

Structure–Activity Relationship Studies of Benzoxazinones and Related Compounds. Phytotoxicity on *Echinochloa crus-galli* (L.) P. Beauv.

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Echinochloa crus-galli (*E. crus-galli*; barnyardgrass) is a weed widely distributed. It constitutes a serious weed problem in 42 countries and has been found in at least 27 more. It is the world's main weed of rice affecting up to 36 crops worldwide. Several biotypes of this plant, with resistance to herbicides with different modes of action have evolved. In our ongoing studies regarding the potential application of benzoxazinones and their soil degradation products for weed control, a complete structure–activity relationships (SARs) study was made by using barnyardgrass as the target plant. Compounds used in this study were previously tested on a wide variety of standard target species (STS), and they include natural allelochemicals 2-*O*- β -D-glucopyranosyl-4-hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIBOA-Glc), 2,4-dihydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIMBOA), and 2,4-dihydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIBOA), together with some degradation derivatives found in wheat crop soil and some synthetic analogues. Their phytotoxicity on *E. crus-galli* is discussed and compared with the results obtained from previous screening. This work constitutes the next step in the search for natural herbicide models based on benzoxazinones and their degradation products. The most active compounds were the degradation product 2-aminophenol (APH) and the synthetic analogue 4-hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (D-DIBOA). Their activities confirm the direction proposed in our previous SAR study, which establishes D-DIBOA to be the best lead for natural herbicide model development with benzoxazinone structure.

KEYWORDS: Rice; *Echinochloa crus-galli*; barnyardgrass; benzoxazinones; DIMBOA; DIBOA; 4-hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one; bioassay; phytotoxicity

INTRODUCTION

Barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.; *E. crus-galli*) is a plant originally from Europe and India. It constitutes a serious weed problem in 42 countries and has been found in at least 27 more (1). It is the world's main weed of rice (2) and, likely, has been for a long time: barnyardgrass is recognizable in Chinese drawings from 1590 (3).

Also, barnyardgrass has been detected in 36 crops worldwide. It is particularly problematic for rice crops all over the world. It is among the three worst weeds in cotton in Australia, Russia, and Spain; in corn in Australia and the former Yugoslavia; and in sugarbeets in the United States. Also, it is a main weed in many other crops such as cotton, corn, and potatoes, for example, in the United States (2). Barnyardgrass has ecological preferences similar to rice, and young plants look similar (4);

several thousand years of intensive handweeding of rice in Asia may have selected for rice mimicry (5). Many plants infesting rice fields may be transplanted in by accident (2).

Heavy infestations may remove 60–80% of the nitrogen from the soil (2), as well as considerable amounts of other macronutrients (6). Fertilizer applications favor the weed over rice (2). It reduces rice tillering by 50% and also reduces the number of panicles, height, weight of grains, the and number of grains per panicle; rice yields may be reduced by 2000–4000 kg ha⁻¹ (2). Barnyardgrass has been proven to reduce the yields of potatoes (7), snap beans (8), corn (9), grain sorghum (10), sugarbeets, green peas, and melons (11). Barnyardgrass interferes with harvesting of row crops and increases labor costs: the crop must be separated from the weed clumps (6).

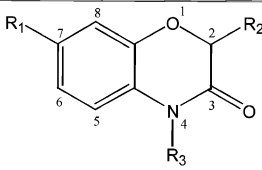
Barnyardgrass is also a host to many viruses of rice and other grass crops (2), as well as a host of *Striga asiatica* (L.) Kuntze, which infests sorghum, corn, millet, sugar cane, rice, and tobacco in India, Africa, and the United States (3).

The success of barnyardgrass is attributed to prolific seeding, seed dormancy, the ability to grow rapidly, flowering in a wide range of photoperiods, and relative resistance to herbicides (6).

