JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Concentration of Benzoxazinoids in Roots of Field-Grown Wheat (*Triticum aestivum* L.) Varieties

ANNA STOCHMAL,† JAN KUS,‡ STEFAN MARTYNIUK,§ AND WIESLAW OLESZEK*,†

Department of Biochemistry, Department of Systems and Economics of Crop Production, and Department of Microbiology, Institute of Soil Science and Plant Cultivation, ul. Czartoryskich 8, 24-100 Pulawy, Poland

Benzoxazinones are naturally occurring secondary metabolites of some Gramineae plants, responsible for their resistance to some pathogenic fungi and for their allelopathic action. Six varieties of winter wheat grown in fields under organic or conventional systems and 11 old accessions were tested for two consecutive seasons and three plant development stages for the concentration in their roots of cyclic hydroxamic acids and their degradation products. This is the first report of six benzoxazinones analyzed in plants grown in the field. An analytical technique employing LC-DAD was used for determination. It was shown that 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one, its degradation product 6-methoxybenzoxazolin-2-one, and the lactam 2-hydroxy-7-methoxy-1,4-benzoxazin-2-one were predominant compounds in all tested samples. Their concentrations significantly differed with plant development stage and season, but no significant differences were found between varieties and between plant cultivation systems. The concentrations of 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) and its degradation product benzoxazolin-2-one (BOA) were much lower, ranging from 60 to 430 mg/kg of dry matter, depending on accession, stage of development, and season. There was no significant difference found between plants grown in different cultivation systems, but there were significant differences between old and new varieties; concentrations of DIBOA and its derivatives were significantly lower in old accessions. It was concluded that the concentrations of DIBOA and BOA, which are precursors of highly fungicidal 2-aminophenol, 2-amino-3*H*-phenoxazin-3-one, and 2-acetylamino-3*H*-phenoxazin-3-one, are theoretically high enough to protect plants against some soilborne pathogens.

KEYWORDS: Wheat; hydroxamic acids; benzoxazinones; concentration

INTRODUCTION

Benzoxazinoids (cyclic hydroxamic acids, lactams, benzoxazolinones, and methyl derivatives of hydroxamic acids) are naturally occurring secondary plant metabolites reported mainly in the family Gramineae (wheat, corn, rye, triticale) (*1*−*3*) but also in some other plant species (*4*−*8*). Their occurrence was proved also in some wild species of the genus *Hordeum* but not in cultivated barley, *Hordeum vulgare* (*9*). Also, seedlings of rice, barley, oat, and sorghum did not contain any detectable amount of benzoxazinoids (*10*).

Benzoxazinoids were suggested to be the major factor responsible for the resistance of rye seedling to fungal pathogens (*11*). Their contribution to resistance of some other Gramineae species to pathogens and insects has also been documented (*2*, *12*, *13*). Hydroxamic acids and their degradation products were also shown to be allelopathic (*1*, *14*−*16*). Besides, in Gramineae, lines with a high concentration of benzoxazinoids showed high resistance to triazine herbicides, whereas lines with low concentrations were more sensitive (*17*).

Seeds of wheat did not contain hydroxamic acids (*18*), but their synthesis began at a very early stage of germination (*19*). It was documented that 2-day-old seedlings already contained aglucones 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) and 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA); glucosides at this stage are not present. In 7-day-old seedlings the concentration of aglucones droped by 10 times, whereas the concentration of glucosides increased. On day 13, the concentration of the DIBOA glucoside decreased and in 18-day-old seedlings it disappeared completely. At the same time the concentration of DIMBOA glucoside was highest in 7-day-old seedlings and gradually decreased in older seedlings (*19*, *20*). Comparison of the concentration of hydroxamic acids in 6-day-old seedlings of wheat varieties grown in Sweden during 1992−1993 showed that older varieties had higher concentrations of these compounds than the youngest one (*21*). Similar studies with *Triticum* species (*22*), Chilean cultivars (*23*), and a worldwide collection of cultivars (*24*) showed significant

* Corresponding author (telephone 48-81-8863421; fax 48-81-8864547; e-mail wo@iung.pulawy.pl).
† Department of Biochemistry.
‡ Department of Economics of Crop Production.
§ Department of Microbiology.

