Allelopathic Evidence in *Brachiaria decumbens* and its Potential to Invade the Brazilian Cerrados

Elizabeth Gorgone Barbosa¹, Vânia Regina Pivello¹* and Sérgio Tadeu Meirelles¹

¹Departamento de Ecologia, Instituto de Biociências; Universidade de São Paulo; Rua do Matão, Travessa 14; Cidade Universitária Armando de Salles Oliveira; vrivel@usp.br; 05508-900; São Paulo - SP - Brasil

**ABSTRACT**

The aim of this study was to look for evidence of allelochemicals in *B. decumbens*, in parts of the plant from where they could easily be released to the environment. The germination inhibition of *Phalaris canariensis*, *Lactuca sativa* (standard species) and *Melinis minutiflora*, another invasive African grass, was tested using *B. decumbens* germinating seeds and aqueous leachates of the roots, green and senescent leaves, at 5, 10 and 20% w/v. Both the germinating seeds and the aqueous leachates of *B. decumbens* reduced the germination of the species tested; the effectiveness of the aqueous leachates increased according to concentration. Apparently, the competitive advantage of *B. decumbens* in the cerrados could be amplified via allelopathy.

Keywords: allelopathy, cerrado, biological invasion, *Brachiaria decumbens*, *Melinis minutiflora*

**INTRODUCTION**

Biological invasion has been recognized worldwide as one of the worst environmental problems that lead to biodiversity loss (Vitousek et al., 1997; Wilcove et al., 1998; Pivello et al., 1999a; Mack et al., 2001). Invasive plant species combine a set of characteristics that enable them to outcompete the native species and dominate the invaded ecosystems. Some of these attributes are: vegetative and sexual reproduction, high productivity and high growth rates due to efficient photosynthetic processes and the effective use of nutrients, large production of easily dispersed seeds with high germination rates, short life cycles, and allelopathy (D’Antonio and Vitousek, 1992; Cronk and Fuller, 1995; Rejmánek and Richardson, 1996; Williamson, 1996; Kolar and Lodge, 2001). Allelopathy, simply defined as “the release of phytotoxins by plants” (Bais et al., 2003) can enhance the competitive success of the plant invaders, since the release of these phytotoxins in the environment may affect the growth and life processes of other community species (Whittaker and Feeney, 1971; Rice, 1984; Ridenour and Callaway, 2001; Callaway, 2002). The Brazilian cerrado is recognized as the richest savanna in the world concerning plant species, and it integrates the list of global hot spots (Myers et al., 2000; Mittermeier et al., 2004). About 3,000 to 7,000 vascular plant species have been estimated for the cerrado sensu lato (following Coutinho’s [1978] classification) (Castro et al., 1999) and more than 6,400 vascular species have been registered for the cerrado region (Mendonça et al., 1998), which includes more than 20

* Author for correspondence
physiognomies of savanna, forest, and grassland (Ribeiro and Walter, 1998). However, the great biodiversity of the cerrado biome is being severely threatened by a number of invasive exotic species, namely the African grasses Brachiaria spp., Melinis minutiflora Beauv., Andropogon gayanus Kunth, Panicum maximum Jacq., Hyparrhenia rufa (Nees) Stapf., once brought to the country as cattle forage. These grasses have spread in such a magnitude that they occur in virtually every cerrado fragment, outcompeting native herbs (Klink, 1996a; 1996b; Pivello et al., 1999a; 1999b).

The high dominance of Brachiaria grasses over cerrado native herbs has recently raised investigations on the presence of phytotoxins in some species. In cerrados, B. decumbens (Nees) Stapf. has advanced massively throughout the native vegetation and formed monospecific patches with no other species growing below or close to it. In situ studies in São Paulo State cerrado areas have shown that even M. minutiflora, another East-African grass that invaded the cerrado, could have been displaced by B. decumbens (Pivello et al., 1999a; 1999b), revealing a high competitive advantage of the latter species.

The present study aimed at looking for evidence of allelopathy in Brachiaria decumbens through bioassays, and exploring parts of the plant from where the supposed allelopathic substances could be easily released to the environment: seeds, senescent and green leaves, and roots. The inhibition of seed germination of Lactuca sativa L. cv. Grand Rapids (Asteraceae) – a standard species used in the tests of germination and allelopathy due to its high responsiveness and rapid germination (Ferreira and Aquila, 2000). Phalaris canariensis L. (Poaceae) is a grass species not usually present in the cerrados but considered as an invader elsewhere in the world (ISI/TNC, 2005). Here, it was used as a Poaceae reference due to its high germination rates and availability.

