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Absorption and Elimination of Allelochemicals MBOA for Weed During Seedling Growth

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Abstract

6-Methoxy-2-benzoxazolinone (MBOA) is an allelochemical found in Poaceae, generally associated with monocotyledon species. This compound is formed from the glycosylated form of 2, 4-dihydroxy-2H-1, 4-benzoxazin-3 (4H)-one (Gly-DIMBOA) by a two-stage degradation process. The MBOA detoxification capacity of three weed species *Echinochloa crus-galli*, and *Lolium rigidum*, including a resistant biotype of *Lolium rigidum* (SLR31) was studied both qualitatively and quantitatively. For all three species, the product of metabolism is similar. This should indicate that all weeds metabolize xenobiotics through an identical route, since the product detected is the same. Kinetic studies of absorption and translocation to the shoot, show differences in these processes depending on the species. The analysis of treated plants, later transplanted to a growth medium without xenobiotic, showed that the weeds studied are capable of transmitting to the medium the previously absorbed compound, by root exudate. This evidence shows that this process is another route of defense of plants facing external stressors and is important to explain the differential behavior of weeds in field studies.

Key words: 6-Methoxy-2-Benzoxazolinone, absorption, weed, elimination, allelochemical.

Absorción y eliminación de Aleloquímicos MBOA por Malezas Durante el Crecimiento de Plántulas

Resumen

6-metoxi-2-benzoxazolinona (MBOA) es un aleloquímicos encontrado en las Poaceae, generalmente asociada con especies monocotiledóneas. Este compuesto se forma a partir de la forma glicosilada de 2, 4-dihidroxi-2H-1, 4-benzoxazin-3 (4H) -ona (Gly-DIMBOA) por un proceso de degradación en dos etapas. La capacidad de desintoxicación de MBOA de tres especies de malezas *Echinochloa crus-galli* y *Lolium rigidum*, incluido un biotipo resistente de *Lolium rigidum* (SLR31) se estudió de forma cualitativa y cuantitativa. Se encontró que el producto de metabolismo es similar, donde todo indica que las malas hierbas metabolizan el xenobióticos a través de una ruta idéntica, ya que el producto detectado es el mismo. Los estudios cinéticos de la absorción por la raíz y su translocación al tallo, muestran diferencias en estos procesos dependiendo de la especie de mala hierba. El análisis de las plantas mostró que las malas hierbas estudiadas son capaces de transmitir al medio el compuesto absorbido previamente, por el exudado de la raíz. Esta evidencia muestra que este proceso es otra ruta de defensa de las plantas que enfrentan factores de estrés externos y es importante para explicar el comportamiento diferencial de las malezas en los estudios de campo.

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Palabras clave: 6-metoxi-2-benzoxazolinona, absorción, mala hierba, eliminación.

Introduction

Cyclic hydroxamic acids and related benzoxazolinone compounds are an important group of allelochemicals in Gramineous plants. They are found in corn, rye, and wheat but not in rice, barley, and oats (1). The maximum recorded level of hydroxamic acids in cultivated wheat is 11 mmol/kg fresh weight (2) Root exudation of these compounds also has been observed (3). In the plant, the hydroxamic acids 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (DIMBOA) and 2,4-dihydroxy-2H-1,4-benzoxazin-3(4H)-one (DIBOA), are sequestered and stabilized as (2R)-2-β-D-glucosides. The more toxic aglucones are produced in response to tissue damage or pathogen attack. In plants after cells are damaged, in aqueous solution and in soil, cyclic hydroxamic acids decompose rapidly to form the respective ring-contracted compounds 6-methoxy-benzoxazolin-2(3H)-one (MBOA) and benzoxazolin-2(3H)-one (BOA) (1). The degradation products BOA and MBOA are produced by means of spontaneous degradation in aqueous solution (4), as well as by biological processes (5). BOA and MBOA have been isolated from plants of agronomic and non-agronomic interest. There are interesting works about their bioactivity (6) and mode of action (7). These chemicals transformations depend on chemical and biological conditions and some of these transformation products are more biologically active than the original ones (8). Until recently, secretion to the soil environment of hydroxamic acid from living plants had only been reported from rye (9). However, Wu (10) showed that selected varieties of wheat were able to exude hydroxamic acids as well. Important research on the structure of the hydroxamic acids, the role of the compounds in the plants, and their biosynthesis has been published (11). This degradation product has been cited as responsible of phytotoxic activity observed (12). Few studies have effectively quantified the allelochemicals' interactions between plants at molecular level. Regardless of this fundamental lack of understanding, an increased interest in allelopathy in agricultural and natural ecosystems continues to be observed. There is still difficulty in fully demonstrating such chemical interactions. One of the main mechanisms through which allelopathic potential is expressed is the exudation of active plant metabolites from the roots into the soil. These metabolites and/or their related degradation products should be absorbed and translocated into the target plant before inducing physiological disturbances. Although the benzoxazolinone allelochemicals and their degradation products in soil have been extensively studied in phytotoxic activity against different standard target species, little is known about the characteristics of absorption, translocation and detoxification phenomena. Among the few studies found, BOA is the derivative that has been most studied, demonstrating its absorption and translocation in 8-day seedlings of Raphanus sativus L. (12).. Little has been reported on the last step of such allelochemical interferences, specifically the absorption of allelochemicals from the soil into the target plant. This crucial step in the assessment of phytotoxic interactions between plants in agro systems has to be clarified in order to determine the active chemical species. Regarding the phenomenon of detoxification, it is this same compound that has attracted more attention, where the products generated in these reactions for biotransformation have been determined. Studies carried out on different plant families, which include weeds such as *Avena fatua* L. and *A. sativa*, show that the detoxification reactions involve the introduction of a hydroxyl group at position 6 of BOA, to generate BOA-6-OH, and glycosylation reactions on this group to generate BOA-6-O-glucoside, or reaction directly on the nitrogen atom to generate BOA-N-glucosides. Moreover, in the case of MBOA there is no data on the dynamics of absorption and translocation, and only recently the products of their detoxification reactions have been published. Since numerous weeds are associated naturally with the hydroxamic acid-producing cereals rye and wheat, the question is how those