10.1021/jf050899+ CCC: $33.50
Published on Web 01/24/2006

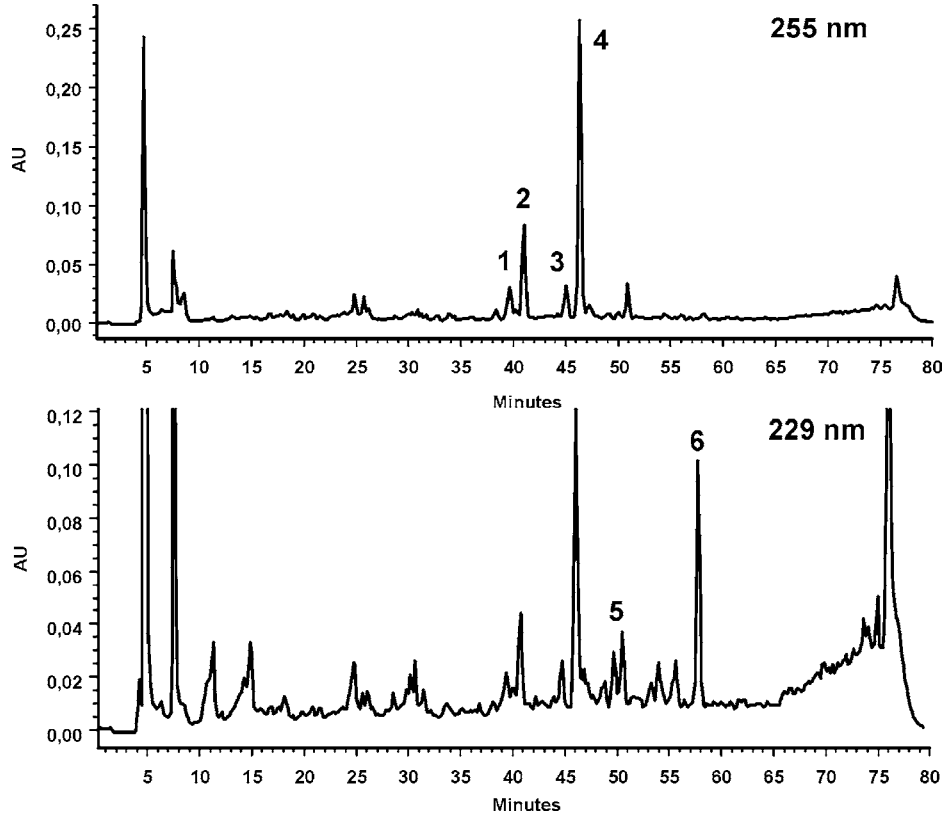

Figure 1. HPLC profiles of wheat root benzoxazinoids registered at 255 and 229 nm: **1**, DIBOA; **2**, DIMBOA-Glc; **3**, HMBOA; **4**, DIMBOA; **5**, BOA; **6**, MBOA.

differences among varieties. The range of the concentration at seedling stage was quite broad and, depending on the study, varied from 0.21 to 16.0 mmol/kg of fresh weigh (fw) (*22*), from 1.4 to 10.9 mmol/kg of fw (*23*), from 0.99 to 8.07 mmol/kg of fw (*24*), and from 1.14 to 2.37 mmol/kg of fw (*21*). Distribution between seedling foliage and roots was also differentiated, with some varieties showing a much higher root/foliar concentration ratio (*25*).

Most of the published research on the concentration of benzoxazinoids was performed at seedling stage. No comprehensive studies have been performed in field conditions at growth phases that might be crucial for pathogen resistance. A high concentration in roots could at later stages of plant development prove to be effective in the control of soilborne root pests, for example, *Gaeumannomyces graminis* var. *tritici* (*Ggt*), *Cephalosporium gramineum*, or *Fusarium culmorum*. In the present research we thus deemed it to be of interest to study for the first time six benzoxazinoid concentrations in the roots of 6 Polish wheat varieties being actually registered for cultivation and 11 old cultivars and accessions grown under field conditions during two seasons. The influence of cultivation systems (organic versus conventional) and of cultivation seasons on the accumulation of benzoxazinoids was also studied.

MATERIALS AND METHODS

Plant Material. Two field experiments were performed. The first one included six varieties actually under cultivation: Kobra, Roma, Korweta, Sukces, Zyta, and Mewa. These varieties were grown in two cultivation systems—organic and conventional—located in an experimental farm of the Institute of Soil Science and Plant Cultivation in Pulawy, Poland. These two systems were located on the same type of soil and have been under organic or conventional cultivation over 10 years. Each system included ~1 ha field (3 plots × 0.05 ha for each variety). Additionally, seven old varieties of *Triticum aestivum* (Wysokolitewka Sztywnosloma, Ostka Kazimierska, Banatka Kresowa, Magnatka Rogalinska, Dankowska Zachodnia, Blondynka, and Kujawianka Wieclawicka) and four *T. aestivum* ssp. *spelta* (Alefeldii, Tiroler Roter Dinkel, Spelt eng. Droogendijk, and Rottweiler Fruehkorn) were grown in 1 × 1m (three replicates) plots in a conventional system. Samples were collected from each variety at three growing stages, BBCH scale (*26*): two-leaf (phase 11−12), four-leaf (phase 21−22), and tillering (phase 31−32). Samples were separated into roots and aerial parts, freeze-dried and powdered, and stored in glass containers at −15 °C until analyzed.

Determination of Benzoxazinoids in Plant Material. Powdered plant (0.1 g) material was extracted with 5 mL of acidified MeOH (MeOH/HOAc, 99:1) by sonication for 5 min followed by overnight cold extraction (refrigerator, 4 °C). The next day, samples were additionally sonicated for 5 min and filtered. The residues were extracted twice with 5 mL of acidified MeOH by sonication for 10 min. Supernatants from three extractions were combined and evaporated under reduced pressure at 30 °C. The dry residue was dissolved in 5 mL of acidified water (H_2O/OHAc, 99:1), and the solution was loaded on a C18 Sep-Pak cartridge preconditioned with acidified water and then washed with 6 mL of acidified water (eluate 1). Then the cartridge was washed with 5 mL of MeOH/H_2O/AcOH (59:40:1) (eluate 2). Eluates 1 and 2 were combined, evaporated to dryness, dissolved in 2 mL of MeOH, and used for HPLC determination.

