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Influence of *Eucalyptus spp.* essential oils and tannins on herbivory

A influência dos óleos essenciais e taninos de *Eucalyptus spp*. na herbivoria

Influencia de los aceites essenciales y taninos de *Eucalyptus spp*. em la herbivoría

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Abstract
Eucalyptus (family Myrtaceae), a native plant from Australia, is a genus cultivated due to its oil, gum, pulp and timber used worldwide for different commercial applications. It is a plant with significant economic importance and possibly its cultivation, in Brazil and other countries, has reduced the exploitation of native trees. The secondary metabolism of *Eucalyptus*, as usually found in plants, produces essential oils and tannins, which may interfere in the consumption of plants by wild animals. The goal of the present work was to investigate the influence of essential oil and tannins concentration on the herbivory of seven *Eucalyptus* taxa. Essential oils composition for all species investigated were characterized by CG MS HS-Trap analysis. The lowest hebivory rate were associated to Urocam, Grancam and *Eucalyptus benthamii*. Those taxa were associated with the presence of mix oil compounds, α/β-Pinene, Pinocarveol, α-Terpineol and Tannins. Tannins concentrations and essential oil are an important factor that contributes to the plant protection against herbivory.
Keywords: Astringency of plants; Phenolic compounds; Protection against herbivory.

Resumo

O *Eucalyptus* (família Myrtaceae) é uma planta nativa da Austrália, sendo um gênero cultivado devido ao seu óleo de goma, celulose e a madeira, utilizada mundialmente para diversas aplicações comerciais. É uma planta de grande relevância econômica e possivelmente seu cultivo, no Brasil, bem como em outros países, tem reduzido a exploração de árvores nativas. O metabolismo secundário do *Eucalyptus*, comumente encontrado nas plantas, produz óleos essenciais e taninos que podem interferir no consumo das plantas pelos animais silvestres. O objetivo do presente trabalho foi investigar a influência da concentração de óleo essencial e taninos na herbivoria de sete táxons de *Eucalyptus*. A composição dos óleos essenciais para todas as espécies investigadas foi caracterizada pela análise CG MS HS-Trap. As menores taxas de herbivoria foram associadas a Urocam, Grancam e *Eucalyptus benthamii*. Esses táxons foram associados à presença de compostos de óleos mistos, α/β-Pinene, Pinocarveol, α-Terpineol e Taninos. As concentrações de taninos e óleo essencial são um fator importante que contribui para a proteção das plantas contra a herbivoria

Palavras-chave: Adstringência de plantas; Compostos fenólicos; Proteção contra herbivoria.

Resumen

Eucalyptus (familia Myrtaceae) es una planta originaria de Australia, siendo un género cultivado debido a su aceite de goma, celulosa y madera, utilizado en todo el mundo para varias aplicaciones comerciales. Es una planta de gran importancia económica y su cultivo, tanto en Brasil como en otros países, posiblemente ha reducido la explotación de árboles nativos. El metabolismo secundario del eucalipto, que se encuentra comúnmente en las plantas, produce aceites esenciales y taninos que pueden interferir con el consumo de plantas por parte de los animales salvajes. El objetivo del presente trabajo fue investigar la influencia de la concentración de aceite esencial y taninos sobre la herbivoría de siete taxones de Eucalyptus. La composición de los aceites esenciales para todas las especies investigadas se caracterizó mediante análisis CG MS HS-Trap. Las menores tasas de herbivoría se asociaron con Urocam, Grancam y Eucalyptus benthamii. Estos taxones se asociaron con la presencia de compuestos oleosos mixtos, α/β-pineno, pinocarveol, α-terpineol y taninos. Las concentraciones de taninos y aceite esencial son un factor importante que contribuye a la protección de las plantas contra la herbivoría.

Palabras clave: Astringencia de plantas; Compuestos fenólicos; Protección contra la herbivoría.