species and species occurring in other communities cope with benzoxazolinone? Therefore, the present study addresses the ability to metabolize MBOA of weeds associated with rye and wheat, in comparison with some other associated species. The aim of this study was to perform a dynamic study of absorption, translocation and elimination of MBOA allelochemicals for three weed species: *Echinochloa crus-galli*, and *Lolium rigidum*, including a resistant biotype of *Lolium rigidum* (SLR31), which has developed ability to metabolize imidazolinone and sulfonylurea herbicides (13).

Materials and Methods

Allelochemicals: 6-Methoxy-2-benzoxazolinone (MBOA), commercial compounds, were purchased from Lancaster Synthesis, and used as received.

Bioassay

Target Species: *Lolium rigidum* L. and *Echinochloa crus-galli* seeds were purchased from Herbiseed Co. (Twyford, England). Herbicide resistant *Lolium rigidum* SRL31 was purchased from Phyto seed S.A (Barcelona-Spain).

Seed: The caryopsides of three weed species were initially washed with liquid detergent, followed by surface sterilization with calcium hypochlorite 10% w/v, with three drops of Tween 20 (Aldrich) for thirty minutes. After this, the seeds were washed with sterile distilled water (120 °C, 1 atm., 25 min) x 4.

Germination seed: Germination seeds were placed in a glass Petri dish (90 mm Ø), previously sterilized (120ºC, 1 atm, 25 min), filled with 10 ml of solidified agar. After adding seeds, Petri dishes were sealed with Parafilm. Seeds were further incubated at 25ºC in a Memmert ICE 700 controlled-environment growth chamber, with a photoperiod of 16 h of light/8 h of darkness. Granulated agar was used free of microbial inhibitors (Merck) and basal nutrient solution (Hoaglands No.2, Sigma), to a pH of 5.5. The medium was prepared by dissolving 0.7 g of granulated agar in 100 ml of basal solution, autoclaved (1 atm, 120 °C, 20 min.) and discharged before solidification in Petri dishes.

MBOA quantitation by HPLC analysis

Seedling growth: The seedling growth was conducted with MBOA at 3mM Treatment or Hoaglands No.2 Sigma solutions (control).

MBOA extraction: At the end of each incubation time (treatment time: 1, 2, 3 and 6 days; time without treatment: 3 days) the weeds seedlings were washed three times in ethanol. For the extraction, a fresh sample of shoots and/or roots from 10 seedlings was used. This sample was extracted with 50 mL of solvent by maceration. The resulting extract was put in an ultrasound bath for 15 minutes at 25 ºC and then filtered (<11 µm). This procedure was repeated twice more with the solid material residue. The resultant solutions were collected, centrifuged, filtered (<0.22 µm) and concentrated to a volume of 3 mL for analysis by liquid chromatography after pre-purification in solid phase (Sec-Pak® C18, 400 mg MERCK).

MBOA in Solutions: To determine the MBOA released by the seedlings after treatment time (Tt), a sample of the culture solution in transplanted plants is analyzed by HPLC. The culture solution and the roots washing are centrifuged, filtered (<0.22 µm), concentrated and dissolved in 1 mL of methanol with 1% acetic acid for analysis by High Pressure Liquid Chromatography (HPLC) Merck HITACHI HPLC equipped with a LaChrom L-7100 quaternary gradient pump, an L-7455 LaChrom diode array detector, and an L-7200 LaChrom autoinjector. Data were collected and processed by using an HPLC Merck HITACHI D7000 data system. Instrument conditions for separation were Lichrospher 100 RP-18 (250cm, 4.0 mm, 5 µm) reversed-phase column at 25 °C. Mobile phases were water: 1% AcOH (A) and methanol: 1% AcOH (B) at a flow rate of 1 mL min. Injection volume was 50 µL. All analytical procedures were validated by means of inter-laboratory calibration study (14).

Bioassay Design

Absorption Studies for Seedling: For the growth of seedlings in MBOA inoculation studies, 10 seedlings were transferred (radicle length > 3 mm), to a transparent polycarbonate-polypropylene (Mangenta Vassel GA-7), with 10 mL of semisolid agar containing MBOA at a concentration of 3 mM. The controls were transplanted into the same growth containers, but without MBOA in a semisolid medium. At the end of the treatment time (2, 4, 6, 8 and 10 days), treated samples and controls were removed from the growth medium. Their roots were carefully washed with water and dried with filter paper. Shoots and roots are extracted with MeOH (1% v / v acetic acid) by ultrasound (x3) for subsequent HPLC analysis.

Absorption and Elimination of MBOA by Weed Seedling: Seedlings were cultured in a hydroponic system, using the same type of growth solution used in translocation studies in agar, using 3 mm-diameter glass beads as the culture medium in this case. The seedlings were treated with 10 mL of basal growth medium (Sigma-Hoaglands No.2) at pH 5.7, and MBOA at 3 mM concentration. The control samples were cultured in the same solution, but without MBOA. The seedlings were submitted to different treatment times (Tt), after this time, the plants were removed from the containers, their roots carefully washed with distilled water and sterilized, and then transplanted to another growth container with 10 mL of nutrient solution, and allowed to grow for another three days (Tst). Both controls and treated plants were process at the end of Tt for subsequent HPLC analysis.

