Available online at www.sciencedirect.com
ScienceDirect
Biochemical Systematics and Ecology 36 (2008) 245–259
biochemical systematics and ecology
www.elsevier.com/locate/biochemsyseco

Benzoxazinoid concentrations show correlation with Fusarium Head Blight resistance in Danish wheat varieties

Malene Søltoft [a,b,c], Lise N. Jørgensen [a], Bo Svensmark [b], Inge S. Fomsgaard [a,*]

[a] University of Aarhus, Faculty of Agricultural Sciences, Department of Integrated Pest Management, Research Centre Flakkebjerg, 4200 Slagelse, Denmark
[b] Department of Chemistry, University of Copenhagen, Universitetsparken 5, 2100 Copenhagen Ø, Denmark
[c] Department of Food Chemistry, National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark

Received 14 July 2007; accepted 21 October 2007

Abstract

Fusarium Head Blight (FHB) is a destructive disease that affects the grain yield and quality of cereals. The relationship between the natural defense chemicals benzoxazinoids and the FHB resistance of field grown winter wheat varieties was investigated. FHB resistance was assessed by the inoculation of wheat ears with mixtures of *Fusarium avenaceum*, *Fusarium culmorum*, *Fusarium graminearum*, and *Microdochium nivale*.

The benzoxazinoids detected in the highest concentration were 2,4-dihydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (3.7–9.4 μmol/kg DW) and 2-hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (HMBOA, 2.0–11 μmol/kg DW). The cultivars most susceptible to FHB were cvs. Hanseat, Asketis, and Ritmo, while cvs. Petrus, Terra, and Hattrick showed high resistance.

2-*O*-β-D-Glucopyranosyloxy-4,7-dimethoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (HDMBOA-glc) and 2-*O*-β-D-glucopyranosyloxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (HMBOA-glc) were detected. HMBOA-glc was found in higher concentrations than 2-*O*-β-D-glucopyranosyloxy-2,4-dihydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIMBOA-glc). Principal component analyses demonstrated correlation between the susceptibility to FHB and the concentrations of DIMBOA-glc, HMBOA-glc, HMBOA, 2-*O*-β-D-glucopyranosyloxy-4-hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIBOA-glc), 2-*O*-β-D-glucopyranosyloxy-1,4-benzoxazin-3(4*H*)-one, and 2-*O*-β-D-glucopyranosyloxy-4-dihydroxy-(2*H*)-7,8-dimethoxy-1,4-benzoxazin-3(4*H*)-one (DIM_2BOA-glc).

Keywords: Benzoxazinoids; DIMBOA; Fusarium Head Blight; HMBOA; Susceptibility; Winter wheat

1. Introduction

Allelochemicals are compounds produced by plants, microorganisms, and fungi that influence the growth and development of agricultural and biological systems (Torres et al., 1996). They are secondary metabolites with a low molecular mass (Rizvi et al., 1992), for instance benzoxazinoids, phenolic acids, and terpenoids (Rice, 1984). In this study, the term benzoxazinoids covers benzoxazinones (hydroxamic acids and lactams), benzoxazolinones, and

* Corresponding author. Tel.: +45 89993610; fax: +45 89993501.
E-mail address: inge.fomsgaard@agrsci.dk (I.S. Fomsgaard).

0305-1978/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bse.2007.10.008

methyl derivates of benzoxazinones. Benzoxazinoids are primarily found in cereals like wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.), and maize (*Zea mays* L.) (Niemeyer and Perez, 1995; Sicker et al., 2004) and the most abundant are shown in Table 1.

In wheat, 2-*O*-β-D-glucopyranosyloxy-4-hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIMBOA-glc) and 2,4-dihydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIMBOA) are the most frequently identified hydroxamic acids (Niemeyer, 1988a; Kumar et al., 1993), while 2-*O*-β-D-glucopyranosyloxy-4-hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIBOA-glc) and 2,4-dihydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIBOA) are often only detected in low concentrations (Hofman and Hofmanova, 1969). 2-*O*-β-D-Glucopyranosyloxy-4-hydroxy-7,8-dimethoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIM_2BOA-glc) has previously only been detected in maize, but not in wheat or rye (Niemeyer, 1988a). The methyl derivative 2-*O*-β-D-glucopyranosyloxy-4,7-dimethoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (HDMBOA-glc) has previously been detected as the main component together with DIM_2BOA-glc in maize (Cambier et al., 2000). 2-Hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (HMBOA-glc) has been detected in significant concentrations only in maize, and 2-*O*-β-D-glucopyranosyloxy-1,4-benzoxazin-3(4*H*)-one (HBOA-glc) has only been found in rye and maize (Hofman and Hofmanova, 1969).

Microbial or mechanical injury of plants leads to the production of aglucones (Cambier et al., 2000), but aglucones have also been detected in undamaged wheat (Leszczynski and Dixon, 1990; Nakagawa et al., 1995; Mogensen et al., 2006). Hydroxamic acids are unstable and degrade rapidly to benzoxazolinones in aqueous and organic solvents (Virtanen and Hietala, 1960; Bredenberg et al., 1962; Woodward et al., 1978), and in sterile and non-sterile soils (Kumar et al., 1993).

Benzoxazinoid concentration depends on plant age (Nakagawa et al., 1995) and growing conditions, including light (Ahman and Johansson, 1994), temperature (Gianoli and Niemeyer, 1997), and water stress (Richardson and Bacon, 1993). In 52 Chilean and British wheat varieties DIMBOA and DIBOA concentrations (sum of aglucones and hydrolyzed glucosides) were 1.4–10.8 and 0–1.1 mmol/kg fresh weight (FW), respectively (Copaja et al., 1991). The average DIMBOA concentration in the shoots of 58 wheat cultivars was (sum of aglucones and hydrolyzed glucosides) 0.468 mmol/kg FW, assuming that shoot water content was 80% (Wu et al., 2001a). The content of DIMBOA was 0.21–16.0 mmol/kg FW in various tested wheat cultivars in a study by Niemeyer (1988b).

Fusarium Head Blight (FHB) is a destructive disease that occurs in all cereal-growing countries (Parry et al., 1995; Pirgozliev et al., 2003). It is caused by plant pathogenic *Fusarium* spp., favored by high temperature and humidity (Parry et al., 1995; McMullen et al., 1997; Xu, 2003). In Denmark, the most common species associated with FHB are *Fusarium avenaceum*, *Fusarium culmorum*, and *Fusarium graminearum* (Thrane, 2000); worldwide *Microdochium nivale* has been associated with FHB (Pirgozliev et al., 2003; Xu, 2003).

FHB affects grain yield and quality (Parry et al., 1995; McMullen et al., 1997), typically causing yield reduction (Parry et al., 1995; McMullen et al., 1997; Chelkowski, 1989) and accumulation of mycotoxins (Parry et al., 1995; McMullen et al., 1997). The mycotoxins can cause various toxicoses and other health disorders and are of great concern in animal and human health (McMullen et al., 1997). Crop rotation, tillage methods, and resistant cultivars are the most important control strategies for FHB (Nielsen and Jørgensen, 2001). Research has been conducted in several wheat-growing countries to exploit host plant resistance (Pirgozliev et al., 2003), which can be due to resistance to initial infection or to pathogen spread, as well as increased breakdown or tolerance of mycotoxins (Parry et al., 1995).

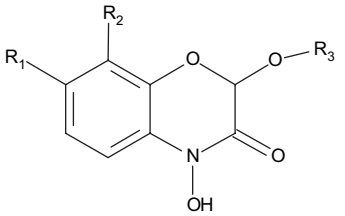
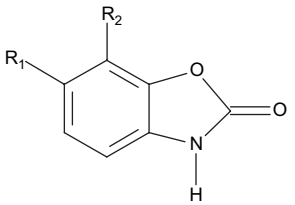
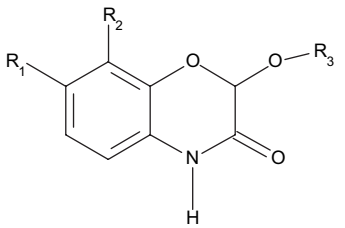
Benzoxazinoids can inhibit fungal activity associated with FHB (Virtanen and Hietala, 1955; Friebe et al., 1998; Wilkes et al., 1999; Zikmundova et al., 2002; Martyniuk et al., 2006). The objective of the present research was to investigate whether the benzoxazinoid concentration in wheat ears can partially explain resistance to FHB in Danish wheat cultivars.

