



## Article

## AUTOTOXICITY IN TOUGH LOVEGRASS (*Eragrostis plana*)

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### *Autotoxicidade em Capim-annoni (Eragrostis plana)*

**ABSTRACT** - Autotoxicity in tough lovegrass has been analyzed in the field, but has never been tested in allelopathy bioassays. Therefore, this study aimed to verify and compare the autotoxicity of leaves and roots from aqueous extracts of tough lovegrass on its germination and early seedling growth. Extracts of green leaves (GL), senescent leaves (SL), and roots (R) at concentrations of 0.05, 0.15, and 0.25 g mL<sup>-1</sup> were analyzed on how they affected seed germination and seedling growth. The extracts, regardless of the source or concentration of the material used, have significantly reduced germination percentage, index of germination speed, and root growth of seedlings of tough lovegrass. Effects on mean germination time and shoot length varied according to the origin and concentration of the extracts. GL, at concentrations 0.15 and 0.25 g mL<sup>-1</sup>, showed the most damaging effect. In tough lovegrass, autotoxicity is a mechanism that may be involved in the control of germination and plant growth, which could explain the distance between their clumps in the field.

**Keywords:** allelopathy, aqueous extracts, *Eragrostis plana*, germination.

**RESUMO** - A autotoxicidade no capim-annoni é observada no campo, mas nunca foi testada em bioensaios de alelopatia. Portanto, este estudo teve como objetivo verificar e comparar a autotoxicidade de extratos aquosos de folhas e raízes de capim-annoni na germinação e crescimento inicial de plântulas da mesma espécie. Extratos de folhas verdes, folhas senescentes e raízes de capim-annoni nas concentrações de 0,05, 0,15 e 0,25 g mL<sup>-1</sup> foram avaliados quanto ao seu efeito na germinação e crescimento inicial. Os extratos, independentemente da origem ou da concentração do material utilizado, diminuíram significativamente a porcentagem de germinação e o índice de velocidade de germinação e crescimento de raízes de plântulas de capim-annoni. O efeito no tempo médio de germinação e no comprimento da parte aérea variou de acordo com a origem e a concentração dos extratos. Extratos de folhas verdes nas concentrações de 0,15 e 0,25 g mL<sup>-1</sup> apresentaram o efeito mais nocivo. No capim-annoni, a autotoxicidade é um mecanismo que pode estar envolvido no controle da germinação e crescimento das plantas, o que poderia explicar a distância entre as touceiras no campo.

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**Palavras-chave:** alelopatia, *Eragrostis plana*, extratos aquosos, germinação.

Received: May 18, 2016  
Approved: August 2, 2016

Planta Daninha 2017; v35:e017164046

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## INTRODUCTION

Allelopathy is a phenomenon in which a plant produces and releases chemical compounds into the environment that influences the development of other organisms surrounding it (Rice, 1984). In general, allelopathy is interspecific, that is, the chemicals released by one species are phytotoxic to another species; this is called heterotoxicity. Whereas, when a plant releases chemicals into the environment inhibiting its own germination and growth, allelopathy becomes intraspecific; this is called autotoxicity (Miller, 1996). This phenomenon, also known as autoallelopathy or autointoxication, occurs for a large number of weeds and crops in natural and agricultural ecosystems (Singh et al., 1999).

The secondary metabolites involved in allelopathic interactions are called allelochemicals (Reigosa et al., 2013). In the case of autotoxicity, they are also called autochemicals or simply phytotoxins and can be released by leaves, roots, stems, fruits, seeds, cotyledons, rhizomes, inflorescences, leaf litter, and plant residues (Singh et al., 1999). The chemical nature of these compounds include simple organic acids, straight-chain alcohols, aldehydes or ketones, lactones unsaturated, fatty acids, naphthoquinones, quinone complex, simple phenols, tannins, terpenoids, amino acids, polypeptides, alkaloids, glucosinolates, purines and nucleotides (Einhellig, 1995; Rice, 1995).

Autotoxicity has been found in natural and artificial ecosystems, such as grasslands, wastelands, fields, natural forests, and orchards, causing ecological and economic implications. Species that experience such autotoxic phenomenon are known to regulate their populations over space and time, avoiding intraspecific competition, guaranteeing self-perpetuation, allowing for a better geographical distribution (Singh et al., 1999). Moreover, such invasive characteristics provide selective advantage to autotoxic plants over other species (Picman and Picman, 1984; Edwards et al., 1988).