HPLC Analysis. Analyses were carried out on an HPLC system (Waters, Milford, MA) consisting of a model 616 pump, a model 600s controller, and a model 996 diode array detector. The Millenium Chromatography Manager was used to monitor chromatographic parameters and to process the data. Separations were performed on a 250 × 4.6 mm Synergi 4μ MAX-RP 80A (Phenomenex, Torrance, CA) column. Chromatographic runs were carried out using a mobile phase (1% H_3PO_4 → 40% CH_3CN in 1% H_3PO_4, 1 mL/min, during 80 min) linear gradient. Integration of chromatograms was performed at 229 nm for BOA and MBOA and at 255 nm for DIBOA, DIMBOA-Glc,

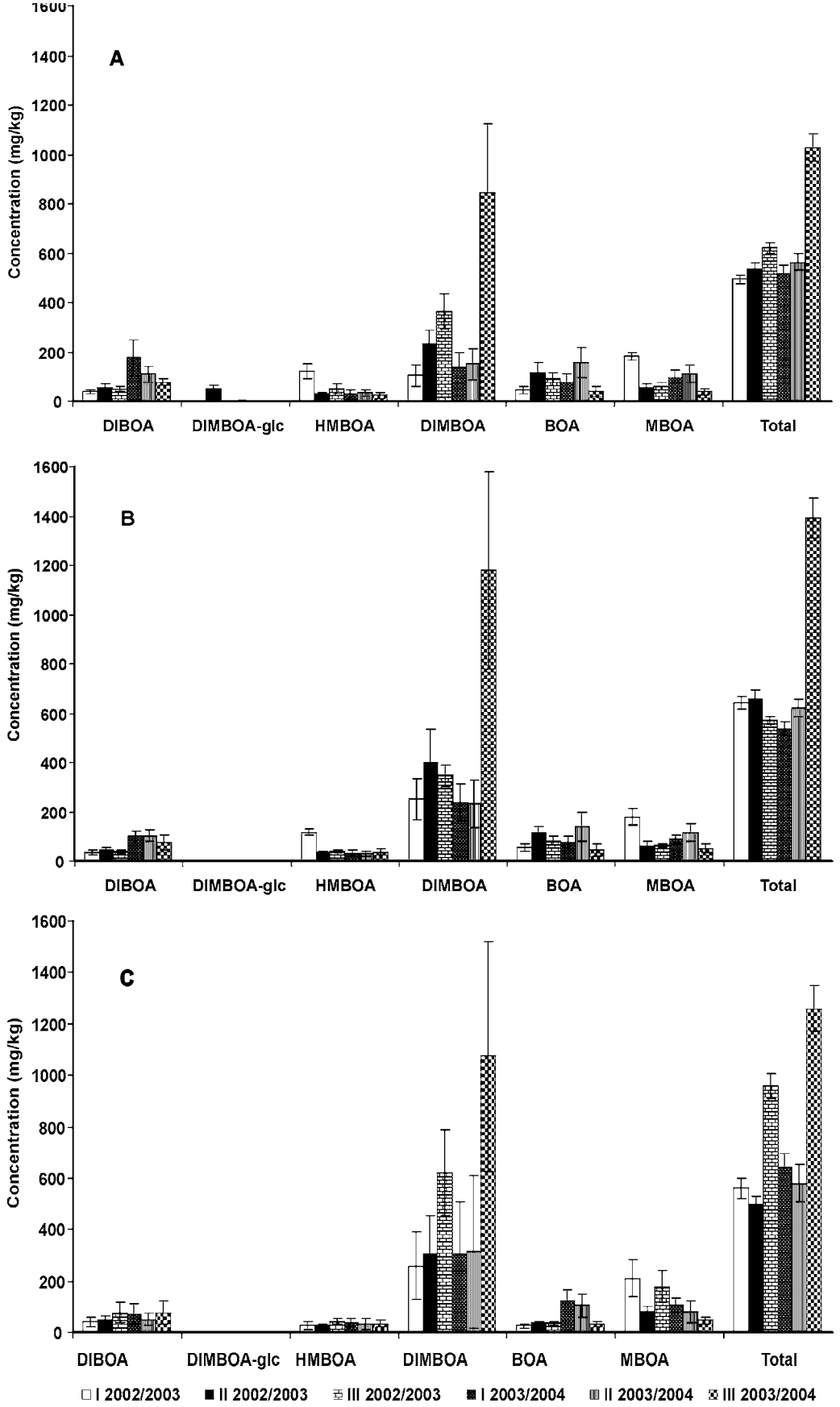

Figure 2. Concentration of measured benzoxazinoids: (**A**) average for 6 varieties from organic system; (**B**) average for 6 varieties for conventional system; (**C**) average for 11 old accessions.

2-hydroxy-7-methoxy-1,4-benzoxazin-2-one (HMBOA), and DIMBOA. The calibration curves were performed for each individual compound, and they were used for data calculation.

Statistical Analysis. The data were analyzed with ANOVA, and the treatment means were tested for least significant difference (LSD) at a 5% level of probability.

Table 1. Concentration of DIMBOA in Wheat Roots at Two Stages of Development [Milligrams per Kilogram (Millimoles per Kilogram) of Dry Matter]

