Weed Science 2012 60:360–365

Enhanced Exudation of DIMBOA and MBOA by Wheat Seedlings Alone and in Proximity to Wild Oat (*Avena fatua*) and Flixweed (*Descurainia sophia*)

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The allelochemicals 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and 6-methoxy-benzoxazolin-2-one (MBOA) in wheat are considered to have a role in plant defense against weeds. This study explored the effect of proximity to two weeds, wild oat and flixweed, on DIMBOA/MBOA production in wheat seedlings under hydroponic culture to identify whether the breeding of modern wheat varieties with higher concentrations of these compounds could ensure plant-mediated weed control. MBOA was detected and was noted to exert a significant response; its exudation by some wheat seedlings was significantly increased irrespective of whether the roots were in contact with or separate from those of the weeds. The weeds were a source of biotic stress to wheat when grown in proximity to it, and the stress resulted in production of higher levels of MBOA in wheat seedlings, although the concentration varied with the wheat cultivar. Therefore, the synthesis and exudation of DIMBOA/MBOA in wheat seedlings appears to be an active metabolic process influenced by the environment, particularly the presence of weeds.
Nomenclature: Flixweed, *Descurainia sophia* (L.) Webb. ex Prantl; wild oat, *Avena fatua* L.; wheat (*Triticum aestivum* L.).
Key words: DIMBOA/MBOA, enhanced exudation, wheat seedling, root, weed.

The chemical interactions between plants, plants and microbes, and plant secondary metabolites and pest wounds are induced by secondary metabolites (Dayan 2006), which are a natural treasure of biologically active chemicals, most of which have been well recognized as allelochemicals. Crop plants have the ability to produce and exude allelochemicals into their surroundings to suppress nearby weed growth (Wu et al. 2001c; Pickett et al. 2007; Vidotto et al. 2008). This phenomenon among crop plants as a biological means for integrated weed management (Putnam et al. 1983; Weston 1996; Wu et al. 1999) is known as allelopathy, which is identified as a category of active agents in the study on chemical ecology. The suppression of weeds varies with the type and number of allelochemicals present in different cultivars (Kong and Xu 2002). Because the use of these unstable allelochemicals as natural herbicides is rather limited (Barnes and Putnam 1987; Sicker et al. 2003), efforts have focused on allelopathy approaches in which degradation is compensated for by a continuous release.

Secondary metabolites produced by many cereals, such as maize (*Zea mays* L.), wheat, and rye (*Secale cereale* L.), have been the subject of numerous investigations as possible natural defense systems against pests, diseases, and weeds. Among cereals, benzoxazinoids have gotten the most attention as possible natural pesticides because of their broad-spectrum toxicity (Sicker and Schulz 2002). The most abundant chemical among the benzoxazinoid aglucones in wheat extracts is 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) (Nakagawa et al. 1995). DIMBOA has been studied extensively as the most important hydroxamic acid to understand the allelopathic phenomena in wheat and maize (Macias et al. 2004, 2005). DIMBOA is involved in the defense of wheat against various pests, and it is most notable for its allelopathic effects that inhibit the germination and growth of weeds associated with wheat (Niemeyer 2009). Perez (1990) found that DIMBOA, at a concentration of 0.7 mM, inhibited root growth of wild oat by 50%. When exuded and leached into the soil, DIMBOA is rapidly converted into 6-methoxy-benzoxazolin-2-one (MBOA), which is more resistant to degradation in soil (Macias et al. 2004). A wealth of information is available on the herbicidal potential and soil transformation of DIMBOA and MBOA (Coja et al. 2006; Etzerodt et al. 2008; Krogh et al. 2006; Macias et al. 2004; Mathiassen et al. 2006). Further research revealed that wheat accessions vary significantly in the production of DIMBOA and certain phenolic acids in shoots and roots (Wu et al. 2000b, 2001a,b).

The concentrations of allelochemicals in crops can potentially be increased by biotic and abiotic factors, particularly chemical and biotic forms of stress (Bi et al. 2000; Fang et al. 2009; Kong et al. 2004; Rizvi et al. 1992; Wu et al. 2000c). We have studied the effects of chemicals on DIMBOA concentration from root exudates and aerial parts of wheat seedlings and found that chemicals such as methyl jasmonate, methyl salicylate, and triadimefon can increase DIMBOA content in wheat seedlings under hydroponic culture (Zheng et al. 2008). However, there are no reports of whether growth of weeds in the vicinity of wheat leads to higher levels of synthesis and exudation of DIMBOA/MBOA in seedlings of certain wheat cultivars. The major objective of the study was to assess the potential of wild oat and flixweed to increase DIMBOA/MBOA production in wheat seedlings. Further, we compared their potential under two conditions: with and without direct contact between the two root systems (weed and crop). In addition, chemical aspects of the interaction were examined to further our understanding of the molecular mechanisms of allelopathy.