Allelopathy, as a whole, favors the impact and success of invasive species in new environments (Meiners et al., 2012), and it is considered one of the reason for the high infestation of tough lovegrass (*Eragrostis plana*) in ecosystems. This alien African grass is the most abundant and aggressive invasive plant in the Pampa Biome, responsible for causing widespread economic impacts on livestock by modifying the structure of plant communities and by affecting ecological balances. The heterotoxicity of tough lovegrass was been observed in bioassays of seed germination with white clover (*Trifolium repens*), Italian ryegrass (*Lolium multiflorum*), African bristlegrass (*Setaria sphacelata*), and bahiagrass (*Paspalum notatum*) (Coelho, 1986; Ferreira et al., 2008; Favaretto et al., 2011). However, it was assumed that tough lovegrass may also display autotoxicity, because in the field, the clumps are separated from each other by empty spaces with bare soil radius of 5–10 cm (Coelho, 1986). Therefore, this study aimed to verify and compare the autotoxicity of leaves and roots from aqueous extracts of tough lovegrass in its germination and early seedling growth.

## MATERIAL AND METHODS

The extracts used in the germination and growth bioassays were prepared with the material from tough lovegrass plants, in their vegetative state, and collected throughout April 2013, in the city of Passo Fundo, located in the state of Rio Grande do Sul, Brazil (28°152 S, 52°242 W). Plants were separated into green leaves (GL), senescent leaves (SL), and roots (R). They were then dried in an oven at 40 °C and ground into a powder. Aqueous extracts of these components were prepared in three concentrations (0.05, 0.15, and 0.25 g mL<sup>-1</sup>, respectively corresponding to 5, 15 and 25 g in 100 mL of distilled water) using the static maceration method (Soares and Vieira, 2000) wherein, plant material was immersed in distilled water for 24 hours at room temperature and under light. After this period, the material was filtered and its pH was determined. Extracts within the normal pH values were used for further experiments (between 4.0 and 7.0) (Table 1) (Oliveira et al., 2012).

Two bioassays were conducted, only once: (i) germination and (ii) initial growth. The germination bioassays consisted of 10 treatments, in factorial 3 x 3 (plant material = green leaf, senescent leaf, and root; extract concentration = 0.05, 0.15, and 0.25 g mL<sup>-1</sup>), more control (distilled

**Table 1** - Characterization of aqueous extracts of tough lovegrass for pH

Source	Concentration (g mL <sup>-1</sup> )	pH
Green leaf	0.05	6.2
	0.15	6.7
	0.25	7.0
Senescent leaf	0.05	6.2
	0.15	6.2
	0.25	4.7
Root	0.05	5.8
	0.15	5.6
	0.25	5.0

water). The design was completely randomized with four replications of 50 seeds. The experimental units were Gerbox boxes containing two Germitest sheets sterilized and moistened with 8 mL of each treatment. To break seed dormancy, they were subjected to a treatment for pre-cooling at 5 °C for 5 days. Subsequently, the boxes were kept in a growth chamber at 20 °C with a photoperiod of 12 hours for 10 days. Germination count was made daily. Seeds with a minimum of 2 mm of root protrusion were considered to have germinated (Ferreira and Aquila, 2000). Germination (G), germination speed index (GSI), and average germination time (GMT) were assessed according to the recommendations of Ranal and Santana (2006).

In the initial growth bioassay, we used the same treatments, experimental design, and number of repetitions of the germination bioassay. However, in each repetition seven pre-germinated seedlings were used in distilled water. Three days after germination and before the application of the extracts, the length of root and shoot of the seedlings was measured, after which they were transferred to boxes containing Germitest paper moistened with the treatments, where they were kept for 15 days. The boxes were placed in a growth chamber under the same conditions described for the germination bioassay. At the end of the experiment, the length of root and shoot was measured again.

The root and shoot length data were expressed as inhibition percentage, according to the Oliveira et al. (2012) equation (Equation 1):

$$\text{Inhibition (\%)} = [(XT - XC) / XC] \times 100 \quad (\text{eq. 1})$$

where xt is elongation average of treatments and xc is the elongation average of control. The value "0" is control. Any positive value implies stimulation of the measured parameters and negative values implies inhibition.

