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Pequi leaves incorporated into the soil reduce the initial growth of cultivated, invasive and native species

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ABSTRACT

Studies have identified the phytotoxicity of many native species of the Cerrado; however, most of them were conducted either in inert substrates, or using exaggerate proportions of plant material. We investigated the phytotoxicity of pequi leaves added to substrate soil in quantities compatible with the litter produced by this species. Pequi leaves were triturated and added to red latosol in concentrations of 0.75%, 1.5% and 3%; the control was constituted of leafless soil. These mixtures were added to pots and irrigated daily to keep them moist. Germinated seeds of the cultivated sorghum and sesame, of the invasive brachiaria and of the native purple ipê, were disposed in the pots to grow for five to seven days at 30 °C within a photoperiod of 12 h. Seedlings of all the species presented a reduction in their initial growth in a dose-dependent way. In general, the root growth was more affected by the treatments than the shoot growth; moreover, signs of necrosis were observed in the roots of the sorghum, sesame and brachiaria. The phytotoxic effects generated by relatively small quantities of leaves, in a reasonable range of species within a soil substrate, suggest potential allelopathy of pequi leaves under natural conditions.

Key words: Allelopathy, *Caryocar brasiliense*, Cerrado, phytotoxicity, *Tabebuia impetiginosa*, *Urochloa decumbens.*

INTRODUCTION

Phytotoxicity and allelopathy are two terms which are frequently considered to be synonyms in Brazilian literature. However, the scientific basis that underlie these two studies differ: first, phytotoxic studies investigate chemical properties and interactions among species that do not necessarily coexist in their regions of origin; second, they normally employ extracts prepared with organic solvents and methodologies which optimize the extraction of the active compounds; finally, they frequently utilize filter paper or other types of inert substrates (Blum 2011, Reigosa et al. 2013). Allelopathy, on the other hand, is the science that studies chemical interactions among plants in their natural environments. Therefore, studies conducted under controlled conditions which attempt to contribute to the deciphering of allelopathic interactions should meet certain minimum criteria: they should employ species that coexist in their natural environments, use water in the preparation of extracts, utilize substrate representative of the location where the phenomena takes place, and be guided by questions and hypotheses which have ecological relevance to the species, population or community (Inderjit et al. 2001, Inderjit 2005, Blum 2011, Reigosa et al. 2013).

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The use of the substrate were the plants occur enables the biotic and abiotic components of the soil to interfere in the activity of the substances liberated by the plant tissues (Tanner and Gange 2013). In the soil, they can be metabolized by the biota, oxidized, volatilized, adsorbed or lixiviated by rain (Weber and Miller 1989, Andrade et al. 2013). Such influences of the surroundings are not added to the experiment when the substrate utilized is neutral.

In addition to the substrate, the quantity of plant biomass employed in the preparation of extracts or solutions to be tested should be representative of the natural quantity of plant material produced by the species under natural conditions. Highly concentrated extracts, or exaggerated quantities of plant tissue used in the experiments, may lead to toxic effects due to the extreme concentration; however, they do not reflect concentration levels which bioactive substances would reach in the environment surrounding the allelopathic species (Blum 2011, Reigosa et al. 2013).

The concentration of substances in the soil depends on several factors: these include the quantity and age of the biomass produced by the plant, the quantity and frequency of rain, the capacity of the substrate to retain bioactive molecules and the quantity of water within the soil. Although the establishment of such parameters could present a reasonable degree of empiricism, there are many publications that present information relevant to setting some guidelines for allelopathic studies; these include the production of plant litter in different ecosystems (p. ex. Santos and Válio 2002, Campos et al. 2008, Silva et al. 2009), the intensity, frequency and volume of rain in the study area (www.inmet.gov.br), among other sources of information to set parameters to conduct allelopathic studies under laboratory conditions.

Plants display considerable variability of responses in similar experiments, and the same agent could lead to different effects in different species (Macias et al. 2000). Consequently, experiments of phytotoxicity should test the effects of extracts and active substances in a wide range of species, preferably of different functional groups, (cultivated, invasive, monocotyledons, pioneers, for example). Futhermore, the use of coexisting species is crucial in order to make laboratory experiments relevant to the understanding of allelopathic interactions in the field. The use of such procedures ensures more reliable results, lead to findings of a higher order of magnitude (Macias et al. 2000), and enable laboratory studies to be placed within more representative contexts of (supposed) chemical interactions of neighbor species in the environment.

