

Interspecific variation in the allelopathic potential of the family Myrtaceae

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Submitted: 14 August, 2012. Accepted: 10 October, 2012

ABSTRACT

Allelopathy is a type of biotic interference wherein a plant releases bioactive metabolites into an environment, thereby affecting the adjacent biota. Stressful environments stimulate the production of these metabolites. The present study tests the novel weapons hypothesis, which postulates that species belonging to the same genus and from the same environment have similar allelopathic effects. The aim of this study was to assess the allelopathic effects that the aqueous leaf extracts of 15 species belonging to five genera of the Myrtaceae family have on the seed germination and initial seedling growth of lettuce (*Lactuca sativa* L.), tomato (*Solanum lycopersicum* L.) and onion (*Allium cepa* L.). Germination rates, average germination times, informational entropy of germination and allelopathic effects, as quantified with a response index, were calculated. A taxonomic distance matrix based on Gower dissimilarity and a Euclidean distance matrix were constructed. The results revealed that all extracts from donor species significantly increased average germination time or reduced the germination rate of eudicotyledonous plant species. The only extracts that showed no effect on monocotyledonous seeds were those of *Campomanesia pubescens* O. Berg and *Psidium cinereum* Mart. We conclude that eudicotyledonous and monocotyledonous plants were both significantly affected by the presence of all extracts tested. Our results make it clear that each species behaves distinctly in relation to allelopathic activity, with no apparent grouping by genus or subtribe. Therefore, the hypothesis was rejected, because plants from the same environment and with taxonomic proximity do not necessarily display similar production of secondary metabolites.

Key words: Allelopathy, Aqueous leaf extract, Genus similarity, Germination

Introduction

In the neotropical savanna of Brazil (the *cerrado*), plants grow in nutrient-poor soils (Haridasan 2008). Therefore, the replacement of predated leaves represents a high cost (Fine *et al.* 2006) and competition for nutrients is intense. Consequently, plant species in this ecosystem have developed defense mechanisms, such as the production of leaves only in propitious periods; the production of coriaceous leaves; the lowering of nitrogen and water content; and the elevation of quantities of phenolic compounds (Marquis *et al.* 2002). Predation and competition for natural resources restrict the distribution of plants, acting as an environmental filter by selecting species with similar traits that allow their survival under environmentally challenging conditions (Fukami *et al.* 2005). Thus, functional traits are generally phylogenetically conserved in plant lineages (Ackerly 2003). Therefore, phylogenetic proximity among species and traits

inherited from a common ancestry should correlate with similar responses to environmental processes (Webb *et al.* 2002; Núñez-Farfán *et al.* 2007). The novel weapons hypothesis postulates that plant species belonging to the same genus and subjected to the same environmental conditions tend to have similar characteristics, principally with respect to their defense mechanisms, which include the production of inhibitory secondary metabolic compounds. To test this hypothesis, we collected samples of 15 species belonging to five genera of the family Myrtaceae, all from the *cerrado* ecosystem. Our objective was to determine whether the allelopathic effect is similar across the genera.

Allelopathy is a type of biotic interference wherein a plant releases bioactive metabolites, known as allelochemicals, into the surrounding environment. The growth of neighboring vegetation might be affected and a selective advantage thus afforded the donor plant (Anaya 1999). Allelochemicals affect various metabolic processes in or-

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ganisms: altering membrane permeability (Bogatek *et al.* 2005) and ion absorption (Gniazdowska & Bogatek 2005); inhibiting electron transport in photosynthesis and respiration (Abraham *et al.* 2000); changing enzyme activity (Singh *et al.* 2009); and impeding cell division (Teerarak *et al.* 2010). Because of these effects, allelopathy is recognized as an important ecophysiological process in ecosystems, influencing primary and secondary plant succession, as well as the structure, composition and dynamics of native or cultivated plant communities (Rizvi *et al.* 1992; Scrivanti *et al.* 2003). In addition, allelopathy plays a key role in the detection of bioactive compounds of commercial importance (Oliveros-Bastidas 2008).