1. Introduction

The genus *Eucalyptus* L'Héritier is native from Australia (Harris, 2003) and was introduced in several continents to be used in different commercial applications. This resulted in significant economic importance and in reducing exploitation of native trees, mainly for providing a fast economic return due to its short cycle (Amazonas et al., 2018) The choice of *Eucalyptus* is justified considering that many species and varieties can easily adapt to various soil and climate conditions (Dutt & Tyagi, 2011; Neiva et al., 2015). Also, *Eucalyptus* is immune to several pests and diseases and its essential oils have great genetic variability and a wide range of physical and chemical properties (Neiva et al., 2015). The essential oil have a high economic importance and is widely used in industry, especially in pharmaceuticals and perfumery, even its medicinal effect was studied after a Covid-19 pandemic (Parys et al., 2022). A very studied species regarding quantitative genetic control of important plant traits, as e.g. including terpenes (Jacq et al., 2022). It is a heteroblastic species and genetic variation has been detected both within and between populations at the juvenile coppice and adult leaf stages, suggesting that the populations may involve quite different ontogenetic trajectories (Wallis et al., 2011).

On the other hand, *Eucalyptus* plant improvement is made by modifying their genetic heritage, leading to hybrids varieties that are able to improve economic efficiency, adaptation to specific environmental conditions and even resistance to pests and diseases (Kullan et al., 2012). For this reason, *Eucalyptus* cloning and hybridization techniques have become increasingly common, one of the best known hybrid is the Urograndis (intersection of *Eucalyptus grandis* W. Hill. ex. Maiden x *Eucalyptus urophylla* S. T. Blake), which comprises the best features of each species (Domingues et al., 2011). Urograndis kept both, fast growing and wood quality from *Eucalyptus grandis* and rapid adaptation and disease resistance from *Eucalyptus urophylla* (Domingues et al., 2011). In this context, hybridization works in favor of providing superior individuals capable of producing genetic gains in forest productivity.

In southern Brazil, Atlantic forest exploitation and its replacement by livestock, agriculture, forestry and cities led to a wild fauna and flora species loss (Oliveira et al., 2022). Atlantic forest shows a high reduction of population size of different animal species coupled with a decrease in food supply resources (Batista et al., 2021). Lower population size was observed especially in periods of low fruit availability, made some animal species to adapt to anthropized environments (Hohenlohe et al., 2021). The use of anthropized environments by wild fauna may leading to the consumption of other resources that may contain large amounts of carbohydrates, proteins and other substances that show priority of ingestion (Baldi et al., 2022). The choice of food for these animals is related to the targeting of individual genetic fitness (Chouvelon et al., 2022). Therefore, changes in food resources by some animal species may also modify their risk of being eaten by predation and energy expenditure involved in foraging strategies to obtain food (Chouvelon et al., 2022).

Wild animals prioritize the intake of nutrients such as proteins and carbohydrates and avoid foods with high concentration of tannins (Prache et al., 2022). Tannins are found in different parts of the plants, such as the bark, wood, fruits, seeds, leaves and roots. It is generally extracted from the bark or heartwood of plants (Hassanpour et al., 2011). Tannins are water-soluble phenolic compounds originated from the plant secondary metabolism, it is responsible for the plant astringency, preventing the attack by predators and herbivores (Cheng et al., 2022). Despite this, tannins are ingested by several mammals herbivorous, thereby reducing the dry matter digestion in some of them, by acting as a toxin or an digestion inhibitor (Rafferty & Lamont, 2021).

Other compounds, such as essential oils, protect plants against herbivores, (Mithöfer & Boland, 2012). Essential oils are originated from the plant secondary metabolism and can be extracted from flowers, leaves, bark, rhizomes and fruits. It is highly volatile, intense and have a pleasant odor with acid and spicy flavor, usually colorless or yellowish, and are in general unstable in the presence of light, heat, humidity and metals (Turek & Stintzing, 2013). The presence of essential oil provides an defense advantage for *Eucalyptus* spp. For example, protecting the leaves against harmful insects and herbivorous attack (Padmanaban et al., 2022). Essential oil composition vary according to the plant species, which makes plants behave differently regarding to the herbivory.

Therefore, based on the presupposed that: i) due to high degradation in Atlantic forest areas led to a wild fauna to adapt to anthropized environments, especially in periods of low fruit availability; and ii) wild fauna change the food resources and prioritize the intake of nutrients such as proteins and carbohydrates and avoid foods with high concentration of tannins. Our first hypothesis is that if the hybridization of *Eucalyptus* species presents higher resistance to pathogens and herbivory, so the hybrids species will present higher concentrations of oils and tannins than non-hybrids species and consequently lower herbivory. To test this hypothesis, we used one way ANOVAs of the herbivory rates and concentration of oils and tannins among species of *Eucalyptus*. Also, if oils and tannins present high efficiency in herbivory control, our second hypothesis is that high oils and tannins concentrations will have a negative relationship with herbivory. To test this, we use linear regressions between herbivory and concentration of oils and tannins. The main goals of this study were: i) to identify which taxa of *Eucalyptus* spp. were preferred by the wild fauna in the subtropical zone of Southern Brazil; ii) to check the effects of different essential oils and tannins concentration on wild animals herbivory avoidance and iii) to describe the characterization of chemical composition and relationships of all the measured compounds with different taxa of *Eucalyptus* spp through a Principal Component Analysis (PCA).