	season 2002/2003		season 2003/2004	
variety	four-leaf stage	tillering	four-leaf stage	tillering
		Organic System		
Kobra	278.9 (1.3)	344.3 (1.6)	146.7 (0.7)	1126.2 (5.3)
Roma	155.3 (0.7)	272.7 (1.3)	70.9 (0.3)	698.7 (3.3)
Korweta	166.4 (0.8)	306.5 (1.5)	211.7 (1.0)	715.9 (3.4)
Sukces	241.0 (1.1)	457.1 (2.2)	109.4 (0.5)	719.4 (3.4)
Zyta	239.7 (1.1)	417.6 (2.0)	155.9 (0.7)	1190.3 (56)
Mewa	301.1 (1.4)	391.8 (1.9)	248.9 (1.2)	1049.6 (5.0)
		Conventional System		
Kobra	294.0 (1.4)	333.0 (1.6)	389.1 (1.8)	1996.1 (9.5)
Roma	315.0 (1.5)	345.4 (1.6)	115.8 (0.5)	1069.0 (5.1)
Korweta	512.1 (2.4)	421.1 (2.0)	276.0 (1.3)	968.2 (4.6)
Sukces	450.2 (2.1)	286.6 (1.4)	226.6 (1.1)	1331.6 (6.3)
Zyta	255.9 (1.2)	341.2 (1.6)	118.7 (0.6)	1196.4 (5.7)
Mewa	583.7 (2.8)	356.2 (1.7)	217.6 (1.0)	888.4 (4.2)
		Old Wheat Varieties		
Wysokolitewka	243.8 (1.2)	649.3 (3.1)	154.3 (0.7)	730.0 (3.5)
Ostka Kazimierska	188.1 (0.9)	366.5 (1.7)	40.3 (0.2)	450.9 (2.1)
Banatka Kresowa	194.1 (0.9)	635.9 (3.0)	255.5 (1.2)	798.8 (3.8)
Magnatka Rogalinska	224.7 (1.1)	718.0 (3.4)	206.7 (1.0)	790.8 (3.7)
Tiroler Roter	292.7 (1.4)	443.9 (2.1)	321.2 (1.5)	1037.1 (4.9)
Alefeldii	186.7 (0.9)	925.1 (4.4)	121.1 (0.6)	1940.1 (9.2)
Spelt Droogendijk	192.7 (0.9)	855.5 (4.1)	255.4 (1.2)	1207.6 (5.7)
Rottweiler	335.6 (1.6)	604.9 (2.9)	290.3 (1.4)	891.2 (4.2)
Dankowska Zachodnia	603.9 (2.9)	592.2 (2.8)	75.9 (0.4)	299.3 (1.4)
Blondynka	289.4 (1.4)	511.1 (2.4)	221.9 (1.1)	1173.4 (5.6)
Kujawianka Wieclawicka	581.4 (2.8)	536.2 (2.5)	261.7 (1.2)	785.1 (3.7)

RESULTS AND DISCUSSION

The extraction and purification method used in this research combined with HPLC photodiode array detection did not ensure full analysis of benzoxazinoids in wheat aerial parts as their peaks were overlapped with the abundance of flavonoids present in the matrix. Some benzoxazinoids (HMBOA and MBOA) could be detected in aerial parts of seedlings at the two-leaf stage but not at the later stages, yet the method worked successfully for root extracts. Because some published studies on the distribution of benzoxazinoids between wheat roots and aerial parts showed that in field conditions at later stages of plant development (stages 21−22 and 31−32) 80−85% of the benzoxazinoids are located in roots (*27*), we concentrated exclusively on these parts of the plant.

The model separation of benzoxazinoids in root extract is shown in **Figure 1**. Six compounds could be determined with this procedure. However, for better integration conditions chromatograms were detected at 229 nm for BOA and MBOA and at 255 nm for DIBOA, DIMBOA-Glc, HMBOA, and DIMBOA, at which these compounds had their absorption maximum. Standard curves prepared for each standard benzoxazinoid showed good linearity ($r^2 = 0.99$) in the range of 0.1−2.5 ng/μL, and detection limits were very close to each other, ranging from 0.02 to 0.06 ng/μL. The correctness of the method in comparison with the LC-MS/MS procedure was additionally confirmed by interlaboratory tests performed among the European Community FATEALLCHEM project partners (*28*).

The distribution of particular compounds in plant samples showed similar patterns in wheat varieties grown under organic and conventional systems as well as in old wheat varieties (**Figure 2**, panels **A**, **B**, and **C**, respectively). The dominant compound in all samples was DIMBOA, whereas its glucoside was found only in a trace amount. Although extraction/purification of the benzoxazinoids in the present research was performed at low temperature, nearly total degradation of DIMBOA-Glc to its aglucone DIMBOA occurred. The other four benzoxazinones, DIBOA, HMBOA, BOA, and MBOA, were present in relatively lower amounts, and the concentration of each of them usually did not exceed 15−20 μg/100 mg of dry matter.

The concentration of DIMBOA in analyzed wheat varieties was very irregular and ranged from 155 to 604 mg/kg (0.73 and 2.86 mmol/kg) and from 40 to 389 mg/kg (0.19 and 1.84 mmol/kg) of dry weight for the four-leaf stage for the 2002/2003 and 2003/2004 seasons, respectively. For the tillering stage these values were higher and ranged from 273 to 925 mg/kg (1.29 and 4.38 mmol/kg) and from 299 to 1996 mg/kg (1.42 and 9.46 mmol/kg) of dry weight for the 2002/2003 and 2003/2004 seasons, respectively (**Table 1**). The present data can be compared to published results on wheat varieties, as DIMBOA was the only compound measured so far for comparison of benzoxazinoids in wheat varieties (*21*−*24*). However, a majority of the work published so far on wheat benzoxazinoids was concentrated on seeking wheat accessions with a high DIMBOA concentration in shoots/leaves for integrated pest management (*21*−*24*). Less attention has been paid in the literature to root concentration. The only data on screening wheat accessions for root DIMBOA concentration were those published by Wu and co-workers for 17-day-old seedlings of 58 wheat accessions from the Australian Winter Cereals Collection (*29*, *30*). The range of concentration in their study remains in good agreement with our present data, although the age of plants was totally different.