Worldwide, the family Myrtaceae includes approximately 3,100 species in approximately 140 genera, divided into two subfamilies, Leptospermoideae and Myrtoideae, outlined in the second edition of the Angiosperm Phylogeny Group classification system, as modified by Judd *et al.* (1999) and later ratified by Watson & Dallwitz (2007). In Brazil, the subfamily Myrtoideae comprises 23 genera and approximately 1,000 species (Cardoso & Sajo 2006). The floral inventory compiled by the Brazilian Institute of Geography and Statistics showed that the *cerrado* contains approximately 211 Myrtaceae species in 14 genera, making this family one of the most representative in the ecosystem. Species of *Eucalyptus* (subfamily Leptospermoideae), the most widely studied genus in the family Myrtaceae, have been reported to show allelopathic effects (Fang *et al.* 2009). However, there have been few studies of the allelopathic potential of Myrtaceae species found in the *cerrado* ecosystem, most of which belong to the subfamily Myrtoideae. In the *cerrado*, some Myrtaceae species have been observed to inhibit the growth of adjacent plants, indicating that the former produce allelochemicals. Therefore, the aim of this study was to assess the allelopathic effects that the aqueous leaf extracts of 15 species belonging to five Myrtaceae family genera found in the *cerrado* ecosystem have on the seed germination and initial seedling growth of *Lactuca sativa* (lettuce), *Solanum lycopersicum* (tomato) and *Allium cepa* (onion).

Materials and methods

Collection area

The plant material used in this study was collected in the *cerrado* (*stricto sensu*) in the state of São Paulo, Brazil (21°58' to 22°00'S; 47°51' to 47°52'W). According to the Köppen climate classification system, the climate in the region is type Cwa, with two well-defined seasons (Monteiro & Prado 2006): a wet season (from October through March) and a dry season (from April through September). The vegetation is characterized by a woody layer composed of trees and bushes that protrude above a clearly defined herbaceous layer (Ribeiro & Walter 1998).

Biological material

The Myrtaceae specimens found on-site were marked and observed until the period of flowering and fruiting, enabling the species to be identified. The leaves of each species were non-systematically collected from at least five plants in the vegetative stage, during the dry season. Leaves were collected from the following donor species (all belonging to the tribe Myrteae): subtribe Myrteinae—*Blepharocalyx salicifolius* Kuth O. Berg, *Campomanesia pubescens* O. Berg, *Psidium australe* Cambess., *P. cinereum* Mart., *P. laruotteanum* Cambess. and *P. rufum* Mart. ex DC.; subtribe Eugeniinae—*Eugenia bimarginata* O. Berg, *E. klotzschiana* O. Berg, *E. myrcianthes* Nied. and *E. puniceifolia* (Kunth) DC.; and subtribe Myrciinae—*Myrcia bella* Cambess., *M. lingua* (O. Berg) Mattos, *M. multiflora* DC., *M. splendens* DC. and *M. tomentosa* DC. Voucher specimens of each species were deposited at the Herbarium of the Federal University of São Carlos (accession nos. 8308, 8309, 8319, 8320, 8321, 8322, 8310, 8311, 8312, 8313, 8314, 8315, 8316, 8317 and 8318, respectively). Leaves were collected and dried in an incubator at 40°C for 48 h, then powdered in an electric grinder and stored in plastic containers at room temperature ($\approx 25^\circ\text{C}$).

The allelopathic effects of these extracts were tested on three target species: two eudicotyledonous species, *Lactuca sativa* L. (Asteraceae) var. Regina (lettuce) and *Solanum lycopersicum* L. (Solanaceae) var. IPA 6 (tomato); and the monocotyledonous species *Allium cepa* L. (Liliaceae) var. Baia Periforme (onion).

Preparation of plant extracts

The extracts were prepared by mixing powdered dried leaves with distilled water in the proportion 1 g powder:10 ml water, at 10% weight/volume. This solution was stored at $\approx 5^\circ\text{C}$ for 12 h. The extract was then filtered by suction through a Buchner funnel covered with filter paper (Gatti *et al.* 2010).