Results and Discussion

A series of bioassays were conducted aimed at determining the capacity of absorption and translocation of allelochemicals in weeds, in order to determine whether significant differences of this property existed among species, and the degree of impact that this shows depending on the duration of treatment. Although there are studies on the metabolism of BOA by weed species, the detoxification capability of MBOA has not been studied. Phytotoxicity studies show a moderate to low activity of MBOA against *Echinochloa crus-galli* and Lolium rigidum, with values of inhibition of root and shoot of about 30% at a concentration of 1 mM for *Echinochloa crus-galli* and 7% and 60% of shoot and root, respectively, in *Lolium rigidum* at the same concentration, but these values decrease dramatically with dilution (8). Levels absorbed during root and shoot growths were measured, and the behavior of the levels measured related to the root/shoot ratio, in order to determine the ability of MBOA translocation of these species. MBOA was selected for this study, since it is a compound that shows moderate to low phytotoxic levels for *Lolium rigidum* and *Echinochloa crusgalli* (15); weed species that show a certain resistance to this compound. Besides this, MBOA, unlike BOA, has not been characterized in terms of its ability to be absorbed, translocate and eliminated by seedlings.

Qualitative analysis

MBOA occurrence in control of Weed Seedlings: No MBOA was detected in the untreated culture medium, in root organelles, and seedlings during the incubation process (figure 1). This confirmed that MBOA is not naturally synthesized by these weeds. Therefore, the MBOA quantities detected in target seedlings could be attributed completely to MBOA absorption. Moreover, MBOA is not detected in the analysis of the solution, confirming that the weeds do not produce MBOA through root exudates.

Absorption of MBOA by weeds: The chromatographic analysis conducted on the shoots and roots of the weeds, shows that MBOA can be detected in the seedlings. For *Echinochloa crus-galli* (Figure 1) was found that MBOA is located in the root, increasing during seedling development. Upon addition of MBOA, another peak in the chromatographic analysis appeared, of unknown structure, at 26 min. retention time.

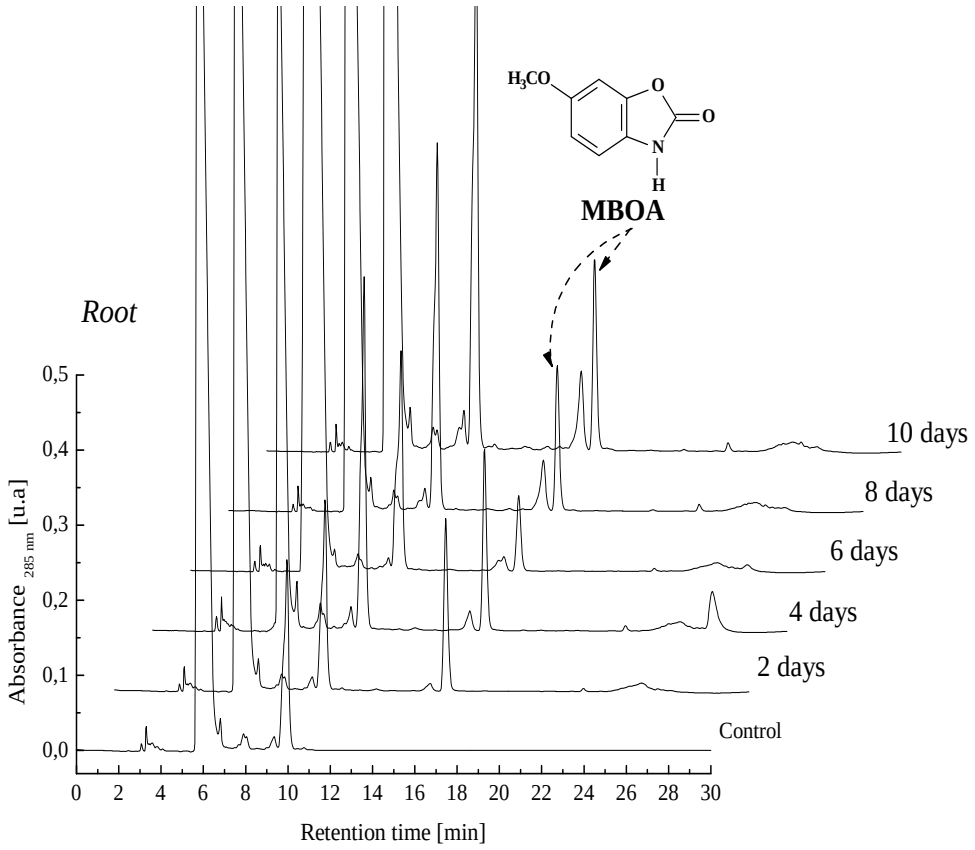

Figure 1. Chromatography analysis of root and shoot in *Echinlochloa cruss-galli* seedling at different times of incubation with 3mM **MBOA**

This fact is especially interesting in the case of Lolium rigidum L., where this peak is detected in both shoots and roots, being greater in the latter case.

