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Herbicidal Effects of Soil-Incorporated Wheat

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The hydroxamic acid 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and the benzoxazolinones benzoxazolin-2-one (BOA) and 6-methoxybenzoxazolin-2-one (MBOA) have been identified as important allelochemicals in wheat. This study examines the possibility of exploiting the allelopathic properties of wheat as a weed control strategy by cultivating wheat as a precrop and incorporating plant residues into the soil before the next crop is sown. Different wheat varieties were cultivated in field plots during two seasons in both conventional and organic farming systems. Plants were sampled at various growth stages, and their contents of DIMBOA, MBOA, and BOA were determined by chemical analyses. The wheat samples were incorporated into soil, and the effect on germination and growth of 12 different weed species was examined in pot experiments under controlled conditions. In some cases significant effects were obtained, but the results were inconsistent and the effects were not correlated to the content of DIMBOA, MBOA, and BOA in the incorporated wheat plants. ED_{50} doses of the pure compounds were estimated in dose−response experiments in Petri dishes, and these turned out to be much higher than the predicted maximum concentrations of DIMBOA, MBOA, and BOA in the soil water following incorporation. The study shows that a prerequisite for exploiting the incorporation of wheat residues as a weed control strategy is the development of wheat varieties with an increased content of allelochemicals.

KEYWORDS: Wheat; herbicidal effect; DIMBOA; MBOA; BOA; allelopathy; soil incorporation; hydroxamic acid

INTRODUCTION

In recent years there has been an increasing focus on the prospects of exploiting allelopathy as an alternative strategy for controlling weeds, in particular, but also insects and diseases (*1*, *2*). Weeds can be controlled by allelochemicals either by growing a crop with the ability to exude allelochemicals or by incorporating plant residues with a high content of allelochemicals into the soil. The interest in allelopathy has been especially pronounced in organic farming systems and in conservation tillage systems.

Several groups of allelochemicals have been identified in wheat. These include phenolic acids (*3*, *4*), benzoxazinones and their metabolites (*5*−*8*), and short-chain fatty acids (*9*). The most studied of these compounds are the hydroxamic acids 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA), the benzoxazolinones 6-methoxybenzoxazolin-2-one (MBOA) and benzoxazolin-2-one (BOA), and the lactams 2-hydroxy-7-methoxy-1,4-benzoxazin-3(2*H*)-one (HMBOA) and 2-hydroxy-1,4-benzoxazin-3(2*H*)-one (HBOA).

DIMBOA is the most abundant hydroxamic acid in wheat, and several studies have shown that the content varies between wheat varieties (*5*, *10*, *11*). DIMBOA is present in shoots, leaves, and roots. Wu et al. found a higher content of DIMBOA in roots compared with shoots of young seedlings, and root exudation was detected in some varieties (*12*). In other studies a higher content of DIMBOA was found in foliage than in roots (*13*, *14*). Several external factors such as the amount of nutrients in the soil, temperature, precipitation, radiation, stress, and the use of synthetic pesticides may influence the content of allelochemicals (*15*).

The hydroxamic acid DIMBOA is chemically unstable in aqueous solutions and is rapidly decomposed to related benzoxazolinones. The half-life of DIMBOA is ≤1 day. In water and soil it is transformed to MBOA (*16*−*18*), which has a half-life of 4−6 days. MBOA is further transformed to 2-amino-7-methoxy-3*H*-phenoxazin-3-one (AMPO), which is stable for several months (*16*). The degradation of BOA is dependent on concentration (*19*, *20*). Understrup et al. found a half-life of BOA of 0.6 days at a concentration of 3 nmol of BOA/g of soil, whereas at 3000 nmol/g of soil the half-life was up to 31 days (*19*). At low concentrations of BOA, Gents et al. found only one transformation product, whereas several metabolites were found in an experiment with high concentrations of BOA

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Table 1. Growth Stages and Time of Year for Sampling in Field Plots and Amount of Plant Material Incorporated in Each Pot in the 2 Years

	2001–2002				2002–2003			
growth stage (BBCH)	month	organic	conventional	plant material (g of fresh wt)	month	organic	conventional	plant material (g of fresh wt)
9–10	10	X	X	0.4	10	X	X	0.4
12	11	X		0.8	10	X	X	0.8
21	03	X		1.6	12	X	X	0.8
31					05	X	X	1.6

(*20*). One among these was 2-amino-3*H*-phenoxazin-3-one (APO), which is much more phytotoxic than BOA (*21*, *22*).