Germination data were submitted to the analysis of variance, considering the differentiated two-factor model (3x3)+1. To verify the allelopathic activity of extracts, we have applied the Dunnett test ( $p \leq 0.05$ ), comparing the treatment of interest in test (extracts) with the control (Dunnett, 1995). For comparing averages of the extracts, the Tukey test ( $p \leq 0.05$ ) was used.

## RESULTS AND DISCUSSION

This work is the first investigation of the autotoxic activity of tough lovegrass. Results show that aqueous extracts can reduce seed germination and early seedling growth of the species, agreeing with what is found in the field, where tough lovegrass clumps are far apart.

### Germination bioassays

There was interaction between the plant material and concentration to the percentage of germination, germination speed index, and average germination time. The aqueous extracts of tough lovegrass affect seed germination in all concentrations tested. The GL concentration of 0.25 g mL<sup>-1</sup> was more harmful than others (Table 2).

GSI was reduced by all the extracts when compared to the control. GL extracts at concentrations 0.15 and 0.25 g mL<sup>-1</sup> proved to be more harmful when compared to SL and R extracts (Table 3). GMT was only affected by GL extract at 0.25 and 0.15 g mL<sup>-1</sup> concentrations, whereas GL at 0.25 g mL<sup>-1</sup> concentration showed the most harmful effect (Table 4).

**Table 2** - Germination of tough lovegrass seeds submitted to aqueous extracts of different types of plant material originating from the plant itself and prepared in different concentrations

Plant material	Concentration (g 100 mL <sup>-1</sup> )		
	5	15	25
	(%)		
Green leaf	*33±9.8 aA	*25±11.8 aA	*6±11.8 bB
Senescent leaf	*24±11.9 aA	*31±5.3 aA	*23±5.0 aA
Root	*33±9.1 aA	*27±16.1 aA	*33±10.2 aA
Control	63±12.1		

Means followed by the same letter, lowercase in the column and uppercase in the line, do not differ by Tukey test ( $p \leq 0.05$ ). \* Value differs from the control by the Dunnett test ( $p \leq 0.05$ ).

**Table 3** - Germination speed index of tough lovegrass seeds submitted to aqueous extracts of different types of plant material originating from the plant itself and prepared in different concentrations

Plant material	Concentration (g 100 mL <sup>-1</sup> )		
	5	15	25
	(%)		
Green leaf	*4±1.3 aA	*2±0.9 bB	*0±0.7 bC
Senescent leaf	*3±1.4 aA	*4±0.8 aA	*3±0.9 aA
Root	*4±1.2 aA	*4±0.8 aA	*4±0.9 aA
Control	8±1.5		

Means followed by the same letter, lowercase in the column and uppercase in the line, do not differ by Tukey test ( $p \leq 0.05$ ). \* Value differs from the control by the Dunnett test ( $p \leq 0.05$ ).

**Table 4** - Germination mean time of tough lovegrass seeds submitted to aqueous extracts of different types of plant material originating from the plant itself and prepared in different concentrations

Plant material	Concentration (g 100 mL <sup>-1</sup> )		
	5	15	25
	(days)		
Green leaf	4±0.1 aC	*8±0.8 aB	*9±0.8 aA
Senescent leaf	4±0.4 aA	4±0.4 bA	5±0.9 bA
Root	4±1.6 aA	4±0.3 bA	4±0.6 bA
Control	4±0.2		

Means followed by the same letter, lowercase in the column and uppercase in the line, do not differ by Tukey test ( $p \leq 0.05$ ). \* Value differs from the control by the Dunnett test ( $p \leq 0.05$ ).