Germination bioassay

The seeds of the target species were placed in Petri dishes (9 cm in diameter) lined with two sheets of filter paper moistened with 5 ml of an aqueous leaf extract or with the same volume of distilled water (control). The experiments were carried out with four replicates of 20 seeds per dish for each extract. Petri dishes containing lettuce and tomato seeds were maintained in a biochemical oxygen demand germination chamber at 25°C and Petri dishes containing onion seeds were maintained at 20°C. After preliminary tests performed to determine the optimal germination conditions for each target species, all of the Petri dishes were maintained on a 12/12-h light/dark cycle. Germination counts were made every 12 h for 15 days. Seeds that sprouted a 2-mm primary root were classified as having germinated (Borghetti & Ferreira 2004).

Seedling growth bioassay

The seeds used in this bioassay were first germinated in water (until presenting a root length of 2–4 mm), then moved to transparent plastic boxes (500 ml, 14 × 10 cm) lined with two sheets of filter paper and moistened with 13 ml of extract or distilled water (control). The boxes were kept in biochemical oxygen demand germination chambers, under the same conditions described for the germination bioassay. The length of the shoot and primary roots, in mm, were measured with a digital caliper after seven days.

Physicochemical characteristics of the extracts

The osmotic potential of the extracts was measured with an osmometer (5004 MICRO-OSMETTE; Precision Systems, Natick, MA, USA). Osmolality was measured in mOsm kg⁻¹ and converted to MPa (Larcher 2004). The germination and growth bioassays of lettuce, onion and tomato were carried out with polyethylene glycol (PEG) 6000 solutions in order to simulate the osmotic potential recorded in the extracts. The PEG solution was prepared in accordance with specifications given by Sun (2002), and the germination and growth bioassays followed the method described above.

The pH of the extracts was measured with a pHmeter (PM608; Analion, Ribeirao Preto, Brazil). Because the pH of all the samples was within the range recommended for germination and plant growth (Larcher 2004), bioassays to evaluate the influence of pH were not carried out.

Mathematical and statistical analysis

We calculated proportional germination, expressed as a percentage; average germination time, in days; informational entropy of germination, in bits (Ranal & Santana 2006); and the response index for the allelopathic effect (Zhang *et al.* 2010). The response index was calculated as follows:

$$RI = (T \cdot C^{-1}) - 1$$

where RI is the response index, T is the germination rate (seeds germinated per day) for seeds exposed to the leaf extract, and C is the germination rate (seeds germinated per day) for the control seeds.

The design of the laboratory experiments was completely randomized, with four replications for each treatment. The statistical significance of the differences between the treatments and the control were investigated by Student's t-test, for normal data, or by the Wilcoxon test, for non-normal data, both at the 5% level.

A taxonomic distance matrix of Gower dissimilarity based on genus and subtribe was constructed for the 15 donors. Subsequently, a Euclidean distance matrix of donors was created based on the mean values of each variable (proportional germination, average germination time, informational entropy of germination, shoot length and root length) for all target species. The correlations between donors, in terms of the taxonomic and Euclidean distances, were

tested with Mantel's test (Manly 2000). A dendrogram was built from the Euclidean distances, using the group average method, to define the clusters formed by the donor species with similar allelopathic effects. Clusters were identified by a dissimilarity ≥ 50 . We used ANOVA, followed by Tukey's test, in order to illustrate which variables influence the clustering. All statistical analyses were performed with the program R, version 2.9.1 (R Development Core Team 2009).

Results

The osmotic potential of the aqueous leaf extracts ranged from –0.1 to –0.2 MPa. The bioassays using PEG 6000 showed that the osmotic potential did not influence germinability, average germination time or seedling length (Fig. 1 and 2).

The results obtained in the germination bioassay are shown in Fig. 1. Regarding the first eudicotyledonous species (lettuce), the aqueous leaf extracts of *C. pubescens* and *P. cinereum* did not have significant effects on the germination rate, although they did significantly increase the average germination time. In addition, lettuce seeds displayed high values for information entropy when subjected to *C. pubescens* extract, indicating low synchrony of the germination process. Regarding the second eudicotyledonous species (tomato), the aqueous leaf extracts of *C. pubescens*, *E. myrcianthes* and *P. cinereum* did not significantly reduce proportional germination, although they did significantly increase the average germination time. In the monocotyledon species (onion), the effects of the aqueous leaf extracts of *E. myrcianthes*, *E. puniceifolia*, *M. multiflora*, *M. splendens*, *M. tomentosa* and *P. laruotteanum* had significant effects on the germination rate, and all of the extracts except *C. pubescens*, *E. myrcianthes*, *P. cinereum* and *P. laruotteanum*, had significant effects on average germination time (Fig. 1). Hence, all of the extracts inhibited the germination of eudicotyledon and monocotyledon seeds, with the exception of the extracts of *C. pubescens* and *P. cinereum*, which did not inhibit the germination of monocotyledon seeds. All of the target species seeds had a negative response index, indicating the presence of allelopathic activity on the part of all donor species (Fig. 1).