2. Methodology

2.1 Study area

Eucalyptus spp. sampling was performed in an experimental area of agricultural research and Rural Extension of Santa Catarina state (Epagri) located in the municipality of Guatambu (27° 07 ' 55 "S and 52° 44 ' 04" W), South of Brazil.

2.1.1 Experimental design

Was analyzed seven taxa of *Eucalyptus* sp.: clones of hybrids of Urograndis (*Eucalyptus urophylla* x *Eucalyptus grandis*), Grancam (*Eucalyptus grandis* x *Eucalyptus camaldulensis* Dehnh) and Urocam (*Eucalyptus urophylla Eucalyptus camaldulensis*), clones of *Eucalyptus grandis* and *Eucalyptus saligna Smith* and *Eucalyptus benthamii* Maiden & Cambage and *Eucalyptus dunnii* Maiden produced from seeds. The experiment was organized in blocks with four repetitions, in portions of 45 plants, with 21 useful plants for each repetition. The planting spacing of the experiment is 3x2 m.

2.2 Data collection

2.2.1 Herbivory data

Twenty-one *Eucalyptus* plants, of each selected taxa, were inspected for herbivory in each experimental plot for each one of the 4 replicates. Each *Eucalyptus* tree evaluated was considered consumed when it had the apex tip uprooted, which impairs the plant`s growth. Plants with no damaged apex tip were considered not consumed. The tip of the damaged apex was considered as an indication that the plant was consumed by wild animals. It was not possible to observe the herbivory in the field because the experiment was found with signs of consumption by wild animals during a routine visit to the experiment.

To recognize the mark left by animals in the consumed Eucalyptus, were given plant branches for animals in captivity, such as *Sapajus nigritus* (black-horned tufted capuchin) and *Amazona aestiva* (turquoise-fronted parrot). A branch of *Eucalyptus benthamii*, *Eucalyptus dunnii*, *Eucalyptus saligna*, *Eucalyptus grandis*, Grancam, Urocam and Urograndis for each of the four black-horned tufted capuchin nurseries and five branches for the turquoise-fronted parrot are offered *Eucalyptus benthamii*, *Eucalyptus dunnii*, *Eucalyptus grandis*, Grancam, and Urograndis.

2.2.2 Eucalyptus* spp. *sampling

The *Eucalyptus* plant collection was performed in December 2015th, at that time each sampled plant was 38 months old. We cut down 4 plants of each one of the seven taxa leading to a total of 28 plants sampled. After taking down the trees the end portion of the tips (barks) were manually removed. The sampled plant parts were stored in plastic bags and duly identified.

2.3 Chemical analysis

2.3.1 Essential oil extraction and quantification

In order to extract the essential oil, a 50g amount of crushed bark was submitted to a two hours steam distillation process using a Clevenger system (500 mL. The aqueous phase was extracted with dichloromethane [3 x 50 mL]). The organic phase was sodium sulphate dried and then filtered. The oil mass was determined after complete solvent evaporation following Sartoratto et al. (2004) methods.

2.3.2 Sample preparation for CG MS HS-Trap analysis

An amount of 0.2 g of crushed bark was weighed and transferred to a HS vial (20 ml), then 1 mL of methanol and 9 mL of ultrapure water was added. The gas chromatography/mass spectrometry (GC MS) analyses were performed by the HS-trap method using an Agilent gas chromatograph (7890B) coupled to a quadrupole mass spectrometer (5977A) (Agilent Technologies, Palo Alto, CA, USA). A CTC PAL sample injector (Agilent Technologies) was used as the HS sampler. The temperature of HS-Trap oven was 70 °C by 10 min.