There are increasing numbers of phytotoxic and allelopathic studies conducted with native species. The preparation of aqueous extracts obtained from different plant tissues, the use of filter paper as a substrate, and the selection of cultivated or invasive species as target, have however, been the most common procedure (Gatti et al. 2004, Oliveira et al. 2004, Aires et al. 2005, Pina et al. 2009, Reigosa et al. 2013). Less common is the use of crushed leaves added to the soil (Souza et al. 2007, Pina et al. 2009). In most studies conducted in Brazil, the concentration rate of the extracts has generally been below 5% in proportions between weight/weight, weight/volume or volume/volume (Reigosa et al. 2013).

Caryocar brasiliense Camb., popularly known as pequi, is a very common species in the Cerrado (Lima et al. 2007). Preliminary studies have revealed that aqueous extracts of pequi leaves at low concentrations significantly inhibit the initial growth of sesame in substrate filter paper (Borghetti et al. 2005), which suggests that lixiviates of pequi leaves could have some allelopathic effect on the growth of neighbor species in the field.

In this context, this study attempted to evaluate the effects of crushed pequi leaves on the initial growth of four target species in substrate soil. The species were the cultivated *Sesamum indicum* and *Sorghum bicolor*, the invasive *Urochloa decumbens*, and the native *Tabebuia impetiginosa.* In order to contextualize these results with the possible allelopathic effects of this tree species in the field, the triturated leaves were directly added to substrate soil, and the quantities of leaves employed in the experiments were compatible with the production of plant litter by adult trees under natural conditions; moreover, this study has made use of a native species that coexists with pequi in a large range of phytophysionomies of the Cerrado.

MATERIALS AND METHODS

Mature and totally expanded leaves of a minimum of five adult pequi individuals were collected in an area of the Cerrado *sensu stricto* located at the campus of the University of Brasilia (UnB, 15° 46´ 13´´ S, 47° 52´ 8´´ W). In the laboratory the leaves were oven-dried at 50 °C for 24 hours. Then, they were lightly triturated in a blender with quick intermittent pulsing; the triturated leaves were then homogeneously mixed with the soil in different proportions (see below).

For the bioassays of initial growth, four species were used: Sesame - *Sesamum indicum* L. (Eudicotyledon, cultivated), sorghum - *Sorghum bicolor* (L.) Moench (Monocotyledon, cultivaded), purple ipê - *Tabebuia impetiginosa* (Mart. ex DC.) Standl (Eudicotyledon, native) and brachiaria - *Urochloa decumbens* (Stapf) R.D. Webster (Monocotyledon, invasive). Purple ipê seeds were collected from species encountered in the same area where the pequi leaves were collected. Sesame seeds were harvested from individuals cultivated in the Laboratory of Thermobiology (UnB) and the sorghum and bachiaria seeds were obtained from a retail store.

To serve as the substrate, red latosol, gathered in the same collection area as the leaves and seeds, was sifted to remove stones and remnants of roots and branches, and to make them more homogenous. After drying at room temperature of ~24 °C to remove excess humidity, the triturated leaves were added to the soil in proportions of 0.75%, 1.5% and 3% (w/w). These proportions were established in accordance with the monthly quantity of plant litter produced by individuals of *C. brasiliense* during a year, which ranges from 10 to 80 grams per square meter (Oliveira 1999). Assuming a depth of 1 cm, the depth at which the radicle of most seedlings are found in the first days of growth, there would be a plant litter production of 10 to 80 grams per $0.01m^3$ of soil ($1m^2$ x 0.01m), or 10-80g/10 L of soil. Considering that 1 L of red latosoil weighs about 1.090 Kg (unpublished results), this results in a proportion between 0.1 to 0.8% ($w_{plant\ litter}/w_{soil}$). In addition to the treatment of 0.75%, within this range, its double (1.5%) and quadruple (3%) were also considered, with the objective of include in the experiment effects of litter accumulation throughout the months, similar to what could possibly occur during the dry season, when litter accumulates for months in the absence of fire.

The mixtures of crushed leaves and soil were placed in plastic pots (capacity 200 mL) for planting seedlings. The pots were placed in plastic boxes and watered daily with distilled water in order to maintain the humidity of the substrate. For the growth experiments, the seeds were first germinated at 30 °C in Petri dishes with filter paper moistened with distilled water. New germinated seeds were then selected according to their size and placed in the pots, totaling 32 seedlings per treatment, for each target species.

The experiment was conducted in a Marconi germination chamber at 30 °C within a photoperiod of 12 h (white light) for five (sorghum and brachiaria) and seven days (sesame and purple ipê). At the end of the experiments, the lengths of the shoots and roots of the seedlings were measured with a digital calypter (Mitutoyo). Effects of the treatments on the seedling's morphology were also considered for comparisons.