The results of the growth bioassay are shown in Fig. 2. Among the eudicotyledonous species, the extracts inhibited shoot and root growth for lettuce seedlings. Regarding tomato, all of the tested extracts caused significant inhibition of root growth, whereas shoot growth was reduced by 11 of the 15 extracts. In the monocotyledon species (onion), all extracts reduced root growth; however, only eight extracts altered shoot growth.

No correlation was found between the variables of Euclidean and taxonomic distances (Mantel's $r = 0.15$, $p = 0.057$). Cluster analysis of the allelopathic effects identified four clusters of donor species—clusters A, B, C and D, comprising 1, 5, 4 and 6 species, respectively (Fig. 3). Cluster A

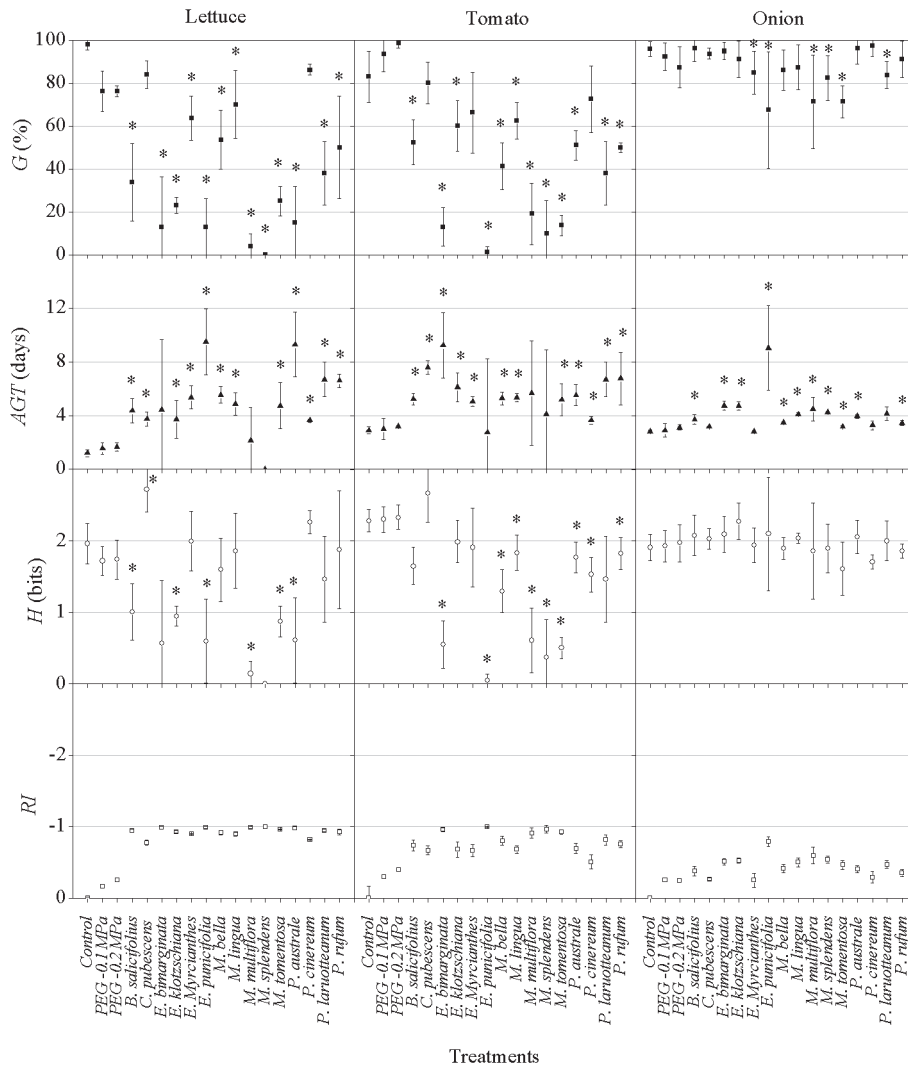


Figure 1. Average values for proportional germination (G , ■); average germination time (AGT , ▲); informational entropy of germination (H , ○) and allelopathic effect, as quantified with a response index (RI , □), in lettuce, tomato and onion seeds exposed to aqueous leaf extracts of the donor species or to distilled water (control). PEG – polyethylene glycol; B. – *Blepharocalyx*; C. – *Campomanesia*; E. – *Eugenia*; M. – *Myrcia*; P. – *Psidium*. *significantly different from control ($p < 0.05$).

(control) differed from the other clusters in terms of seed germination for lettuce and tomato; shoot length for onion, tomato and lettuce; and root length for onion and tomato. Cluster C did not differ from cluster A in terms of lettuce seed germination or root length (Table 1). In cluster B, the extracts strongly inhibited lettuce and tomato germination, compared with only slight inhibition of such germination in cluster C. Cluster D species extracts moderately inhibited lettuce and tomato germination (Table 1).