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Table 1. Natural Allelochemicals and Synthetic Analogues with Benzoxazinone Skeleton Employed for SAR Study^a

		
Natural Allelochemicals	<p>R₁=H; R₂=O-β-D-glucose; R₃=OH: 2-O-β-D-glycopyranosyl-4-hydroxy-(2<i>H</i>)-1,4-benzoxazin-3(4<i>H</i>)-one DIBOA-Glc</p> <p>R₁=H; R₂=OH; R₃=OH: 2,4-dihydroxy-(2<i>H</i>)-1,4-benzoxazin-3(4<i>H</i>)-one DIBOA</p> <p>R₁=OCH₃; R₂=OH; R₃=OH: 2,4-dihydroxy-7-methoxy-(2<i>H</i>)-1,4-benzoxazin-3(4<i>H</i>)-one DIMBOA</p>	A
	<p>R₁=H; R₂=H; R₃=H: (2<i>H</i>)-1,4-benzoxazin-3(4<i>H</i>)-one D-HBOA</p> <p>R₁=OCH₃; R₂=H; R₃=H: 7-methoxy-(2<i>H</i>)-1,4-benzoxazin-3(4<i>H</i>)-one D-HMBOA</p> <p>R₁=H; R₂=H; R₃=OH: 4-hydroxy-(2<i>H</i>)-1,4-benzoxazin-3(4<i>H</i>)-one D-DIBOA</p> <p>R₁=OCH₃; R₂=H; R₃=OH: 4-hydroxy-7-methoxy-(2<i>H</i>)-1,4-benzoxazin-3(4<i>H</i>)-one D-DIMBOA</p>	B

^aFunctionalization, systematic name, and acronym are shown for each compound.

Its first case of herbicide resistance was described in Maryland in 1978 evolving the Photosystem II inhibitor herbicides (HRAC group C1). Later, a number of resistant biotypes have been described in the United States, Brazil, Spain, Canada, France, Italy, China, and other countries affecting different herbicide groups such as synthetic auxines, thiocarbamates, or dinitroanilins as well as different modes of action as Photosystem II or ACCase inhibitors (12–17). Several cases of multiple resistances have also been investigated (18).

Benzoxazinones containing the hydroxamic acid moiety acquired high relevancy on phytochemistry research after the isolation of 2,4-dihydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIBOA) (19) in 1959 and 2,4-dihydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIMBOA) in 1962 (20) (Table 1A). Benzoxazinones have been described as important allelochemicals from Gramineae, as well as Acanthaceae, Ranunculaceae, and Scrophulariaceae. The necessity of obtaining new herbicides with new and multiple modes of action that could prevent resistant phenomena lead us to study the influence of benzoxazinones and related compounds on the growth of barnyardgrass.

Interesting bioactivity was observed for both compounds, some of their degradation products, and also some synthetic analogues, being antimicrobial (21), antifeedant, insecticidal (22), and phytotoxic (23, 24) and widely described. The DIBOA natural glycoside (2-*O*-β-D-glucopyranosyl-4-hydroxy-(2*H*)-1,4-

benzoxazin-3(4*H*)-one (DIBOA-Glc) (Table 1A) is the form in which DIBOA is preserved inside the plant prior to its release (25). It has also been detected in plant exudates (26). Benzoxazolin-2-one (BOA) and 6-methoxybenzoxazolin-2-one (MBOA) (Table 2A) are the first chemicals in DIBOA and DIMBOA degradation series, respectively (27–29). There are interesting precedents regarding their bioactivity on different systems (30, 31). According to these previous evaluations, DIMBOA and DIBOA (Table 1A) were the most active compounds. Their degradation products MBOA and BOA (Table 2A) are much less active, and 2-deoxy analogues of DIMBOA and DIBOA (Table 1B) have different behaviors depending on the different species assayed.

In addition to this bioactivity research, the low stability of DIMBOA and DIBOA and the moderate degradability of their related benzoxazinones in several conditions, such as biotransformation by fungi (32) and degradation in crop soil (28, 29, 33, 34) and in aqueous solution (27), have been investigated. After the characterization of conversion dynamics of these compounds in model wheat crop soils, 2-aminophenoxazin-3-one (APO) and 2-amino-7-methoxyphenoxazin-3-one (AMPO) (Table 2B) were the final products for DIBOA and DIMBOA degradation routes found by us (28, 29). Their *N*-acetyl derivatives, 2-acetamidophenoxazin-3-one (AAPO) and 2-acetamido-7-methoxyphenoxazin-3-one (AAMPO) (Table 2B) have been proposed as detoxification compounds produced by nonpathogenic organisms associated with Gramineae (32, 35). APO has been already described as a potent phytotoxic agent for barnyardgrass (33). We recently reported a complete structure–activity relationship (SAR) study dealing with 21 chemicals, including natural benzoxazinones, a wide variety of synthetic analogues of them, and degradation products belonging to four different structural types (36).