For each target species, a completely randomized approach was employed. Comparisons were made between the average growth in different treatments for each target-species. For parametric data, Analysis of Variance (ANOVA) followed by the Tukey test was applied. For non-parametric data, the Kruskall-Wallis test was employed, followed by the Student-Newman-Keuls test. The tests were conducted at 5% probability, and the statistical program used for the analyses was the BioEstat 5.0.

RESULTS AND DISCUSSION

We have not tested the effects of the treatments on seed germination of the target species because, as a rule, plant extracts do not significantly interfere with the germination of seeds (Ferreira 2004, Reigosa et al. 2013). Nevertheless, initial growth has shown to be a critical stage in the development of plants due to its high sensitivity to the effects of plant extracts, isolated molecules and allelopathic compounds (Ferreira 2004, Adkins et al. 2007, Reigosa et al. 2013). So, in order to separate any possible effect of the treatments on germination from the effects on the growth of seedlings, this study made use of seeds which had been previously germinated in water for the experiments of initial growth of the target-species.

In the present study, both sorghum (Figure 1) and sesame (Figure 2) revealed a progressive reduction in shoot and root growth with an increase in the quantity of leaves added to the soil.

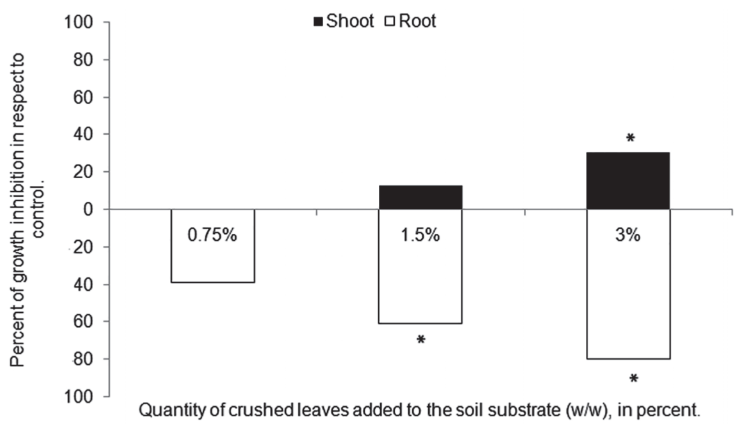

Figure 1 - Percent of inhibition (in respect to control) of shoot and root growth of *Sorghum bicolor* seedlings cultivated for five days in red latosol mixed with crushed leaves of *Caryocar brasiliense* at different proportions. The experiment was conducted for five days at 30 °C and 12 h light cycle. N=32 seedlings per treatment. * indicates statistical differences among treatments and control by the ANOVA – Tukey test (shoots) or Kruskal-Wallis – SNK test (roots), p< 0.05.

The effects were dose-dependent and were more pronounced in the roots than in the shoots, a pattern which has been identified in a wide range of studies (Gatti et al. 2004, Oliveira et al. 2004, Pina et al. 2009). In these two species, necrosis was evident in the tips of the roots, especially in treatments of higher concentration, a manifestation which had been previously observed in other studies involving sesame (Pina et al. 2009). The use of cultivated species has traditionally been carried out as one of the first approaches in phytotoxic and allelopathic studies. Cultivated species are highly sensitive to extracts, show rapid and uniform growth, and respond quickly to the treatments (Ferreira 2004).

In our study, the growth of *U. decumbens* was progressively reduced by the addition of crushed pequi leaves in the soil, reaching an inhibition of 60% of the root growth in comparison to the control (Figure 3).

The presence of triturated leaves also reduced the quantity of secondary roots and increased the number of necrotic roots. These findings suggest that lixiviation of plant litter present below and around individuals of pequi may generate a suppressive effect on the establishment of *U. decumbens* under natural conditions.

The species of the genus *Urochloa* were brought to America from Africa and were widely used in the conversion of native vegetation to