Discussion

The osmotic potentials of the aqueous leaf extracts evaluated significantly influenced neither seed germination nor seedling development (Fig. 1 and 2), indicating that the detrimental effects exerted by the extracts on the germi-

nation and growth of the target species resulted from the presence of bioactive substances. According to Grisi *et al.* (2012), osmotic potentials below -0.3 MPa do not interfere in seed germination or in the initial growth of seedlings.

The results showed that the monocotyledon and eudicotyledon plants respond differently to the extracts (Fig. 1 and 2). The influence of the extracts on germination depends on the size and permeability of the seed coat (Hanley & Whiting 2005). The species-dependent response to the allelochemicals shows that they are fundamental not only in natural environments, where they influence floristic composition, but also in agriculture, where they can be used as selective herbicides.

Allelopathic chemicals alter plant growth and seed germination through a multiplicity of effects on physiological and biochemical processes, because there are hundreds of different structures and many of the compounds have

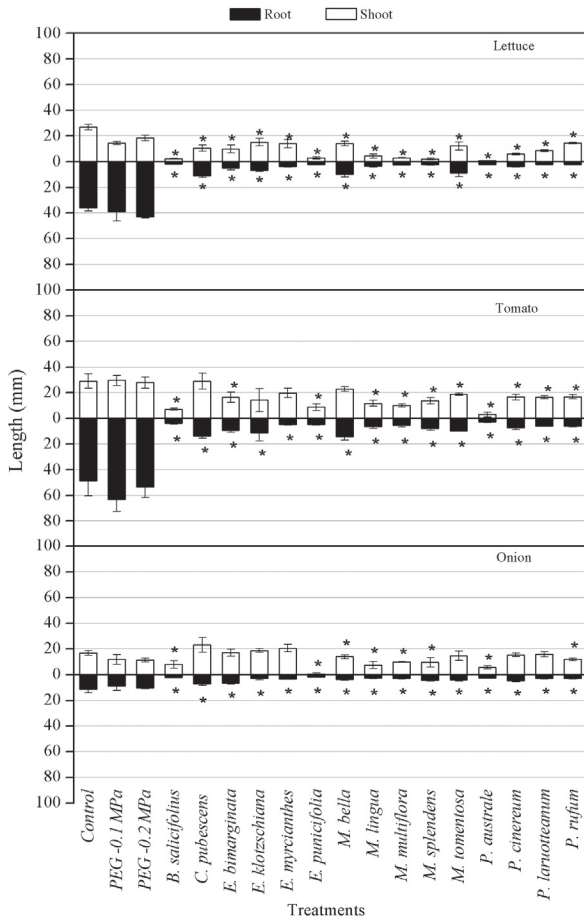


Figure 2. Average lengths of shoots (□) and roots (■) of lettuce, onion, and tomato seedlings exposed to aqueous leaf extract of the donor species or to distilled water (control). PEG – polyethylene glycol; B. – *Blepharocalyx*; C. – *Campomanesia*; E. – *Eugenia*; M. – *Myrcia*; P. – *Psidium*. *significantly different from control (p<0.05).