Helium was the carrier gas with a column head pressure of 150 kPa. The GC temperature program was from 40 °C (held for 2 min) up to 200 °C at a rate of 5 °C/min and further up to 300 °C at a rate of 40 °C/min (held for 3 min). The MS transfer line temperature was set up to 250 °C and the ion sources temperature was set up to 230 °C. For GC-MS detection, an electron ionization system was used with ionization energy set at 70 eV, and mass range at m/z 40-400. The analytes were detected in time windows and identified by the NIST MS library search. The compounds of essential oil were identified on the basis of comparison of their mass spectra with a computer matching with National Institute of Standards and Technology (NIST 5.01) libraries provided with computer controlling the GC MS system (Agilent P/N G1033A).

2.3.3 Tannin quantification

For tannins extraction from the crushed bark, 100 mL of bi-distilled water was added to 1g of dried leaves and heated with total reflux during 1h. Tannin content was determined spectrophotometrically using the Folin - Denis reagent (Mossi et al., 2009). The calibration curve for the analysis was prepared from 0.01 solutions; 0.02; 0.04; 0.1 and 0.2 g/ml of tannic acid diluted in water. The absorbance was measured at 760 nm. The concentration was calculated through a standard curve, prepared with a standard solution of tanic acid. The quantification was carried out in triplicate.

2.4 Statistical analysis

The normality of the biological variables was tested using a Kolmogorov-Smirnov test, and the variables were transformed as necessary (log10 x+1). The data was analyzed by analysis of variance (ANOVA). We used one way Anova test to evaluate both, the *Eucalyptus* spp. consumption and the difference in the total amount of essential oil and tannins among the different *Eucalyptus* taxa (Crawley, 2007). Differences among the categorical variables (*Eucalyptus* taxa) were assessed through orthogonal contrast analysis (Crawley, 2007). In this analysis, the dependent variables for the different taxa of *Eucalyptus* were ordered (increasingly) and tested pairwise (with the closest values) and sequentially by adding to the model values with no differences and testing with the next model in a stepwise model simplification process (Crawley, 2007). The relationship among consumption of *Eucalyptus* spp., essential oil and total tannins was assessed through linear regression (Crawley, 2007). A we used Principal Component Analysis (PCA) based on a covariance matrix to evaluate the relationship between essential oil chemical concentration and total amount of tannins among the different taxa of *Eucalyptus* (Legendre and Legendre, 2012). The significance of the axes was measured according to the model of Broken-Stick.

3. Results

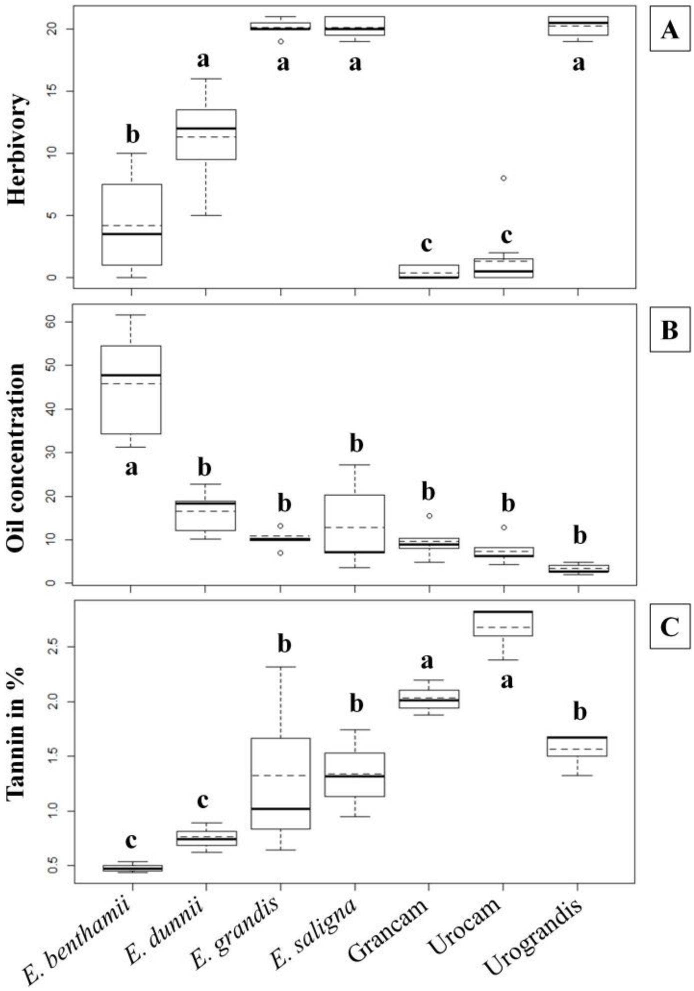
3.1 Consumption and chemical compounds variation among *Eucalyptus spp*.