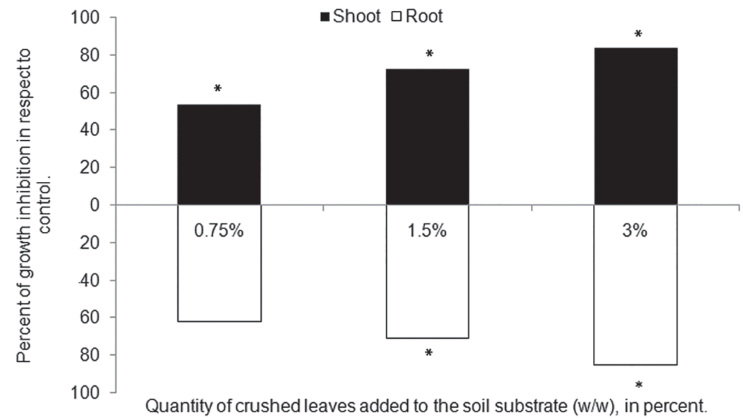

Figure 2 - Percent of inhibition (in respect to control) of shoot and root growth of *Sesamum indicum* seedlings cultivated for five days in red latosol mixed with crushed leaves of *Caryocar brasiliense* at different proportions. The experiment was conducted for five days at 30 °C and 12 h light cycle. N=32 seedlings per treatment. * indicates statistical differences among treatments and control by the ANOVA – Tukey test (shoots) or Kruskal-Wallis – SNK test (roots), $p < 0.05$.

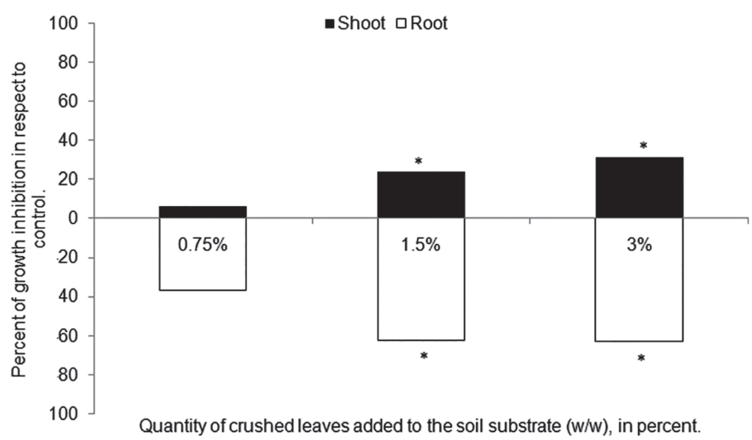

Figure 3 - Percent of inhibition (in respect to control) of shoot and root growth of *Urochloa decumbens* seedlings cultivated for five days in red latosol mixed with crushed leaves of *Caryocar brasiliense* at different proportions. The experiment was conducted for five days at 30 °C and 12 h light cycle. N=32 seedlings per treatment. * indicates statistical differences among treatments and control by the ANOVA – Tukey test (shoots) or Kruskal-Wallis – SNK test (roots), $p < 0.05$.

pasture for livestock grazing; however, they quickly turned into an invasive species of native areas and, due to their negative effects on the recruitment of local species, have affected the dynamics of natural ecosystems (Pivello et al. 1999a, b). Nowadays, these invading species have spread to most regions of Brazil; they are especially prevalent in savanna physiognomies of the Cerrado. The identification of phytotoxicity in the tissues of *Urochloa* spp. (Souza et al. 2006, Barbosa et al. 2008, Rodrigues et al. 2012) suggests that these invasive species could benefit from their phytotoxic effects on other species in their expansion over natural as well as degraded areas (Barbosa et al. 2008).

Taking into consideration that leaf tissues of a wide range of tree species of the Cerrado have demonstrated phytotoxicity against the growth of several species (Reigosa et al. 2013), including species of the genus *Urochloa* (Souza Filho 2006), these results suggest that the tree cover and natural production of plant litter could reduce the establishment of these invasive species in physiognomies where the tree component of the vegetation is present. Indeed, these results might help explain why this African grass is potentially invasive of degraded areas as well as ecosystems in which the natural vegetation has been removed (Williams and Baruch 2000).

In this study it was observed that the presence of triturated leaves of pequi in the soil inhibited the initial growth of purple ipê seedlings in a dose-dependent way (Figure 4).

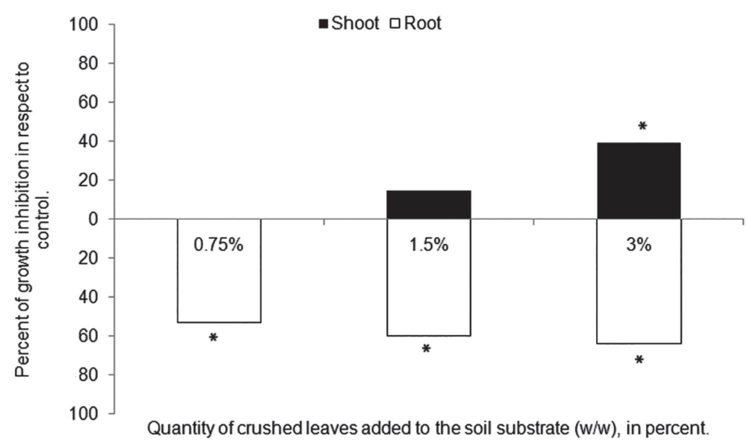

Figure 4 - Percent of inhibition (in respect to control) of shoot and root growth of *Tabebuia impetiginosa* seedlings cultivated for five days in red latosol mixed with crushed leaves of *Caryocar brasiliense* at different proportions. The experiment was conducted for five days at 30 °C and 12 h light cycle. N=32 seedlings per treatment. * indicates statistical differences among treatments and control by the ANOVA – Tukey test (shoots) or Kruskal-Wallis – SNK test (roots), $p < 0.05$.