multiple phytotoxic effects. According to Singh *et al.* (2009), phytotoxins can affect enzyme activities or plant hormones, increasing amylase activity and promoting a greater release of reserves that would otherwise be provided to the embryo, extending oxidative stress and seed dormancy through the increase of abscisic acid production and inhibiting water absorption via alterations in membrane permeability. The same author also observed a reduction in protein content and nitrate reductase activity in corn root tissues exposed to *Nicotiana plumbaginifolia* leachates. Einhellig (1995) also reported that alteration in the enzymatic activity of seeds affects the mobility of stored compounds, thus increasing germination inhibition, or sometimes completely suppressing it. Therefore, the observed differences between the control and treatment groups, in terms of the number of germinated seeds, might be attributed to the presence of allelopathic compounds. The seedling growth results for almost all of the target species showed that the roots were as sensitive to the leaf extracts as were the shoots. Allelochemicals can affect hydrogen adenosine triphosphatase in

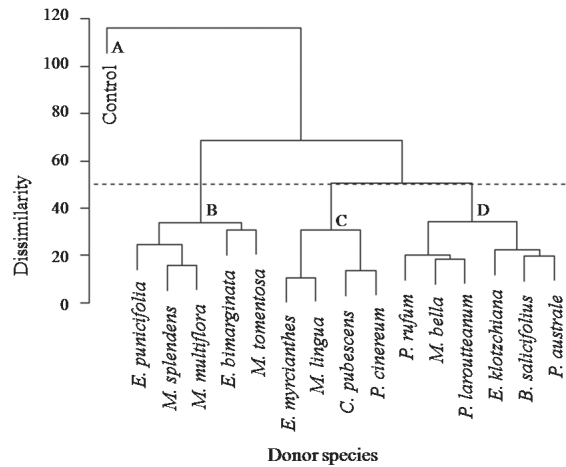


Figure 3. Dendrogram of the allelopathic activity of 15 donor species of the family Myrtaceae. The main clusters were designated at a dissimilarity ≥ 50 . E. – *Eugenia*; M. – *Myrcia*; C. – *Campomanesia*; P. – *Psidium*; B. – *Blepharocalyx*.

the plasma membrane, which is responsible for generating the electrochemical proton gradient and thus provides the driving force for the uptake and efflux of ions and metabolites across the plasma membrane. Hydrogen adenosine triphosphatase inhibition results in reduction of the uptake of minerals and water by roots and, consequently, has a significant effect on essential plant functions such as photosynthesis, respiration and protein synthesis, culminating in growth reduction (Gniazdowska & Bogatek 2005). In addition, allelochemicals have been associated with the inhibition of mitosis and disruption of organelle structure (Zhang *et al.* 2010), due to their effects on chromatin organization, altering the physical and chemical properties of DNA (Teerarak *et al.* 2012). Cell growth in plants is dependent on the mitotic process. When cell divisions are disturbed during germination, most seedlings either die before emergence or show abnormalities.

The effects of allelochemical activity can be detected at the molecular, structural, biochemical, physiological and ecological levels of plant organization (Gniazdowska & Bogatek 2005). Allelopathic compounds can induce secondary oxidative stress, manifesting as increased production of reactive oxygen species (ROS), as demonstrated by Weir *et al.* (2004). It is known that ROS act as signaling molecules, indicating biotic and abiotic stress (Foyer & Noctor 2005), as well as being major regulators of plant growth and development (Gapper & Dolan 2006). Certain ROS—mainly ethylene and abscisic acid—have been shown to act as second messengers in plant hormone responses (Kwak *et al.* 2006). Ethylene and abscisic acid are both regarded as common stress hormones involved in the regulation of seed dormancy and germination (Kucera *et al.* 2005). Some allelochemicals of *Artemisia annua* have been shown to decrease protein content and increase superoxide dismutase activity (Lixiao *et al.* 2012). According to Oracz *et al.*

Table 1. Allelopathic responses of three target species to the donor species extracts. Clusters of donor species were formed by hierarchical ordering of allelopathic activity. The significance of differences among groups was tested by ANOVA.*