The number of plants tips damaged by herbivores differed among the samples ($F =_{(6,49)}$ 126.63; $p < 0.001$). Lower herbivory was observed in Urocam and Grancam followed to *Eucalyptus benthamii* compared to other *Eucalyptus* taxa (Contrast Analysis; $p < 0.05$). Oppositely, almost all the 21 individuals evaluated from *Eucalyptus saligna* (20.12±0.83), *Eucalyptus grandis* (20.12±0.64) and Urograndis (20.25±0.88) suffered herbivory. *Eucalyptus dunnii* (11.37±3.54) and *Eucalyptus benthamii* (4.25±3.84) had only a few individuals damaged. Urocam (1.50±2.72) and Grancam (0.37±0.51) suffered virtually no attack (Figure 1A).

In this study, Iit was not possible to identify the animal that consumed the plants of the Eucalyptus. Eucalyptus branches were given to *Sapajus nigritus* (black-horned tufted capuchin) and *Amazona aestiva* (turquoise-fronted parrot) in captivity. All the branches were preyed upon by both species and the pattern of consumption (mark left by animals) was the same for both.

The essential oil concentration differed among the *Eucalyptus* ($F =_{(6,28)}$ 21.33; $p < 0.001$; Figure 1B). *Eucalyptus benthamii* displayed the highest concentration of essential oil reaching 12 times more oil than Urograndis, species with less concentration. The other taxa did not show any statistically significant difference (Contrast Analysis; $p < 0.05$).

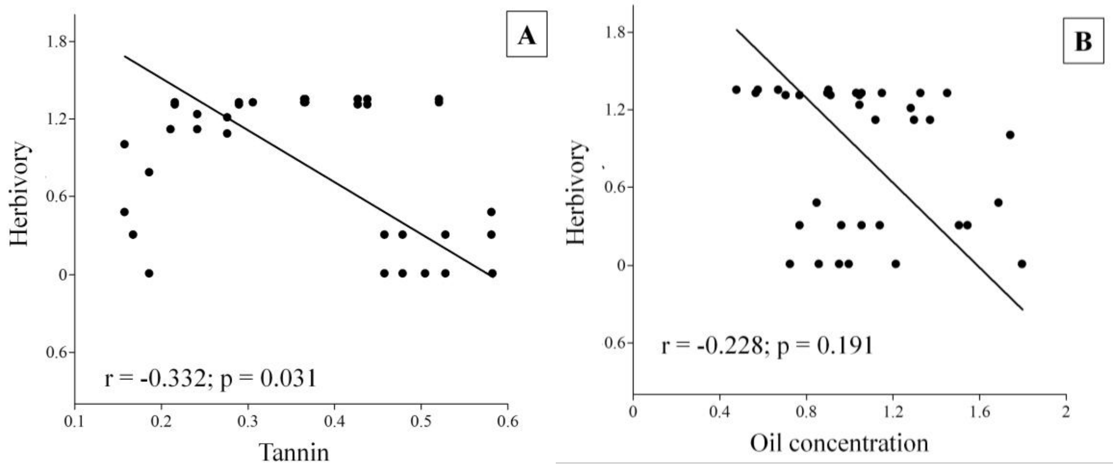
Figure 1. Mean values of number of plants consumed (A), concentration of essential oil ($mg.g^{-1}$; B) and tannin concentration (%; C) in the barks of seven taxa of *Eucalyptus*. Different letters ("a" and "b") indicate significant differences. Boxes represent the quartiles, the bold line represents the median, the horizontal dashed line the mean, the vertical dashed line represents the upper and lower limits and circles the outliers.



Source: Authors.

Different concentrations were registered as percentage of tannins. Urocam (2.67%±0.21) and Grancam (2.02%±0.13) displayed the highest tannin concentrations ($F =_{(6,14)} 10.17$; $p < 0.001$; Figure 1C), differing from all other *Eucalyptus* species (Contrast Analysis; $p < 0.05$), [e.g. *Eucalyptus benthamii* (0.48%±0.04) and *Eucalyptus dunnii* (0.75%±0.101)]. Our correlations study comparing herbivory against tannin level (Figure 2A) and essential oil concentrations (Figure 2B) reveled a strong negative relationship for both tannin and oil, however the significant relationship was only for tannins.