Materials and Methods

Chemicals and Materials. DIMBOA, used as a standard reference was isolated from maize seedlings by the method

DOI: 10.1614/WS-D-11-00119.1
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developed by Larsen and co-workers (Larsen and Christiansen 2000). Aboveground parts of maize seedlings grown in the dark were harvested 7 d after sowing, and DIMBOA was extracted from shoots that were homogenized in a blender with water. A filtrate obtained following pressing of the homogenate was purified by the addition of Amberlite XAD-7 (Anland Biotechnology Co., Ltd., Shanghai) nonionic polymeric as an adsorbent. The mixture was stirred for 1 h and refiltered. This filtrate, after vacuum distillation, was dissolved by methylene chloride and subsequently recrystallized by ice-cold methylene chloride and hexane to yield pale yellow amorphous crystals, which were composed of pure DIMBOA.

The MBOA standard was obtained from Alfa Aesar China (Tianjin) Co. Ultra-performance liquid chromatography (UPLC)-grade solvents, namely, methanol and formic acid (chromatography grade), were purchased from Honeywell International Inc. (Morristown, NJ). High performance liquid chromatography (HPLC)-grade water was obtained by purifying demineralized water in a Milli-Q Integral 3 water system (Millipore, Bedford, MA). The mobile-phase solvents were distilled and passed through a filter (pore size 0.22 μm) before being used.

Three cultivars of winter wheat (Lumai168, Nongda211, and Duokang1) were selected for the experiment. This selection was made on the basis of their commercial importance and popularity in the local wheat industry. Seeds of wild oat and flixweed with high germination ability were collected from Shandong Province prior to seed dispersal during the autumn of 2009. The seeds were dried under sunlight and then stored in sealed glass jars. All seeds were stored at room temperature and sterilized with 10% NaClO for 5 min and rinsed three times with distilled water before use. Before sowing, the seed viability of all cultivars was examined using germination percentage (> 98%). When the experiment was initiated, soil was collected from a field in Beijing (China), air-dried, mixed, and then sieved (2-mm pore size mesh) to remove plant tissues and other impurities. Soil was a silty loam with a pH of 6.94, organic matter content of 19.9 g kg^{-1}, and fertility status of available N, 101.9 mg kg^{-1}; available P, 17.0 mg kg^{-1}; and available K, 205.8 mg kg^{-1}.

Identification of DIMBOA. The pale yellow amorphous crystals had a melting point (mp) of 163 to 164 C; UV in absolute ethanol: λmax = 288 (shoulder), 262 nm (ε = 10,300); ^{1}H NMR in deuterated acetone (300 MHz), δ 7.25 (d, 1 H, J = 8.8 Hz), 6.68 (dd, 1 H, J = 8.8 Hz), 6.61(s, 1 H), 5.72 (s, 1 H), 3.57 (s, 3 H), 2.85 (br s, OH); HPLC/MS (ES+): *m/z* $(M+H)^+$ 211.95 (100), $(M+Na)^+$ 233.95 (48), $(M-OH)^+$ 193.92 (36), $(M-COOH)^+$ 165.93 (19). The mass spectrum of DIMBOA is depicted in Figure 1. These characteristics are in accordance with those previously reported for DIMBOA (Atkinson et al. 1991; Lyons et al. 1988; Woodward et al. 1978).

Experimental Design. Seeds of wheat and wild oat were sown uniformly in boxes (37 by 30 by 15 cm) filled with moist vermiculite and covered with a 1- to 2-cm-thick layer of vermiculite, and seeds of flixweed were sown in soil. All boxes were placed in a greenhouse, the ambient conditions were as follows: day/night temperature at 28/20 C for wheat and wild oat, 20/15 C for flixweed, and 80% relative humidity. Wheat