2. Materials and methods

2.1. Assessment of susceptibility to FHB

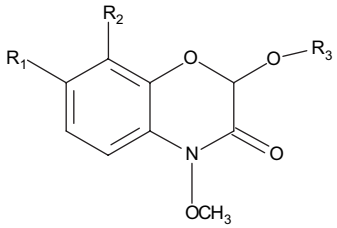
At the end of September 2003, 54 winter wheat (*T. aestivum*) varieties were sown; they were inoculated twice during the flowering period (growth stage BBCH 60–69). The experiment was a randomized block design, plots were 2 m rows, and there were four replicates. During the trial experiment, plants were inoculated with a mixture of *Fusarium* spp. (*F. avenaceum*, *F. culmorum*, and *F. graminearum*) and *M. nivale* to ensure development of FHB, using a knapsack sprayer to apply 1 L of inoculum per 30 m of plot rows. The symptoms appeared approximately 2–3 weeks after

Table 1
The acronyms, systematic names and structural formulae for the most abundant benzoxazinoids in cereals

			Acronym	Systematic name	Molecular mass
Hydroxamic acids (benzoxazinones):					
$R_1 = H$	$R_2 = H$	$R_3 = Glc$	DIBOA-glc*	2-*O*-β-D-Glucopyranosyloxy-4-hydroxy-(2*H*)-1, 4-benzoxazin-3(4*H*)-one	343
$R_1 = H$	$R_2 = H$	$R_3 = H$	DIBOA	2,4-Dihydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one	181
$R_1 = OCH_3$	$R_2 = H$	$R_3 = Glc$	DIMBOA-glc*	2-*O*-β-D-Glucopyranosyloxy-4-hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one	373
$R_1 = OCH_3$	$R_2 = H$	$R_3 = H$	DIMBOA*	2,4-Dihydroxy-7-methoxy-(2*H*)-1, 4-benzoxazin-3(4*H*)-one	211
$R_1 = OCH_3$	$R_2 = OCH_3$	$R_3 = Glc$	DIM_2BOA-glc*	2-*O*-β-D-Glucopyranosyloxy-4-dihydroxy-7, 8-dimethoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one	403
$R_1 = OCH_3$	$R_2 = OCH_3$	$R_3 = H$	DIM_2BOA	2,4-Dihydroxy-7,8-dimethoxy-(2*H*)-1, 4-benzoxazin-3(4*H*)-one	241
Benzoxazolinones:					
$R_1 = H$	$R_2 = H$		BOA*	Benzoxazolin-2-one	135
$R_1 = OCH_3$	$R_2 = H$		MBOA*	6-Methoxy-benzoxazolin-2-one	165
$R_1 = OCH_3$	$R_2 = OCH_3$		M_2BOA	6,7-Dimethoxy-benzoxazolin-2-one	195
Lactams:					
$R_1 = H$	$R_2 = H$	$R_3 = Glc$	HBOA-glc*	2-*O*-β-D-Glucopyranosyloxy-l,4-benzoxazin-3(4*H*)-one	327
$R_1 = H$	$R_2 = H$	$R_3 = H$	HBOA*	2-Hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one	165
$R_1 = OCH_3$	$R_2 = H$	$R_3 = Glc$	HMBOA-glc*	2-*O*-β-D-Glucopyranosyloxy-7-methoxy-(2*H*)-1, 4-benzoxazin-3(4*H*)-one	357
$R_1 = OCH_3$	$R_2 = H$	$R_3 = H$	HMBOA*	2-Hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one	195
$R_1 = OCH_3$	$R_2 = OCH_3$	$R_3 = Glc$	HM_2BOA-glc	2-*O*-β-D-Glucopyranosyloxy-7,8-dimethoxy-(2*H*)-1, 4-benzoxazin-3(4*H*)-one	387
$R_1 = OCH_3$	$R_2 = OCH_3$	$R_3 = H$	HM_2BOA	2-Hydroxy-7,8-dimethoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one	225

(*continued on next page*)

Table 1 (*continued*)

			Acronym	Systematic name	Molecular mass
Methyl derivates:					
					
$R_1 = OCH_3$	$R_2 = H$	$R_3 =$ Glc	HDMBOA-glc*	2-*O*-β-D-Glucopyranosyloxy-4,7-dimethoxy-(2*H*)-1, 4-benzoxazin-3(4*H*)-one	387
$R_1 = OCH_3$	$R_2 = H$	$R_3 = H$	HDMBOA	2-Hydroxy-4,7-dimethoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one	225

The compounds marked with an asterisk (*) were quantified in the present study. Modified from Cambier et al. (1999).

inoculation and the varieties were ranked for susceptibility to FHB by scoring the percentage of prematurely bleached spikelets.

2.2. Sampling of plants for analysis of benzoxazinoids

The wheat ears (growth stage BBCH 65) were sampled by removal in June 2004, before the plants were inoculated with FHB. Five ears from each replicate were sampled, giving a total of 20 ears per variety; these were amalgamated into one sample per variety. The following varieties were analyzed (with assigned cv. number according to our internal numbering system in parentheses): cvs. Ritmo (1), Bill (4), Grommit (6), Biscay (10), Hattrick (13), Smuggler (41), Terra (47), Asketis (48), Petrus (53), and Hanseat (54). The varieties covered a broad range of susceptibilities to FHB and there were two reference varieties, resistant cv. Petrus and susceptible cv. Hanseat (Jørgensen and Olsen, 2004; Meier, 1997). The samples were placed in liquid nitrogen immediately after sampling and stored at −20 °C until freeze-drying. The samples were freeze-dried for 24–30 h at −96 °C and 0.5 kPa and stored at −20 °C until analysis. The content of dry matter in the ears was approximately 20% prior to freeze-drying.

2.3. Reagents and chemicals

The following were purchased: HPLC-grade methanol and HPLC-grade acetonitrile (Rathburn Chemicals Ltd, Walkerburn, Scotland), glacial acetic acid (Merck, Darmstadt, Germany), 2-benzoxazolinone (BOA) purity 98% (Acros Organics, Geel, Belgium), and 6-methoxy-2-benzoxazolinone (MBOA) purity 98+% (Lancaster Synthesis, Lancashire, UK). DIMBOA was kindly provided by F. Macías, University of Cádiz (Macías et al., 2006), DIBOA-glc and DIMBOA-glc were kindly provided by Prof. Dr. Hajime Iwamura (Kyoto University). DIMBOA-glc was also kindly provided by Prof. Dr. Lisbeth Jonsson (Södertörn University College). HMBOA-glc and HDMBOA-glc were kindly provided by Dr. Ishihara (Kyoto University). 2-Hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (HBOA) and 2-hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (HMBOA) were synthesized in our laboratory as described by Krogh et al. (2006).

2.4. Standard solutions

Individual stock solutions of each benzoxazinoid were made by dissolving the solid compound in methanol. The stock solution concentrations were: DIBOA-glc: 102 mg/L (0.297 mM), DIMBOA-glc: 2.40 g/L (6.43 mM), DIMBOA: 1.06 g/L (5.01 mM), MBOA: 4.00 g/L (24.2 mM), BOA: 0.984 g/L (7.29 mM), HBOA: 1.06 g/L (6.42 mM), HMBOA: 1.31 g/L (6.69 mM), HMBOA-glc: 49.6 mg/L (0.139 mM), and HDMBOA-glc: 45.5 mg/L (0.118 mM). Standard curves were prepared using serial dilutions of the stock solutions. The final solutions for the standard curve

were prepared in 50% methanol and 50% water (v/v) containing 20 mM glacial acetic acid in the following concentrations: 3.13, 6.25, 12.5, 25.0, 50.0, 100, 200, and 400 μg/L.

2.5. Extraction of benzoxazinoids

The freeze-dried samples were crushed and homogenized with a Waring blender before extraction on an Accelerated Solvent Extraction 200 system (Dionex) (ASE). Five grams of glowed chemically inert Ottawa sand (particle size 20–30 mesh, Fisher Chemicals) was added to the 33 mL extraction cells. Subsequently 0.1 g of the freeze-dried and homogenized sample was transferred to the extraction cell and a filter was placed on top of the sample, and the extraction cell was filled with glowed glass balls. The eluent was 80% methanol, 19% water, and 1% glacial acetic acid (v/v). The protocol for the ASE extraction was the following: preheat for 5 min, heat for 5 min, static for 3 min, flush 80%, purge for 50 s, four cycles, pressure 10^7 Pa, and temperature 80 °C. Extracts were collected in vials, which were filled with eluent to maintain 44 g weight for all extracts, and stored at −20 °C until chemical analysis.

2.6. Chemical analysis of benzoxazinoids

The extracts were filtered on a Sartorius SRP 15 0.45 μm filter (PTFE membrane) and diluted with water in a 1:1 ratio. An Applied Biosystems/MDS Sciex API 2000 liquid chromatography–triple quadrupole mass spectrometer (LC/MS/MS) with turbo electrospray ionization in a positive multiple reaction monitoring (MRM) mode was used for the chemical analysis. The chromatographic separation was performed at a flow rate of 0.2 mL/min at 30 °C with an injection volume of 20 μL. The column was a Hypersil BDS C18 (2.1 × 250 mm, 5 μm). The A-eluent contained 10% methanol and 90% filtered milliQ water (v/v) with 20 mM glacial acetic acid. The B-eluent was methanol containing 20 mM glacial acetic acid. The gradient contained the following: 90% A for 1 min followed by a linear gradient to 30% A for 8 min and isocratic elution for the following 7 min, and subsequently a 1-min ramp back to 90% A and re-equilibration for 7 min. The total run time of the analysis was 23 min. The first 8 min were run to waste.