Figure 2: Linear regression of number of plants consumed (herbivory) between tannin (A) and concentration of essential oil (B) in the barks of seven taxa of *Eucalyptus*.



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3.2 Combined effect of chemical compounds of *Eucalyptus* spp.

The essential oils of crushed bark of different plants were identified and quantified by GC-MS analysis. Table 1 shows the chemical composition and concentration of the major compounds present in the seven taxa studied. We identified and quantified those higher concentration compounds present in the oil of all *Eucalyptus* taxa. We found the alpha-pinene compound in all plants, however it had a higher concentration in *Eucalyptus benthamii* (78.28%). The compound p-cymene was found in *Eucalyptus grandis* and Urograndis at concentrations of 50.57% and 45.12%, respectively. Eucalyptol had higher concentrations in Urocam (54.54%), Grancam (22.1%) and *Eucalyptus saligna* (21.96%).

Table 1. Chemical composition of the essential oil present in the barks of seven taxa of *Eucalyptus*. (UG=Urugrandis, GD=*Eucalyptus grandis*, SL=*Eucalyptus saligna*, DN=*Eucalyptus dunnii*, BT=*Eucalyptus benthamii*, GC=Grancam, UC=Urocam).

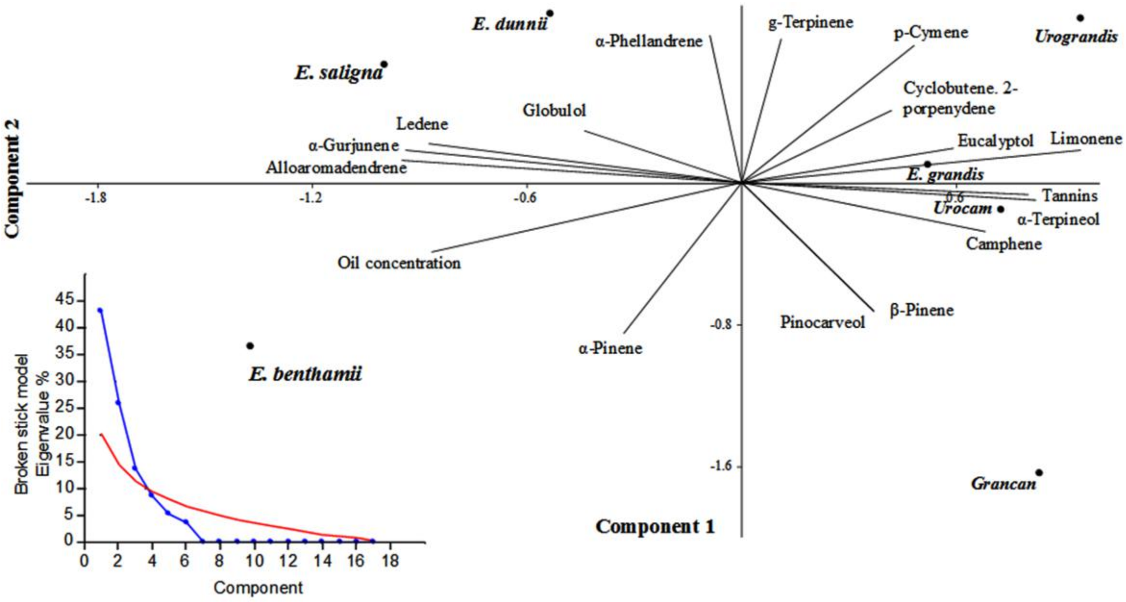
	Area (%)						
Chemical Compound	UG	GD	SL	DN	BT	GC	UC
α-Pinene	10.73	21.87	12.04	10.59	78.28	48.24	80.08
Camphene	10.56	20.92				20.57	
p-Cymene	45.12	50.57	14.49	16.66		20.46	70.79
Limonene	90.33	50.96	30.21	50.78	10.71	80.24	80.18
α-Phellandrene	40.99	10.47	30.84	38.15			10.50
Eucalyptol	70.64	80.13	21.96	80.59		22.10	54.54
α-Terpinene	60.38	20.96	10.43	60.9			
α-Gurjunene			30.20	10.49	10.94		
Alloaromadendrene			13.85	5.42	11.91		10.18
Ledene			40.02	10.08	10.43		
Globulol			2.35				
α-Terpineol	10.41	12.00				10.27	40.90
Cyclobutene 2-porpenydene	20.18						
β-Pinene						10.99	
Pinocarveol						10.61	
Total	88.69	95.08	80.39	96.15	96.98	88.48	86.17

Source: Authors.

