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Physiological, biochemical and growth responses of Italian ryegrass to butachlor exposure

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ABSTRACT

A dose–response experiment was conducted to examine the effects of butachlor on the growth, physiology and biochemistry of Italian ryegrass. The plant suffered a >50% reduction in fresh biomass when exposed to a butachlor dose of 5 mg L^{-1}. However, a significant further biomass reduction only occurred when the dosage level of butachlor was increased to 40 mg L^{-1}. The root was more sensitive than the shoot to butachlor toxicity. It appears that the inhibition of fine root development under butachlor stress was the upstream cause for the retarded plant growth. A butachlor dose of 5 mg L^{-1} was sufficiently high to cause significant H_2O_2-induced oxidative damage in the plant cells, as indicated by the increased MDA and the lower production rate relative to consumption rate of CAT. The plants tended to maintain sufficiently high levels of root activity and photosynthesis, and possibly relied on these mechanisms for survival in such a stressed environment. Butachlor had a stimulatory effect on the release of dissolved organic carbon but not amino acids from the plant roots. There were 5 types of organic acids in the root exudates, which all exhibited a trend to decrease with increasing level of butachlor.

1. Introduction

Butachlor (*N*-(Butoxymethyl)-2-chloro-*N*-(2,6-diethylphenyl)acetamide) is a selective systemic herbicide that has been widely used since 1970's to control annual grass and broadleaf weeds in paddy rice fields [1,2] and dryland crop systems [3,4], as well as submerged macrophytes in freshwater fish ponds [5]. Like other agricultural herbicides, butachlor poses a threat to non-target biota in the affected areas [6–11]. In areas where butachlor has been used over an extended period of time, the weeds tend to develop resistance to the herbicide. To achieve the effective weed control goals, farmers usually increase the dose of herbicide application. This invariably results in increased concentration of residual herbicide in both soil pore water and draining water, which poses a significant threat to the adjacent aquatic ecosystems.

Italian ryegrass (*Lolium multiflorum* Lam.) is a herbaceous annual or biennial grass that is grown for silage and as a cover crop. It can be used as a winter rotation crop in rice production areas [12]. Ryegrass-rice rotation has the potential effects on improving soil structure, minimizing non-point pollution of nitrogen during the period between two rice crops and enhancing nutrient supply for the subsequent rice crop [13–16]. It has been demonstrated that the growth of ryegrass enhanced the degradation of certain herbicides in the soils [17–19]. Some researchers also found that ryegrass can be used to remove water-borne pollutants in a constructed macrophyte floating bed system [20–22].

Biodegradation is an important pathway for elimination of organic pollutants in the environments. It has been demonstrated that plant growth enhances in-soil degradation of butachlor in the rhizosphere [23,24]. Phytoremediation of butachlor represents a potentially cost-effective method for the clean-up of butachlor-contaminated environments. Understanding the tolerance of a candidate plant for butachlor toxicity is necessary for evaluating its suitability for being used as a bio-remediating plant species.

While conventional biotoxicity data are available in pesticide database (e.g. http://www.pesticideinfo.org/), detailed research is limited to certain organism groups such as amphibians, fish, crustaceans, green algae and microbes [11,25–29]. Currently the available information on the phytotoxicity of butachlor is scarce. In this study, we used Italian ryegrass as a test organism to examine the physiological, biochemical and growth responses of this plant species to butachlor exposure. The main objective was to establish a dataset to systematically show the biotoxicity of butachlor to an example of vascular plants. This information also has implications for further study to explore the possibility of using Italian ryegrass as a bio-remediating plant species in soil and water environments that are heavily contaminated by

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butachlor such as in the areas when excess amounts of butachlor were applied or at butachlor spill sites.

2. Materials and methods

2.1. Materials

The seeds of *L. multiflorum* Lam were purchased from the Guangdong Academy of Agricultural Sciences. Prior to the experiment, the seeds were germinated in a biological incubator with the temperature set at 20 °C during the night time and at 25 °C during the day time. The daily light exposure period was set at 16 h. After 4 days of incubation, the healthy seedlings were selected for the experiment.

All the organic acid standards used for gas chromatography analysis in this study were the products of SUPELCO.

Hogland nutrient solutions were used as the liquid growth media for the experiment. The ingredients of the Hoagland nutrition solutions used in the experiment were provided in Table 1.

2.2. Experimental design

Given that butachlor can be strongly adsorbed by soil colloids and therefore it is difficult to quantify the concentration of bioavailable butachlor in a soil system, a solution culture experiment was chosen for better examination of the dose–response relationships between butachlor and various test parameters. Since the main objective was to generate a phytotoxicity dataset for butachlor, the dose range of butachlor was set at levels that are likely to cause acute phytotoxicity of the plant based on the pre-experimental tests. One control (no added butachlor) and four treatments were set for the experiment: (a) Treatment 1 (T1): 5 mg L^{-1} of butachlor; (b) Treatment 2 (T2): 10 mg L^{-1} of butachlor; (c) Treatment 2 (T3): 20 mg L^{-1} of butachlor; and (d) Treatment 4 (T4): 40 mg L^{-1} of butachlor. The experiment was in triplicate.