Products of MBOA metabolism: Several products have been detected in root extracts of Vicia fava, Avena sativa, and A. fatua treated with BOA (11). For MBOA, methoxy-glycoside carbamate,1-(2-hydroxy-4-metoxyphenilamino)-1-deoxy-β-glucoside-1,2-carbamate, besides BOA-6-O-glucoside, has been found as products of biotransformation in *Zea mays* L. Other studies have detected other detoxification products by HPLC, primarily promoted by fungi. Comparison of the UV-vis spectra between MBOA and this new chromatographic signal, transformed MBOA (MBOA-tr), shows high coincidence between maxima and minima (figure 2), where a degree of structural similarity between the two compounds could be inferred, perhaps maintaining the same basic structure, with modifications due to detoxification reactions. This unknown signal also appears in the analysis of shoot and root extracts of *Lolium SLR31* resistant biotype. For the species studied, there is a similarity in the elimination of MBOA compounds, perhaps indicating a similar elimination mechanism for all three species.

MBOA detoxification: The dynamics of accumulation of MBOA-tr were studied in order to determine the differential ability of MBOA transformation. The detoxification of allelochemicals is a mechanism that operates in order to reduce or completely inactivate

their phytotoxic action, and this detoxification ability has been primarily concerned with the benzoxazolinones, and is a characteristic of the resistance of these species towards the phenomenon of self-toxicity. So, *Echinochloa crus-galli* has been cited as a species that synthesizes this compound, which makes it interesting to compare its ability to remove medium-provided MBOA, with *Loilium rigidum*, which shows no production of these compounds. Thus, the dynamics of transformation of MBOA for *Echinochloa crus-galli* and two biotypes of *Lolium* were studied, where active absorption of compounds from the culture medium is estimated, a pre-requisite for maximum expression of detoxification.

To carry out this comparative study the peak area for relationship MBOA/MBOA-tr is considered as the unit of measurement and it is normalized to the fresh weight of shoot or root. Figure 3 summarizes the results obtained by species of weed and organ of the seedling (shoot or root). These results verified the three species' ability to absorb the allelochemicals from the culture medium, and that the three species show mechanisms for metabolizing the allelochemicals. Considering that this product has the same chromatographic characteristics (retention time and ultraviolet spectrum), it is indicative of a common mechanism of MBOA metabolization for the three species studied.

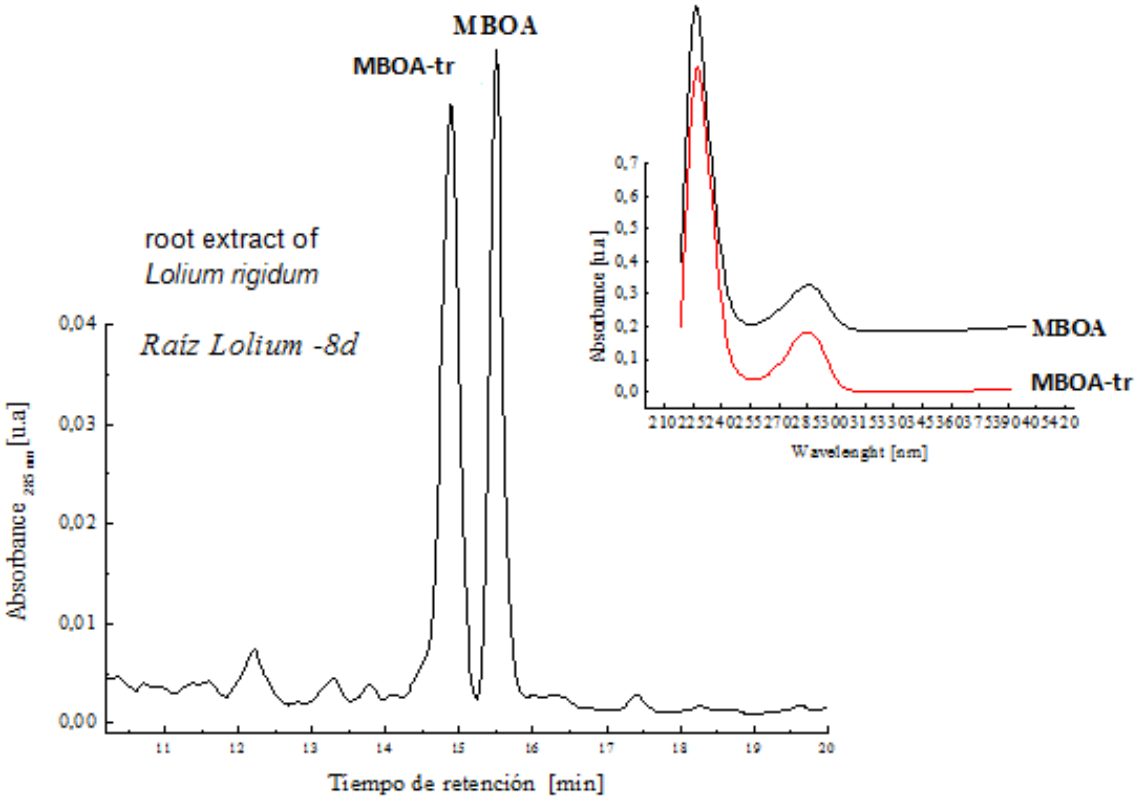

Figure 2. Chromatographic analysis of root extract of *Lolium rigidum* L. and comparative analysis of UV-Vis spectrum of MBOA and MBOA-tr. Conditions: 8 days of growth, 3 mM MBOA