DIMBOA and MBOA inhibit seed germination of *Avena fatua* (*23*), and one study concluded that MBOA was more potent than its precursor DIMBOA and inhibited germination, radicle, and hypocotyl length of *Trifolium incarnatum* and *Ipomoea hereracea* (*24*). In contrast, DIMBOA was recently found to be more phytotoxic to five standard target species than MBOA and BOA (*22*).

Wheat allelopathy is genetically inheritable, and a multigenic model has been proposed. Genetic transfer of allelopathic potential and development of allelopathic cultivars for weed control have been reviewed recently (*25*).

Most studies in wheat allelopathy have been made in laboratory tests in Petri dishes using water extract from plants or wheat soil, or they have been carried out in field experiments by studying the effect of wheat residues on weed emergence and growth in the subsequent crop. In the first case the conditions are far from being natural, with no interference with soil factors, and the transformation of the active compounds in extracts may be different from the transformation under field conditions. In field experiments, on the other hand, many factors may vary at the same time, influencing the efficacy of allelochemicals and their tranformation and making it difficult to elucidate possible allelopathic effects. Efficacy studies have seldom been accompanied by quantitative chemical analysis of allelopathic compounds in the extracts.

The aim of this study was to determine if the effect of soil incorporation of wheat on weed germination and weed growth was correlated to the content of DIMBOA, MBOA, and BOA in the wheat samples, simulating natural conditions as closely as possible. The research activities included (1) estimation of the potential activity of pure compounds of DIMBOA, MBOA, and BOA in dose−response experiments in Petri dishes without interference of external factors; (2) sampling of wheat with varying contents of DIMBOA, MBOA, and BOA, which was obtained by sampling plants of different varieties and at different growth stages from field plots in two farming systems (the contents of DIMBOA, MBOA, and BOA were quantified by chemical analyses); and (3) testing of the efficacy of soil-incorporated wheat samples on weed germination and weed growth in pot experiments.

MATERIALS AND METHODS

Winter wheat varieties were grown in field plots in both organic and conventional farming systems for two years. The farming systems were established in fields where soil texture and cultivation history differed, as the organic fields were located in an area that had been cultivated organically for more than 10 years. In 2001−2002, six varieties were included in the experiment (cv. Astron, Stakado, Ritmo, Portal, Bill, and Solist) with two replicate plots. Part of the field experiment was repeated in 2002−2003 including only three of the wheat varieties (cv. Astron, Stakado, and Ritmo) in four replicate plots. In both years, a completely randomized design was used. All fields were located at Research Centre Flakkebjerg. Plant samples were collected from the field plots at different growth stages as shown in **Table 1**. Subsamples were taken for quantification of the content of hydroxamic acids and benzoxazolinones by chemical analyses (*14*) and for studying the herbicidal effect (germination and growth) of incorporating wheat plants into the soil in pot experiments.

The herbicidal effect was examined in pot experiments in a glasshouse. The fresh plant material was cut into 0.5 cm long pieces and mixed carefully with 300 g (dry weight) of a sandy loam soil (1.9% humus, 9% clay, 30% silt, 61% sand, pH 6.3). The soil was sampled in noncultivated plots in the organic field in 2001 and used for all pot experiments in the two years. The amount of incorporated plant material at the different growth stages (**Table 1**) was equal to ∼3 times the amount that would have been incorporated in the field if the wheat crop were mulched into the upper 5 cm of soil at the specific growth stages. Dry soil without incorporation of plant material was used as control. The soil was filled into 9 × 9 × 9 cm pots.

