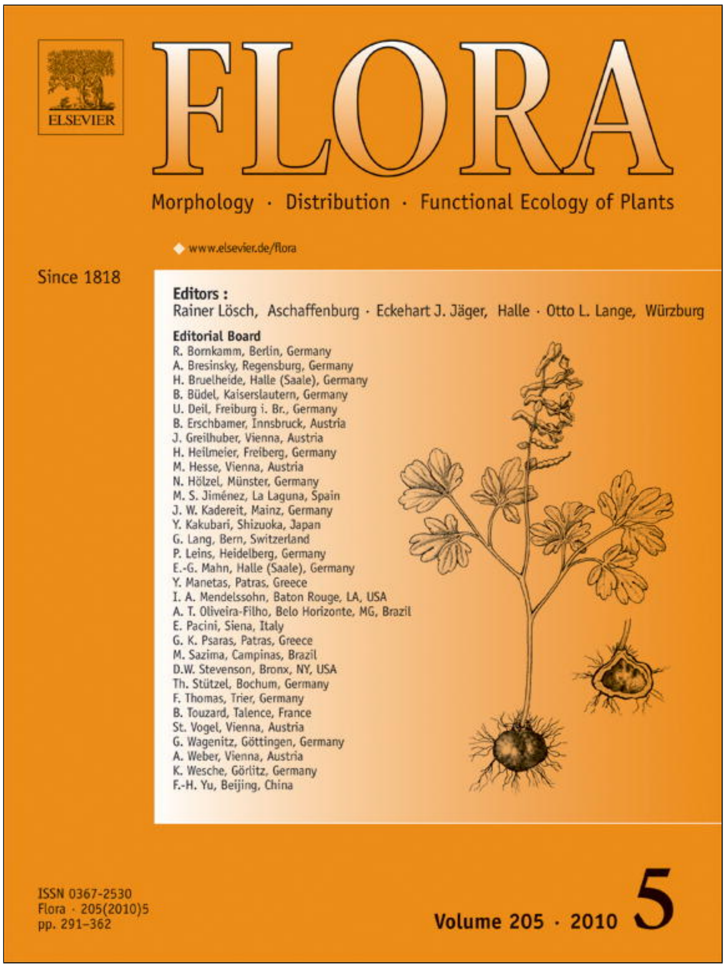
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Allelopathic potential of *Bothriochloa laguroides* var. *laguroides* (DC.) Herter (Poaceae: Andropogoneae)

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ABSTRACT

Bothriochloa laguroides var. *laguroides* (DC.) Herter, a native bluestem of America, has been shown to produce many biologically active compounds. The allelopathic potential of aqueous extracts from roots, stems and leaves was examined. Lettuce seeds (*Lactuca sativa*) and maize (*Zea mays*), the common allelopathy bioassay systems, as well as seeds from two native species, wintergreen paspalum (*Paspalum guenoarum*) and lovegrass (*Eragrostis curvula*), were germinated in the presence of aqueous extracts. Percent seed germination, root and shoot elongation were measured. After 4 and 7 days root, stem and leaf extracts caused inhibition of root and shoot elongation in all four species tested. Aqueous extracts were generally less inhibitory to seed germination. Aqueous extracts from different parts of *B. laguroides* var. *laguroides* show therefore allelopathic effects inhibiting, in particular, growth of competing plants.

Introduction

Bothriochloa laguroides var. *laguroides* (DC.) Herter (Poaceae: Andropogoneae) is a common native bluestem of America. This species is perennial and can be found growing on the prairies from North America and South America (Vega, 2000). The plants generally are caespitose, culms 30–100 cm long, leaf blades 3–20 cm long, glabrous; the 6–10 cm long inflorescences are oblong-lanceolate. Chasmogamous and cleistogamous spikelets coexist in the same panicle, and in the Southern Hemisphere anthesis occurs from November to May.

B. laguroides var. *laguroides* produces essential oil, rich in sesquiterpenes and monoterpenes. The major oxygenated sesquiterpenes are the *E*, *E*-farnesol and hexadecene (Scrivanti et al., 2009). Among the found hydrocarbons, n-dodecane and 1-tetradecene had the highest presence.