A black plastic cup (height: 12.5 cm; inner diameter of the top: 8.6 cm; and inner diameter of the bottom: 6.0 cm) was used to contain 300 mL of the liquid growth medium. Each cup was covered by a piece of black foam board with 10 holes to support 10 seedlings. The 10 seedlings in each cup were allowed to initially grow in deionized water for 2 days, followed by growing in Hogland Solution A for 3 day. The liquid growth medium was then changed to Hogland Solution B to allow the plants to grow for 7 days before adding the butachlor into the liquid growth medium in each cup. The plants were exposed to butachlor for 12 days prior to harvest.

During the entire period of the experiment, the plants were allowed to grow in a biological incubator with the temperature set at 20 °C during the night time and at 25 °C during the day time. The daily light exposure period was set at 16 h.

Table 1
Chemical composition of the Hoagland nutrition solutions used for the liquid culture experiment.

Chemical	Solution A	Solution B
$Ca(NO_3)_2{\cdot}4H_2O$	0.59 g/L	1.18 g/L
$MgSO_4{\cdot}7H_2O$	0.25 g/L	0.49 g/L
KH_2PO_4	0.07 g/L	0.14 g/L
KNO_3	0.26 g/L	0.51 g/L
H_3BO_3	2.86 mg/L	2.86 mg/L
$MnCl_2{\cdot}4H_2O$	1.81 mg/L	1.81 mg/L
$ZnSO_4{\cdot}7H_2O$	0.22 mg/L	0.22 mg/L
$CuSO_4{\cdot}5H_2O$	0.08 mg/L	0.08 mg/L
EDTA–Fe–Na	8.42 mg/L	8.42 mg/L
$(NH4)_6Mo_7O_{24}{\cdot}2H_2O$	0.09 mg/L	0.09 mg/L

2.3. Extraction and condensation of root exudates

Extraction of root exudates was performed at harvest. The roots of the 10 plants from the same cup were soaked in 500 mL of 0.5 mmol L^{-1} $CaCl_2$ that were contained in a black cup after removing the endosperm with the plants and gently rinsing the roots with deionized water. Collection of root exudates lasted for 4 h. The root exudate-containing solution was passed through an ion exchange resin column containing 5 g of anion exchange resins (201 × 7, Type 717, Shanghai Zhanyun Chemicals Ltd.) in the lower part of the column and 5 g of cation exchange resins (001 × 7, Type 732, Shanghai Zhanyun Chemicals Ltd.) in the upper part of the column. The root exudates retained by the anion exchange resins were extracted by 10 mL of 1 mol L^{-1} HCl. The HCl extracts were then condensed on a rotary evaporator (with temperature set at 40 °C) to nearly dryness. After adding 5 mL of deionized water to dilute the exudates, the solution was passed through a 0.45 μm membrane filter. The filtrates were stored in a fridge at 4 °C prior to analysis.

2.4. Analytical methods

2.4.1. Plant growth parameters

Upon harvest, the shoot portion and root portion of the plants from each cup were separated, washed with deionized water and surface-dried with moisture sorbents (filter papers) prior to weighing to obtain the fresh biomass of both portions. Total root length (TRL) of fresh plant samples was determined using an STD 1600 flatbed scanner (Seiko Epson Corporation, Japan), coupled with the WinRHIZO 3.1 computer software (Regent Instruments, Inc., Quebec, Canada).

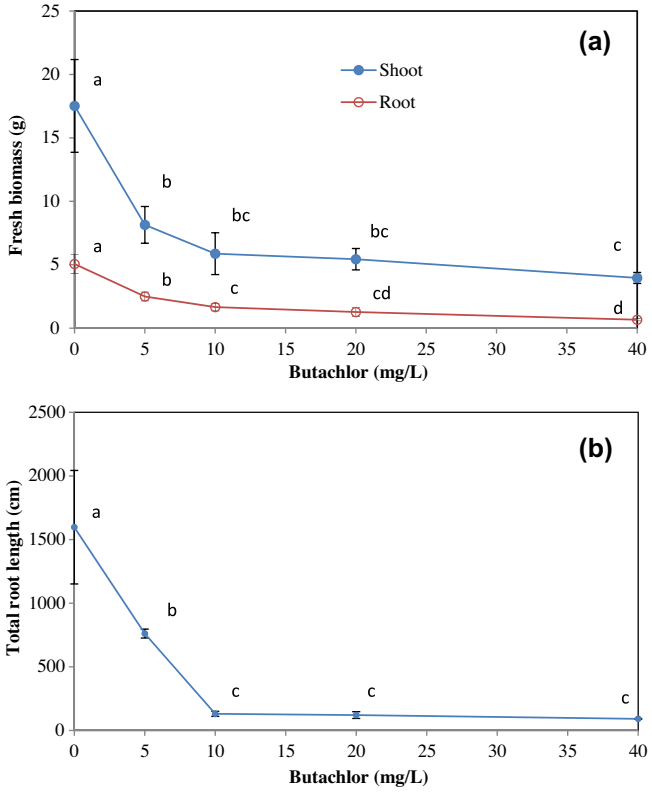

Fig. 1. Comparison of (a) fresh biomass, and (b) total root length of the Italian ryegrass among the control and various treatments. Different letters indicate statistically significant ($P < 0.05$) difference.