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The seeds of B. decumbens, P. canariensis and L. sativa were commercially acquired, whereas M. minutiflora seeds were field collected (July 2004).

**Plant extracts**

*B. decumbens* was cultivated in 8-l plastic pots with commercial garden soil and maintained in a greenhouse at room temperature (around 28°C), 50% relative humidity, and natural light intensity reaching 1000 μmol maximum. Three months after the seeding, green leaves and roots were collected to make the extracts used in the tests. Senescent leaves were collected (September 2004) in the Cerrado Pé-de-Gigante Reserve (Vassununga State Park, SP).

Aqueous leachates of *B. decumbens* green leaves, senescent leaves and roots were prepared using 12.5, 25 and 50 g of the fresh material in 250 ml of distilled water. The plant material was fitted into Erlenmeyer tubes and then shaken for 12 h. The extracts were double filtered, resulting in 5, 10 and 20% w/v solutions, stored at 7°C.
Tests
The effects of the extracts and germinating seeds were tested adopting a full randomized design with six repetitions for each treatment and control groups. The seeds of *L. sativa* were allowed to germinate for three days while *P. canariensis* and *M. minutiflora* were allowed 10 days (following Brasil, 1992). To test for the allelopathic effects of *B. decumbens* germinating seeds, 30 of these and 30 seeds of the test species (*M. minutiflora*, *L. sativa* and *P. canariensis*) were put in Petri dishes (diameter = 11 cm) with moistened filter paper and incubated in germination chambers at 26º C, with 12 h light/darkness cycle. All the seeds had been previously sterilized with 1% sodium hypochlorite for 30 min and rinsed with distilled water (Yamamoto et al., 1999; Higashinakasu et al., 2004). Petri dishes and filter paper were sterilized in boiling water for 30 min. Germinated seeds were considered those with root equal or bigger than 1 cm (Copeland, 1976). The significance of the effects of *B. decumbens* germinating seeds on other species was tested using a Mann-Whitney U test (Siegel and Castellan-Jr, 1988).

The allelopathic effect of the aqueous leachates of *B. decumbens* organs (green leaves, roots and senescent leaves) at different concentrations (Table 1). The inhibitory response to the aqueous
dishes with filter paper moistened with 2 ml of the aqueous extracts at 5, 10 and 20% (w/v). The control dishes were moistened with distilled water. The Petri dishes were then incubated in germination chambers at the same conditions as in the previous test. The percentages of germinated seeds in each Petri dish were angle-transformed prior to statistical treatment. The results of the tests using the aqueous leachates were submitted to a General Linear Model Analysis of Variance (GLM ANOVA). Significant effects were submitted to the post hoc pairwise tests of Tukey and Dunnet (Sokal and Rohlf, 2000).

RESULTS
In the presence of *B. decumbens* seeds, the germination of *L. sativa*, *P. canariensis* and *M. minutiflora* seeds decreased from 29.6 ± 0.51, 29.0 ± 1.26 and 29.6 ± 0.51 to 27.6 ± 1.86, 25.8 ± 4.26 and 22.3 ± 5.16, respectively. Although there was a general decrease in the germination, results were only significant for *M. minutiflora* (p <0.01) (mean reduction = 23%). An overall increase of the standard deviation dispersion was another noticeable effect (Fig. 1).

The number of germinated seeds of the standard species (*L. sativa* and *P. canariensis*) was reduced when exposed to the aqueous leachates of *B. decumbens* organs (green leaves, roots and senescent leaves) at different concentrations (Table 1). The inhibitory response to the aqueous

![Figure 1](image-url)
leachates of *B. decumbens* on seed germination was more pronounced in *P. canariensis* (Table 1). However, the effects of the leachates at different concentrations were only significant against water alone (concentration 0% versus other concentrations) (Tables 1, 2) indicating that the effects were not proportional to the leachate concentration. Differential effects could not be attributed to different plant organs or to different leachate concentrations. The germination of *M. minutiflora* and *L. sativa* seeds was also significantly inhibited by the aqueous leachates of *B. decumbens* green leaves, roots and senescent leaves but only when comparing the results of distilled water and all the leachates (no proportional effect of the leachate concentration was observed) (Tables 1, 2).