The pure reference compounds were used for identification of the benzoxazinoids based on a comparison of fragmentation patterns and retention times (Table 2).

The standard curves were applied to a quadratic function with a weighting of $1/x$ since there were more data points at the lower part of the curve (correlation coefficient > 0.99). The detection limits were determined according to Miller and Miller (1993) based on the recovery experiment at the lowest concentration, 25 mg/kg dry weight (DW). The calculations were based on three replicates with the same concentration. The detection limits were as follows: DIBOA-glc: 1.4 μmol/kg DW (0.48 mg/kg DW), DIMBOA-glc: 13 μmol/kg DW (4.7 mg/kg DW), DIMBOA: 38 μmol/kg DW (8.1 mg/kg DW), BOA: 20 μmol/kg DW (2.7 mg/kg DW), MBOA: 14 μmol/kg DW (2.2 mg/kg DW), HBOA: 72 μmol/kg DW (12 mg/kg DW), and HMBOA: 6.1 μmol/kg DW (1.2 mg/kg DW). The detection limits of HMBOA-glc and HDMBOA-glc were not determined due to limited quantities of pure standards.

The matrix effects were investigated by comparing a standard curve prepared in 50% methanol and 50% water (v/v) containing 20 mM glacial acetic acid with a standard curve prepared in 50% plant matrix and 50% water (v/v).

Table 2
Retention time and *m/z* ratio for the benzoxazinoids quantified in wheat ears by application of LC/MS/MS

Compound	*m/z* Ratio	t_R (min)
DIM_2BOA-glc	404/242	14.6
DIMBOA-glc	374/166	13.7
DIBOA-glc	344/136	12.9
HDMBOA-glc	388/226	14.6
HMBOA-glc	358/196	13.3
HBOA-glc	328/166	12.2
DIMBOA	212/166	14.3
HMBOA	196/178	14.2
MBOA	166/110	15.6
BOA	136/80	15.3
HBOA	166/148	13.6

2.7. Recovery experiment

A recovery experiment was performed in plant tissue sampled at a time when no benzoxazinoids were present (growth stage 51). This experiment was carried out as described for the extraction and chemical analysis of benzoxazinoids in order to validate the method. Each freeze-dried sample was transferred to the extraction cells and spiked with each standard solution at a low and a high concentration corresponding to 25 and 50 mg benzoxazinoids/kg DW. Recovery experiments with HMBOA-glc and HDMBOA-glc were not performed because the amount of pure standards was insufficient.

The recoveries of the benzoxazinoids were acceptable at 59–118% (Table 3). The recovery estimates were not used for data correction, as use of correction factors generally leads to higher relative uncertainties (Thompson et al., 1999). The freeze-drying step was not included in the recovery experiment, but Villagrasa et al. (2006a) showed that freeze-drying did not reduce benzoxazinoid recovery in wheat.

2.8. Statistical analysis

Tukey's multiple comparison test and a two-way ANOVA were applied to differences ($P < 0.05$) in the total concentration of benzoxazinoids between cultivars using MINITAB® Release 14 Statistical Software, while a Student-Newman–Keuls test ($P < 0.05$) was applied to differences in FHB resistance between cultivars. Principal component analyses (PCA) were performed using the Unscrambler® v8.0 Software to investigate relations between the content of benzoxazinoids in wheat and FHB resistance; the data were standardized using the weighting 1/standard deviation.

3. Results and discussion

3.1. Matrix effects in the analytical method

Matrix effects occurred for some compounds, but the degree varied between compounds (Figs. 1 and 2). However, matrix effects were primarily at high concentrations, with suppression or enhancement of the signal for several compounds due to the presence of the plant matrix; for example, BOA signal was intensified in the plant matrix at higher concentrations (Fig. 2). The results were not corrected for matrix effects due to the low concentrations in the samples.

3.2. Identification of HBOA-glc and DIM_2BOA-glc

The compound with retention time of 12.2 min and *m/z* 328 was identified as the protonated molecular ion $[M + H]^+$ of HBOA-glc based on a comparison with HMBOA-glc. Fragments at *m/z* 166 and *m/z* 148 corresponded

Table 3
Percentage of recovery of benzoxazinoids in a spiked wheat matrix, with means and relative standard deviation ($N = 3$)

Compound	Concentration of compound added to plant matrix		Recovery (%)
	mg/kg DW	μmol/kg DW	
DIBOA-glc	26	76.0	78 ± 0.4
	51	149	116 ± 5
DIMBOA-glc	25	67.0	59 ± 5
	50	134	86 ± 3
DIMBOA	25	118	87 ± 6
	50	237	62 ± 11
BOA	25	185	113 ± 2
	50	370	98 ± 4
MBOA	25	152	111 ± 1
	50	303	96 ± 2
HBOA	25	152	118 ± 7
	50	303	98 ± 5
HMBOA	25	128	60 ± 1
	50	256	98 ± 4

The molar masses (g/mol) of the compounds are as follows: DIMBOA-glc: 373, DIBOA-glc: 342, DIMBOA: 211, HMBOA: 195, MBOA: 165, BOA: 135, and HBOA: 165.

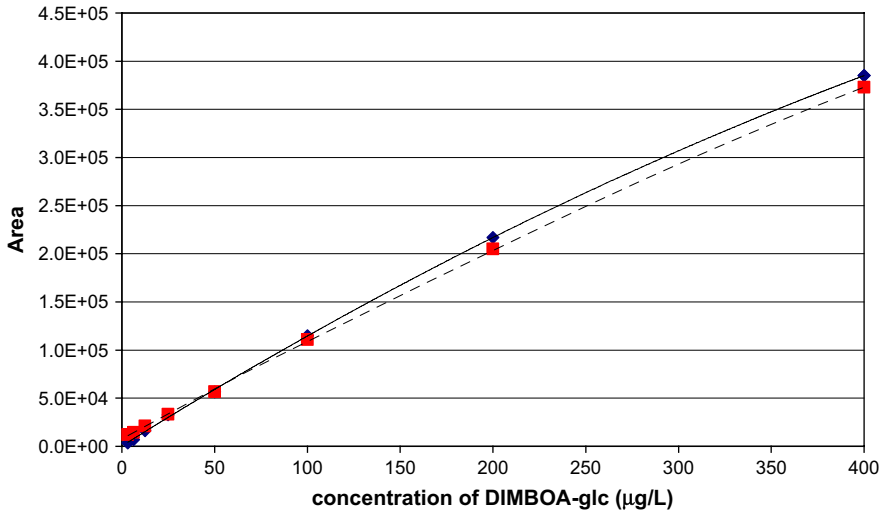


Fig. 1. The matrix effects on DIMBOA-glc. ♦: DIMBOA-glc in 50% methanol and 50% water (v/v) containing 20 mM glacial acetic acid, ■: DIMBOA-glc in 50% plant matrix and 50% water (v/v).

to the loss of a glucose moiety and glucose plus a water molecule, respectively. The neutral loss fragments were in accordance with the signals for the pure HMBOA-glc standard, where the fragments were 30 amu higher, due to the presence of the methoxy-group. The retention time of HMBOA-glc was longer (13.3 min, Table 2) than HBOA-glc due to the methoxy-group in the chemical structure. A reference standard for HBOA-glc was not available. For statistical evaluation of differences between varieties, HBOA-glc was quantified on the HMBOA-glc standard curve.