Variable	Target	Cluster A (n = 1)	Cluster B (n = 5)	Cluster C (n = 4)	Cluster D (n = 6)	P
Germination (%), mean ± SD	Lettuce	98 ± 2.3 ^a	11 ± 9.7 ^c	76 ± 10.9 ^a	35.6 ± 15.0 ^b	<0.001
	Onion	96 ± 3.3	77.5 ± 11.3	90.9 ± 5.7	90.8 ± 5.1	0.04
	Tomato	83 ± 11.9 ^a	11.4 ± 6.5 ^d	70.3 ± 7.7 ^b	48.8 ± 8.8 ^c	<0.001
Average germination time (days), mean ± SD	Lettuce	1.2 ± 0.3	4.2 ± 3.6	4.4 ± 0.8	6.0 ± 2.0	0.29
	Onion	2.8 ± 0.1	5.1 ± 2.3	3.3 ± 0.5	3.9 ± 0.5	0.22
	Tomato	2.9 ± 0.3	5.4 ± 2.4	5.4 ± 1.6	5.9 ± 0.7	0.45
Shoot length (mm), mean ± SD	Lettuce	26.5 ± 2.1	7.0 ± 6.6	11.4 ± 2.1	7.3 ± 4.1	0.01
	Onion	34.2 ± 5.1 ^a	7.5 ± 5.7 ^b	11.5 ± 3.8 ^b	7.4 ± 3.1 ^b	<0.001
	Tomato	67.6 ± 9.1 ^a	13.7 ± 5.8 ^b	15.0 ± 4.3 ^b	13.2 ± 5.1 ^b	<0.001
Root length (mm), mean ± SD	Lettuce	27.4 ± 2.2 ^a	7.3 ± 5.8 ^b	12.1 ± 5.0 ^{ab}	9.5 ± 5.5 ^b	0.03
	Onion	31.4 ± 0.9 ^a	7.6 ± 4.4 ^b	12.5 ± 6.5 ^b	8.2 ± 3.8 ^b	0.004
	Tomato	63.4 ± 15.9 ^a	14.2 ± 4.6 ^b	14.5 ± 4.4 ^b	12.8 ± 6.1 ^b	<0.001
Entropy (bits), mean ± SD	Lettuce	2 ± 0.3	0.8 ± 0.7	1.8 ± 1.1	1.3 ± 0.5	0.24
	Onion	1.9 ± 0.2	1.9 ± 0.2	1.9 ± 0.1	2.0 ± 0.1	0.6
	Tomato	2.3 ± 0.2	0.9 ± 0.9	1.2 ± 0.8	1.8 ± 0.5	0.15

Cluster A – control; Cluster B – *Eugenia puniceifolia*, *Myrcia splendens*, *M. multiflora*, *E. bimariginata* and *M. tomentosa*; Cluster C – *E. myrcianthes*, *M. lingua*, *Campomanesia pubescens* and *Psidium cinereum*; Cluster D – *P. rufum*, *M. bella*, *P. laruooteanum*, *E. klotzschiana*, *Blepharocalyx salicifolius* and *P. australe*.

*Values on the same row and followed by same superscript letter do not differ significantly from each other.

(2012), myrigalone, extracted from *Myrica gale*, inhibits the seed germination of *Lepidium sativum* by impeding the metabolism of gibberellins, the metabolism of abscisic acid, and apoplastic superoxide production, all of which are required for endosperm rupture and consequent embryo growth by elongation.

Few studies have focused on the allelopathic potential of Myrtaceae species in Brazil. In a study of sesame and radish (Pina *et al.* 2009), the leaf extracts of *E. dysenterica* (Myrtaceae) were found to have no influence on the germination of seeds but drastically reduced the seedling elongation rate. Fresh leaf extracts of *Blepharocalyx salicifolius* (Myrtaceae) have been shown to reduce the survival and germination of lettuce seeds (Mairesse *et al.* 2007). Souza-Filho *et al.* (2006) observed that methanolic and hexane extracts of *M. guianensis* inhibited seed germination of the weeds *Senna obtusifolia* and *Mimosa pudica*. Crushed leaves and aqueous leaf extract of the species *Campomanesia adamantium* and *E. dysenterica*, tested in soil, have been found to reduce the length of sesame seedlings (Souza *et al.* 2007). Species of *Eucalyptus*—a genus originating mainly from Oceania—have been widely examined for allelopathic potential. Currently, approximately 38 *Eucalyptus* species of Australia have proven to display inhibitory activity against various organisms (Willis 1999).