In spite of several reports about volatile components in different species of *Bothriochloa* (Bhandari et al., 1993; De Wet and Scott, 1965; Kaul and Vats, 1998; Melkani et al., 1984; Pinder and Kerr, 1980; Scrivanti et al., 2009; Zalkow et al., 1980), only one study of their allelopathic potential has been published (Hussain et al., 1982). Nothing is known of the role of allelopathy in the normal development of communities with *B. laguroides* var. *laguroides*, nor whether a greater variability in chemical composition of individual plants plays an evolutionarily significant role. Visual observations of wild populations suggest that this variability may influence interactions between *B. laguroides* var. *laguroides* plants and potential competitors (Scrivanti, 2007). In order to demonstrate that allelopathic potential exists in this species, a series of experiments was conducted testing for the allelopathic potential of *B. laguroides* var. *laguroides*.

Material and methods

Plant material

Plants of *B. laguroides* var. *laguroides* were collected in April 2008 from Province of Córdoba, Argentine. Vouchers specimen (*Scrivanti and Nagahama 308, 318*) are on deposit at the Herbarium of the Museo Botánico de Córdoba (CORD). The plants were separated into stem, leaf, and root portions and air dried. Water extracts were produced and examined to determine the allelopathic potential of the different plant parts.

Seed of test plants

Seeds of four species, lettuce (*Lactuca sativa* L.), maize (*Zea mays* L.), lovegrass (*Eragrostis curvula* (Schard.) Nees) and wintergreen paspalum (*Paspalum guenoarum* Arechav.) were used for germination assays. Lettuce and maize function as standard test plants because they are recognized as a sensitive and reliable species commonly used in bioassays (Lin et al., 2004; Scrivanti et al., 2003; Shafer and Garrison, 1986). The other two grasses coexist with *B. laguroides* var. *laguroides* and are supposed to be affected by allelopathic substances from bluestem grass.

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Germination of these seeds was tested before use and ranged from 80% to 100%.

Aqueous extracts

Five grams of dried culms, leaf-blade and root materials from each *B. laguroides* var. *laguroides* plant were coarsely cut with a razor and gently crushed using a mortar and pestle to create openings in the surface tissues. Each sample was placed in a tube containing 50 ml of deionized water and the mixture was kept in a refrigerator for 2 h and then stirred on a rotary shaker for 1 h and centrifuged at 1500 rotations min^{-1} for 15 min. The supernatant was recovered and stored in a refrigerator until it was used as a crude water soluble extract.

Bioassay

To evaluate the allelopathic effect of water soluble extracts, 25 seeds of lettuce, maize, lovegrass and wintergreen paspalum were placed in each Petri dish, which contained two layers of filter paper moistened with 4 ml of aqueous *B. laguroides* var. *laguroides* extracts. The Petri dishes were maintained at 23 °C under low light for 7 days. Plates were illuminated with 300 μmol photons $m^{-2}\,s^{-1}$ photosynthetically active radiation (PAR), provided by fluorescent lamps. The experiments were conducted in the greenhouse for 7 days and the treatment was replicated three times.

Controls

Seeds were placed in Petri dishes on filter paper, moistened with 4 ml of deionized water, and the dishes were maintained under the conditions previously described.

Growth measurements

Three days after seeding, germinated seeds were counted and root and shoot lengths were measured and recorded for the lettuce, maize, lovegrass and wintergreen paspalum plants. At the 7th day, root and shoot lengths were measured again. Percentages of growth inhibition of root and shoot lengths under the influence of the aqueous *Botriochloa* extract were calculated from the equation:

$$I(\%) = [1 - T/C] \times 100$$

where T=length of treatment organs (mm) and C=length of control organs (mm). The experiments were conducted using a randomized complete block design with three replications. Analysis of variance 25 of the data was made using ANOVA with InfoStat program version 1.1 (Group InfoStat, 2002). Differences between main effects were compared using Duncan's multiple range option of the program, with $P < 0.05$ used as the level of acceptance.

Results

Effect of aqueous extracts of root, stem and leaf on seed germination

The inhibitory activity of water soluble compounds from *B. laguroides* var. *laguroides* on seed germination of lettuce, maize, lovegrass and wintergreen paspalum is shown in Table 1. The root and stem aqueous extracts significantly reduced germination of maize and wintergreen paspalum ($P \leq 0.05$). However, no significant reduction in seed germination was seen in lettuce and lovegrass. A maximum reduction occurred with root extracts, which inhibited germination more in wintergreen paspalum than in the other plants tested at day 3 of the experiment. Nevertheless, seven days after seeding some wintergreen paspalum seeds germinated. The leaf aqueous extract significantly reduced germination of lettuce, maize and wintergreen paspalum ($P \leq 0.05$). However, no significant reduction in seed germination was seen in lovegrass.