The effect of soil incorporation was examined on the following 12 common weed species representing broad-leaved as well as grass weed species: *Echinochloa crus-galli* L. (Beauv.), *Setaria viridis* L. (Beauv.), *Poa annua* L., *Apera spica-venti* L., *Alopecurus myosuroides* (Huds.), *Stellaria media* L., *Abutilon theophrastis* (Medik), *Galium aparine* L., *Tripleurospermum inodorum* (Mérat) Laínz, *Chenopodium album* L., *Papaver rhoeas* L., and *Amaranthus retroflexus* L.

In 2001−2002, two replicate pots of each weed species were prepared with plant material from each field plot. In 2002−2003, three replicates of each weed species were prepared for each variety and farming system. Nine seeds of each weed species were sown per pot. The pots were placed on a gravimetric watering table in a heated glasshouse (minimum temperature, 12 °C) and subirrigated several times daily. Supplemental light was applied to extend the day length to 16 h.

Four weeks after sowing, the number of germinated plants and fresh and dry weight of the plants were recorded. The data were analyzed using PROC GLM and the least-squares means test in SAS.

The herbicidal effects on selected weed species of pure compounds of DIMBOA, MBOA, BOA, and APO were assessed in Petri dish experiments. DIMBOA and APO were provided by Dr. Francisco Macías, Universidad de Cádiz, whereas the other compounds were purchased commercially (MBOA from Lancaster Chemicals, BOA from ACROS Organics). Stock solutions were prepared in methanol, and six doses were applied to Petri dishes containing two pieces of filter paper. The methanol was allowed to evaporate, and deionized water was subsequently added to the Petri dishes. The day of germination was recorded for each seed, and the radicle length was measured when the experiments were terminated at 5−7 days after initiation. The results were expressed as average radicle growth per day following germination, and ED_{50} doses were estimated using a log−logistic dose−response model (*26*):

$$U_i = \frac{D - C}{1 + \exp[2b_i(\log(ED_{50i}) - \log(z))]} + C \qquad (1)$$

In eq 1, U is the radicle growth, z is the dose, D and C are the upper and lower asymptotes at zero and very high doses, ED_{50} is the dose resulting in a 50% reduction in radicle growth, and b is the slope around ED_{50i}. The assumption that logistic dose−response curves could be fitted to the data was assessed by a test for lack of fit comparing the residual sum of squares of an analysis of variance and the nonlinear regression.

Table 2. Number of Germinated Plants per Pot in Control Pots (No Wheat Incorporated)[a]

	2001–2002			2002–2003			
weed species	BBCH 9–10	BBCH 12	BBCH 21	BBCH 9–10	BBCH 12	BBCH 21	BBCH 31
E. crus-galli	0.0 (0.0)	0.0 (0.0)	2.5 (2.4)	7.4 (1.3)	6.0 (1.1)	6.3 (1.7)	8.0 (0.6)
S. viridis	0.0 (0.0)	0.0 (0.0)	1.0 (0.0)	5.8 (1.0)	6.0 (1.3)	6.8 (1.2)	7.8 (1.1)
P. annua	7.5 (1.9)	5.8 (2.9)	8.8 (0.5)	8.3 (0.5)	8.0 (0.6)	7.3 (1.6)	5.3 (3.8)
A. spica-venti	6.5 (0.6)	7.0 (1.4)	7.3 (0.6)	5.7 (2.0)	6.5 (1.0)	7.0 (1.3)	2.8 (2.1)
A. myosuroides	1.5 (0.6)	2.8 (0.5)	7.5 (1.3)	6.2 (1.5)	5.8 (1.6)	6.0 (2.1)	7.3 (1.9)
S. media	5.5 (1.0)	5.8 (1.3)	2.8 (1.0)	4.4 (2.6)	4.8 (0.8)	0.0 (0.0)	1.8 (0.8)
A. theoprastis	0.3 (0.5)	1.0 (1.4)	1.5 (1.0)	4.8 (0.8)	5.7 (1.0)	5.0 (1.9)	0.0 (0.0)
G. aparine	2.5 (1.3)	6.3 (4.2)	0.0 (0.0)	6.3 (1.5)	5.2 (1.6)	1.8 (1.1)	0.0 (0.0)
T. inodorum	2.5 (1.7)	6.0 (1.2)	1.3 (0.6)	4.8 (2.6)	5.0 (2.3)	3.3 (1.5)	3.8 (2.8)
C. album	3.5 (3.3)	5.0 (2.7)	5.0 (1.6)	3.8 (2.3)	4.7 (2.3)	3.7 (1.5)	1.5 (0.5)
P. rhoeas	4.3 (2.1)	5.3 (1.5)	4.3 (1.3)	7.5 (1.2)	7.0 (1.7)	4.2 (1.8)	5.8 (3.4)
A. retroflexus	0.0 (0.0)	2.8 (2.4)		6.0 (1.3)	5.2 (2.0)	4.0 (1.2)	4.2 (2.9)