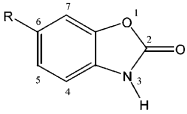
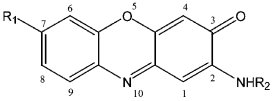
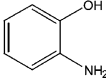
The bioactivity profiles shown by benzoxazinones suggested 2-deoxy derivatives of natural allelochemicals DIBOA and DIMBOA (D-DIMBOA and D-DIBOA) (Table 1B) to be the best leads for new herbicides with this structural base. Amino-phenoxazine APO was the most active degradation compound, with high phytotoxicity levels at both high and low concentrations. The lack of phytotoxic effect observed for its structurally related compounds such as AMPO and AHPO was related to their lipophilia.

In this context, evaluation of these compounds on problematic weeds constitutes the next step for natural herbicide model development. Thus, the main objective of this work was to evaluate phytotoxicity of benzoxazinones and related compounds on barnyardgrass, in the search for new compounds able to be applied as part of strategies directed to weed control on barnyardgrass-affected crops. Statistical treatment of the acquired data would allow discovering structure–activity relationships useful in further development of highly phytotoxic compounds based on natural product structures.

MATERIALS AND METHODS

General Methods. The purity of the assayed compounds was determined by ¹NMR and HPLC analyses and was found to be >98%. ¹H and ¹³C NMR spectra were recorded at 399.99 and 100.577 MHz, respectively, by using MeOH-*d*₄ and CDCl₃ as solvents in a Varian INOVA spectrometer. The resonance of residual solvents for ¹H NMR was set to δ_H = 3.30 (methanol) and δ_H = 7.25 (chloroform). Solvent peaks for ¹³C NMR were set at δ_C = 49.0 (methanol) and 77.0 (chloroform). For HPLC analysis, HPLC PDA detector (diode array UV–vis system), column Phenomenex SYNERGI 4 micron Fusion RP-80 (250 × 460 mm) and Varian 1200L quadrupole MS/MS detector were used.

Table 2. Base Structures, Functionalizations, Systematic Names, and Acronyms for the Degradation Products Employed for the SAR Study

A Benzoxazolinones	B Aminophenoxazines	C Miscellaneous
		
<p>R=H Benzoxazolin-2(3<i>H</i>)-one BOA</p> <p>R=OCH₃ 6-methoxybenzoxazolin-2(3<i>H</i>)-one MBOA</p>	<p>R₁=H; R₂=H 2-aminophenoxazin-3-one APO</p> <p>R₁=OCH₃; R₂=H 2-amino-7-methoxyphenoxazin-3-one AMPO</p> <p>R₁=H; R₂=OAc 2-acetamidophenoxazin-3-one AAPO</p> <p>R₁=OCH₃; R₂=OAc 2-acetamido-7-methoxyphenoxazin-3-one AAMPO</p>	<p>2-aminophenol APH</p>

Isolation and Synthesis of Natural Allelochemicals, Degradation Products, and Their Derivatives. *Natural Benzoxazolinones (Table 1A).* DIBOA and DIMBOA were obtained from natural sources by means of previously reported isolation procedures (37) modified by us. The DIBOA natural glycoside (DIBOA-Glc) was isolated from natural sources. Its isolation protocol, adapted from literature, has been already described by us regarding its degradation study in wheat crop soil (28, 29).

Synthetic Benzoxazolinones. They were obtained in our laboratory by adapting methods from literature (38) as it was previously described by us (36).

Degradation Products (Table 2). They have been selected according to the precedents mentioned above, belonging to four different structural types:

Benzoxazolinones (Table 2A). Both of them are commercial compounds. They were purchased from Fluka Chemika and Lancaster Synthesis, respectively. They were used as received.

Aminophenoxazin-3-ones (Table 2B). They were obtained in our laboratory by previously reported synthesis procedures (36).

Additional Compounds Evaluated. To characterize its bioactivity and to discuss the phytotoxicity of DIBOA degradation route chemicals on barnyardgrass, 2-aminophenol (**Table 2C**; purchased from Sigma-Aldrich Co. and used as received) was evaluated.

Phytotoxicity Bioassays. *Methodology.* Bioassays used Petri dishes (90 mm diameter) with one sheet of Whatman No.1 filter paper as substrate. Germination and growth were conducted in aqueous solutions at controlled pH by using 10^{-2} M 2-(*N*-morpholino)ethanesulfonic acid (MES) and addition of solution of NaOH 1 M to reach pH = 6.0. Solutions (0.2, 0.1, 0.02, 0.01, and 0.002 M) of the compounds to be assayed were prepared in dimethyl sulfoxide (DMSO) and then diluted with buffer (5 μ L of DMSO/(mL of buffer)) to reach the test concentrations for each compound (1, 0.5, 0.1, 0.05, and 0.01 mM).