Principal Components Analysis (PCA) separated the species of *Eucalyptus* into 2 distinct groups: hybrid (with the exception of *Eucalyptus grandis*) and not hybrid (Figure 3). The first two axes of the PCA together explained 68% of the variation of the chemical composition parameters. The first axis of the PCA explained 44% of the variation, and was negatively correlated with α-Pinene, Oil concentration, Alloaromadendrene, α-Gurjunene, Ledene, Globulol and α-Phellandrene (*Eucalyptus benthamii, Eucalyptus saligna* and *Eucalyptus dunnii*), and positively with Camphene, p-Cymene, Limonene, Eucalyptol, α-Terpinene, α-Terpineol, cyclobutene. 2-porpenydene, β-Pinene, Pinocarveol (Urograndis, Grancam, Urocam and *Eucalyptus grandis*).

The second axis explained 26% of the variation, and had a positive relation with Alloaromadendrene, α-Gurjunene, Ledene, Globulol, α-Phellandrene, α-Terpinene, p-Cymene, cyclobutene. 2-porpenydene, Limonene and Eucalyptol (Urograndis, *Eucalyptus grandis*, *Eucalyptus saligna* and *Eucalyptus dunnii*), and negatively with α-Pinene, Oil concentration, Camphene, α-Terpineol, β-Pinene and Pinocarveol (Urocam, Grancam and *Eucalyptus benthamii*). The first and second axis differed when compared to the Broken-Stick model (Figure 3).

Figure 3. PCA analisys of chemical composition of the essential oil present in the barks of seven taxa of *Eucalyptus.* Small figure corresponds to the model of broken stick (red line) among component 1 and 2 (blue line).



Source: Authors.

4. Discussion

The values found in the analysis of essential oil suggest that the factor that prevents the wildlife attack to *Eucalyptus benthamii* is the largest concentrations of essential oil. Some essential oil features, such as acid and spicy flavor, and the total amount of tannins are responsible for the plant astringency (Felton et al., 2009). Essential oils can play an important role in pest control, acting as a defense weapon against herbivory pressure caused by wild life, i.e. protection against predators and herbivores (Batish et al., 2008). Undesirable and toxic organoleptic effects can contribute to the plant defenses against herbivory. Essential oils are a complex mixture of components, acting synergistically inside the plant as a defense strategy. The chemical composition of essential oil of bark of *Eucalyptus bentamii* presented 78.28% α-pinene, which is an important monoterpene that in high concentrations can be toxic to mammals (Rider, 2016) and this can be the main factor to prevent the herbivory for this plant. The α-pinene is a chemical compound with recognized antibacterial and fungicidal activity (Silva et al., 2012), being widely used by the pharmaceutical industry as a fragrance in cleaning products and perfumes (Mokrzycki et al., 2009).

The hybrid varieties Grancam and Urocam displayed the largest tannins concentrations, compared to the other taxa. Those were taxa that presented the lowest predation rate, which suggests that tannins, due to its chemical characteristics, were the main factor that triggered the herbivores and predators feeding avoidance. It is well-known that tannins present bitter taste (Lesschaeve & Noble, 2005), i.e. act as a toxin, being less digestible by inhibit enzymes involved in digestion (Mehansho et al., 1987; Mueller-Harvey, 2006). Some tannins can have positive effects in some animals and can reduce the amount of protein that is digested in the stomach, which increases the amount of protein available for digestion in the small intestine, thus eliminating parasites and decreasing the bloat frothy as in the case of cattle (Mueller-Harvey, 2006). The hybrids Grancam and Urocam bring together the best features of *Eucalyptus grandis* vs. *Eucalyptus camaldulensis* and *Eucalyptus urophylla* vs. *Eucalyptus camaldulensis*, respectively (*GUIA DO EUCALIPTO: oportunidades para um desenvolvimento sustentável*, 2008). The species *Eucalyptus camaldulensis* and *Eucalyptus urophylla* present high content of tannins (Trugillho et al., 1997). The resistance or susceptibility of plants to the attack of predators is predominantly inherited and can be expressed in both, pure species and in hybrids (Bailey et al., 2006). The hybrids less attacked in the experiment holds the genetic characteristics of the pure species that have high concentrations of tannins in the barks. It was less consumed by wild fauna, reinforcing the importance of plant genetics on the ecological control of the *Eucalyptus* spp. predation by wildlife.