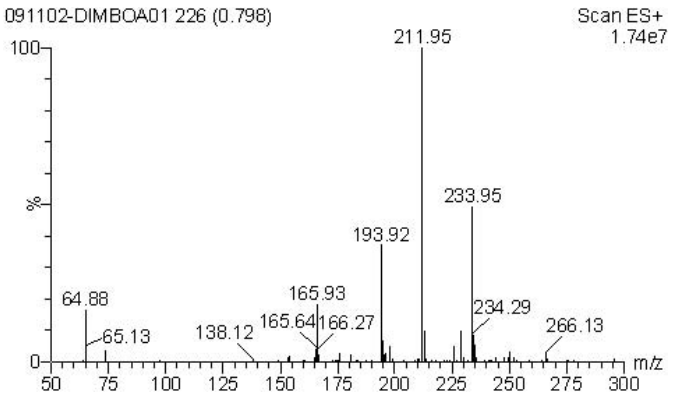

Figure 1. The mass spectrum of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA).

and weeds were irrigated daily with approximately 100 ml of water. Well-grown seedlings, 6 d old in the case of wheat and wild oat and 2 mo old in the case of flixweed, were uprooted. The two-leaf wheat and wild oat seedlings (Chen et al. 2010) and flixweed seedlings at the stage of eight to nine leaves were selected as experimental material. The roots were rinsed twice with distilled water and washed under running water to remove residual vermiculite and transferred to glass containers as described in the following sections. Experiments were repeated three times under identical conditions.

Experiment 1: Direct Contact between the Two Root Systems. A glass container (8 cm in diameter and 6.5 cm in height) filled with sterilized deionized-distilled water and covered, with three uniformly distributed holes (2 cm in diameter), were used for the experiment. The water was kept well aerated using an air compressor throughout the experiment. A bunch of wheat seedlings at the two-leaf stage was wrapped in sponge and inserted into one of the holes and another bunch, similarly wrapped, was inserted into a second hole; a bunch of weed seedlings (two-leaf stage of wild oat seedling or eight to nine leaves stage of flixweed), also wrapped in sponge, went into the third hole. Each bunch comprised 15 seedlings.

The outer sidewall of the glass container was wrapped up in tin-foil paper to ensure the roots remained in the dark. This experiment was conducted with five replications. A similar set-up, but with separate containers for wheat seedlings and weed seedlings, was maintained as a control. In this study, MBOA accumulated gradually in wheat roots and reached a stable maximum in 5 to 7 d. So in each case, the seedlings were maintained for 6 d (Zheng et al. 2010).

Experiment 2: No Direct Contact between the Two Root Systems. The experimental set-up used was the same as that described above, with the exception that the weed's root system was enclosed in a 4-cm-wide and 6-cm-deep bag of nylon mesh (300 mesh, or a pore size of 48 μm). The bag prevented any direct contact between the two root systems although other chemicals, nutrients, and microorganisms could circulate freely. This experiment was designed to study the potential ability of weeds to increase the production of DIMBOA in the seedlings of wheat.

Collection of DIMBOA/MBOA from the Aqueous Solution. The volume of water was maintained at 200 ml by

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Table 1. Retention time and *m/z* ion selected for quantification and confirmation of DIMBOA and MBOA.[a]

			UPLC-MS/MS (ESI)					
Compound	t_R (min)	Mol wt	Precursor ion (*m/z*)	Confirmation ion (*m/z*)	Quantification ion (*m/z*)	Dwell time (sec)	Cone voltage (V)	Collision Energy (V)
MBOA	2.07	165	166	110	95	0.1	35	23/20
DIMBOA	1.82	211.2	212.2	194	166	0.1	20	10/10

[a] Abbreviation: DIMBOA, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one; ESI, electrospray ionization; MBOA, 6-methoxy-benzoxazolin-2-one; t_R, retention time.

adding water in adequate quantities; the water was replaced and sampled every day. The collected samples (5 ml each) were dried in a rotational vacuum concentrator maintained at 35 C, redissolved in 1 ml of methanol, and filtered through syringe filters (pore size 0.22 μm) before being subjected to UPLC-tandem mass spectroscopy (MS/MS) analysis.

UPLC-MS/MS Analysis. The samples were directly analyzed in a Waters Acquity UPLC binary solvent manager equipped with a Waters Acquity UPLC BEH C_{18} Column (2.1 by 50 mm, 1.7 μm particle size; Milford, MA). Gradient UPLC elution was performed with LC-grade methanol as mobile phase A and 0.2% formic acid in ultra-pure water as mobile phase B. Mobile phase solvents were distilled and passed through a filter (pore size 0.22 μm) before use. The gradient elution process consisted of the following steps: 0 to 3.0 min, linear gradient 10 to 90% A; 3.0 to 3.1 min, linear gradient 90 to 10% A; return to the initial composition and equilibration for 1.9 min before the next injection. Separation and stabilization were completed in 5.0 min. The sample volume injected was maintained at 3 μl. The components were separated in the Acquity UPLC BEH C_{18} column at a flow rate of 0.3 ml min^{-1}.