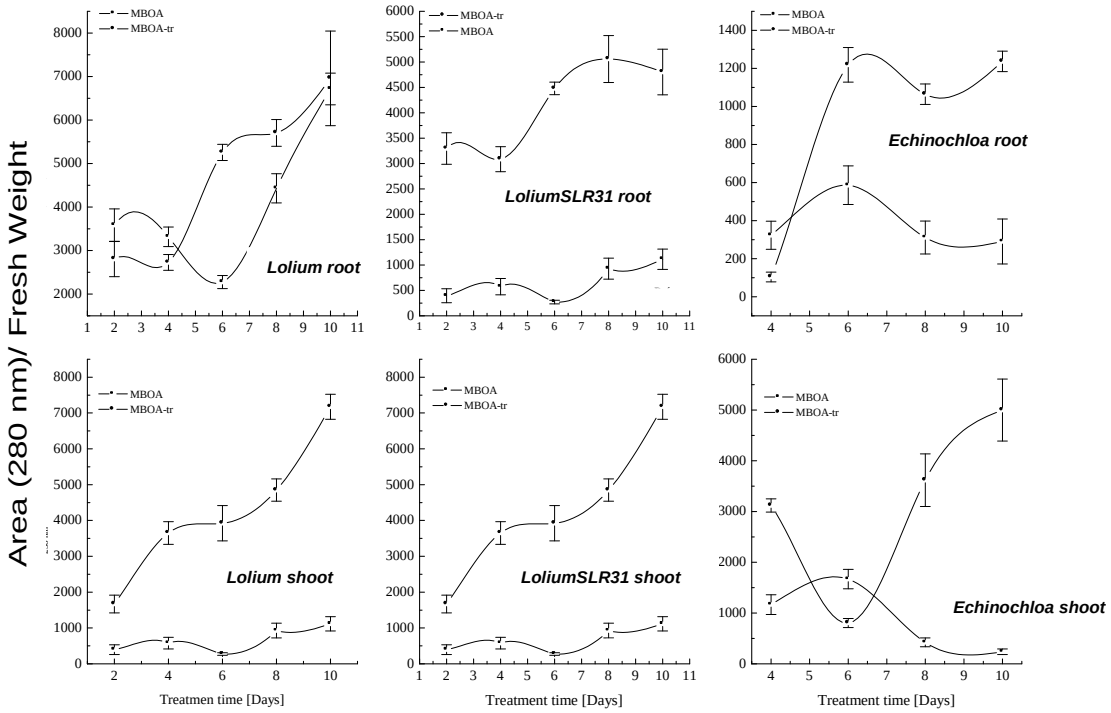

Figure 3. Levels of MBOA and MBOA transformed (MBOA-tr) according to weed species and organ of the seedling (root and shoot) at various treatment times. [MBOA]= 3 mM. Error bars represent standard deviation for n = 3

Two general characteristics are observed: first, MBOA-tr levels are higher than those of free MBOA in all species and organs. Second, the behavior over time is similar for both compounds. This similarity in the levels of both compounds over time may be due to the plants' capacity of absorption and transformation of the compound. Clearly, a constant concentration of MBOA, except for the root of *Lolium*, may be a consequence of rapid MBOA absorption from the culture medium. Although there are significant amounts of MBOA-Tr, this rate of absorption of MBOA allows its concentration to remain constant. Therefore, it can be concluded that the levels of MBOA and MBOA-tr, are a function of the absorption rate and the rate of the detoxification reaction. Interesting results were obtained for levels of both compounds in the roots of the two biotypes of *Lolium rigidum* L.: for its wild biotype comparable levels of both compounds were observed, while for the resistant biotype (SLR31), there was a marked difference between the levels found, MBOA-tr being much higher. This may be a consequence of the resistance acquired by this plant. Such resistance may be a consequence of an increased detoxification capacity, showing a higher rate of metabolism of the herbicide, in contrast with the wild biotype. However, the resistant biotype shows lower levels of MBOA than the wild biotype. An interesting case is also shown by *Echinochloa crus-galli*, where differences in levels of the two compounds were not found at two days of growth; in contrast, the process of MBOA detoxification increases with the growth of the seedling. This could indicate that, although the mechanism of detoxification is similar in all three species, in the case of *Echinochloa crus-galli*, the reaction shows slower kinetics, increasing with seedling development. Figure 4 shows the relationship between levels of

MBOA-tr and MBOA in the three species of weeds. For both wild and resistant (SLR31) *Lolium rigidum* MBOA-tr and MBOA levels show a similar behavior, while for *Echinochloa crus-galli*, an increase in MBOA-tr/MBOA ratio from the six days of growth is observed, verifying the increased detoxification of MBOA with the growth of the plant, being the species that shows the greatest detoxification capability. This result is consistent with the metabolic characteristic of *Echinochloa crus-galli*, which is cited as a weed capable of producing benzoxazolinones. It is logical that it presents an efficient mechanism of detoxification and/or inactivation of these compounds, as a measure to avoid the phenomenon of auto toxicity.