On the other hand, *Eucalyptus saligna* (higher Alloaromadendrene and Globulol), *Eucalyptus grandis* (higher Limonene and α-Terpinene) and Urograndis (higher Eucalyptol) were intensely attacked. We associate it to the low tannins or essential oil concentration found on it, suggesting that this two chemicals might determine those *Eucalyptus* species consumption by wildlife (Batish et al., 2008). The plant chemical compounds associated with these species (listed above), present low toxicity when compared to others as Pinene (α and β) (Silva et al., 2012) and tannins (Mueller-Harvey, 2006). The lower toxicity of this specific chemical mixtures increases the litter palatability too many herbivorous (Rider, 2016). These chemicals are also contributing to lowest herbivory rate in Urocam (higher α-Terpineol and cyclobutene. 2-porpenydene), Grancam (higher α/β-Pinene and Pinocarveol) and *eucalyptus benthamii* (higher α-Pinene and Total oil) associated whit most toxic mixed compounds. Our results may indicate that mixtures in chemicals compounds in a synergistic effect may be the answer for a the founded biological response, such as herbivory. Therefore, future studies should emphasize on understanding the role of mixed compounds and less on only individual response of just one compound, looking at the synergistic effect that might provide a clearance on role of those chemicals on environmental service (e.g. herbivory) and wild life biology and behavior. Understanding the role of exotic species on animal food preferences maybe a keystone to understand and contribute to wildlife preservation.

However, our experimental design did not allow us to identify the animal that feed on the *Eucalyptus* plants. On the other hand, our objective was to identify which taxa of *Eucalyptus* spp. was to describe the characterization of chemical composition and relationships to herbivory with different taxa of *Eucalyptus*. Mikich and Liebsch (2014) results lead us to suggest that the damage caused to the *Eucalyptus* tips could have been done by *Sapajus nigritus* (Goldfuss, 1809), which is shown as removing the plant`s bark to feed on the sap. The authors discuss that the sap drawn up is not the preferred food item in the diet of the monkey, but it does feed on the plant`s sap due to low fruits availability (Canale et al., 2009). Our results may indicated that *Pionus maximiliani* (Scaly-headed parrot) (Kuhl, 1820) or *Amazona vinacea* (Vinaceous-breasted Parrot) (Kuhl, 1820), two bird species frequently found on the region where the study was carried on, could have fed on the plants. Birds of the Psittacidae family have are known as feeding on fruits, such as guava and orange trees, eating the sprouts, flowers and tender leaves, including different *Eucalyptus* spp. (Galetti, 1993). Also, the *Eucalyptus* are used as dormitories for parrots, such as *Amazona aestiva* (Turquoise-fronted Parrot) (Linnaeus, 1758) and *Alipiopsitta xanthops* (Yellow-faced parrot) (Spix, 1824), the physical structure of *Eucalyptus* trees enhances the birds camouflage, protecting them against predators (Carrara et al., 2007). In addition, in periods of low food availability, they *Eucalyptus* can be used as a surrogate to the above described foods (Galetti, 1993).

5. Conclusion

The lowest hebivory rate were associated to Urocam, Grancam and *Eucalyptus benthamii*. Those taxa were associated with the mix compounds of Oil concentration, α/β-Pinene, Pinocarveol, α-Terpineol and Tannins. On the other hand, *Eucalyptus saligna*, *Eucalyptus grandis* and Urograndis were associated with the Alloaromadendrene, Globulol, Limonene, α-Terpinene and Eucalyptol, indicating that this chemical composition is inefficient against a part of the herbivory. The hybrids, Grancam and Urocam, hold the genetic characteristics of the pure species, presenting a high tannins level in the bark. *Eucalyptus Benthamii* had low tannins concentrations, however, it displayed high essential oil concentration in the bark. Our results support our hypothesis that that the mix of compounds, tannins and essential oil levels are important factors that contributes to exotic *Eucalyptus* plants to face herbivory.

As a suggestion for future research, new studies could be carried out based on the lethal action of Urocam on vectors that transmit aboviruses, such as *Aedes aegypti*. Associating in this way, the low herbivory in this eucalyptus with the possible potential of Urocam to control vectors in a sustainable way.

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