Samples were analyzed using a Triple–Quadruple Mass Spectrometer (TQD; Waters Corp., Milford, USA) in the multiple reaction monitoring mode and the positive electrospray ionization mode. The capillary voltage was 3.0 kV and the extractor voltage was 35 V; the source temperature was 120 C and the desolvation temperature was 350 C; and the flow rate of desolvation gas was 600 L h^{-1} and of cone gas was 50 L h^{-1}. The flow rate of the collision gas, that is, high-purity argon, was maintained at 0.15 ml min^{-1}. MS/MS parameters were optimized in direct flow-injection mode. The precursor and the corresponding product ions for multiple reaction monitoring detection are listed in Table 1, together with other optimal mass spectrometric parameters. Under the above conditions, the mass detector was operated in the multiple ions monitoring mode to acquire the most abundant LC-MS/MS adducts for each active ingredient. Chromatograms of standard samples obtained under the above conditions are shown in Figure 2. From Figure 2, it can be seen that there was no interference at the retention times (t_R) of the compounds of interest. The t_R of DIMBOA was approximately 1.82 min and that of MBOA was approximately 2.07 min. Electrospray ionization fragmentation patterns and t_R were the criteria used to identify the compounds, and the corresponding values of the standard mixtures were used for comparison.

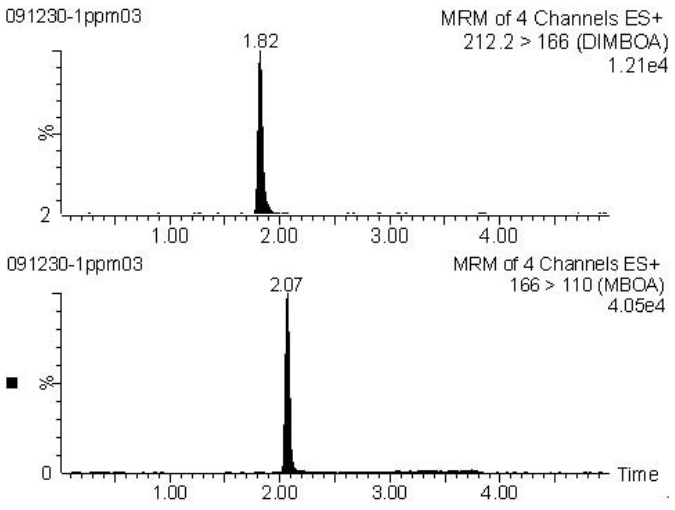

Figure 2. Chromatograms of a standard of 2,4-dihydroxy-7-methoxy-1, 4-benzoxazin-3-one (DIMBOA) and 6-methoxy-benzoxazolin-2-one (MBOA).

Validation Method. Standard stock solutions of DIMBOA and MBOA (100 mg L^{-1}) were prepared in methanol, and the standard solutions required for constructing a calibration graph (0.05, 0.1, 0.5, 1, 5, and 10 mg L^{-1}) were prepared from the stock solutions by serial dilution with methanol. All solutions were stored in a refrigerator at 4 C. Pure solvent standards based on methanol-calibration curves of graphs were generated by plotting the peak area against the concentration (0.05 to 10 mg L^{-1}) of the component. Equations for the standard curve were as follows: $y = 802.88x + 37.50$ ($R^2 = 0.9945$) for DIMBOA; and $y = 3{,}789.20x - 207.92$ ($R^2 = 0.9967$) for MBOA.

Statistical Analysis. Total concentrations of MBOA between wheat seedling alone, wild oat alone, and wheat seedling coexisting with weed (wild oat or flixweed) in the 6 d after transfer to the glass containers were sources of variation. These data are presented as mean ± standard deviation (SD) of three independent experiments for each replication. The increase in the levels of DIMBOA/MBOA due to the presence of weeds was ascertained using analysis of variance (ANOVA), and statistical significance specified at $P < 0.05$. All data were analyzed using the Statistical Package for Social Sciences (SPSS; version 17.0 for Windows, Bizinsight Information Technology Co., Ltd., Beijing.).