Effect of aqueous extracts of root, stem and leaf on root length

The inhibitory effect of aqueous extracts from *B. laguroides* var. *laguroides* on root growth is shown in Table 1. Root growth of lettuce, maize, lovegrass and wintergreen paspalum seedlings was

Table 1
Effects of aqueous extracts (AE) of *Bothrichloa laguroides* var. *laguroides* on seed germination and root (R) and shoot (S) growth of lettuce, maize, wintergreen paspalum and lovegrass.

Treatment	Seed germination (%)[a]	Inhibition (%)[a]			
		3 day		7 day	
		R	S	R	S
AE Root					
Lettuce	100	52.40*	62.38*	41.35*	32.26*
Maice	20.00*	86.76*	100*	3.85	42.00*
Wintergreen paspalum	0*	100*	100*	48.44*	62.45*
Lovegrass	90.00	68.60*	65.08*	38.53*	8.33
AE Stem					
Lettuce	87.88	55.29*	56.93*	46.12*	35.48*
Maice	60.00*	44.85*	100*	32.02*	56.20*
Wintergreen paspalum	16.22*	89.68*	95.73*	14.58	64.26*
Lovegrass	90.00	58.45*	55.55*	48.62*	4.76
AE Leaf					
Lettuce	75.00*	53.36*	50.99*	42.54*	23.66*
Maice	26.67*	78.67*	100*	34.75*	85.95*
Wintergreen paspalum	7.00*	94.84*	100*	26.04*	54.62*
Lovegrass	85.00	53.14*	72.22*	32.41*	0

*Indicate values significantly less than the respective control ($P \leq 0.05$).

[a] Data are expressed as percentage means from experiments with three replicates of 25 seeds each.

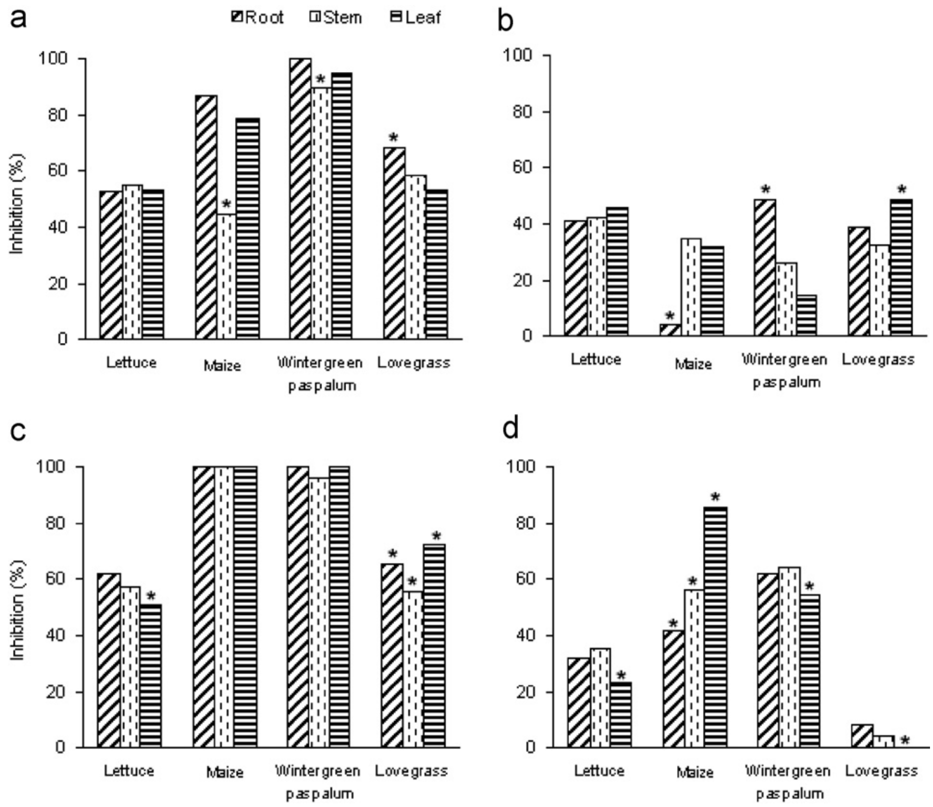

Fig. 1. Inhibitory effects of aqueous extracts of plants parts – root, stem and leaf – from *Bothriochloa laguroides* var. *laguroides* on lettuce, maice, wintergreen paspalum and lovegrass root and shoot growth. (a) Root growth to 3 days, (b) root growth to 7 days, (c) shoot growth to 3 days, (d) shoot growth to 7 days. Data are the means of three replicates. * indicates values significantly different from each other according to Duncan's multiple range test at $P \leq 0.05$ (n=25).

visibly affected by *B. laguroides* var. *laguroides* root, stem and leaf extracts after 3 days from start of the experiment. After 7 days, aqueous extracts of root, stem and leaf were effective reducing ($P \leq 0.05$) the root length at least of some of the assay plants (Fig. 1). However, reduction in root growth was not significant in maize and wintergreen paspalum treated with root and stem aqueous extracts, respectively.