The mass spectrum of the compound at 14.6 min with a protonated molecular ion $[M + H]^+$ at *m/z* 404 was investigated further and was suggested to be DIM_2BOA-glc. A loss of 162 amu was observed, corresponding to glucose less a water molecule. There was an additional loss of 46 amu corresponding to HCOOH. There were identical neutral loss fragments for pure DIMBOA-glc and DIBOA-glc standards. The two methoxy-groups in DIM_2BOA-glc resulted in a longer retention time than for DIMBOA-glc and DIBOA-glc. Hence, the compound was provisionally characterized as DIM_2BOA-glc. The position of the additional CH_3O-group (compared with DIMBOA-glc) can only be investigated

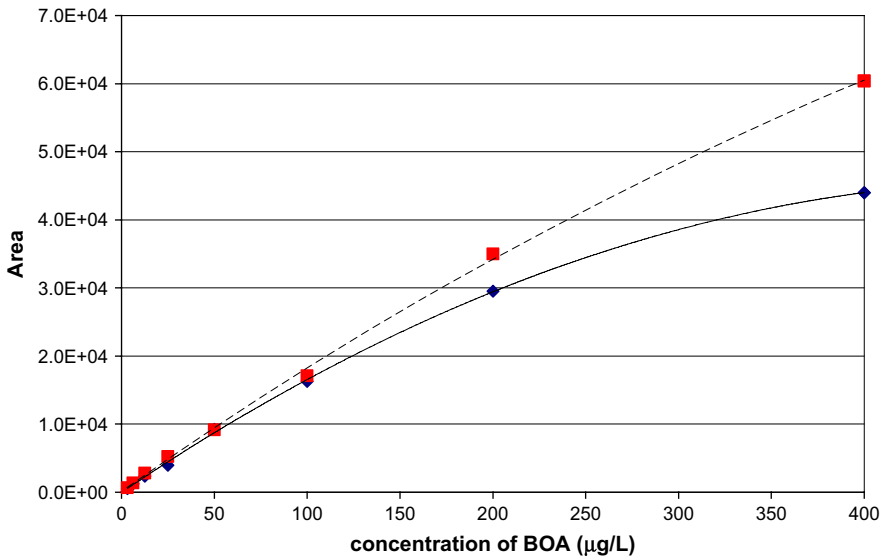


Fig. 2. The matrix effects on BOA. ♦: BOA in 50% methanol and 50% water (v/v) containing 20 mM glacial acetic acid ■: BOA in 50% plant matrix and 50% water (v/v).

further using a pure standard. For statistical comparison between varieties DIM_2BOA-glc was quantified using the DIMBOA-glc standard curve.

3.3. Contents of benzoxazinoids in wheat ears

The benzoxazinoids detected in the highest concentration in the ears of 10 cultivars were the aglucones DIMBOA (3.7–9.4 μmol/kg DW) and HMBOA (2.0–11 μmol/kg DW). The concentration range of HMBOA-glc was 0.95–6.4 μmol/kg DW and was especially high in cvs. Terra, Grommit, and Bill, while the concentration of HDMBOA-glc was 0.27–2.0 μmol/kg DW (Fig. 3). The glucosides HBOA-glc and DIM_2BOA-glc have not previously been detected in wheat. The values of DIMBOA were high as related to DIMBOA-glc. Whether the reason for this is degradation due to age, low glucosyltransferase activity especially in the wheat ears, or an increased DIMBOA synthesis is not possible to conclude. Recent microarray analysis has shown that a number of genes in the biosynthetic pathway for benzoxazinoid biosynthesis are upregulated during leaf senescence (Gregersen et al., 2005; Gregersen and Holm, 2007). Explanation to the relation between DIMBOA and DIMBOA-glc in the wheat ears in our study would require similar gene expression studies.

Our results confirmed previous reports that of the two most often quantified hydroxamic acid glucosides, DIMBOA-glc and DIBOA-glc, DIMBOA-glc is the main hydroxamic acid glucoside in wheat (Niemeyer, 1988a; Kumar et al., 1993; Richardson and Bacon, 1993) and that DIBOA-glc is normally present in low concentrations (Hofman and Hofmanova, 1969). The concentrations of DIM_2BOA-glc (quantified on basis of a DIMBOA-glc standard) in wheat ears in our study were even higher than the concentrations of DIMBOA-glc. This results need to be confirmed on basis of an isolated and purified DIM_2BOA-glc standard. The differences in the concentration of DIMBOA-glc (0.16–2.9 μmol/kg DW) between the cultivars were larger than the variation in DIBOA-glc (0.19–0.96 μmol/kg DW). The same trend has previously been observed between their aglucones (Macías et al., 2004, 2005).

DIMBOA has previously been detected in wheat at various growth stages: 0.199–0.794 mmol/kg FW in foliage at growth stage BBCH 9–10 (Mogensen et al., 2006), 0.04–0.572 mmol/kg FW in entire wheat plants at growth stage BBCH 21–22 (Stochmal et al., 2006), and 0.74–3.60 mmol/kg FW in the youngest leaf of wheat plants (growth stage not known) (Zuñiga et al., 1983). A dry matter content of 20% was assumed, where it was not specified. In the present study only the ears were analyzed, and the DIMBOA concentration was considerably lower (0.74–1.88 μmol/kg FW equal to 156–397 μg/kg FW). The concentration differences between the previously quoted studies and the present study could be a growth dilution effect (Nicol et al., 1992; Gianoli and Niemeyer, 1997; Copaja et al., 1999), since in the present study sampling was at a later growth stage.

Similar to the present study, very low concentrations of benzoxazinoids have been found in wheat ears at growth stage BBCH 60–62 (undetectable to 0.05 mmol/kg FW, detection limit not given) (Nicol and Wratten, 1997).

In the past, HMBOA-glc has been primarily detected in maize (Hofman and Hofmanova, 1969; Cambier et al., 2000; Oikawa et al., 2001), however, in the present study it was detected in wheat at appreciable concentrations of 0.95–6.4 μmol/kg DW, higher than the concentrations of DIMBOA-glc in all cultivars (Fig. 3). HMBOA-glc, DIMBOA-glc, and HDMBOA-glc have previously been identified as the main 1,4-benzoxazinone derivatives in wheat leaves of cv. 417/65 (Grambow et al., 1986). In the present study the concentration of HDMBOA-glc was 0.27–2.0 μmol/kg DW; previously it has been found in maize (Cambier et al., 2000; Oikawa et al., 2001). HDMBOA-glc was thought to be very susceptible to degradation, forming MBOA (Grambow et al., 1986). However, MBOA was not detected in the present study.

BOA, MBOA, and HBOA were not detected in wheat ears in the present study, despite having previously been found in wheat (Mogensen et al., 2006; Nicol et al., 1992; Villagrasa et al., 2006b). This could be related to the late sampling in the present study; HBOA and BOA were not detected later than growth stage 12 by Mogensen et al. (2006), and HMBOA and MBOA were found in the aerial parts of seedlings at and not later than growth stage BBCH 11–12 (Nicol et al., 1992). Villagrasa et al. (2006b) found that DIMBOA and HMBOA were the predominant benzoxazinoids in wheat foliage at growth stages BBCH 10 and 12 (similar to the present study, despite the later sampling of ears).

It has been suggested that detection of aglucones in non-injured plants is a product of a sampling procedure that does not inhibit β-glucosidase activity (Cambier et al., 1999). However, aglucones and gluco-conjugated compounds have been found in wheat tissue when β-glucosidase activity was inhibited with liquid nitrogen and methanol (Zuñiga and Massardo, 1991; Nakagawa et al., 1995). Mogensen et al. (2006) also utilized immediate freezing with dry ice