Results and Discussion

MBOA was detected in aqueous extracts of the three wheat cultivars and wild oat but not in the aqueous extract of flixweed. However, because of significant genetic variation, MBOA concentrations varied markedly with the cultivar. Lumai168 and Duokang1 plants exuded considerable amounts of MBOA from their roots (Figures 3 and 4).

When the root systems of wheat and the weed were in direct contact, the concentration of MBOA was always higher than that in the control plants for both weeds. The average concentration in the range 120.7 to 172.5 mg L^{-1} with

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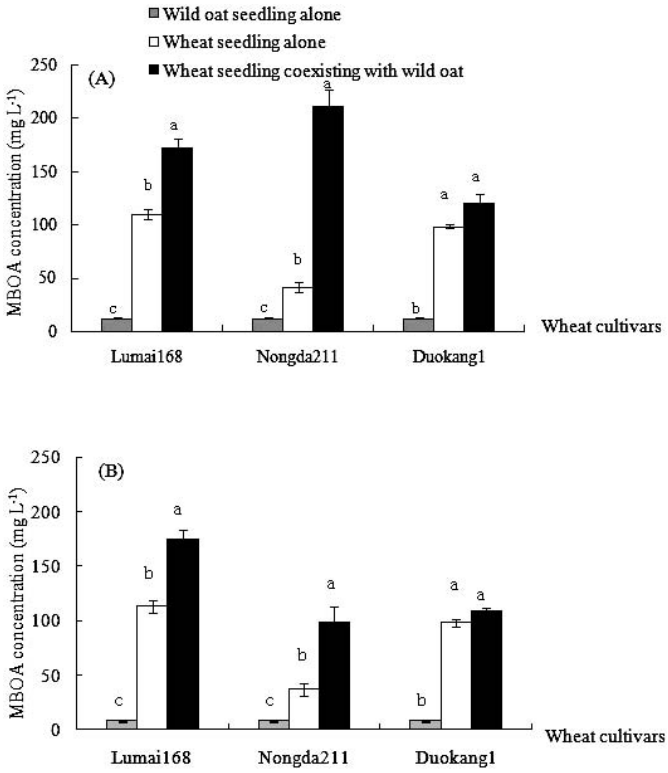

Figure 3. Response of 6-methoxy-benzoxazolin-2-one (MBOA) production in aqueous solutions for each wheat seedling cultivar (30 plants/6 d) tested associated with wild oat as a biological inducing factor. (A) Direct contact between the two root systems. (B) No direct contact between the two root systems. Vertical bars represent SD. Columns with the same letters are not significantly different at $P < 0.05$.

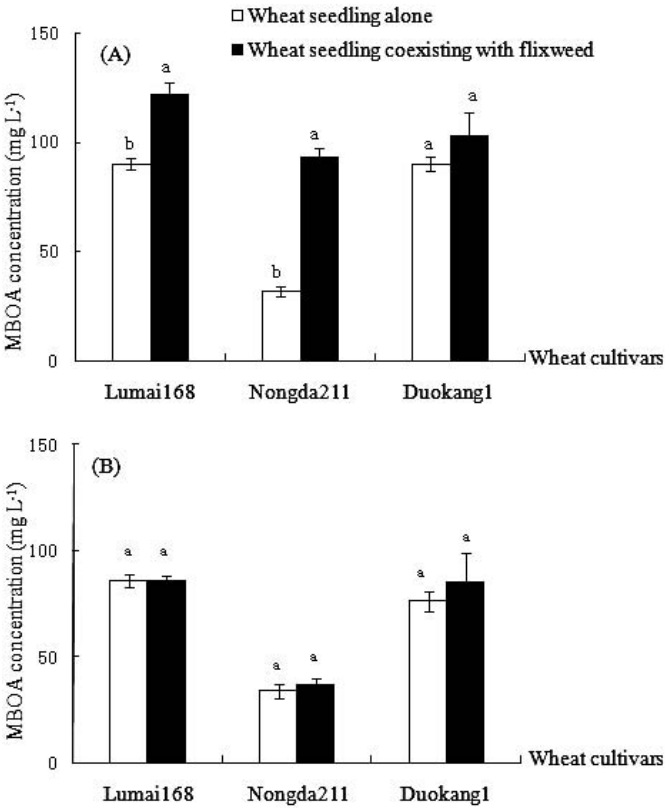

Figure 4. Response of 6-methoxy-benzoxazolin-2-one (MBOA) production in aqueous solutions for each wheat seedling cultivar (30 plants/6 d) tested associated with flixweed as a biological inducing factor. (A) Direct contact between the two root systems. (B) No direct contact between the two root systems. Vertical bars represent SD. Columns with the same letters are not significantly different at $P < 0.05$.

relative standard deviations (RSDs) in the range of 7.41 to 14.93% for all five replications when the weed was wild oat and 93.0 to 121.8 mg L^{-1} with RSDs in the range of 3.84 to 10.94% for all five replications when the weed was flixweed. The concentrations of MBOA exuded by the Lumai168, Nongda211, and Duokang1 cultivars were, respectively, 1.4, 4.0, and 1.1 times those in control plants (Figure 3A) when the weed was wild oat; the corresponding values for flixweed were 1.4, 2.9, and 1.1 (Figure 4A).