[a] Figures in parentheses are standard deviations

RESULTS AND DISCUSSION

The organic and conventional field experiments were located in different fields, and to avoid having winter wheat as precrop in the experiment in the second year the experiments were not carried out in the same fields in the two growth seasons either. Consequently, factors such as soil type, availability of nutrients in the soil, soil microflora, and the presence of weeds, diseases, and pests varied between the experiments, and climatic factors were not similar in the two years of experiments. In 2001−2002, wheat plants at growth stages 9−10 and 12 were sampled in the autumn and winter, whereas the sampling at growth stage 21 was done in the spring. In 2002−2003, the wheat crop was sown early and due to favorable growth conditions the first three samplings were made in the autumn/winter and one additional sampling was made in the spring (**Table 1**). The pot experiments were carried out immediately after sampling of the wheat and accordingly at different times of the year. It is known that biotic and abiotic factors influence the production and transformation of allelochemicals (*15*) as well as growth of the weeds, and therefore the experimental design allows comparisons only between varieties and growth stages within each farming system in each year.

Effects on Weed Germination. Benzoxazinones and their metabolites have previously been reported to inhibit germination of different plant species (*3*, *23*). The number of germinated plants of each weed species in the control pots of all experiments is shown in **Table 2**. The germination was very poor for *E. crus-galli*, *C. album*, *S. viridis*, *A. theophrastis*, and *A. retroflexus* in 2001−2002. Consequently, the results of these weed species were excluded from the subsequent analyses. In 2002−2003 the germination was improved by raising the temperature in the glasshouse and using new seed stocks of the above-mentioned species.

Soil incorporation of the different wheat varieties had no significant influence on the germination of the weed species in the experiment in 2001−2002. In 2002−2003, the most pronounced effects were observed after incorporation of wheat at growth stage 9−10. The wheat varieties from the organic farming system reduced germination of *S. viridis* (Stakado and Ritmo), *G. aparine* (Astron and Ritmo, not shown), and *P. rhoeas* (Astron), and conventionally grown Astron reduced germination of *P. annua* (**Figure 1**). At growth stage 21, the germination of *S. viridis* and *A. spica-venti* was reduced by organically grown Ritmo and Astron, respectively, and at growth stage 31 the germination of *S. viridis* was reduced by organically grown Stakado and conventionally grown Astron, whereas organically grown Astron inhibited germination of