Barnyardgrass seeds were purchased from Herbiseed Co. (Twyford, England). They were used as received. The number of seeds in each Petri dish was 25, and 5 mL of treatment, control or internal reference solution was added to each Petri dish. Four replicates were used (100 seeds).

After adding seeds and aqueous solutions, Petri dishes were sealed with Parafilm to ensure closed-system models. Seeds were further incubated at 25 °C in a Memmert ICE 700 controlled environment growth chamber, in the absence of light. The bioassay took 5 days.

After growth, plants were frozen at -10 °C for 24 h to avoid subsequent growth during the measurement process. This helped the handling of the plants and allowed for a more accurate measurement of root and shoot lengths.

The commercial herbicide Logran, a combination of *N*-(1,1-dimethylethyl)-*N*-ethyl-6-(methylthio)-1,3,5-triazine-2,4-diamine (Terbutryn, 59.4%) and 2-(2-chloroethoxy)-*N*-[[4-methoxy-6-methyl-1,3,5-triazin-2-yl]amino]carbonyl]benzenesulfonamide (Triasulfuron, 0.6%) was used as internal reference, according to a comparison study previously reported (39). It was used at the same concentrations (1, 0.5, 0.1, 0.05, and 0.01 mM) and in the same conditions as the compounds in study. Buffered aqueous solutions with DMSO and without any tested compound were used as control samples.

Bioassay Data Acquisition. Evaluated parameters (germination rate, root length, and shoot length) were recorded by using a Fitomed system (39) that allowed automatic data acquisition and statistical analysis by its associated software.

Statistical Analysis. Data were statistically analyzed using Welch's test, with significance fixed at $P = 0.01$ and 0.05 . They are presented as percentage differences from control. Zero represents control, positive values represent stimulation of the studied parameter, and negative values represent inhibition (39, 40).

Once the germination and growth data were acquired, cluster analysis was used to group compounds with similar phytotoxicity behaviors and associate them with their molecular structure. Complete linkage was used as the amalgamation rule, and the distance measurement was based on squared Euclidean distances (41), given by this equation:

$$d(x,y) = \sum_i (x_i - y_i)^2$$

where $d(x,y)$ is the squared Euclidean distance (i -dimensional), i represents the number of variables, and x and y are the observed values.

The cluster was obtained by using Statistica v. 5.0 software. The lack of consistent effects over germination enforced us to make cluster analysis on the basis of growth parameters (root length and shoot length). Both parameters were used together to perform the analysis. The data belonging to all the concentrations employed in the bioassay were included in the cluster, and they had the same statistical weight.

EC₅₀ values (concentrations in which half of the observed effect is reached) were obtained after adjusting phytotoxicity data to concentra-