When the two root systems were not in direct contact, wild oat alone had a significant effect on the wheat seedlings (Figure 3B), their MBOA concentration ranging from 97.89 to 174.83 mg L^{-1} with RSDs in the range of 3.52 to 10.49% for all five replications. MBOA concentrations were 1.4, 2.2, and 1.0 times those observed in the control plants in Lumai168, Nongda211, and Duokang1 cultivars, respectively. Proximity to flixweed did not have a significant effect on the concentration of MBOA in the wheat seedlings (Figure 4B). Further, MBOA concentration in the exudate from wheat roots varied with the weed: proximity to wild oat led to greater concentrations of MBOA than proximity to flixweed (Figures 3 and 4).

The presence of allelochemicals in plant tissues does not necessarily indicate that these compounds are released into the growth environment to affect neighboring plants. In wheat seedlings, allelopathic effects are evident only when allelochemicals in the shoots and roots are eventually excreted through living and intact roots into the growth environment. Phenolic acids present in shoots and roots have been shown to be exuded by young wheat seedlings through their roots (Zheng et al. 2008). DIMBOA was not detected in aqueous extracts of the wheat cultivars tested and, although the DIMBOA released from wheat roots could be detected in root exudates and agar media (Wu et al. 2000a, 2001b), once it was released into aqueous solution or soil it decomposed rapidly into MBOA (Macias et al. 2004; Michael et al. 1978). We found that, if the hydroponic solution in which the seedlings had been growing was sampled and detected immediately without evapotranspiration, UPLC-MS/MS could simultaneously detect both DIMBOA and MBOA, although the DIMBOA concentration was lower and significantly different. For higher sensitivity and more accurate data, the aqueous solution was concentrated and tested. The rate of decomposition of DIMBOA was first studied in a bacterial growth medium (pH 6.75), and the half-life of DIMBOA at three different concentrations was determined using first-order kinetics. At 28 C, half-lives were 5.3, 5.1, and 5 h when the initial concentrations of DIMBOA were 0.1, 0.05, and 0.025 mM, respectively (Michael et al. 1978). Therefore, it was MBOA rather than DIMBOA that was assayed in this study after the exudates from roots of wheat seedlings had been vacuum-dried.

The present study supports the idea that exudation of DIMBOA is an active metabolic process. Data generated in this study are consistent with those reported from several other studies that have shown that the production of allelochemicals is stimulated by the presence of competing weeds (Dayan 2006; Kong et al. 2004, 2006). Further, these

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results are consistent with the report by Chen et al. (2010) that living wheat seedlings could detect the presence of certain weeds and respond by exuding increased amounts of MBOA into the rhizosphere soil. DIMBOA synthesis in wheat seedlings was significantly increased by the presence of two weeds even when the two root systems were not in direct contact. The reason for this effect could be attributed to microbial interactions in the rhizosphere. Further, it is possible that wheat seedlings, like rice (*Oryza sativa* L.) (Kong et al. 2006), produce biological signals stimulating the synthesis and release of DIMBOA. However, detection and identification of a signaling process in wheat remains obscure. Such an induction effect is more significant when the two root systems are in direct contact than when they are separated, possibly because the direct contact leads to greater microbial interaction, which results in the production of a chemical that induces DIMBOA synthesis and, in turn, in wheat seedlings releasing greater amounts of DIMBOA/MBOA into the growth medium.