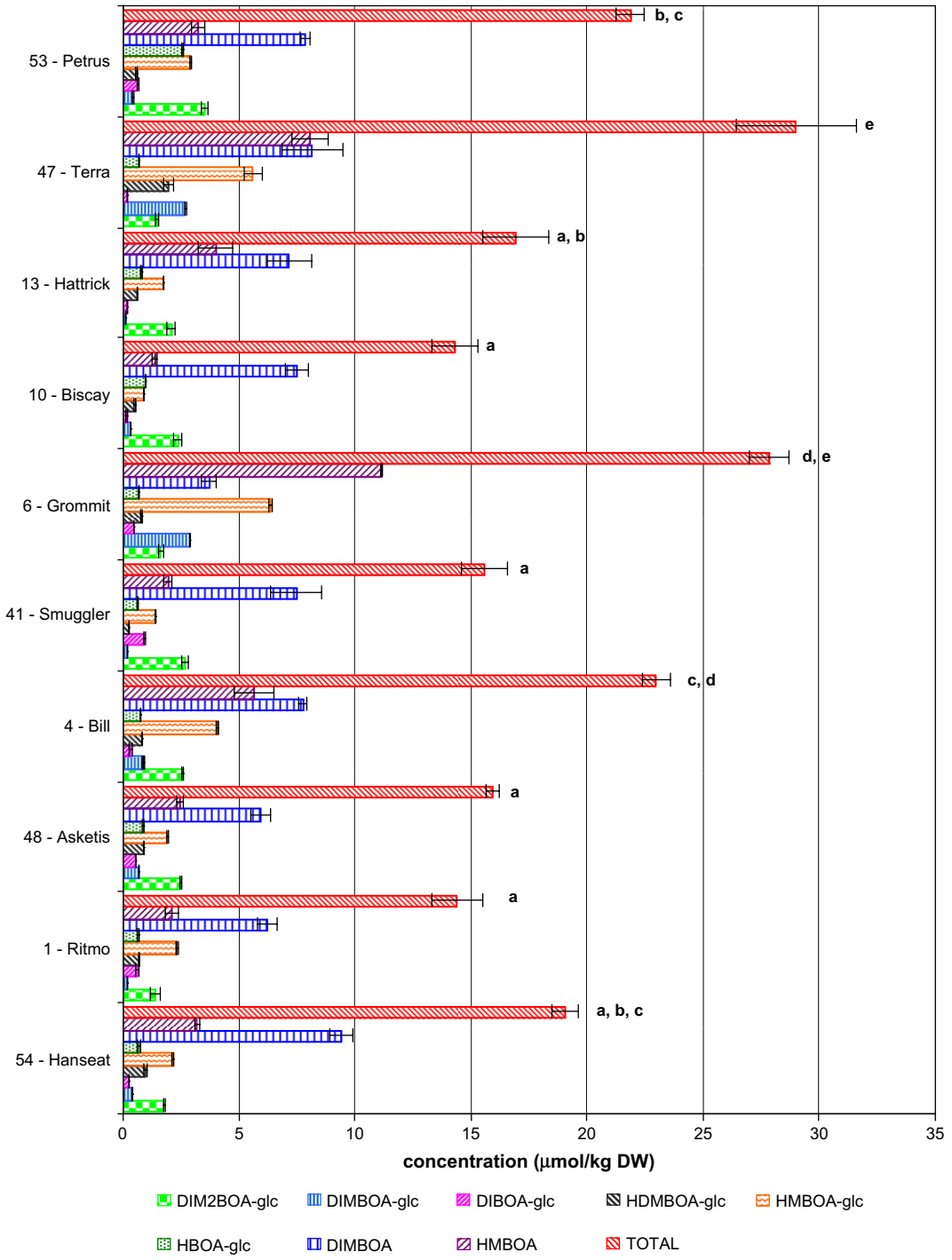


Fig. 3. The content of benzoxazinoids in winter wheat (*Triticum aestivum*) ears of 10 cultivars. The error bars indicate the standard error based on three replicate analysis of the amalgamated sample. The value of total benzoxazinoid concentration bars marked with the same letter is not significantly different according to Tukey's multiple comparison test ($P < 0.05$). The cultivars are arranged according to their susceptibility to Fusarium Head Blight provided in Fig. 4.

after sampling, and DIMBOA-glc was well preserved during the ensuing sampling preparation because non-correlating variations in the content of DIMBOA-glc, DIMBOA, and MBOA were observed. The detection of aglucones in the present study, where immediate freezing in liquid nitrogen after sampling was performed, should thus be expected not to be consequence of the sample preparation.

Differences in benzoxazinoid content between varieties were observed (Fig. 3) in consistency with previous studies of a wide range of wheat cultivars (Copaja et al., 1991; Nicol et al., 1992; Wu et al., 2001b; Mogensen et al., 2006; Villagrasa et al., 2006b). The total content of benzoxazinoids in the cvs. Grommit and Terra was significantly higher than in all the other cultivars except cv. Bill; a non-significant difference in the total concentration of benzoxazinoids was seen within a group that included cvs. Hattrick, Biscay, Smuggler, Asketis, Ritmo, and Hanseat.

3.4. Relationship between concentrations of individual benzoxazinoids

The biosynthetic pathway for the most described benzoxazinoids (DIBOA, DIMBOA, DIBOA-glc, DIMBOA-glc, and HBOA) has been elucidated in maize (Gierl and Frey, 2001; Glawischnig et al., 1999) and wheat (Nomura et al., 2002). The biosynthesis of HMBOA, HDMBOA, and their glucosides was never described. DIMBOA-glc has been proposed as the main benzoxazinoid component in maize and wheat (Hofman and Hofmanova, 1969; Niemeyer, 1988a), while HMBOA-glc was mainly detected in maize (Hofman and Hofmanova, 1969). In the present study HMBOA-glc concentration was 0.95–6.4 μmol/kg DW. In general, the concentration of HMBOA-glc was higher than DIMBOA-glc, and both compounds were found in especially high concentrations in cvs. Grommit and Terra. There was a strong correlation between the two glucosides DIMBOA-glc and HMBOA-glc (Fig. 4A) similar to what was shown by Hofman and Hofmanova (1969). There was a strong correlation between DIMBOA-glc and HMBOA ($R^2 = 0.85$), and between HMBOA-glc and HMBOA ($R^2 = 0.92$) (Fig. 4B and C). The cvs. Grommit and Terra contained high levels of DIMBOA-glc, HMBOA, and HMBOA-glc, in comparison with the other varieties. Such strong correlations could indicate that the biosynthesis of DIMBOA-glc, HMBOA, and HMBOA-glc is connected. Correlations were not significant between DIMBOA-glc and HDMBOA-glc and between HMBOA-glc and HDMBOA-glc. Thus it could be supposed that the intrinsic biosynthesis of HDMBOA-glc follows a pathway that is not connected to DIMBOA-glc pathway or that the activity of the gene that methylates in the N–OH position is low in the wheat ears under normal circumstances.

3.5. Resistance to FHB in relation to concentrations of benzoxazinoids

The percentage incidence of FHB on the 10 cultivars investigated in the year 2004 is shown in Fig. 5. The known variation in susceptibility to FHB between cultivars (Nielsen and Jørgensen, 2001) was seen in the present study and confirmed trends in equivalent studies of 2002 and 2003 (Jørgensen and Olsen, 2004). The difference in susceptibility is from different resistance mechanisms – some varieties have common features, while others are unique (Crute et al., 1985).

The cv. Hanseat was the most susceptible to FHB; cvs. Asketis and Ritmo were also more susceptible than other cultivars. In contrast, cv. Petrus was highly resistant to FHB, while a group consisting of the cvs. Biscay, Grommit, Smuggler, and Bill showed moderate susceptibility with the incidence of FHB in the range of 2.7–3.9%. The cvs. Terra and Hattrick were placed in a group between the cultivars with low and moderate susceptibility. In Denmark, cv. Smuggler is the most commonly cultivated variety, while cvs. Bill, Ritmo, Hattrick, Biscay, and Grommit are less common.

There was no obvious correlation between benzoxazinoid content and FHB susceptibility when comparing Figs. 3 and 5. However, various trends and relationships were demonstrated by principal component analysis (PCA). The PCA model consists of a set of orthogonal axes determined as maximum variance directions from a common origin. Through this, a score and a loading plot illustrate a map of samples and variables, respectively, which provides graphical information about the relationship between the samples or variables and the principal components (PC). The least-squares optimization principle was applied to generate the first PC (PC1), which is the line that is the best simultaneous fit to all points. PC2 is orthogonal to PC1 in the direction of the second largest variance, while PC3 is orthogonal to both PC1 and PC2 in the direction of the third largest variance, and so on for the desired number of PCs. The score and loading plot are any two plots of PCs against each other, usually PC1 and PC2 as they describe the largest amount of variance (Esbensen, 2002).