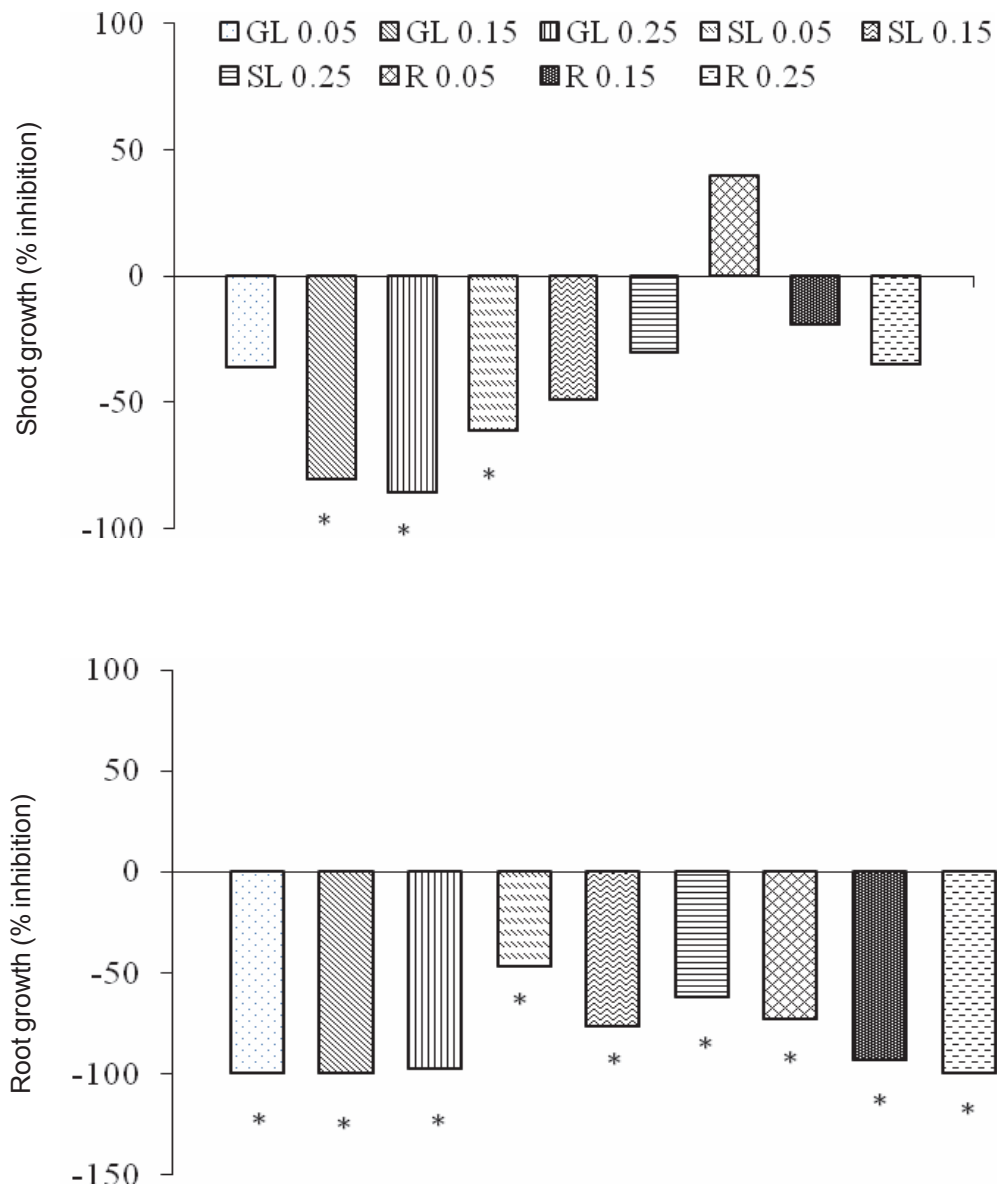
### Initial growth bioassay

Tough lovegrass extracts caused the appearance of several abnormalities in seedlings, emphasizing the appearance of necrotic, dark, thin and twisted roots, absence of secondary roots, chlorosis, and necrosis in the shoot.

Besides causing abnormalities, tough lovegrass extracts used in this work have affected its own root and shoot growth (Figure 1). There was no interaction between plant material and concentration of extracts. Only GL 0.15 g mL<sup>-1</sup>, GL 0.25 g mL<sup>-1</sup>, and SL 0.05 g mL<sup>-1</sup> extracts have significantly inhibited the growth of shoots of tough lovegrass seedlings. However, all extracts have significantly reduced root growth (Figure 1).