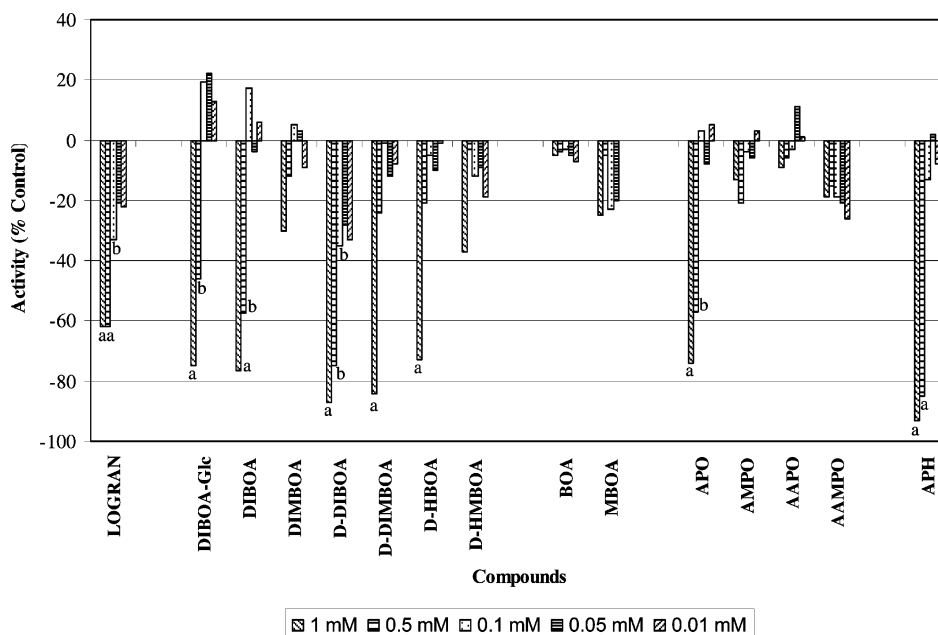


Figure 1. Phytotoxicity bioassay results (root length, % from control) for *Echinochloa crus-galli*. If it is not indicated, $P > 0.05$ for Welch's test. (a) Values significantly different with $P < 0.01$. (b) Values significantly different with $0.01 < P < 0.05$.

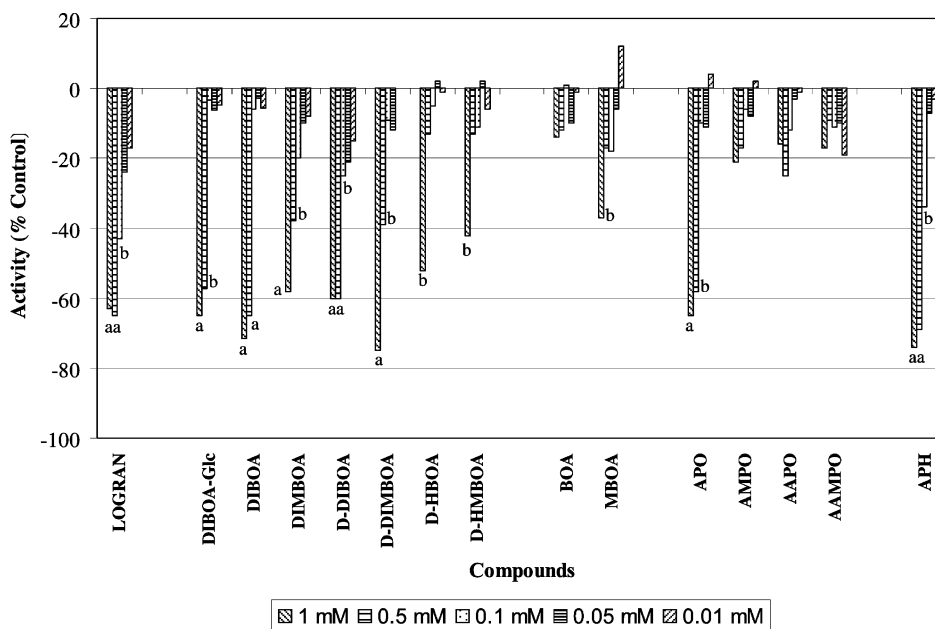


Figure 2. Phytotoxicity bioassay results (shoot length, % from control) for *Echinochloa crus-galli*. If it is not indicated, $P > 0.05$ for Welch's test. (a) Values significantly different with $P < 0.01$. (b) Values significantly different with $0.01 < P < 0.05$.

tion (logarithmic scale), to a sigmoidal dose–response curve, defined by the equation:

$$Y = Y_{\min} + \frac{Y_{\max} - Y_{\min}}{1 + 10^{\log EC_{50} \cdot X}}$$

where X indicates the logarithm of the concentration, Y indicates the response (phytotoxicity), and Y_{\max} and Y_{\min} are the maximum and minimum values of the response, respectively. Goodness of fit is described by the determination coefficient (r^2). The adjustment and the r^2 were obtained by using GraphPad Prism software v. 4.00.

Lipophilicity Calculations. Lipophilicity, expressed as the $\log P$ (water/*n*-octanol partition coefficient) value, was obtained by computational methods according to Ghose et al. (42). This algorithm is implemented in Hyperchem v. 7.0 software.

RESULTS AND DISCUSSION

Bioactivity Profiles Observed. Results for phytotoxic activity bioassay on barnyardgrass are shown in **Figure 1** (root length) and **Figure 2** (shoot length). The compounds which had significant activity levels were inhibitory for both parameters.

Benzoxazinones (Table 1). All assayed benzoxazinones showed important inhibitory effects on growth parameters, some of them being equal or higher than the ones shown for the internal reference. DIBOA-Glc and its aglycon DIBOA exhibited very similar phytotoxicity, with high inhibition at the two more concentrated treatments. Although DIMBOA shows an interesting profile on shoot length, it did not inhibit root growth in a significant manner.