### Table 1 - Number of *Lactuca sativa* (Asteraceae), *Phalaris canariensis* (Poaceae) and *Melinis minutiflora* (Poaceae) seeds germinated when in contact with the aqueous leachates of *Brachiaria decumbens* (Poaceae) green leaves, senescent leaves and roots, at 5, 10 and 20% w/v (mean of six repetitions ± standard deviation).

<table>
<thead>
<tr>
<th>Extract concentration</th>
<th>0%</th>
<th>5%</th>
<th>10%</th>
<th>20%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactuca sativa</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>green leaves</td>
<td>29.6 ± 0.21</td>
<td>28.6 ± 1.21</td>
<td>28.5 ± 1.68</td>
<td>28.6 ± 1.21</td>
</tr>
<tr>
<td>roots</td>
<td>29.6 ± 0.21</td>
<td>28.3 ± 0.81</td>
<td>26.5 ± 2.25</td>
<td>28.6 ± 1.21</td>
</tr>
<tr>
<td>senescent</td>
<td>29.6 ± 0.21</td>
<td>27.6 ± 0.51</td>
<td>28.6 ± 1.03</td>
<td>28.1 ± 1.47</td>
</tr>
<tr>
<td><em>Phalaris canariensis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>green</td>
<td>29.0 ± 1.26</td>
<td>25.0 ± 4.04</td>
<td>24.0 ± 4.28</td>
<td>19.3 ± 1.86</td>
</tr>
<tr>
<td>roots</td>
<td>29.0 ± 1.26</td>
<td>21.6 ± 4.50</td>
<td>22.0 ± 4.93</td>
<td>21.8 ± 4.32</td>
</tr>
<tr>
<td>senescent</td>
<td>29.0 ± 1.26</td>
<td>21.8 ± 7.05</td>
<td>20.0 ± 7.20</td>
<td>17.0 ± 2.09</td>
</tr>
<tr>
<td><em>Melinis minutiflora</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>green leaves</td>
<td>29.3 ± 0.81</td>
<td>25.5 ± 0.83</td>
<td>27.6 ± 2.16</td>
<td>27.0 ± 2.28</td>
</tr>
<tr>
<td>roots</td>
<td>29.3 ± 0.81</td>
<td>25.8 ± 1.94</td>
<td>25.1 ± 2.63</td>
<td>25.5 ± 2.88</td>
</tr>
<tr>
<td>senescent</td>
<td>29.3 ± 0.81</td>
<td>27.8 ± 1.32</td>
<td>24.5 ± 2.42</td>
<td>25.3 ± 1.5</td>
</tr>
</tbody>
</table>

### Table 2 - Results from GLM ANOVA considering the effects of aqueous leachates of *Brachiaria decumbens* (Poaceae) green leaves, senescent leaves and roots at different concentrations (5, 10 and 20% w/v) on the germination of *Lactuca sativa* (Asteraceae), *Phalaris canariensis* (Poaceae) and *Melinis minutiflora* (Poaceae), as well as the effects of the interactions among these factors.

<table>
<thead>
<tr>
<th>Factor</th>
<th><em>L. sativa</em></th>
<th><em>P. canariensis</em></th>
<th><em>M. minutiflora</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>p</td>
<td>F</td>
</tr>
<tr>
<td>Organ</td>
<td>1.583</td>
<td>0.214</td>
<td>1.060</td>
</tr>
<tr>
<td>Leachate concentration</td>
<td>9.098</td>
<td>0.000</td>
<td>23.216</td>
</tr>
<tr>
<td>Organ X Leachate concentration</td>
<td>1.328</td>
<td>0.259</td>
<td>0.607</td>
</tr>
</tbody>
</table>