In the present research significant differences ($\alpha = 0.05$) in DIMBOA concentration could be observed between plants at different development stages and between seasons (**Table 1**). No significant differences were observed between varieties and cultivation systems, yet our findings show that environmental influences on DIMBOA concentration can be higher than those from genetic background. This may question the possibilities of predicting in practice the concentration of this chemical in wheat plants. In general, the present findings show that

Table 2. Σ DIBOA and BOA in Wheat Roots at Two Stages of Development (Milligrams per Kilogram of Dry Matter)

	season 2002/2003		season 2003/2004	
variety	four-leaf stage	tillering	four-leaf stage	tillering
		Organic System		
Kobra	227.5 ± 11.6	202.3 ± 12.3	429.9 ± 27.1	173.0 ± 6.2
Roma	161.9 ± 7.2	141.6 ± 5.0	209.7 ± 11.1	108.3 ± 7.2
Korweta	87.0 ± 3.1	115.7 ± 9.6	205.6 ± 9.9	100.0 ± 8.1
Sukces	163.0 ± 6.4	137.4 ± 8.7	336.6 ± 12.3	122.8 ± 10.0
Zyta	208.7 ± 9.0	110.7 ± 5.3	232.5 ± 11.0	112.6 ± 9.8
Mewa	169.5 ± 5.8	144.1 ± 6.1	267.8 ± 15.6	118.9 ± 8.1
		Conventional System		
Kobra	176.0 ± 4.9	162.9 ± 6.3	373.8 ± 18.0	222.8 ± 16.4
Roma	156.6 ± 5.6	112.0 ± 4.0	229.7 ± 9.5	107.3 ± 9.6
Korweta	142.1 ± 2.7	90.2 ± 3.6	196.9 ± 9.1	71.6 ± 3.1
Sukces	177.7 ± 4.4	129.5 ± 2.2	264.9 ± 13.2	124.8 ± 4.2
Zyta	108.2 ± 3.8	95.8 ± 2.1	202.1 ± 10.2	134.8 ± 4.4
Mewa	204.8 ± 10.3	134.2 ± 2.8	297.5 ± 9.5	105.8 ± 3.5
		Old Wheat Varieties		
Wysokolitewka	117.7 ± 8.5	111.6 ± 3.0	229.9 ± 8.9	132.0 ± 3.9
Ostka Kazimierska	81.7 ± 3.6	151.2 ± 1.6	116.0 ± 6.1	105.6 ± 3.0
Banatka Kresowa	71.0 ± 0.9	107.5 ± 1.4	202.1 ± 11.6	113.6 ± 2.1
Magnatka Rogalinska	70.4 ± 1.1	87.6 ± 1.9	178.8 ± 7.8	129.1 ± 1.9
Tiroler Roter	77.5 ± 1.2	67.9 ± 0.5	218.3 ± 7.2	92.0 ± 1.8
Alefeldii	69.2 ± 0.6	197.5 ± 9.7	106.0 ± 4.2	199.8 ± 1.3
Spelt Droogendijk	61.3 ± 0.8	107.3 ± 3.5	105.5 ± 6.5	186.3 ± 1.6
Rottweiler	87.9 ± 0.8	122.4 ± 3.9	123.1 ± 4.6	139.4 ± 1.0
Dankowska Zachodnia	88.3 ± 1.1	114.2 ± 2.1	80.6 ± 3.2	28.1 ± 0.2
Blondynka	99.0 ± 0.9	76.8 ± 1.7	105.2 ± 5.9	61.6 ± 0.2
Kujawianka Wieclawicka	113.2 ± 2.0	83.1 ± 0.8	114.3 ± 1.9	83.3 ± 0.4

Table 3. Σ HMBOA, DIMBOA, and MBOA in Wheat Roots at Two Stages of Development (Milligrams per Kilogram of Dry Matter)