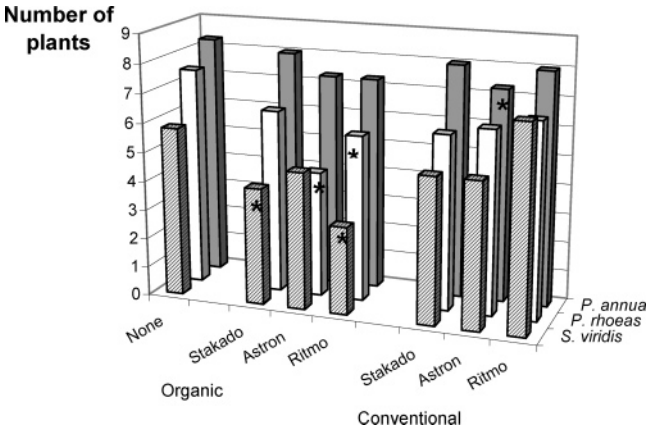

Figure 1. Effect of soil incorporation of wheat varieties at growth stage 9–10 on the number of germinated plants of selected weed species. Wheat varieties were cultivated in both organic and conventional farming systems in 2002–2003. Significant effects (0.05) within the farming system compared with control are marked with an asterisk.

A. myosuroides (data not shown). Inhibitory effects seemed to be more frequent when the incorporated wheat varieties were cultivated in the organic farming system compared with the conventional; however, it was not possible to identify any general trend in the response concerning differences in the susceptibility of weed species or in the allelopathic properties of wheat varieties.

Effects on Weed Growth. All weed biomass data were expressed as average fresh weight per plant relative to control (= 100). An LS-MEANS test revealed that there were no significant differences in responses of biomass between the two field plot replicates in the 2001−2002 experiment; hence, the two pot replicates from each of the field plots were combined as four replicates in the statistical analyses.

Incorporation of wheat caused growth inhibition as well as growth stimulation of the different weed species. In **Table 3** each weed species responding with significant growth inhibition following the different treatments is marked. Obviously in 2001−2002, the number of weed species with significant responses was much higher at BBCH 9−10 than at later growth stages. None of the weed species were significantly influenced by incorporation of wheat varieties sampled at growth stage 12, whereas organically grown Ritmo and Solist sampled at growth stage 21 inhibited the growth of *T. inodorum*. **Figure 2** shows the relative fresh weights per plant of the different weed species following incorporation of the wheat varieties at BBCH 9−10. The organically grown wheat varieties inhibited the

Table 3. Significant Herbicidal Effects of Incorporated Wheat Varieties at Different Growth Stages[a]

	2001–2002				2002–2003							
	org, 9–10	conv, 9–10	org, 12	org, 21	org, 9–10	conv, 9–10	org, 12	conv, 12	org, 21	conv, 21	org, 31	conv, 31
Astron	XXX				X				X		X	X
Bill	XXXX	XX										
Portal	XXXXX	X										
Ritmo	XXXXX	X		X						X		XXX
Stakado	XXX	X				X			X		X	X
Solist	XXXX			X								

[a] X's represent a weed species with significant growth reduction (5% probability level) compared with control pots. Org, organic; conv, conventional; 9–10, 12, 21, 31, BBCH growth stage.