Earlier research work has shown that strongly allelopathic wheat accessions, such as Tasman, exuded higher levels of allelochemicals than weakly allelopathic accessions, such as HY-65 (Wu et al. 2000a). Mattice et al. (1998) found that rhizosphere soil around allelopathic rice accessions contained higher levels of allelochemicals than those around nonallelopathic accessions. Our study corroborates these results in that the average levels of MBOA exuded by seedlings of the three cultivars were significantly different, indicating a strong genetic basis to the difference in the quantities of DIMBOA/MBOA secreted by different wheat accessions. Exudation of allelochemicals by living plants is one of the important processes necessary for crop allelopathy to occur. Some accessions produced high levels of DIMBOA, while others had no detectable DIMBOA in shoots or roots (Wu et al. 2001b). The substantial genetic variation of DIMBOA production in wheat germplasm implies that it may be possible to manipulate DIMBOA levels by means of modern biotechnology, thereby reducing the dependence on synthetic pesticides. Weeds may not be able to grow fast and allelochemicals may not work as rapidly as common chemical inducers, thus extending the duration before their effects are obvious. However, the role of weeds as a type of adverse factor that stimulates the production of MBOA cannot be excluded. At the same time, it must be admitted that the role of potential signaling, which increased the levels of MBOA in exudates from roots of wheat, remains to be resolved and awaits clear-cut detection and identification.

Genetic enhancement of the production and exudation of DIMBOA/MBOA has implications for future weed management. Further research is under way to investigate the close association between the allelopathy shown by wheat seedlings and DIMBOA. Chemical analysis coupled with DNA technology will facilitate the identification of genetic markers conferring the allelopathic trait, and our study provides theoretical and technical support to the task of breeding wheat cultivars resistant to weeds.

Acknowledgments

This work was supported by National Natural Science Foundation of China (30900951), Public service sector R & D Project (200903033), and National Key Technology R & D Program (2009BADB7B03).

Literature Cited

Atkinson, J., P. Morand, J. T. Arnason, H. M. Niemeyer, and H. R. Bravo. 1991. Analogues of the cyclic hydroxamic acid 2,4-dihydroxy-7-methoxy-2*H*-1, 4-benzoxazin-3-one: decomposition to benzoxazolinones and reaction with β-mercaptoethanol. J. Org. Chem. 56:1788–1800.

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

Bi, H. H., R. S. Zeng, L. M. Su, M. An, and S. M. Luo. 2007. Rice allelopathy induced by methyl jasmonate and methyl salicylate. J. Chem. Ecol. 33:1089–1103.

Chen, K., Y. Zheng, C. Kong, S. Zhang, J. Li, and X. Liu. 2010. 2, 4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and 6-methoxy-benzoxazolin-2 -one (MBOA) levels in the wheat rhizosphere and their effect on the soil microbial community structure. J. Agric. Food Chem. 58:12710–12716.

Coja, T., J. Idinger, and S. Blumel. 2006. Influence of the soil composition on the effects of benzoxazinoid allelochemicals on two soil nontarget organisms. J. Agric. Food Chem. 54:1093–1098.

Dayan, F. E. 2006. Factors modulating the levels of the allelochemical sorgoleone in *Sorghum bicolor*. Planta 224:339–346.

Etzerodt, T., A. G. Mortensen, and I. S. Fomsgaard. 2008. Transformation kinetics of 6-methoxybenzoxazolin-2-one in soil. J. Environ. Sci. Health B 43:1–7.

Fang, C. X., J. Xiong, L. Qiu, H. B. Wang, and B. Q. Song. 2009. Analysis of gene expressions associated with increased allelopathy in rice (*Oryza sativa* L.) induced by exogenous salicylic acid. Plant Growth Regul. 57:163–172.

Kong, C. H., H. B. Li, F. Hu, X. H. Xu, and P. Wang. 2006. Allelochemicals released by rice roots and residues in soil. Plant Soil 288:47–56.

Kong, C. H. and X. H. Xu. 2002. Allelopathic potential and chemical constituents of volatiles from *Ageratum conyzoides* under stress. J. Chem. Ecol. 28:1173–1182.

Kong, C. H., X. H. Xu, B. Zhou, F. Hu, C. X. Zhang, and M. X. Zhang. 2004. Two compounds from allelopathic rice accession and their inhibitory activity on weeds and fungal pathogens. Phytochemistry 65:1123–1128.

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

Larsen, E. and L. P. Christensen. 2000. Simple method for large scale isolation of the cyclic arylhydroxamic acid DIMBOA from maize (*Zea mays* L.). J. Agric. Food Chem. 48:2556–2558.

Lyons, P. C., J. D. Hipskind, K. V. Wood, and R. L. Nicholson. 1988. Separation and quantification of cyclic hydroxamic acids and related compounds by high-pressure liquid chromatography. J. Agric. Food Chem. 36:57–60.