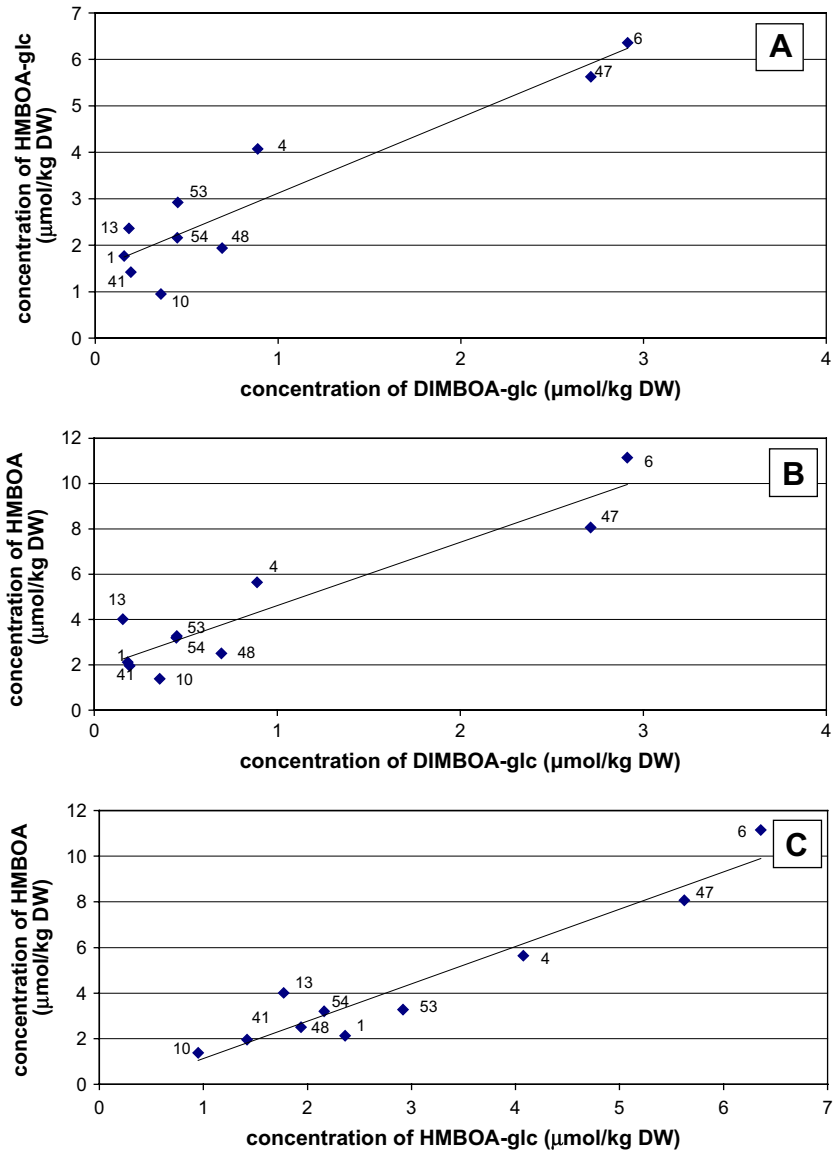


Fig. 4. Correlations between the wheat ear content of (A): DIMBOA-glc and HMBOA-glc ($R^2 = 0.86$), (B): DIMBOA-glc and HMBOA ($R^2 = 0.86$), and (C): HMBOA-glc and HMBOA ($R^2 = 0.91$) in cvs. Ritmo (no. 1), Bill (no. 4), Grommit (no. 6), Biscay (no. 10), Hattrick (no. 13), Smuggler (no. 41), Terra (no. 47), Asketis (no. 48), Petrus (no. 53), and Hanseat (no. 54).

PCA was applied to all cultivars, benzoxazinoids, and the susceptibility to FHB (Fig. 6). The variables were normalized and PC1 and PC2 explain 44 and 24% of the variance, respectively. The relationship between the scores and loadings was that cultivars with an elevated score of one PC had a high content of variables with high loadings for the same PC; for example cv. Grommit had a high concentration of DIMBOA-glc, HMBOA-glc, and HMBOA.

The cultivars with high susceptibility to FHB (cvs. Hanseat and Ritmo) are in the upper right corner of the plot, and cultivars with lower susceptibility are to the left or at the bottom of the plot (cvs. Terra, Grommit, and Petrus). This means that Terra and Grommit on the left and Petrus on the bottom of the plot have a low susceptibility to FBH but for slightly different reasons.

The cvs. Terra and Grommit in the left part of the plot (Fig. 6) had the highest concentrations of benzoxazinoids, while those cultivars to the right had lower concentrations, so the first principal component (PC1) mainly described the total concentration of benzoxazinoids ($R^2 = 0.71$, $P = 0.002$ for total concentration vs. PC1). The second principal

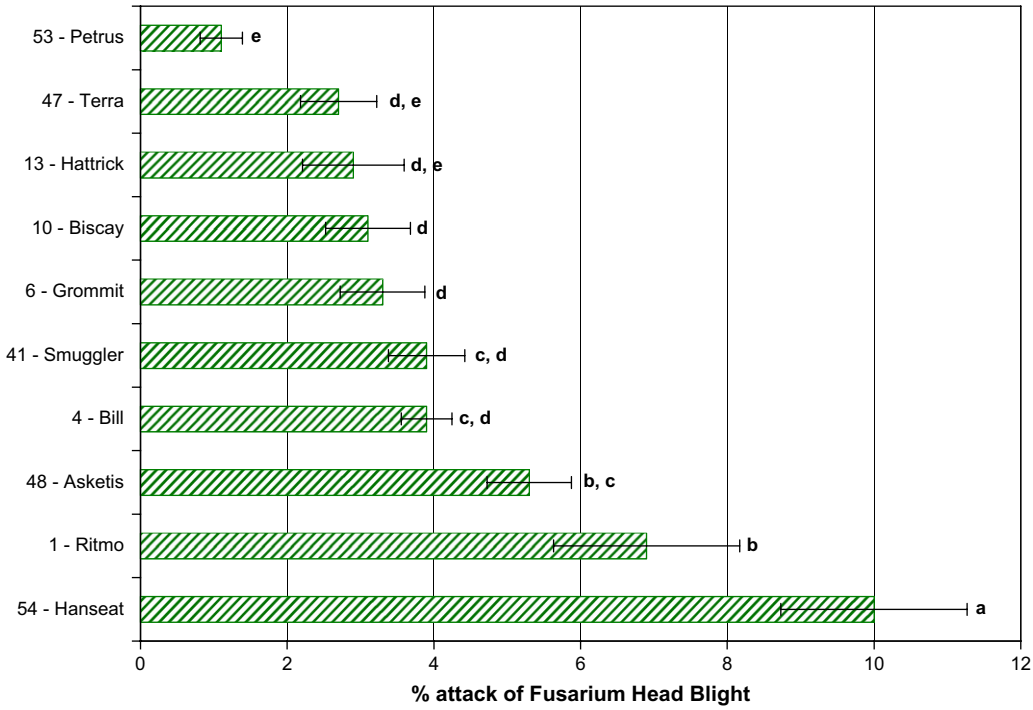


Fig. 5. The percentage of attack of Fusarium Head Blight of 10 winter wheat (*Triticum aestivum*) cultivars. The error bars indicate the standard error based on three replicates. The results of the bars indicated with the same letter are not significantly different according to a Student-Newman–Keuls test ($P < 0.05$).

component (PC2) described susceptibility to FHB for cultivars in the right part of the score plot, since incidence of FHB was generally low for cultivars with zero and negative values of PC2 ($R^2 = 0.71$, $P = 0.002$ for % incidence of FHB vs. PC2).

Another PCA analysis of the 10 cultivars, with the variable Fus (% incidence of FHB) removed, gave virtually identical PCA plots, i.e. the grouping was controlled by the chemical variables.

Fig. 6 showed that susceptibility to FHB and the content of the compounds DIMBOA-glc, HMBOA-glc, HMBOA, DIBOA-glc, HBOA-glc, and DIM_2BOA-glc were negatively correlated on PC2, i.e. a high concentration of these compounds could explain a part of the resistance towards FHB. DIBOA-glc, HBOA-glc, and DIM_2BOA-glc seemed to be most effective since Petrus with the lowest susceptibility has the highest concentration of these compounds. The agluconic compounds are the toxic compounds and they are produced from the glucosides upon microbial or mechanical injury of the plant. The fact that we find the correlation between high glucoside concentrations and high FHB resistance is not strange. Our study focuses on the constitutive benzoxazinoids, since we sampled the wheat ears before inoculation with FHB. If we had made additional sampling after the infection we probably would have found increased concentrations of aglucones.

Although a high concentration of some benzoxazinoids increased resistance, high concentrations of DIMBOA did not seem to offer an advantage. The high negative correlation between DIMBOA-glc and DIMBOA could be a consequence of DIMBOA being a degradation product of DIMBOA-glc. Hence, a high content of DIMBOA-glc indicates a low content of DIMBOA due to lack of degradation and vice versa.

A correlation between DIMBOA-glc and the resistance to stem rust (*Puccinia graminis* var. *tritici* Erikss. and Henn.) has been shown (Elnaghy and Linko, 1962), and benzoxazinoids were involved in resistance to *Helminthosporium turcicum* Pass (Long et al., 1978). In contrast, production of the benzoxazinoids DIMBOA, DIBOA, MBOA, and BOA in maize did not appear to be a highly effective resistance mechanism against *Fusarium verticillioides* (Glenn et al., 2002).

The concentrations of benzoxazinoids in the ears of wheat varieties in the present study were low in comparison with the concentrations inhibitory to fungal species related to FHB (Martyniuk et al., 2006). This could be partly due

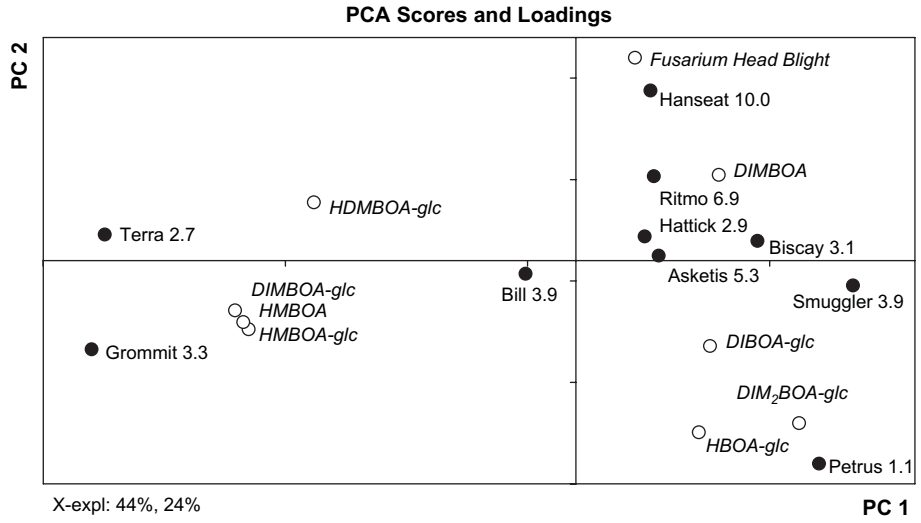


Fig. 6. Results from principal component analysis of benzoxazinoids and susceptibility to Fusarium Head Blight in all cultivars. Filled circles are scores for the cultivars and open circles are *loadings* for the *variables*. The numbers in the score plot indicate the percentage of attack of Fusarium Head Blight for each cultivar.

to a lack of focus in Danish wheat breeding on increasing benzoxazinoid concentrations. It was not possible to explain the resistance to FHB solely by a correlation with benzoxazinoids; several other allelopathic compounds and resistance mechanisms could influence resistance (Einhellig, 1996; Wu et al., 2002; Chunghong et al., 2006; Fomsgaard, 2006).