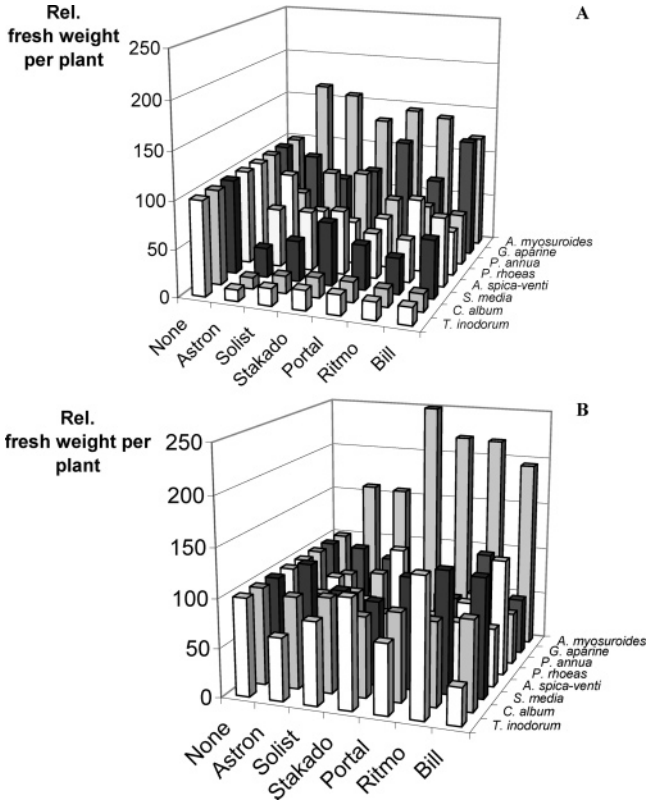

Figure 2. Effect of soil incorporation of organically (**A**) and conventionally (**B**) cultivated wheat varieties at BBCH 9–10 on the growth of weed species (2001–2002).

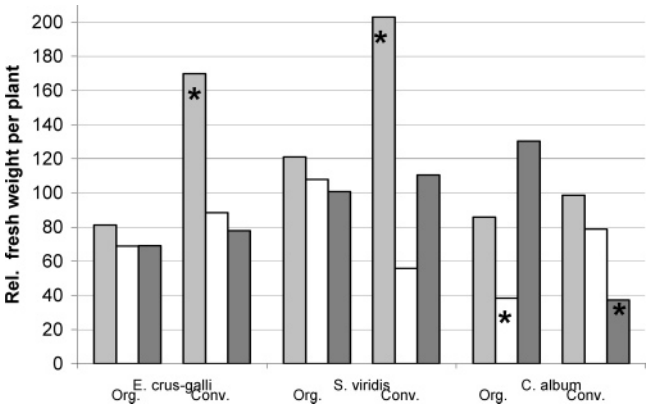

Figure 3. Effect of soil incorporation of different wheat varieties at BBCH 21 on the growth of selected weed species: (light gray bars) Stakado; (white bars) Astron; (dark gray bars) Ritmo. Wheat varieties were cultivated in both organic and conventional farming systems in 2002–2003. Significant effects (0.05) within the farming system compared with control are marked with an asterisk.

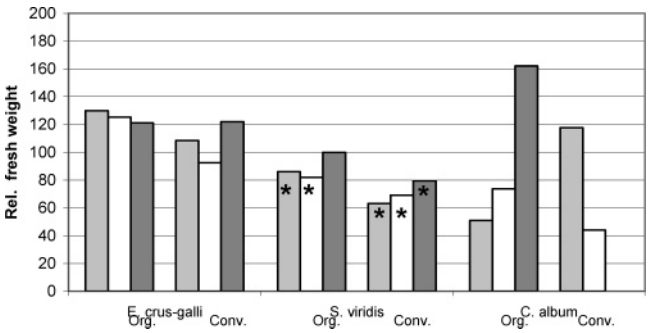

Figure 4. Effect of soil incorporation of different wheat varieties at BBCH 31 on the growth of selected weed species: (light gray bars) Stakado; (white bars) Astron; (dark gray bars) Ritmo. Wheat varieties were cultivated in both organic and conventional farming systems in 2002–2003. Significant effects (0.05) within the farming system compared with control are marked with an asterisk.

growth of *P. annua*, *A. spica-venti*, *S. media*, *T. inodorum*, *C. album*, and *P. rhoeas* (**A**), whereas varieties from the conventional farming system reduced the growth of only *A. spica-venti* and *G. aparine* (**B**). All varieties tended to stimulate the growth of *A. myosuroides*; however, biomasses were not significantly different from the control. *T. inodorum*, *C. album*, and *S. media* were more susceptible to incorporated wheat irrespective of variety than the other species, with growth reductions ranging from 55 to 88%. *P. annua*, *A. spica-venti*, and *P. rhoeas* responded significantly to some of the wheat varieties, whereas the growth of *G. aparine* was unaffected.