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

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

Mathiassen, S. K., P. Kudsk, and B. B. Mogensen. 2006. Herbicidal effects of soil-incorporated wheat. J. Agric. Food Chem. 54:1058–1063.

Mattice, R., T. Lavy, B. Skulman, and R. Dilday. 1998. Search for allelochemicals in rice that control ducksalad. Pages 81–98 *in* M. Olofsdotter, ed. Allelopathy in Rice. Los Banos, Philippines: IRRI.

Michael, D. W., J. C. Luis, P. H. John, and D. U. Christed. 1978. Decomposition of 2,4-dihydroxy-7-methoxy-2H-l,4-benzoxazin3(4H)-one in aqueous solutions. Plant Physiol. 61:796–802.

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

Niemeyer, H. M. 2009. Hydroxamic acids derived from 2-hydroxy-2H-1,4-benzoxazin-3(4H)-one: key defense chemicals of cereals. J. Agric. Food Chem. 57:1677–1696.

Perez, F. J. 1990. Allelopathic effect of hydroxamic acids from cereals on *Avena sativa* and *A. fatua.* Phytochemistry 29:773–776.

Pickett, J. A., M. A. Birkett, M.C.B. Moraes, T.J.A. Bruce, and K. Chamberlain. 2007. Cis-Jasmone as allelopathic agent in inducing plant defence. Allelopathy J. 19:109–117.

Putnam, A. R., J. Defrank, and J. P. Barnes. 1983. Exploitation of allelopathy for weed control in annual and perennial cropping systems. J. Chem. Ecol. 9:1001–1011.

Rizvi, S.J.H., H. Haque, V. K. Singh, and V. Rizvi. 1992. A discipline called allelopathy. Pages 1–38 *in* S.J.H. Rizvi and V. Rizvi, eds. Allelopathy: Basic and Applied Aspects. London: Chapman and Hall.

Sicker, D., H. Hao, and M. Schulz. 2003. Benzoxazolin-2(3H)-oness generation, effects and detoxification in the competition among plants. Pages 77–102 *in* F. A. Macias, J.C.G. Galindo, J.M.G. Molinillo, and H. G. Cutler, eds. Allelopathy: Chemistry and Mode of Action of Allelochemicals. Boca Raton, FL: CRC Press.

Sicker, D. and M. Schulz. 2002. Benzoxazinones in plants: occurrence, synthetic access, and biological activity. Pages 185–232 *in* U. A. Atta, ed. Studies in Natural Products Chemistry. Amsterdam: Elsevier.

Vidotto, F., F. Tesio, and A. Ferrero. 2008. Allelopathic effects of *Helianthus tuberosus* L. on germination and seedling growth of several crops and weeds. Biol. Agric. Hortic. 26:55–68.

Weston, L. A. 1996. Utilization of allelopathy for weed management in agroecosystems. Agron. J. 88:860–866.

Woodward, M. D., L. J. Corcuera, J. P. Helgeson, and C. D. Upper. 1978. Decomposition of 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one in aqueous solutions. Plant Physiol. 61:796–802.

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

Wu, H., T. Haig, J. Pratley, D. Lemerle, and M. An. 2000b. Allelochemicals in wheat (*Triticum aestivum* L.): variation of phenolic acids in root tissues. J. Agric. Food Chem. 48:5321–5325.

Wu, H., T. Haig, J. Pratley, D. Lemerle, and M. An. 2001a. Allelochemicals in wheat (*Triticum aestivum* L.): variation of phenolic acids in shoot tissues. J. Chem. Ecol. 27:125–135.

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

Wu, H., J. Pratley, D. Lemerle, and T. Haig. 1999. Crop cultivars with allelopathic capability. Weed Res. 39:171–180.

Wu, H., J. Pratley, D. Lemerle, and T. Haig. 2000c. Evaluation of seedling allelopathy in 453 wheat (*Triticum aestivum*) accessions by Equal-Compartment-Agar-Method. Aust. J. Agric. Res. 51:937–944.

Zheng, Y., X. Liu, F. Dong, J. Li, Y. Gong, and G. Zhu. 2010. Biological induction of DIMBOA in wheat seedlings by weeds. Allelopathy J. 25:433–440.

Zheng, Y., Y. Zhao, X. Liu, J. Yao, and F. Dong. 2008. Chemical inducement of 2,4-dihyroxy-7-methoxy-1, 4-benzoxazin-3-one (DIMBOA) in wheat seedlings. Allelopathy J. 21:263–271.

Received July 17, 2011, and approved March 5, 2012.