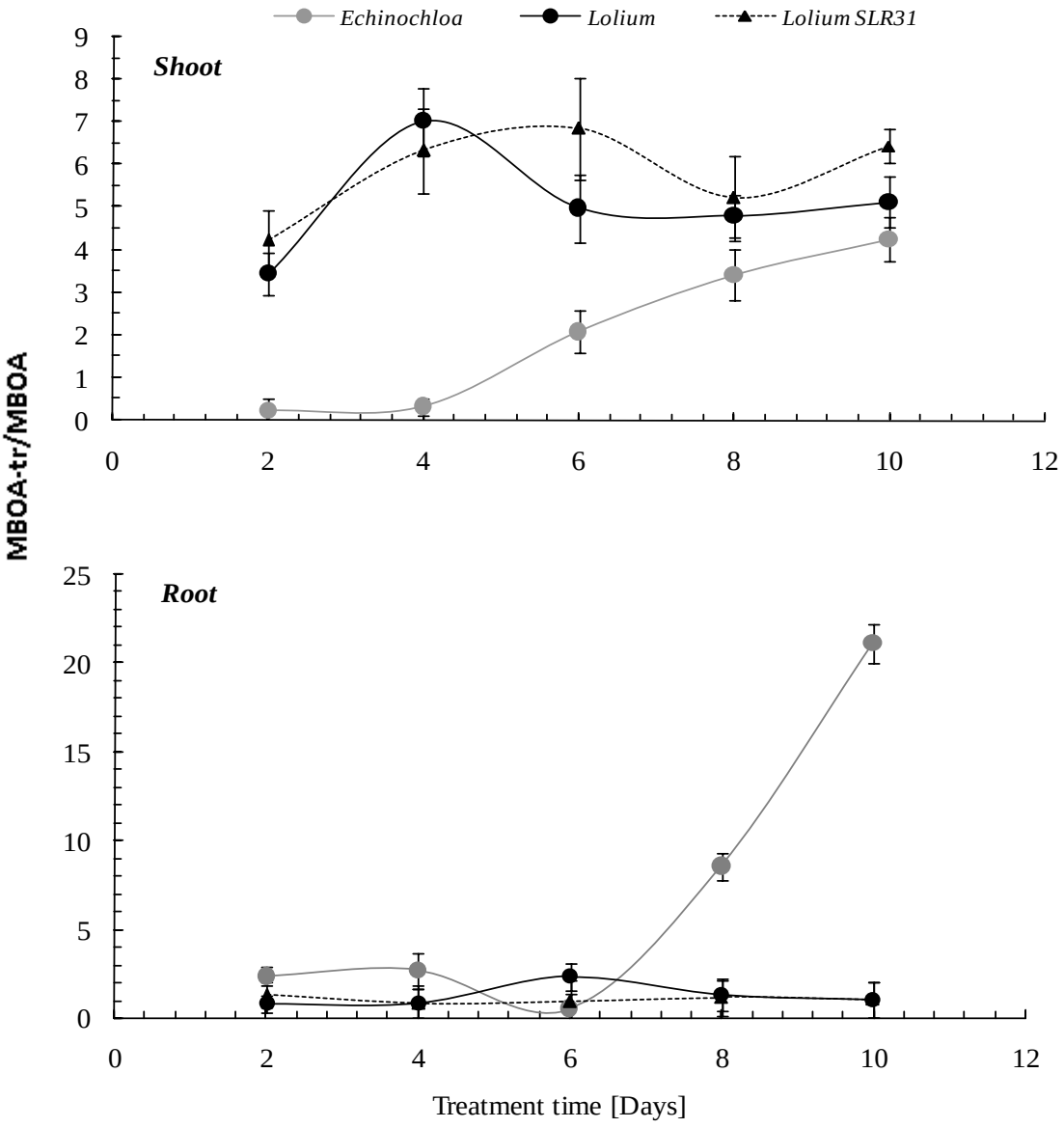

Figure 4. Relationship [MBOA-tr]/[MBOA] in root extract of *Echinochloa crus-galli*, *Lolium rigidum* L, and *Lolium rigidum SLR31* at various treatment times.[MBOA]= 3 mM. Error bars represent standard deviation for n = 3

MBOA translocation: The capacity of absorption and translocation of MBOA from the culture medium to a shoot is difficult to estimate, since there are many variables that take part in this process, including the concentration levels of compounds that are eliminated. However, the concentration of the compound in its free state is a good indicator to estimate the translocation ability. This balance was studied for MBOA content in shoot/root of three weeds at different times of treatment. The results are summarized in Figure 5. There is shown a behavior usually found in the dynamics of absorption of xenobiotics by plants (17), with an increase in concentration to relatively constant levels in the root, gradually increasing concentration in the shoot, although this behavior may vary depending on the species studied and the compound being absorbed. *Echinochloa crus-galli*, which has been the species with the greatest potential to detoxify BOA (18) shows the lowest concentration levels of these compounds, in both shoot and root; however, the concentration levels translocated to the shoot are interesting, although it is possible that the two factors, the amount absorbed and translocated, are correlated. An estimated capacity of translocation may be found from the levels of allelochemicals in shoot and root. As shown, all values of this ratio are smaller tan one, showing higher concentrations of compound in the roots than in shoots. Both Lolium sp biotypes increase this value with treatment time, it being higher in the wild biotype, while for Echinochloa crus-galli this ratio remains relatively constant, except at one day of treatment. The variations in the concentration of allelochemicals in shoot and root can result from several factors, but generally the capacity to absorb nutrients is an intrinsic property of the plant. However, at different times of treatment, the phytotoxic effects of the absorbed agent can modify the measured values of free MBOA. So, the species of weed that shows a higher degree of affectation by MBOA, will have less detoxification capability of the compound, increasing the concentration of free MBOA. Allelochemical concentration measured at the root, and the shoot is a time-dependent phytotoxic effect. It is generally considered that maximum rates of absorption and detoxification are measured during the early stages of development of the plant (17). Given this fact, the values of this ratio for different weeds for a treatment time of one day (Figure 5, bar graph) were compared. According to the values obtained, *Echinochloa crus-galli* shows the highest ratio [MBOA] shoot / [MBOA] root, indicating that this species is showing a greater capacity for translocation of MBOA from the root to the shoot.