In 2002−2003, inhibitory effects of incorporated wheat varieties were—in contrast to the previous year—more frequent when sampling and incorporation were done at late growth stages (BBCH 21 and 31) compared with early growth stages. Incorporation of organically grown Astron at growth stage 9−10 significantly inhibited the growth of *A. spica-venti*, whereas the growth of *S. media* was reduced by conventionally grown Stakado (growth stage 9−10). Similar to the first year, we did not find any significant influence of incorporated wheat varieties sampled at growth stage 12, whereas at growth stage 21, significant effects were observed on *C. album* with conventionally grown Ritmo, on *A. spica-venti* with organically grown Stakado, and on *C. album* with organically grown Astron (**Figure 3**). Incorporation of wheat varieties at growth stage 31 significantly inhibited the growth of *S. viridis* (conventionally grown Stakado, Ritmo, and Astron; organically grown Stakado and Astron), *S. media* (conventionally grown Ritmo), and *T. inodorum* (conventionally grown Ritmo) (**Figure 4**).

In summary, the results show that in some cases soil incorporation of wheat varieties affected weed growth. However, the results were not consistent concerning the phytotoxic effects

Table 4. Calculated Concentrations (Millimolar) of DIMBOA, MBOA, and BOA Using Assumptions on Distribution of Weight Ratios between Foliage and Root Parts As Described in the Text and the Amounts of Plant Material Shown in **Table 1**[a]

	2001–2002				2002–2003							
	org, 9–10	conv, 9–10	org, 12	org, 21	org, 9–10	conv, 9–10	org, 12	conv, 12	org, 21	conv, 21	org, 31	conv, 31
Astron	0.0011	0.0008	0.0019	0.0018	0.0019	0.0041	0.0027	0.0021	0.0007	0.0003	0.0054	0.0063
Bill	0.0012	0.0017	0.0014	0.0021								
Portal	0.0013	0.0007	0.0021	0.0021								
Ritmo	0.0030	0.0022	0.0023	0.0018	0.0021	0.0026	0.0036	0.0033	0.0007	0.0005	0.0032	0.0040
Stakado	0.0064	0.0033	0.0014	0.0005	0.0058	0.0065	0.0069	0.0053	0.0007	0.0004	0.0031	0.0029
Solist	0.0031	0.0011	0.0022	0.0020								

[a] Org, organic; conv, conventional; 9–10, 12, 21, 31, BBCH growth stages.

of varieties and the susceptibility of weed species. The results indicated that inhibition of growth was more frequent from wheat varieties sampled in the organic compared to the conventional farming system (**Table 3**).

In the present study, the contents of DIMBOA, MBOA, and BOA in foliage and roots were quantified by chemical analyses (*14*), and we expected to find a positive correlation between the content of these compounds and the efficacy on weeds. In both years the highest concentrations of the allelochemicals were found at the early growth stage. Soil incorporation included whole plants, and to predict the content of allelochemicals in the soil water of the pots, we had to make some assumptions. Weighing of the plants showed that the dry weight of the plants was 10% of the fresh weight, and the ratios between foliage and roots were approximately 25:75 at growth stage 9−10, 70:30 at growth stage 12, 75:25 at growth stage 21, and 80:20 at growth stage 31. On the basis of these figures and assuming that the allelochemicals were not absorbed to the soil colloids but fully dissolved in the soil water (60 mL/pot), the concentrations of DIMBOA + MBOA + BOA in pot soil water following incorporation of the different samplings were estimated to levels between 0.0002 and 0.0065 mM (**Table 4**). The highest concentrations were obtained following incorporation of organically grown Stakado at growth stage 9−10 in 2001−2002 and incorporation of Stakado at growth stages 9−10 and 12 and Astron at growth stage 31 from both farming systems in 2002−2003. We did find significant effects on the growth of three weed species of organically grown Stakado at growth stage 9−10 in 2001−2002 and on one weed species in 2002−2003 following incorporation of conventionally grown Stakado at stage 9−10 and Astron from both farming systems at growth stage 31, respectively (**Table 3**). However, in other cases with the same detectable level of the compounds we did not obtain significant effects on the weed species, and simultaneously we had significant effects in samples with low contents of the compounds (2001−2002, growth stage 9−10 of Astron, Bill, and Portal). Consequently, the efficacy on weeds was not positively correlated to the estimated levels of DIMBOA, MBOA, and BOA in the soil water.