	season 2002/2003		season 2003/2004	
variety	four-leaf stage	tillering	four-leaf stage	tillering
		Organic System		
Kobra	381.6 ± 27.0	506.5 ± 21.2	346.4 ± 11.2	1218.2 ± 88.8
Roma	232.5 ± 19.0	365.5 ± 15.1	165.1 ± 5.1	739.2 ± 33.6
Korweta	214.6 ± 15.2	381.6 ± 12.4	363.7 ± 7.2	768.4 ± 25.6
Sukces	318.5 ± 17.3	590.9 ± 23.9	248.4 ± 3.1	791.9 ± 24.4
Zyta	342.3 ± 14.2	529.3 ± 22.2	312.5 ± 9.5	1275.3 ± 72.9
Mewa	403.1 ± 19.5	498.0 ± 20.8	436.2 ± 9.9	1121.8 ± 62.5
		Conventional System		
Kobra	365.0 ± 12.4	431.5 ± 17.7	566.3 ± 14.2	2125.9 ± 89.9
Roma	402.0 ± 18.6	443.0 ± 18.7	203.8 ± 7.8	1153.0 ± 56.1
Korweta	613.3 ± 28.8	511.0 ± 19.6	375.0 ± 12.0	1046.0 ± 34.6
Sukces	552.5 ± 16.2	398.8 ± 14.5	413.0 ± 16.5	1429.5 ± 58.2
Zyta	323.2 ± 13.3	450.1 ± 15.5	296.6 ± 8.4	1293.6 ± 53.1
Mewa	722.5 ± 24.1	469.9 ± 15.7	385.3 ± 11.0	975.0 ± 23.9
		Old Wheat Varieties		
Wysokolitewka	332.8 ± 14.2	827.7 ± 31.0	241.1 ± 11.6	790.7 ± 21.5
Ostka Kazimierska	275.9 ± 9.5	487.4 ± 15.2	109.7 ± 4.2	509.4 ± 22.6
Banatka Kresowa	278.6 ± 9.1	816.7 ± 28.1	376.3 ± 6.5	860.1 ± 26.4
Magnatka Rogalinska	319.3 ± 10.0	989.4 ± 26.4	307.4 ± 8.9	864.5 ± 27.1
Tiroler Roter	384.5 ± 13.4	656.4 ± 13.5	476.6 ± 15.0	1130.0 ± 31.0
Alefeldii	265.2 ± 14.7	1259.6 ± 85.0	331.9 ± 11.4	2046.3 ± 30.5
Spelt Droogendijk	319.9 ± 12.7	1147.4 ± 64.2	485.4 ± 12.0	1312.7 ± 12.1
Rottweiler	449.5 ± 19.6	811.8 ± 23.3	418.3 ± 13.6	955.6 ± 3.9
Dankowska Zachodnia	757.1 ± 30.6	798.8 ± 21.8	104.4 ± 3.9	327.8 ± 2.1
Blondynka	413.3 ± 14.9	797.2 ± 31.6	285.8 ± 4.8	1229.3 ± 61.2
Kujawianka Wieclawicka	715.0 ± 24.1	685.8 ± 27.6	368.6 ± 8.5	886.7 ± 21.7

benzoxazinoid concentration in wheat roots remains stable or even increases in later development stages as compared with the juvenile seedling stage. Besides, they prove that under field conditions the concentration of benzoxazinoids is comparable to that of plants grown indoors (*29*).

The concentration of benzoxazinoids in wheat roots can be of great interest due to their allelopathic function and the inhibitory activity against soilborne fungi such as *C. gramineum*, *G. graminis* var. *tritici*, and *F. culmorum*. As shown in previous research, hydroxamic acids are allelopathic to several weed species (*1*, *30*) and together with benzoic and cinnamic acids are concentrated predominantly in the wheat root system. They can be exuded to the surrounding soil, but exudation is accession-specific (*25*).

Previous studies, however, did not recognize all benzoxazinones present in roots and did not consider their degradation products, for example, benzoxazolinones and aminophenoxazines (*31*). The benzoxazines DIMBOA and DIBOA, which

Table 4. Multiple-Range Tests for Σ DIBOA + BOA and Wheat Accession Correlation (95% LSD)

wheat accession	LS mean	homogeneous groups
Dankowska Zachodnia	83.3	X
Blondynka	91.1	XX
Kujawianka Wieclawicka	104.0	XXX
Ostka Kazimierska	119.1	XXXX
Tiroler Roter Dinkel	119.4	XXXX
Spelt Droogendijk	120.6	XXXX
Magnatka Rogalinska	122.0	XXXXX
Rottweiler Fruehkorn	123.7	XXXXX
Korweta	126.1	XXX
Banatka Kresowa	129.1	XXXXX
Alefeldii	148.7	XXXXX
Zyta	150.7	__XXXX
Wysokolitewka Sztywnosloma	153.3	XXXXX
Roma	153.4	____XXX
Mewa	180.3	______XX
Sukces	182.1	________X
Kobra	246.0	_________X

persist for a short time in soil, had low allelopathic activity in wheat, lettuce, and cress bioassays (*32*). Their degradation products, benzoxazolinones BOA and MBOA, had an even lower allelopathic activity than the parent compounds. The further degradation products of MBOA, 7-methoxyphenoxazines AMPO and AAMPO, had the lowest allelopathic activity, but 2-aminophenoxazin-3-one (APO), the degradation product of BOA, was the most inhibitory compound. The same was true when these compounds were tested against soilborne wheat pathogens. Benzoxazines were active at a concentration of 1−3 mmol/L, whereas the ID_{50} values for 2-aminophenol, APO, and AAPO were in the range of 0.0025−0.005 mmol/L, which was similar to the effective doses of synthetic fungicides used to control wheat soilborne diseases (*33*).

The above facts clearly show that DIBOA and its degradation products may have a more important allelopathic and fungicidal function in wheat roots than DIMBOA and its microbial transformation products. Thus, we deemed it to be of interest to find out how these two groups of compounds are distributed over wheat accessions. The data are presented in **Tables 2** and **3**. In general the concentration of Σ DIBOA + BOA, the parent compounds for highly active AP, was 4−5 times lower than the concentration of Σ DIMBOA + MBOA + HMBOA. Significant differences ($\alpha = 0.05$) in Σ DIBOA + BOA could be found between wheat accessions, stages of plant development, and seasons but not between cultivation systems. It was interesting to note that old accessions were lower in Σ DIBOA + BOA concentration and that the new variety Kobra showed the highest concentration (**Table 4**). This may suggest that breeding of wheat for better agronomical traits resulted (not necessary intentionally) in an increase of Σ DIBOA + BOA in the roots. In the case of DIMBOA and related compounds there was a significant difference found between seasons and phases of development, but no significant difference was seen between varieties and growing systems.