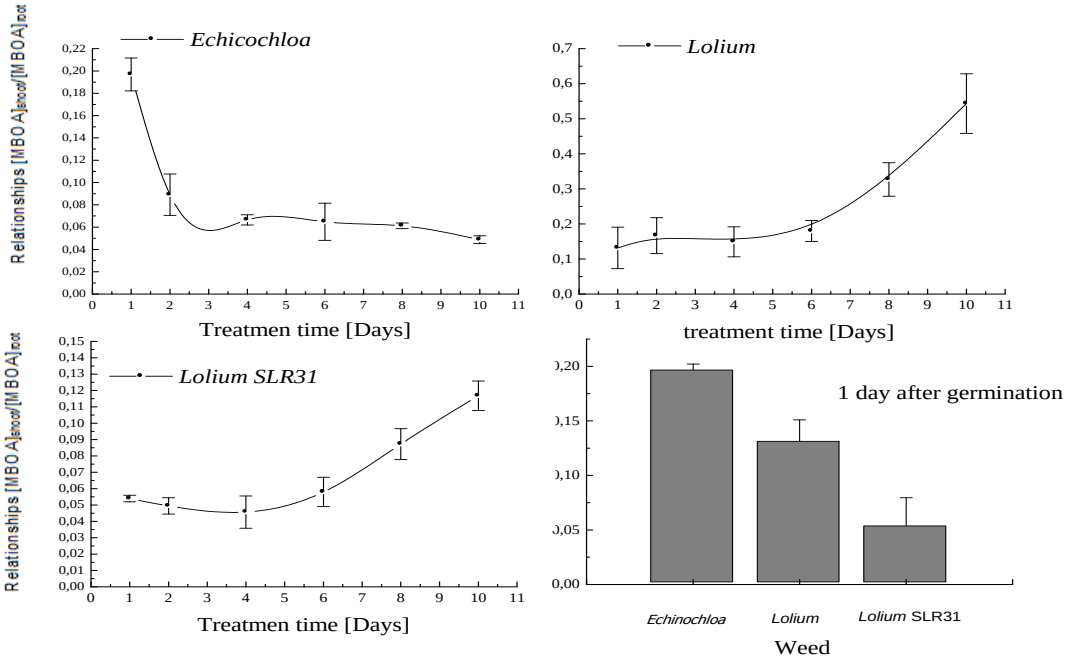

Figure 5. Relationships $[MBOA]_{shoot}/[MBOA]_{root}$ ratio during treatment time with 3 mM MBOA and comparative levels for one day of treatment (Bar Graph). Error bars represent standard deviation for n = **3**

Uptake and elimination of MBOA vs. time: Studies on the transformation of BOA by seedlings have included analysis of whole plants, not discriminating between shoot and root and, in other cases, only the root is considered, where some results show that is at the root where the detoxification is carried out. In the present study, the determination of endogenous levels of free MBOA was considered, as in previous translocation studies. The inoculated allelochemical is detected in both the shoot and the root and, under these conditions; it can be detected after three days of growth without treatment. Qualitatively, after this period of plant growth without MBOA in the culture medium, the endogenous levels of free MBOA clearly decrease both in the shoot and in the root. A similar result is observed for a maximum period of treatment (Tt) of 6 days, showing a decrease of the corresponding chromatographic signal of the inoculated allelochemicals in seedlings growing without treatment, as compared with those growing with treatment (growth conditions Tst and Tt, respectively). The comparative analysis of endogenous levels of MBOA formed at different treatment times (Tt), shows a growing accumulation of compound (Figure 6), both in shoot and root, up to a Tt of three days, noting a decline from the 3-day peak until the end of the six-day treatment. This acquires a relevant interest, as these conditions of progressive accumulation of MBOA, lead us to suppose that during the time of growth after transplanting (Tst), plants contain different levels of endogenous MBOA, so it is possible to estimate the potential for transformation of MBOA in the three weed species. Thus, it is important to perform experiments at various times of treatment, in order to estimate this capacity for detoxification, to different degrees of affectation of the seedlings, especially comparing this ability between different species.

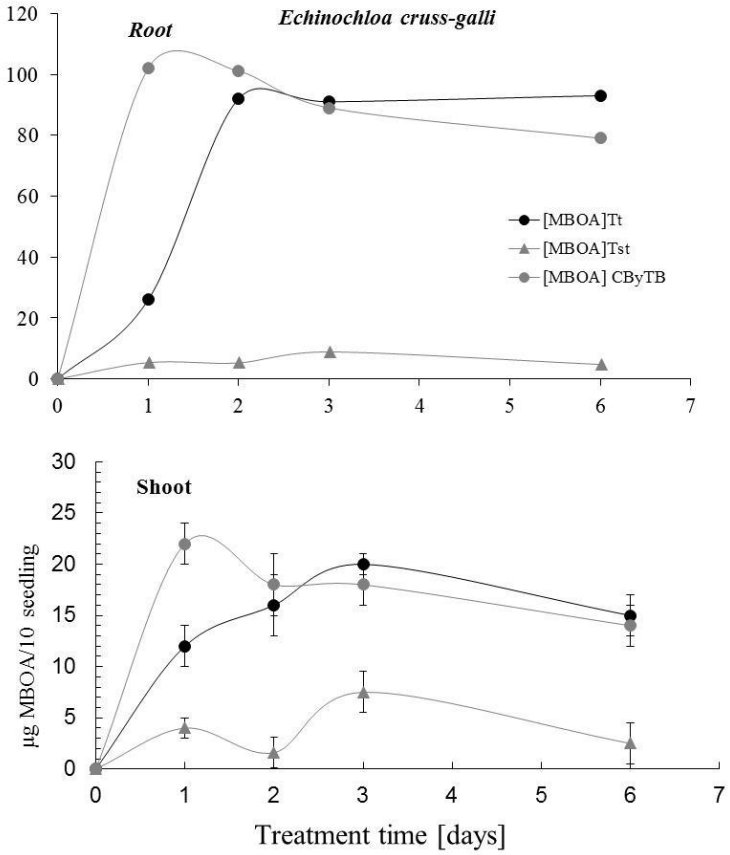

Figure 6. **MBOA** absorbed levels determined by HPLC in *Echinochloa crus-galli* (shoot and root) at different treatment times (Tt), time without treatment by transplantation (Tst) and TB samples (treatment time= Tst + Tt, continuous treatment). Error bars represent standard deviation for n = 3