The potential activity on weeds of DIMBOA, MBOA, BOA, and APO without any interference of soil was examined in Petri dish experiments. The estimated ED_{50} doses revealed only minor differences in the herbicidal effects of DIMBOA, MBOA, and BOA (**Table 5**). *S. media* and *T. inodorum* were more susceptible (ED_{50} of MBOA and BOA = 0.4−0.6 mM) than *S. viridis*, *P. annua*, and *A. spica-venti* (ED_{50} = 0.8−1 mM), supporting the results of the 2001−2002 pot experiment. The most tolerant species was *E. crus-galli*, with estimated ED_{50} doses of 2−3 mM. The activity of APO was 5−50 times higher than the activities of the parent compounds. Macías et al. recorded significant effects on root length of *A. cepa*, *L. aestivum*, and *L. esculentum* by 0.5−1 mM of DIMBOA,

Table 5. Estimated ED_{50} Doses of DIMBOA, MBOA, BOA, and the Degradation Product APO on Radicle Growth per Day of Different Weed Species[a]

	ED_{50} (mM)			
plant species	DIMBOA	MBOA	BOA	APO
E. crus-galli	2.59 (0.18)	1.99 (0.15)	3.16 (0.20)	≈0.2
S. viridis	1.37 (0.07)	0.95 (0.04)	1.09 (0.06)	nd
P. annua	nd	0.91 (0.03)	1.03 (0.09)	≈0.02
A. spica-venti	0.60 (0.05)	0.78 (0.06)	0.94 (0.07)	nd
S. media	nd	0.42 (0.02)	0.61 (0.03)	nd
A. theopratis	nd	1.92 (0.05)	1.97 (0.05)	nd
T. inodorum	nd	0.50 (0.08)	0.51(0.11)	nd
A. retroflexus	0.56 (0.05)	1.09 (0.06)	0.68 (0.04)	≈0.15

[a] Figures in parentheses are standard deviations. nd, not determined.

whereas the phytotoxicity of BOA and MBOA was lower (*22*). The degradation product APO was the most phytotoxic compound in their study, and significant effects were recorded in a concentration of 0.1 mM. The concentration levels for herbicidal effects are very similar to our results, but we did not find indications of DIMBOA being more phytotoxic than MBOA and BOA. The fact that the estimated ED_{50} doses were in the millimolar range indicated a relatively low herbicidal activity compared with many synthetic herbicides.

Calculations showed that the concentrations of DIMBOA, MBOA, and BOA in the pot soil water were at least 65 times lower than the ED_{50} concentrations of the most susceptible weed species, and consequently they cannot explain the effects we obtained in some treatments. According to previous studies the much more phytotoxic transformation product APO will not be formed in the present low concentrations of BOA (*13*, *14*), and even if it was formed, it would not be present in phytotoxic concentrations. The effect of AMPO, which is the transformation product of DIMBOA and MBOA, was not examined in our experiment, but it has been reported to possess a very low phytotoxicity (*22*).

The effects that we obtained in some treatments could be the result of synergism between several allelochemicals being present at the same time. Synergistic effects of allelochemicals have previously been reported (*15*); however, results of detailed studies on the joint effect of mixtures of the allelochemicals showed an additive or less than additive effect of mixtures (*21*). Conclusively, the present study did not reveal a distinct explanation of the obtained effects on germination and growth as neither the amounts of benzoxazinoids in the incorporated wheat plants that were found by chemical analyses (*14*) nor the joint action of the identified allelochemicals (*21*) can explain these effects. It can be concluded that exploiting wheat allelopathy as a weed control strategy implies development of varieties with an enhanced content of benzoxanoids.

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