The present study revealed correlations between the susceptibility to FHB and the concentrations of the benzoxazinoids DIMBOA-glc, HMBOA-glc, HMBOA, DIBOA-glc, HBOA-glc, and DIM_2BOA-glc. Thus the capacity to produce secondary metabolites of the benzoxazinoid group should be considered in addition to other resistance mechanisms when developing resistant wheat varieties.

Acknowledgements

The research was performed as part of the project ‘‘FATEALLCHEM’’, ‘‘Fate and Toxicity of Allelochemicals (natural plant toxins) in Relation to Environment and Consumer’’ (http://www.fateallchem.dk). The project was carried out with financial support from the Commission of the European Communities under the Work Programme Quality of Life, Contract no. QLK5-CT-2001-01967.

We are grateful to Professor F. Macías (University of Cádiz), Prof. Dr. Hajime Iwamura (Kyoto University), Prof. Dr. Lisbeth Jonsson (Södertörn University College) and Dr. Ishihara (Kyoto University) for kindly providing reference compounds for this study. We are also very grateful to Louise Vedel Olsen for the sampling of wheat ears and to the technicians Jette Them Lilholt and Anne Garfield Mortensen for help with the analytical work.

References

Ahman, I., Johansson, M., 1994. Effect of light on DIMBOA-glucoside concentration in wheat (*Triticum aestivum* L.). Ann. Appl. Biol. 124, 569–574.

Bredenberg, J., Honkanen, E., Virtanen, A., 1962. The kinetics and mechanisms of decomposition of 2,4-dihydroxy-1,4-benzoxazin-3-one. Acta Chem. Scand. 16, 135–141.

Cambier, V., Hance, T., de Hoffmann, E., 1999. Non-injured maize contains several 1,4-benzoxazin-3-one related compounds but only as glucoconjugates. Phytochem. Anal. 10, 119–126.

Cambier, V., Hance, T., de Hoffmann, E., 2000. Variation of DIMBOA and related compounds content in relation to the age and plant organ in maize. Phytochemistry 53, 223–229.

Chelkowski, J., 1989. Formation of mycotoxins produced by *Fusaria* in heads of wheat, triticale and rye. In: Chelkowski, J. (Ed.), *Fusarium*. Mycotoxins, Taxonomy and Pathogenicity. Elsevier, Amsterdam, The Netherlands, pp. 63–84.

Chunghong, J., Kudsk, P., Mathiassen, S.K., 2006. Joint action of benzoxazinone derivatives and phenolic acids. J. Agric. Food Chem. 54, 1049–1057.

Copaja, S.V., Nicol, D., Wratten, S.D., 1999. Accumulation of hydroxamic acids during wheat germination. Phytochemistry 50, 17–24.

Copaja, S.V., Niemeyer, H.M., Wratten, S.D., 1991. Hydroxamic acid levels in Chilean and British wheat seedlings. Ann. Appl. Biol. 118, 223–227.

Crute, I.R., De Wit, P.J.G.M., Wade, M., 1985. Mechanisms by which genetically controlled resistance and virulence influence host colonization by fungal and bacterial parasites. In: Fraser, R.S.S. (Ed.), Mechanisms of Resistance to Diseases. Kluwer Academic Publishers, Norwell, MA, pp. 197–309.

Einhellig, F.A., 1996. Interactions involving allelopathy in cropping systems. Agron. J. 88, 886–893.

Elnaghy, M.A., Linko, P., 1962. Role of 4-*O*-glucosyl-2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one in resistance of wheat to stem rust. Physiol. Plant. 15, 764–770.

Esbensen, K.H., 2002. Multivariate Data Analysis – in Practice, fifth ed., CAMO Process AS.

Fomsgaard, I.S., 2006. Chemical ecology in wheat plant–pest interactions. How the use of modern techniques and a multidisciplinary approach can throw new light on a well-known phenomenon: allelopathy. J. Agric. Food Chem. 54, 987–990.

Friebe, A., Vilich, V., Hennig, L., Kluge, M., Sicker, D., 1998. Detoxification of benzoxazolinone allelochemicals from wheat by *Gaeumannomyces graminis* var. *tritici*, *G. graminis* var. *graminis*, *G. graminis* var. *avenae*, and *Fusarium culmorum*. Appl. Environ. Microbiol. 64, 2386–2391.

Gianoli, E., Niemeyer, H., 1997. Environmental effects on the accumulation of hydroxamic acids in wheat seedlings: the importance of plant growth rate. J. Chem. Ecol. 23, 534–551.

Gierl, A., Frey, M., 2001. Evolution of benzoxazinone biosynthesis and indole production in maize. Planta 213, 493–498.

Glawischnig, E., Grün, S., Frey, M., Gierl, A., 1999. Cytochrome P450 monooxygenases of DIBOA biosynthesis: specificity and conservation among grasses. Phytochemistry 50, 925–930.

Glenn, A.E., Gold, S.E., Bacon, C.W., 2002. Fdb1 and Fdb2, *Fusarium verticillioides* loci necessary for detoxification of preformed antimicrobials from corn. Mol. Plant Microbe Interact. 15, 91–101.

Grambow, H.J., Luckge, J., Klausener, A., Muller, E., 1986. Occurrence of 2-(2-hydroxy-4,7-dimethoxy-2H-1,4-benzoxazin-3-one)-β-D-glucopyranoside in *Triticum aestivum* leaves and its conversion into 6-methoxy-benzoxazolinone. Z. Naturforsch. Sect. C J. Biosci. 41, 684–690.

Gregersen, P.L., Brinch-Pedersen, H., Holm, P.B., 2005. A microarray-based comparative analysis of gene expression profiles during grain development in transgenic and wild type wheat. Transgenic Res. 14, 887–905.

Gregersen, P.L., Holm, P.B., 2007. Transcriptome analysis of senescence in the flag leaf of wheat (*Triticum aestivum* L.). Plant Biotechnol. J. 5, 192–206.

Hofman, J., Hofmanova, O., 1969. 1,4-Benzoxazine derivatives in plants. Sephadex fractionation and identification of a new glucoside. Eur. J. Biochem. 8, 109–112.

Jørgensen, L.N., Olsen, L.V., 2004. Aksfusarium. Sortsmodtagelighed og kemisk bekæmpelse. DJF Rapport 98, 73–82.

Krogh, S., Mensz, S.J.M., Nielsen, S.T., Mortensen, A.G., Christoffersen, C., Fomsgaard, I.S., 2006. Metabolic profiling of benzoxazinone allelochemicals in soil after incorporation of wheat and rye sprouts. J. Agric. Food Chem. 54, 1064–1074.

Kumar, P., Gagliardo, R.W., Chilton, W.S., 1993. Soil transformation of wheat and corn metabolites MBOA and DIM_2BOA into aminophenoxazinones. J. Chem. Ecol. 19, 2453–2461.

Leszczynski, B., Dixon, F.G., 1990. Resistance of cereals to aphids: interaction between hydroxamic acids and the aphid *Sitobion avenae* (Homoptera: Aphididae). Ann. Appl. Biol. 117, 21–30.

Long, B.J., Dunn, G.M., Routley, D.G., 1978. Relationship of hydroxamate concentration in maize and field reaction to *Helminthosporium turcicum*. Crop Sci. 18, 573–575.

Macías, F.A., Marín, D., Oliveros-Bastidas, A., Chinchilla, D., Simonet, A.M., Molinillo, J.M.G., 2006. Isolation and synthesis of allelochemicals from Gramineae: benzoxazinones and related compounds. J. Agric. Food Chem. 54, 991–1000.

Macías, F.A., Oliveros-Bastidas, A., Marín, D., Castellano, D., Simonet, A.M., Molinillo, J.M.G., 2004. Degradation studies on benzoxazinoids. Soil degradation dynamics of 2,4-dihydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIMBOA) and its degradation products, phytotoxic allelochemicals from Gramineae. J. Agric. Food Chem. 52, 6402–6413.