The clusters of the donor species investigated in the present study were not aligned by genus or subtribe, as

would be expected. This corroborates the results obtained by Santos & Salatino (2000), who observed the non-clustering of Annonaceae species of the *cerrado* with respect to the chemical composition of the leaf flavonoids. All donor species studied here belong to the Myrteae tribe, whose systematics have been studied in an attempt to determine the best grouping within the tribe (McVaugh 1968; Salywon *et al.* 2002; Snow *et al.* 2003; Wilson *et al.* 2001; 2005). Wilson *et al.* (2001) stated that the Myrteae tribe is paraphyletic. Indeed, analysis of the *matK* gene sequence has demonstrated that the *Syzygium/Acmena* group arose independently. Lucas *et al.* (2007) showed that the Myrteae tribe would be monophyletic if the *Syzygium/Acmena* group were excluded. Among the subtribes studied here, Myrtinae and Eugeniinae are paraphyletic, whereas Myrciinae is monophyletic (Lucas *et al.* 2005). Studies based on nuclear DNA sequencing suggest that the genus *Psidium* (subtribe Myrtinae) may be paraphyletic (Salywon *et al.* 2002), whereas Lucas *et al.* (2005), using nuclear and plastid DNA sequences, demonstrated that this genus is very likely monophyletic. The genus *Blepharocalyx* (subtribe Myrtinae) is extremely isolated, and further research is needed in order to establish the relevance of this group within the tribe (Lucas *et al.* 2005). The genus *Eugenia* (subtribe Eugeniinae) shows signs of being paraphyletic, furthermore, nuclear and plastid DNA sequence data have demonstrated that the genus *Myrcia*

is also paraphyletic (Lucas *et al.* 2005). Reports in the literature show that most of the investigated genera have a tendency to be paraphyletic, which underscores the results obtained in the present study. Another factor that explains the lack of clustering of the genera studied is the variation in composition and quantity of allelochemicals in the family Myrtaceae. Keszei *et al.* (2008) stated that the leaves of Myrtaceae species have high concentrations of terpenes and that these compounds show substantial qualitative and quantitative variations between each taxon, population and individual. Other phytochemical studies of Myrtaceae leaves have identified sesquiterpenes, triterpenes, flavonoids and alkaloids in *Calycorectes psidiiflorus* (Domingues *et al.* 2010); flavonoids, tannins and phenols in *Plinia cauliflora* (Souza-Moreira *et al.* 2010); and flavonoids in *Baeckea frutescens* (Kamiya & Satake 2010).

In the present study, which involved eudicotyledonous and monocotyledonous species, germination and growth were both significantly affected by the aqueous leaf extracts evaluated, demonstrating the phytotoxicity of the donor species. This information might foster the success of agricultural and agro-forestry systems, allowing the identification of interspecific associations. In addition, such extracts could be studied from the perspective of their herbicidal, insecticidal and fungicidal properties, which could make them useful in the battle against the main pests responsible for reducing productivity of those systems.

Our data could also be of use in ecological studies, because these species evaluated here can influence the diversity and spatial distribution of individuals in natural communities. On the basis of these results, it is clear that each donor species has a different phytotoxic effect. There was no clustering by genus or subtribe. Therefore, plants from the same environment and with taxonomical proximity showed no similarities in the production of secondary metabolites. For this reason, the hypothesis tested here was rejected; in relation to the production of bioactive compounds, the characteristics intrinsic to each species supersede environmental conditions, as the environment was the same for all species. It was concluded that both the eudicotyledonous and monocotyledonous plants were significantly affected by the presence of aqueous leaf extracts.

Acknowledgements

This study received financial support from the Brazilian *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES, Office for the Advancement of Higher Education) and *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq, National Council for Scientific and Technological Development; grants to PN and SCJG). We are grateful to D. M. da Silva for assisting in the statistical analyses and to M. Sobral for aiding in species identification.

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