The average concentration of Σ DIBOA + BOA in new varieties, in plants at four-leaf stage, from both organic (6 varieties) and conventional (6 varieties) systems, was nearly double the average concentration (11 accessions) found for the old varieties. It was interesting to observe that the highest differences occurred at the four-leaf stage, which usually sustains overwintering in temperate zones. Winter is the period when soilborne fungi (*Ggt*, *C. gramineum*) infect winter cereals through wounded roots; thus, the highest concentration of DIBOA group in this period can be crucial for natural plant defense systems. This hypothesis was partially supported by our field observations (data unpublished) that old accessions were more susceptible to *Ggt* and *C. gramineum* than the new varieties and that Kobra was the most resistant one. Thus, could the concentration of the DIBOA group benzoxazinones be meaningful in the defense of wheat plants against soilborne pathogens? We can make a simple assumption that for the concentration of 100 mg/kg of dry matter, and dry matter makes up 20% of fresh weight, the real concentration of compounds in cell solution is ∼25 mg/L (∼0.17 mmol/L). *C. gramineum* and *Ggt* infect roots of winter cereals and spread further through water-conducting vessels into stem and leaves. They enter the root system at the sites of root damage, for example, a break of root as a result of frosts. Such root damage creates good conditions for microbial transformation of DIBOA and BOA to APO. If we calculate that from 1 mol of DIBOA + BOA only 0.5 mol of APO is formed, the maximal possible concentration of APO in the cell solution can be at the level of 0.085 mmol/L. Total inhibition of *C. gramineum* and *Ggt* growth by APO occurred at the concentration of 0.02 mmol/L and 0.005 mmol/L, respectively (*33*). Thus, it seems reasonable to justify that the concentration of DIBOA and its degradation products is high enough to protect plant against these fungi.

LITERATURE CITED

(1) Barnes, J.; Putnam, A.; Burke, B.; Aasen, A. J. Isolation and characterization of allelochemicals in rye herbage. *Phytochemistry* **1987**, *26*, 1385−1390.

(2) Niemeyer, H. Hydroxamic acids (4-hydroxy-1,4-benzoxazin-3-ones) defence chemicals in the gramineae. *Phytochemistry* **1988**, *27*, 3349−3358.

(3) Niemeyer, H.; Pesel, E.; Copaja, S.; Bravo, H.; Franke, S.; Francke, W. Changes in hydroxamic acids levels of wheat plants induced by aphid feeding. *Phytochemistry* **1990**, *28*, 447−449.

(4) Nagano, T.; Otsuka, H.; Kohda, H.; Sato, T.; Yamasaki, K. Benzoxazinones of *Coix lachryma-jobi* var *ma-yuen*. *Phytochemistry* **1985**, *24*, 2959−2962.

(5) Wolf, R.; Spencer, G.; Plattner, R. Benzoxazolinone, 2,4-dihydroxy-1,4-beznoxazin-3-one, and its glucoside from *Acanthus mollis* seeds inhibit velvetleaf germination and growth. *J. Nat. Prod.* **1985**, *48*, 59−63.

(6) Özden, S.; Özden, T.; Attila, I.; Kuecuekislamoglu, M.; Okatan, A. Isolation and identification via high-performance liquid chromatography and thin-layer chromatography of benzoxazolinone precursors from *Consolida orientalis*. *J. Chromatogr.* **1992**, *609*, 402−406.

(7) Pratt, K.; Kumar, P.; Chilton, W. S. Cyclic hydroxamic acids in dicotyledonous plants. *Biochem. Syst. Ecol.* **1995**, *23*, 781−785.

(8) Sicker, D.; Hartenstein, H.; Kluge, M. Benzoxazinoid acetal glucosides and aglucones from plants: occurrence, isolation, synthesis, biological performance. *Phytomed.−Jena* **2000**, *7*, 75−76.

(9) Barria, B. N.; Copaja, S. V.; Niemeyer, H. M. Occurrence of DIBOA in wild *Hordeum* species and its relation to aphid resistance. *Phytochemistry* **1991**, *31*, 89−91.

(10) Tang, C.-S.; Chang, S. H.; Hoo, D.; Yanagihara, K. H. Gas chromatographic determination of 2(3)-benzoxazolinones from cereal plants. *Phytochemistry* **1975**, *14*, 2077−2079.

(11) Virtanen, A.; Hietala, P. Precursors of benzoxazolinone in rye plants. I. Precursor II, the aglycone. *Acta Chem. Scand.* **1960**, *14*, 499−502.

(12) Escobar, C. A.; Sicker, D.; Niemeyer, H. M. Evaluation of DIMBOA analogues as antifidants and antibiotics towards the aphid *Sitobion avεane* in artificial diets. *J. Chem. Ecol.* **1999**, *25*, 1543−1554.

(13) Sicker, D.; Frey, M.; Schulz, M.; Gierl, A. Role of natural benzoxazinones in survival strategies of plants. In *International Review of Cytology—A Survey of Cell Biology*; Jeong, K. W., Ed.; Academic Press: San Diego, CA, 2000; Vol. 198, pp 319−346.

(14) Barnes, J. P.; Putnam, A. R. Role of benzoxazinones in allelopathy by rye (*Secale cereale* L.) *J. Chem. Ecol.* **1987**, *13*, 889−906.

(15) Barnes, J. P.; Putnam, A. R.; Burke, B. Allelopathic activity of rye (*Secale cereale* L.). In *The Science of Allelopathy*; Putnam, A. R., Tang, C. S., Eds.; Willey Interscience: New York, 1986; pp 271−286.