Uptake and Elimination: An experimental design was carried out aimed to estimate the capacity of MBOA transformation into MBOA-tr, in presence and in the absence of the allelochemical. For this, the design includes the treatment of seedlings for a time (treatment time, Tt), then the treatment is suspended, by transplanting the plants into a growth medium without the allelochemicals, and the subsequent analysis after a second period of growth time (time without treatment, Tst). Thus, varying Tt, at constant Tst, it is possible to determine phytotoxic activity, resistance of the plant with a suspended treatment, and the amount of MBOA detoxified. The amount of MBOA metabolized, can be calculated using the concentration levels measured during the growth of the plant at times Tt and Tst, according to:

$$[MBOA]_e = [MBOA]_{T_t} - [MBOA]_{T_{st}} \quad (1)$$

Where = Concentration of endogenous MBOA eliminated by the plant during time Tst; = Concentration of endogenous MBOA in the plant at treatment time Tt; = Concentration of endogenous MBOA in the plant at time without treatment Tst. [MBOA]Tt increases with time up to Tt = 3, then remains constant until the sixth day of growth. Moreover, [MBOA] Tst shows a consistent behavior over time and lower concentration levels than those measured for Tt. The concentration levels found for TB seedlings ([MBOA] CB and CT) show a similar behavior for [MBOA] Tt, with higher levels of MBOA up to Tt = 3 days of growth, to reach values similar to the sixth day. This behavior is observed in both shoot and root, with the highest concentration levels in the root. For the two biotypes of Lolium, the same behavior is observed, with differences between the two biotipes in terms MBOA levels in roots, where plants in TB, the wild biotype, contain an MBOA concentration higher than the resistant biotype SLR31. Moreover, just as was obtained for *Echinochloa crus-galli*, the concentration levels remain relatively constant after a Tt of three days. In case of plants growing in TB conditions (continuous allelochemical treatment with no transplant), they reach this constant level after four days of treatment (= Tt + Tst). Quantities of MBOA metabolized by the plant can be estimated from Eq-1 as the difference between the levels of endogenous MBOA under the growth conditions in Tt and Tst periods. The estimated amount of MBOA varies depending on treatment time (Tt). The three species were found to increase this amount up to Tt=3 days and remained relatively constant for *Echinochloa crus-galli* and *Lolium rigidum SLR31*, decreasing for *Lolium rigidum* wild biotype after six days of treatment. According to the calculated levels, *Echinochloa crus-galli* is the species with the highest potential for elimination of MBOA, and this is reasonable, given that this weed produces hydroxamic acids in its tissues. Thus, this weed can provide all the enzymatic mechanism for a lot more tolerance for this type of compound. For the two biotypes of Lolium, was found that the wild species showed higher initial values of eliminated MBOA, but, finally, after 6 days of treatment, it showed the lowest recorded capacity to metabolize it. In this case, there is a strong dependence of the detoxified compound concentration levels with treatment duration, and thus, with the endogenous MBOA concentration after transplant and the degree of affectation to the plant. In general, a slower rate of growth and development is characteristic of the resistant biotypes, so that required nutritional levels are lower, and therefore, the amount absorbed is smaller. The degree of impact is also lower, the seedlings maintaining greater vitality. Thus, the resistant biotype of Lolium, has a higher MBOA elimination capacity at longer times of treatment (Tt =6 days). This argument can also be applied to the case of *Echinochloa crus-galli*: it shows the slowest rate of growth of all three weeds. For example, shoot weight values after three-day growth were 144.2 ± 2.3; 121.3 ± 3.2 and 91.8 ± 2.5 mg (n = 10), for *Lolium*, *Lolium SLR31* and *Echinochloa crus-galli* respectively. This slower rate of growth, together with the characteristic ability of this species to detoxify MBOA, may explain its high detoxification capacity.

Conclusions

These results indicate that the seedlings show signs of recovery, if they are transplanted into a growth medium without test compound. This is an important result because it is a necessary condition for estimating

the ability to remove the endogenous levels of the compound by the seedling. Although phytotoxic effects are observed, seedlings are viable after treatment, which is a requirement for studies of dynamic processes of detoxification. Echinochloa crus-galli and two biotypes of *Lolium rigidum* were evaluated for their absorption, translocation to shoot, and absorbed compound elimination ability. The results show that these species can absorb and translocate to the shoot MBOA inoculated in the growth medium, where usually there are differences in the ability to translocate and metabolize the absorbed compound, depending on the species, as has been previously observed regarding the detoxification ability. Of the three species tested, *Echinochloa crus-galli* emerges with the highest capacity for translocation and removal of MBOA, and this difference is dependent on length of time of treatment. HPLC analysis shows the presence of products which are formed by structural changes of the inoculated compound, where studies of UV spectra and Tr, suggest a structural similarity. The analysis shows that the seedling, especially Echinochloa crus-galli has the ability to transmit to the environment, through root exudation, the compound previously absorbed, showing therefore another route of elimination of xenobiotics, in addition to detoxification. Experimental designs are currently being developed with the aim of structurally characterize the products MBOA transformed.

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