Effect of aqueous extracts of root, stem and leaf on shoot length

The effects of the water soluble extracts on shoot elongation were similar to those seen in the root elongation tests (Table 1). The aqueous extracts inhibited ($P \leq 0.05$) shoot elongation in all four test species after 3 days from start of the experiment (Fig. 1). After 7 days, no significant reduction was detectable in love grass shoot growth treated with the three aqueous extracts.

Discussion

The results indicated that aqueous extracts of *B. laguroides* var. *laguroides* plants contain indeed growth inhibitors that are capable of reducing the growth of lettuce, maize, wintergreen paspalum and lovegrass. Several secondary metabolites have been isolated from *B. laguroides* var. *laguroides*, such as sesquitepenes, monoterpenes, esters and hydrocarbons (Scrivanti et al., 2009) among them are *E, E*-farnesol, hexadecane, n-dodecane and 1-tetradecene. The majority of these compounds have been reported to function as allelopathic substances (Rice, 1984). The reduction in seedling root and shoot length may be attributed to the reduced rate of cell division and cell elongation due to the presence of the allelochemicals (Javaid and Anjum, 2006). Production of a wide range of secondary products of *B. laguroides* var. *laguroides* plants has been shown to be characteristic of the species and has been used as a tool in the taxonomic identification of populations of this species (Scrivanti et al., 2009).

Results of this study showed that the stem extracts were less inhibitory on root growth of test plants than root and leaf extracts, and the inhibitory effect was more important after three days, decreasing to 7 days (Fig. 1). On the other hand, after three days the three extracts did not show significantly different inhibitory effects on shoot growth, except for leaf and stem extracts in lettuce and lovegrass, respectively (Fig. 1). The inhibitory effect on shoot growth was more important after 3 days as compared with the situation after 7 days. In addition, the results for root and shoot growth inhibition indicated that the inhibitory effects of all three extracts generally were greater on shoot than on root growth (Table 1, Fig. 1).

Different plant tissues such as leaves, stems and roots can release different amounts of allelochemicals into the surrounding environment (Chung et al., 2000; Miller, 1983). Nevertheless, Hao et al. (2007) demonstrated that the various plant parts of watermelon (root, stem and leaf), although having different physiological functions, showed no significant differences in the bioassay tests. In the present study, the aqueous root, stem and leaf extracts showed no significant differences on lettuce root growth during the experiment (Fig. 1). Nevertheless, significant differences could be observed among the parts of *B. laguroides* var. *laguroides* affecting root and shoot growth of plants tests during the experiment. The chemical composition of aqueous extracts from different parts (root, stem and leaf) of *B. laguroides* var. *laguroides* plants might be based on organ-specific expression of genes. But, in spite of the high number of allelochemicals known, little or almost nothing is known to date about the genes encoding their biosynthetic pathways (Macías et al., 2007). In spite of this, important advances are expected from genetic engineering using allelopathic traits to reduce herbicide applications (Duke et al., 2001; Scheffler et al., 2001).

From several studies the Poaceae family has been reported to show evidence of allelopathic activity (Sánchez-Moreiras et al., 2004), e.g., *Cenchrus ciliaris* L. and *Bothriochloa pertusa* (L.) A. Camus are known to suppress and preclude the associated species (Hussain et al., 1982). Li et al. (2005) studied the allelopathic effect of *Cymbopogon citratus* volatile compounds on seed germination and seedling growth of corn and *Echinochloa crus-galli* in field. Anjum et al. (2005) and Javaid et al. (2007) reported that *Imperata cylindrica* and *Desmotachya bipinnata* reduce the spread of weeds in the field. *B. laguroides* var. *laguroides* can be added to the group of grasses with high allelopathic potential. The production of allelochemicals, a process under genetic control, is subject to natural selection (Blanco, 2007; Viles and Reese, 1996). Heterogeneity at subpopulation level of expression of this trait should have importance in competition and, hence, further evolution of *B. laguroides* var. *laguroides*.

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