(16) Hofman, J.; Hofmanova, O. 1,4-Benzoxazine derivatives in plants: absence of 2,4-dihydroxy-7-methoxy-2*H*1,4-benzoxazin-3(4*H*)-one from uninjured *Zea mays* plants. *Phytochemistry* **1971**, *10*, 1441−1444.

(17) Hamilton, R. H. Tolerance of several grass species to 2-chloro-*s*-triazine herbicides in relation to degradation and content of benzoxazinone derivatives. *J. Agric. Food Chem.* **1964**, *12*, 14−17.

(18) Copaja, S. V.; Nicol, D.; Wratten, S. D. Accumulation of hydroxamic acids during wheat germnation. *Phytochemistry* **1999**, *50*, 17−24.

(19) Zuòiga, G. E.; Massardo, F. Hydroxamic acid content in undifferentiated and differentiated tissues of wheat. *Phytochemistry* **1991**, *30*, 3281−3283.

(20) Argandoòa, W. H.; Niemeyer, H. M.; Corcurea, L. J. Effect of content and distribution of ydroxamic acids in wheat on infestation by *Schizphis graminum*. *Phytochemistry* **1981**, *20*, 673−676.

(21) Escobar, C. A.; Niemeyer, H. M. Potential of hydroxamic acids in breeding for aphid resistance in wheat. *Acta Agric. Scand.* **1993**, *43*, 163−167.

(22) Niemeyer, H. M. Hydroxamic acid content of *Triticum* species. *Euphytica* **1988**, *37*, 289−293.

(23) Copaja, S. V.; Niemyer, H. M.; Wratten, S. D. Hydroxamic aid levels in Chilean and British wheat seedlings. *Ann. Appl. Biol.* **1991**, *118*, 223−227.

(24) Nicol, D.; Copaja, S. V.; Wratten, S. D.; Niemeyer, H. M. A screen of worldwide wheat cultivars for hydroxamic acid levels and aphid antixenosis. *Ann. Appl. Biol.* **1992**, *121*, 11−18.

(25) Wu, H.; Haig, T.; Pratley, J.; Lemerle, D.; An, M. Distribution and exudation of allelochemicals in wheat *Triticum aestivum*. *J. Chem. Ecol.* **2000**, *26*, 2141−2154.

(26) Meier, U. *Growth Stages of Mono- and Dicotyledonous Plants*; BBCH Monography; Blackwell Wissenschaht-Verlag: Berlin, Germany, 2001; 622 pp.

(27) Fomsgaard, I. S.; Hansen, L. M.; Kudsk, P.; Mathiassen, S.; Benfenati, E.; Lo Piparo, E.; Blümel, S.; Coja, T.; Idinger, J.; Macías, F. A.; Chinchilla, D.; Marín, D.; Molinillo, J. M. G.; Oliveros-Bastidas, A.; Simonet, A. M.; Fritz, J.; Barceló, D.; Eljarrat, E.; Bonnington, L.; Guillamon, M.; Villagrasa, M.; Taberner, A.; Sakaliene, O.; Mogensen, B. B.; Krongaard, T.; Oleszek, W.; Kus, J.; Martyniuk, S.; Stochmal, A. *The Holistic Approach in Allelopathy Research*, Glasgow, Scotland, Nov 1−3, 2005; British Crop Protection Council: Surrey, U.K., 2005.

(28) Eljarrat, E.; Guillamón, M.; Seuma, J.; Mogensen, B. B.; Fomsgaard, I. S.; Olivero-Bastidas, A.; Macías, F. A.; Stochmal, A.; Oleszek, W.; Shakaliene, O.; Barceló, D. First European interlaboratory study of the analysis of benzoxazinone derivatives in plants by liquid chromatography. *J. Chromatogr. A* **2004**, *1047*, 69−76.

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

(30) Wu, H.; Pratley, J.; Lemerle, D.; Haig, T. Allelopathy in wheat (*Triticum aestivum*). *Ann. Appl. Biol.* **2001**, *139*, 1−9.

(31) Fomsgaard, I. S.; Mortensen, A. G.; Carlsen, S. C. K. Microbial transformation of benzoxazolinone and benzoxazinone allelochemicals—a review. *Chemosphere* **2004**, *54*, 1025−1038.

(32) Macías, F. A.; Castellano, D.; Oliveros-Bastidas, A.; Maring, D.; Simonet, A. M.; Molinillo, J. M. G.; Chinchilla, D.; Kudsk, P.; Mathiassen, S.; Taberner, A. Structure−activity relationship (SAR) studies of wheat allelochemicals. *Proceedings of the Second European Allelopathy Symposium*; Institute of Soil Science and Plant Cultivation Press Service: Pulawy, Poland, 2004; p 78.

(33) Martyniuk, S.; Stochmal, A.; Macías, F. A.; Marin, D.; Molinillo, J. M. G.; Oleszek, W. Effect of hydroxamic acids on wheat infesting fungi (*Gaeumannomyces graminis* var *tritici*, *Fusarium* culmorum, *Cephalosporium gramineum*). *Proceedings of the Second European Allelopathy Symposium*; Institute of Soil Science and Plant Cultivation Press Service: Pulawy, Poland, 2004; pp 85−87.

Received for review April 19, 2005. Revised manuscript received June 15, 2005. Accepted December 6, 2005. This work was performed as a part of the project "FATEALLCHEM", "Fate and Toxicity of Allelochemicals in Relation to Environment and Consumer" (http://www.fateallchem.dk), a project carried out with financial support from the Commission of the European Communities under the work program "Quality of Life", Contract QLK5-CT-2001-01967.

JF050899+