Macías, F.A., Oliveros-Bastidas, A., Marín, D., Castellano, D., Simonet, A.M., Molinillo, J.M.G., 2005. Degradation studies on benzoxazinoids. Soil degradation dynamics of (2*R*)-2-*O*-beta-D-glucopyranosyl-4-hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIBOA-Glc) and its degradation products, phytotoxic allelochemicals from Gramineae. J. Agric. Food Chem. 53, 554–561.

Martyniuk, S., Stochmal, A., Macías, F.A., Marín, D., Oleszek, W., 2006. Effects of some benzoxazolinones and their metabolites on in vitro growth of *Cephalosporium gramineum* and other fungi pathogenic to cereals, and on *Cephalosporium* stripe of winter wheat. J. Agric. Food Chem. 54, 1036–1039.

McMullen, M., Jones, R., Gallenberg, D., 1997. Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Dis. 81, 1340–1348.

Meier, U., 1997. Growth Stages of Plants. Blackwell Wissenschafts-Verlag, Berlin, Germany.

Miller, J.C., Miller, J.N., 1993. Statistics for Analytical Chemistry, third ed. Ellis Horwood and Prentice Hall, Englewood Cliffs, NJ.

Mogensen, B.B., Krongaard, T., Mathiassen, S.K., Kudsk, P., 2006. Quantification of benzoxazinone derivatives in wheat (*Triticum aestivum*) varieties grown under contrasting conditions in Denmark. J. Agric. Food Chem. 54, 1023–1030.

Nakagawa, E., Amano, T., Hirai, N., Iwamura, H., 1995. Non-induced cyclic hydroxamic acids in wheat during juvenile stage of growth. Phytochemistry 38, 1349–1354.

Nicol, D., Copaja, S., Wratten, S., Niemeyer, H., 1992. A screen of worldwide wheat cultivars for hydroxamic acid levels and aphid antixenosis. Ann. Appl. Biol. 121, 11–18.

Nicol, D., Wratten, S.D., 1997. The effect of hydroxamic acid concentration at late growth stages of wheat on the performance of the aphid *Sitobion avenae*. Ann. Appl. Biol. 130, 387–396.

Nielsen, G.C., Jørgensen, L.N., 2001. Hvilke faktorer påvirker indholdet af Fusariumtoksiner i korn? DJF Rapport 40, 131–148.

Niemeyer, H., 1988a. Hydroxamic acid content of *Triticum* species. Euphytica 37, 289–293.

Niemeyer, H., 1988b. Hydroxamic acids (4-hydroxy-1,4-benzoxazin-3-ones) defence chemicals in the Gramineae. Phytochemistry 27, 3349–3358.

Niemeyer, H.M., Perez, F.J., 1995. Potential of hydroxamic acids in the control of cereal pests, diseases and weeds. In: Inderjit, Dakshini, K.M.M., Einhellig, F.A. (Eds.), Allelopathy – Organisms, Processes, and Applications. American Chemical Society, Washington DC, pp. 260–270.

Nomura, T., Ishihar, A., Imaishi, H., Endo, T.R., Ohkawa, H., Iwamura, H., 2002. Molecular characterization and chromosomal localization of cytochrome P450 genes involved in the biosynthesis of cyclic hydroxamic acids in hexaploid wheat. Mol. Genet. Genomics 267, 210–217.

Oikawa, A., Ishihara, A., Hasegawa, M., Kodama, O., Iwamura, H., 2001. Induced accumulation of 2-hydroxy-4,7-dimethoxy-1,4-benzoxazin-3-one glucoside (HDMBOA-Glc) in maize leaves. Phytochemistry 56, 669–675.

Parry, D.W., Jenkinson, P., Mcleod, L., 1995. *Fusarium* ear blight (scab) in small-grain cereals – a review. Plant Pathol. 44, 207–238.

Pirgozliev, S.R., Edwards, S.G., Hare, M.C., Jenkinson, P., 2003. Strategies for the control of *Fusarium* head blight in cereals. Eur. J. Plant Pathol. 109, 731–742.

Rice, E.L., 1984. Allelopathy, second ed. Academic Press Inc., London, United Kingdom.

Richardson, M., Bacon, C., 1993. Cyclic hydroxamic acid accumulation in corn seedlings exposed to reduced water potentials before, during, and after germination. J. Chem. Ecol. 19, 1613–1624.

Rizvi, S.J.H., Haqua, H., Singh, V.K., Rizvi, V., 1992. A discipline called allelopathy. In: Rizvi, S.J.H., Rizvi, V. (Eds.), Allelopathy. Basic and Applied Aspects. Chapman & Hall, London, United Kingdom, pp. 1–8.

Sicker, D., Hao, H., Schulz, M., 2004. Benzoxazolin-2(3H)-ones – generation, effects and detoxification in the competition among plants. In: Macías, F.A., Galindo, J.C.G., Molinillo, J.M.G., Cutler, H.G. (Eds.), Allelopathy. Chemistry and Mode of Action of Allelochemicals. CRC Press, pp. 77–102.

Stochmal, A., Kús, J., Martyniuk, S., Oleszek, W., 2006. The concentration of benzoxazinones in roots of field grown wheat (*Triticum aestivum* L.) varieties in two cultivation systems. J. Agric. Food Chem. 54, 1016–1022.

Thompson, M., Ellison, S.L., Fajgelj, A., Willets, P., Wood, R., 1999. Harmonised guidelines for the use of recovery information in analytical measurement. Pure Appl. Chem. 71, 337–348.

Thrane, U., 2000. Mykotoksinproducerende *Fusarium* arter i dansk korn. DJF Rapport 24, 165–169.

Torres, A., Oliva, R.M., Castellano, D., Cross, P.I., 1996. Introduction. In: Proceedings of the First World Congress on Allelopathy – a Science for the Future, p. 7.

Villagrasa, M., Guillamón, M., Eljarrat, E., Barceló, D., 2006a. Determination of benzoxazinone derivatives in plants by combining pressurized liquid extraction–solid phase extraction followed by liquid chromatography–electrospray-mass spectrometry. J. Agric. Food Chem. 54, 1001–1008.

Villagrasa, M., Guillamón, M., Labandeira, A., Taberner, A., Eljarrat, E., Barceló, D., 2006b. Benzoxazinone allelochemicals in wheat: distribution among foliage, root and seed. J. Agric. Food Chem. 54, 1009–1015.

Virtanen, A.I., Hietala, P.K., 1955. 2(3)-Benzoxazolinone, an anti-fusarium factor in rye seedlings. Acta Chem. Scand. 9, 1543–1544.

Virtanen, A., Hietala, P., 1960. Precursors of benzoxazolinone in rye plants. I. Precursor II, the aglycone. Acta Chem. Scand. 14, 499–502.

Wilkes, M., Marshall, D., Copeland, L., 1999. Hydroxamic acids in cereal roots inhibit the growth of take-all. Soil Biol. Biochem. 31, 1831–1836.

Woodward, M., Corcuera, L., Helgeson, J., 1978. Decomposition of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one. Plant Physiol. 61, 796–802.

Wu, H., Haig, T., Pratley, J., Deidre, L., An, M., 2001a. Allelochemicals in wheat (*Triticum aestivum* L.): production and exudation of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one. J. Chem. Ecol. 27, 1691–1700.

Wu, H., Haig, T., Pratley, J., Lemerle, D., An, M., 2001b. Allelochemicals in wheat (*Triticum aestivum* L.): cultivar difference in the exudation of phenolic acids. J. Agric. Food Chem. 49, 3742–3745.

Wu, H., Haig, T., Pratley, J., Lemerle, D., An, M., 2002. Biochemical basis for wheat seedling allelopathy on the suppression of annual ryegrass (*Lolium rigidum*). J. Agric. Food Chem. 50, 4567–4571.

Xu, X.M., 2003. Effects of environmental conditions on the development of *Fusarium* ear blight. Eur. J. Plant Pathol. 109, 683–689.

Zikmundova, M., Drandarov, K., Bigler, L., Hesse, M., Werner, C., 2002. Biotransformation of 2-benzoxazolinone (BOA) and 2-hydroxy-1,4-benzoxazin-3-one (HBOA) by endophytic fungi isolated from *Aphelandra tetragona*. Appl. Environ. Microbiol. 68, 4863–4870.

Zuñiga, G.E., Massardo, F., 1991. Hydroxamic acid content in undifferentiated and differentiated tissues of wheat. Phytochemistry 30, 3281–3283.

Zuñiga, G., Argandona, V., Niemeyer, H., Corcuera, L., 1983. Hydroxamic acid content in wild and cultivated Gramineae. Phytochemistry 22, 2665–2668.