The only compound that preserved significant effect at the lower doses was D-DIBOA on root length ($EC_{50} = 0.72$ mM,

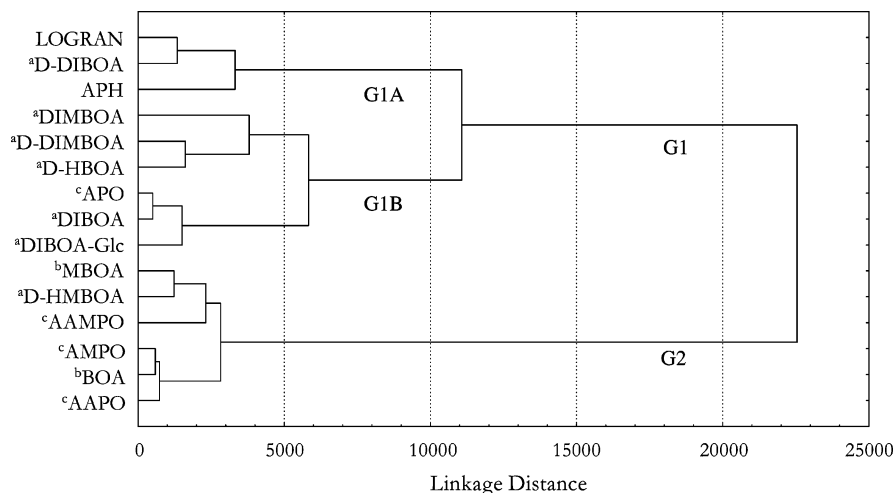


Figure 3. Cluster analysis for *Echinochloa crus-galli* growth inhibition (effects on root length and shoot length): (a) benzoxazinones; (b) benzoxazolinones; (c) aminophenoxazines.

$r^2 = 0.96$), with inhibitions close to 40% at the lowest doses. It was the most active compound of the whole bioassay, considering its effects on roots and shoots. In this compound, the reduction of the dose from 1 to 0.5 mM did not represent a significant reduction of the activity neither for root nor for shoot length. It was more of an inhibitor than its 7-methoxy analogue D-DIMBOA, which did not fit a sigmoidal dose–response curve for this parameter. The synthetic benzoxazinone lactams D-HBOA and D-HMBOA had significant phytotoxicity levels on root and shoot length, but just at the highest concentration.

Regarding the phytotoxicity of benzoxazinone degradation products (**Table 2A**), benzoxazolinone BOA did not show any significant effect on barnyardgrass growth. Shoot length was slightly affected by MBOA (37% inhibition at 1 mM), this effect being lost at any lower dose.

Aminophenoxazines (Table 2B). APO is the only aminophenoxazine that shows inhibitory effects, reaching 60% at the two more concentrated treatments for both growth parameters. There were not significant inhibitory effects caused by the other assayed aminophenoxazines. Nevertheless, its precursor APH showed potent inhibition on both growth parameters, being the effect preserved with treatment dilution, especially on shoot growth ($EC_{50} = 0.17$ mM, $r^2 = 0.97$). Although this compound provoked high root length inhibition with the two first treatments (1 and 0.5 mM), the effects on this parameter were not significant at any lower concentration.

Structure–Activity Relationships. Cluster analysis for growth parameters is shown in **Figure 3**. Compounds can be classified according to their phytotoxic effects in two main groups: G1 for high or moderate effects and G2 for low or null activities. G1 is divided into two subgroups: G1A for the most active compounds and G1B for those which showed moderate effects.

All compounds assayed with benzoxazinone skeleton (**Table 1**) belong to the G1 group, except D-HMBOA. Regarding the behavior of natural allelochemicals, all of them had moderate activity levels, preserved just at the highest concentrations. In fact, all of these compounds belong to the G1B group. Thus, the benzoxazinone skeleton was the most active structure assayed. The synthetic benzoxazinone D-DIBOA was the most active compound of all of the bioassays, with phytotoxicity profiles very similar to the commercial herbicide Logran, which had EC_{50} values of 0.26 ($r^2 = 0.95$) and 0.11 mM ($r^2 = 0.96$) for root and shoot lengths, respectively. As it occurred in our previous phytotoxicity study, its 7-methoxy analogue D-