Although germination is considered less sensitive to the presence of allelochemicals (Oliveira et al., 2012), the interference of tough lovegrass extracts on this parameter was found in this work, which proves the autotoxic effect of these extracts, because germination was affected

even in the lowest concentration used. In general, the preparation of aqueous extracts and subsequent tests of its effects on the germination and growth of target species has been acknowledged as a classic process in the field of allelopathy (Inderjit and Dakshini, 1995). However, there are cases where the allelopathic effect is not only obvious in the germination process, but also in the occurrence of abnormalities, which makes the study of seedling growth a valuable tool in the field of allelopathy (Ferreira and Aquila, 2000). Allelochemicals can affect cytological structures, hormones, membrane permeability, absorption of minerals, stomatal movement, pigment synthesis, photosynthesis, respiration, protein synthesis, enzyme activity, water relations and cause changes in DNA and RNA (Rizvi and Rizvi, 1992; Ferreira and Aquila, 2000). Thus, abnormalities may be secondary consequences of effects that occur primarily at cellular and molecular levels.



\* Indicates significant difference from the control by Tukey test ( $p \leq 0.05$ ). GL 0.05 = green leaf extract at 0.05 g mL<sup>-1</sup>; GL 0.15 = green leaf extract at 0.15 g mL<sup>-1</sup>; GL 0.25 = green leaf extract at 0.25 g mL<sup>-1</sup>; SL 0.05 = senescent leaf extract at 0.05 g mL<sup>-1</sup>; SL 0.15 = senescent leaf extract at 0.15 g mL<sup>-1</sup>; SL 0.25 = senescent leaf extract at 0.25 g mL<sup>-1</sup>; R 0.05 = root extract at 0.05 g mL<sup>-1</sup>; R 0.15 = root extract at 0.15 g mL<sup>-1</sup>; R 0.25 = root extract at 0.25 g mL<sup>-1</sup>.

**Figure 1** - Inhibition percentage and /or stimulation of root growth and shoot the tough lovegrass seedlings grown under influence of different concentrations of aqueous extracts of its leaves and roots.

In this present study, root was the most affected by extracts, which could be due to its closest contact with the plant extract. Possibly, the presence of allelochemicals has inhibited or reduced the metabolic activity by altering the physiology of the radicle protrusion (Chung et al., 2001; Yamagushi et al., 2011). Among all abnormalities, root necrosis is one of the most common symptoms (Ferreira and Aquila, 2000). In addition, curling of radicle axis and discoloration and lack of root hairs were noted (Bhadoria, 2011). The browning and weakening of roots are harmful effects that may indicate the action of toxic substances in the extracts (Yamagushi et al., 2011).

Both in germination and in the initial growth, GL extract was the most autotoxic. Since leaves are the most active plant part, metabolically speaking, it is reasonable that they have greater diversity of allelochemicals and, hence, greater allelopathic effect (Ribeiro et al., 2009). Previous studies have shown greater amount of allelochemicals on the leaves of tough lovegrass (Favaretto et al., 2015), which could explain both a greater heterotoxic or autotoxic effect of this organ. Regarding the allelochemicals, ferulic, vanillic, p-coumaric and caffeic acids were found along with coumarin, catechin and epicatechin (Favaretto et al., 2015). These compounds may be associated with plant allelopathy, acting both individually and together with compounds not yet identified.

Isolated autotoxins from plants can be exploited for weed control (as bioherbicides), pests, or as plant growth regulators and seed savers (Singh et al., 1999). Whereas, allelochemicals are compounds with potential to be used as bioherbicides, added to the fact that the autotoxic effect of tough lovegrass, due to the isolated compounds of this plant, could be tested for their own control, especially those isolated from GL.

Tough lovegrass leaf and root aqueous extracts have negatively affected its own germination and initial growth of seedlings. The degree of autotoxicity varies depending on the source material and concentration, which could explain the distance between their clumps in the field, agreeing with what has been reported by Coelho (1986).

Autotoxicity is an important phenomenon in controlling population density in natural systems (He et al., 2009). In tough lovegrass, it is a mechanism that possibly is involved in controlling the germination and growth of plants of the species itself, which could explain the gaps between their tussocks in the field. In that case, autotoxicity provides a selective advantage in soil occupation, specially in low fertility soil conditions where tough lovegrass grows well. Alternatively, germination may be delayed and that will regulate seedling grow avoiding intraspecific competition. As a result, plant stands will become healthier and more vigorous (Singh et al., 1999).

## ACKNOWLEDGEMENTS

We would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes/Brazil), for funding this research by granting scholarship for the first author, and to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/Brazil), for supporting this work under project N. 471427-6.

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