. poae* and *F. culmorum* may penetrate deeper into the kernels. The potential enniatin-producing species, *F. sporotrichioides* and *F. langsethiae*, do not seem to contribute significantly to the contamination of Norwegian grain with these metabolites. Even if the prevalence of the latter two species is high in some grain species, the infection level is likely too low – or they simply do not produce the metabolites on the substrate under field conditions.

A significant difference in the likelihood of detecting enniatin A1, as well as the concentrations of enniatins B and B1 was found between the growing seasons 2001 and 2002, which was restricted to wheat. This shows that yearly variations in the concentrations of fungal metabolites are not necessarily the same for all grain species in a confined area. Instead, grain species-specific factors may influence these yearly variations. The statistical models detected (in a few cases) significant relations between fungal metabolite and region. However, no distinct regional gradient was found as before for other mycotoxins, for example for the contamination of Norwegian grain with HT-2 toxin (Langseth & Rundberget, 1999).

The significant correlation of enniatins A, A1, B and B1 concentrations with each other and with moniliformin concentrations, from an earlier study of the same samples, may lead to the following conclusions: (1) The enniatins A, A1, B and B1 are (under Norwegian growing conditions) all produced at the same time and in a distinct concentration ratio (enniatin B > B1 > A1 > A). Local differences in climatic conditions do not change this ratio. (2) The production of both moniliformin and the four enniatins is due to the same promoting factor(s) even if the biosynthetic pathways are different. This is important from a toxicological point of view indicating that several different and potentially hazardous fungal metabolites may be present in the same sample. However, the scientific literature contains practically no data about the toxicological effects of the enniatins. It is therefore impossible to assess whether the concentrations, which were found in this survey, are of concern for public and animal health. Investigations must be carried out in the future in order to explore possible toxic effects of the enniatins. Considering the positive correlation between enniatins A, A1, B and B1 and moniliformin concentrations, investigations into the combined effects (namely synergistic, additive, antagonistic) of enniatins and moniliformin have to be included in these studies.

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