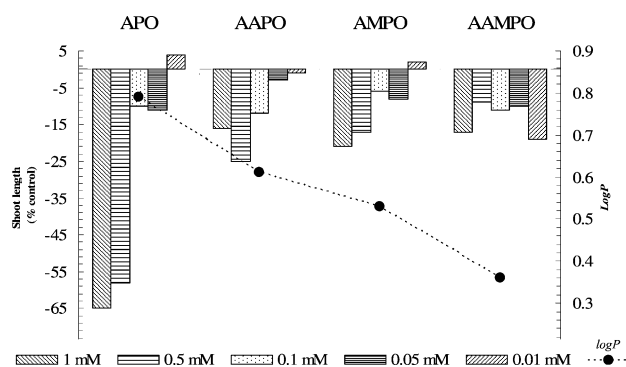


Figure 4. Phytotoxicity (*Echinochloa crus-galli*, shoot length) and lipophilicity ($\log P$) for aminophenoxazines.

DIMBOA showed weaker phytotoxicity. The same occurs with the assayed benzoxazinone lactams, showing D-HBOA higher inhibition than D-HMBOA. The influence of the 7-methoxy moiety seems to be the opposite analyzing phytotoxicity of natural allelochemicals, since DIMBOA effects are stronger than DIBOA and DIBOA-Glc ones.

In our previous study, which included the aminophenoxazines assayed here, we offered a possible explanation for the lack of phytotoxic effect observed for AAPO, AMPO, and AAMPO. In this case, the same behavior is observed, so that these phytotoxicity values can be correlated with aqueous solubility and lipophilicity, in the context of Lipinski (43) and Tice (44) models for bioactivity in pharmaceuticals and agrochemicals. Lipophilicity, calculated as logarithmic water/*n*-octanol partition coefficient ($\log P$) has a value close to 0.8 for APO (**Figure 4**), whereas AMPO, AAMPO, and AAPO roughly reach 0.6, which gives much lower phytotoxic effects.

The fact that DIBOA-Glc, DIBOA, BOA, APO, and AAPO constitute a complete degradation series in wheat crop soil models (29), as well as in other systems (45), allows one to relate the phytotoxicity of these compounds with their persistence in soil, to describe the influence of the complete series in the development of barnyardgrass. Phytotoxic effect and persistence in wheat crop soil recorded by us are shown in **Figure 5**. The most persistent compound of the series (APO) had just moderate effects. Taking into account the phytotoxicities observed, the most active compound (APH) has the shortest half-life of the whole series, while the most persistent compound (APO) had just moderate effects.