### DISCUSSION

The results obtained here indicate possible allelopathic effects of *B. decumbens* on other species. These effects may occur at different stages of the *B. decumbens* life cycle and may come from different plant parts. During the germination stage, *B. decumbens* seeds were able to inhibit the germination of *L. sativa*, *P. canariensis*, and especially of *M. minutiflora*. Plant-to-plant interactions at the germination stage through allelopathic inhibition of root and shoot growth have been reported by Higashinakasu et al. (2004) in seven crop and weed species, and by Laterra and Bazzalo (1999) between two invasive species of the pampa grasslands (Argentina).
*Brachiaria decumbens* allelopathic potential may remain throughout its lifetime including senescence, as the roots, green leaves and senescent leaves also showed allelopathic effects. Maciel et al. (2003) tested for allelopathic effects of *B. decumbens* dry leaves on the initial development of two crop legumes under agriculture conditions and obtained positive results for one of them. Indejit and Duke (2003) highlighted the advantage of the plants able to release phytochemicals from dead tissues, as their incorporation to the soil could be accelerated by leaching, thus facilitating their effects in the field. Root exudates can be synthesized by plants and released to the soil by the live roots (Rice, 1984) and have been reported as an efficient strategy used by some *Centaura* species (Bais et al., 2003; Vivanco et al., 2004) and *Echinochloa crus-galli* (Yamamoto et al., 1999) – plants recognized as very aggressive invaders in North America. Testing for the allelopathic inhibition of *Brachiaria* species on legumes, Almeida et al. (1997) and Fagioli et al. (1997) obtained positive results using the extracts of compressed green leaves and roots of *B. humidicola*, *B. decumbens* and *B. brizantha*. The greatest effects were revealed by *B. decumbens*. However, in the compressed material the cells are smashed and all the substances inside them are released. Therefore, this procedure is far from representing the field conditions. Alternatively, aqueous leachates can represent conditions closer to the natural ones. In the present study, the inhibition effects were stronger over the grasses (Poaceae) *P. canariensis* and *M. minutiflora*, especially on the latter, compared to *L. sativa* (Asteraceae). A number of Poaceae family species have been pointed out as carrying allelopathic activity. Four main groups of phytotoxic compounds have been identified in Poaceae – phenolic acids, hydroxamic acids, alkaloids, and quinones – which can be found in all parts of the plants, from pollen to root exudates (Steinsiek, et al., 1982; Schumacher et al., 1983; Anaya et al., 1992; Einhellig and Souza, 1992; Hoffman et al., 1996; Alsaadawi et al., 1998; Kato-Noguchi and Ino, 2001; Sanchez-Moreiras, et al. 2004), and confer them a great advantage over other species.

Although the results here obtained came from a laboratory essay, they indicate the capacity of *B. decumbens* to release allelopathic substances to the environment. As in natural conditions, where a great number of interactions with the physical environment, as well as with other organisms, can enhance or restrain allelopathic effects, field experiments must be carried out to test the effectiveness of such allelopathic potential under natural conditions (Inderjit and Callaway, 2003). However, the great success of *B. decumbens* on invading cerrados could in part be due to allelopathy, as suggested in other parts of the world by authors who related allelopathy to biological invasions (Hiero and Callaway, 2003; Vivanco et al., 2004; Ridenour and Callaway, 2001; Vaughn and Berhow, 1999).

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**RESUMO**

Invasão biológica é uma das maiores causas atuais da perda de biodiversidade. Várias espécies que se tornam invasoras produzem substâncias fitotóxicas que aumentam sua capacidade competitiva; assim, a alelopatia é uma estratégia capaz de potencializar o sucesso de invasão. *Brachiaria decumbens*, uma gramínea africana, invadiu os cerrados brasileiros e representa, atualmente, uma séria ameaça à biota regional. Neste ensaio, verificamos a presença de aleloquímicos em partes de *B. decumbens* das quais poderiam ser facilmente liberados para o ambiente. Testamos a inibição da germinação de *Phalaris canariensis*, *Lactuea sativa* (espécies-padrão) e *Melinis minutiflora* (outra gramínea africana invasora) usando sementes de *B. decumbens* e soluções aquosas lixiviadas de suas raízes, folhas verdes e folhas senescentes, a 5, 10 e 20% de peso do material por volume de água. Tanto as sementes como as soluções lixiviadas de *B. decumbens* reduziram a germinação das outras espécies; a eficiência dos lixiviados aumentou de acordo com a concentração da solução.
Acreditamos que a grande vantagem competitiva de *Brachiaria decumbens* no cerrado possa ser amplificada pela alelopatia.

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