Inhibition of shoot growth was 40%, whereas the inhibition of root growth reached 65%, within a treatment of 3%, when compared to the control (Figure 4). The use of native plants as target-species in allelopathic studies have been recognized as an essential requirement when the aim is to extrapolate results obtained in the laboratory to allelopathic interactions in the field (Blum 2011). The possible chemical effect of plant litter on the growth of native species (Santos and Válio 2002) could have a role on the natural dynamics of populations and communities in native areas of the Cerrado.

With the exception of sesame, whose inhibition of shoot growth was quite pronounced, for the other target-species the root growth was more affected by the treatments than was the shoot growth. The greater inhibition of root growth in relation to shoot growth has been observed in various studies (Gatti et al. 2004, Oliveira et al. 2004, Pina et al. 2009, Reigosa et al. 2013), and may be the result of more direct contact between the roots and the test-solutions or allelochemicals present in the substrate (Chung et al. 2001). Alternatively, this greater effect on the roots could result simply from the fact that the root system is the way by which the majority of the water as well as the organic and inorganic solutes penetrate the plant. Therefore, phytotoxic and allelopathic effects observed in the shoots of seedlings could be real, but could also be an indirect consequence of these effects on root growth.

The reduction of root growth and/or of the differentiation of secondary roots significantly affects the absorption of nutrients by the seedlings (Raven et al. 2001), which could hamper their development under natural conditions. In the case of the Cerrado, where the soil is characterized by a low availability of nutrients, and the soil surface is seasonality subjected to both water deficit and frequent fires, the initial investment in deep and branched roots becomes essential for the survival of young individuals (Moreira and Klink 2000, Hoffmann 2000, Saboya and Borghetti 2012). In such circumstances, any inhibition of root growth brought about by external agents could hamper the establishment of young individuals in these harsh environments.

Caryocar brasiliense is among the most common and widest-distributed plant species of the Cerrado biome (Ratter et al. 2003). The interference in the seedling growth caused by the presence of leaf residues of pequi in amounts comparable to those recorded in natural conditions (Oliveira 1999) suggest that the litter produced by this species could interfere in the initial establishment of other plant species, among which are the native tree *Tabebuia impetiginosa* and the invasive grass *Urochloa decumbens*. Taking into consideration that the leaves of other native species have presented some level of phytotoxic effect on initial growth of many target-species (Reigosa et al. 2013), these results open up new perspectives for studies that could realistically quantify the allelopathic effects of plant litter in the dynamics of vegetation in the Cerrado.

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RESUMO

Estudos têm identificado potencial fitotóxico de tecidos vegetais de diversas espécies do Cerrado. Entretanto, a maior parte destes estudos foi conduzida em substrato inerte, ou fazendo uso de quantidades exageradas de material vegetal. Neste estudo investigamos o potencial fitotóxico de folhas de pequi adicionadas a substrato solo em quantidades compatíveis ao volume de liteira produzida por esta espécie no campo. Folhas de pequi foram trituradas e incorporadas a latossolo vermelho em proporções entre 0,75 e 3% (p/p), sendo o controle constituído por solo sem folhas. Estas misturas foram adicionadas a vasos plásticos irrigados diariamente. Sementes germinadas das espécies cultivadas sorgo e gergelim, da invasora braquiária, e da nativa ipê-roxo foram dispostas nos vasos e crescidas por cinco a sete dias a 30 °C, com fotoperíodo de 12 h. Plântulas de todas as espécies apresentaram redução dose-dependente no crescimento, sendo o crescimento radicular mais afetado que o aéreo. Sinais de necrose foram observados nas raízes de sorgo, gergelim e braquiária. Os efeitos fitotóxicos gerados por uma quantidade relativamente baixa de tecido vegetal, em substrato solo, e em razoável número de espécies de diferentes grupos funcionais sugerem que a liteira desta espécie possa exercer real potencial alelopático em condições de campo.

Palavras-chave: Alelopatia, *Caryocar brasiliense*, Cerrado, fitotoxicidade, *Tabebuia impetiginosa*, *Urochloa decumbens*.

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