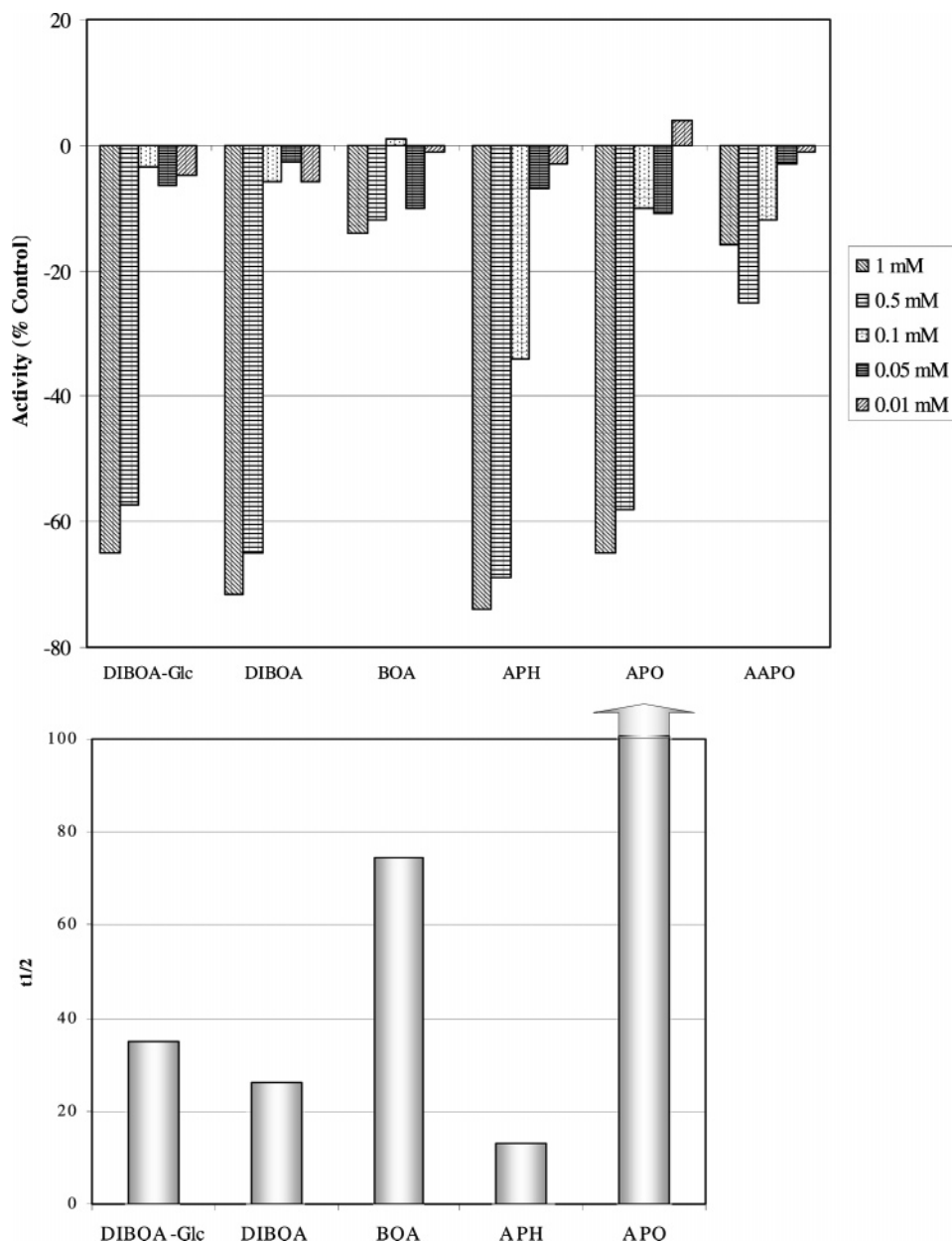


Figure 5. Phytotoxicity (*Echinochloa crus-galli*, shoot length) and persistence in soil (half-life) (29) for DIBOA degradation series compounds.

Conclusion. Regarding to the phytotoxicity of degradation products, the only one that shows moderate effects was APO (Table 2B). The lack of phytotoxicity observed for its analogues could be due to their lack of lipophilicity and the difficulties of diffusing through the cell membrane, according to the Hansch model for bioactivity (46). Further modification directed toward increasing the lipophilicity of these molecules would provide more candidates for natural herbicide models based on their structures.

The high persistence of this compound in wheat crop soil recorded by us (29) makes this compound a weak candidate for new herbicide models. Nevertheless, its stability in soils belonging to rice crops, in which environmental conditions, microbial population, and its dynamics should be very different, needs to be studied. That research will be needed to establish its utility in barnyardgrass management.

D-DIBOA (Table 1B) seems to be an optimal candidate for further natural herbicide model development toward barnyardgrass management, as the SAR study shows. The hydroxyl group at N-4 is a structural requirement for bioactivity, as it

can be observed in DIBOA-Glc, DIBOA, DIMBOA, D-DIBOA, and D-DIMBOA. The lack of this group provided just moderate or null effects (D-HBOA and D-HMBOA). Regarding functionalization at the C-2 position, the phytotoxic effect increased in its absence in the case of DIBOA and D-DIBOA. The opposite is observed for D-DIMBOA and DIMBOA, which have very similar phytotoxicity. This fact can be due to the higher effect shown by MBOA and the possibility of the conversion from DIMBOA to MBOA that could occur during the bioassay. In addition to this, the absence of this group also avoids degradation to benzoxazinones (less active). Thus, 7-methoxybenzoxazinones would not be adequate candidates for the development of compounds useful in barnyardgrass management. This result is parallel to those obtained in STS evaluation (36) and confirms the utility of D-DIBOA in the search for new natural herbicide models.

The phytotoxic effects shown by the DIBOA degradation series (Figure 5) allow one to conclude that the presence of these benzoxazinone glucosides and aglycones in barnyardgrass exudates (47) does not imply autotoxicity. The most persistent

compounds just produce moderate effects, and the most phytotoxic compound is the one that has less half-life. If natural compounds and their synthetic analogues are compared, it can be concluded that the DIMBOA or DIBOA detoxification capacity is not effective when barnyardgrass is exposed to synthetic analogues such as D-DIBOA. Then, the lower effects caused by natural benzoxazinones are in debt to two factors: degradation in the bioassay conditions and potential detoxification capacity. Our current efforts are directed toward the evaluation of the phytotoxic potential of this compound at different barnyardgrass and rice growth stages, to discover its utility for pest management in rice.

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