International Journal of Ecology and Environmental Sciences 43 (1): 17-24, 2017
ISSN: 2320-5199 (Online)

Herbivory and Chemical Defense: How is *Miconia cabucu* Hoehne (Melastomataceae) Reacting on a Remnant of Atlantic Rainforest?

Gabriela Thomaz Da Silva*[1], Alan Da Silva Felisbino[2], Patrícia De Aguiar Amaral[2,3] and Birgit Harter-Marques[3,4]

[1] *Laboratório de Morfologia e Comportamento Animal, Instituto de Biociências, Universidade Federal do Rio Grande do Sul – UFRGS, Av. Bento Gonçalves, 9500 – Porto Alegre, Rio Grande do Sul, Brazil*
E-mail gabi_thomaz@hotmail.com
[2] *Laboratório de Plantas Medicinais, UNASAU, Universidade do Extremo Sul Catarinense - UNESC, Av. Universitária, Criciúma, Santa Catarina, Brazil*; Email: alan_aru@msn.com
[3] *Programa de Pós Graduação em Ciências Ambientais, UNAHCE, Universidade do Extremo Sul Catarinense - UNESC, Av. Universitária, 110, Criciúma, Santa Catarina, Brazil*; Email: paa@unesc.net
[4] *Laboratório de Interação Animal-planta, UNAHCE, Universidade do Extremo Sul Catarinense - UNESC, Av. Universitária, 1105, Criciúma, Santa Catarina, Brazil*; Email: bhm@unesc.net
* Corresponding author:

ABSTRACT

This study examined herbivory rates and chemical defense mechanism in leaves of *Miconia cabucu* Hoene (Melastomataceae) at different development stages and environments. We also identified variations of these data in relation to seasonality and climatic factors. Young and mature leaves from 40 individuals were sampled at the border and the interior of a remnant area for the herbivory analysis. The chemical analysis was accomplished in order to detect secondary metabolites also in young and mature leaves of different development stages and environments. Young individuals were generally more attacked than adults. Higher herbivory rates were observed for mature leaves of young individuals, for both edge and interior. The chemical analysis presented positive results for phenolic compounds, flavonoids, tannins, and coumarins. Mature leaves showed higher phenolic compounds and flavonoids concentration. For the border area,higher contents was detected on summer, and for the interior, higher contents on winter. The relative air humidity and pluviosity were positively correlated with herbivory rates, while insolation and photoperiod were negatively correlated. The results of herbivory and their secondary metabolites demonstrate the importance of these relationships in *Miconia cabucu*, and its value for preservation and biodiversity.

Key Words: Insect-Plant Interaction; Herbivory Rates; Secondary Metabolites; Fragmentation; Seasonality.

INTRODUCTION

Plants and insects are the most representative groups on earth; their interaction dates back for more than 430 million years now, and includes a variety of ecological systems (Schoonhoven et al. 1998).The interactions include mutualisms, pollination, dispersal, predation, competition and herbivory (Del-Claro and Torezan-Silingardi 2012, Herrera and Pellmyr 2002, Schoonhoven et al. 1998). Herbivory is described as an interaction of an animal obtaining its energy by feeding out on plants (Herrera and Pellmyr 2002, Sevegnani 2007).

Different insect species, from different stages of development or environmental conditions may require a high or varied nutritional demand from plants. Some plant characteristics determinewhat is valid for consumption; the most common is leaf hardness, which can affect feeding habits and the growth of herbivorous insects (Schoonhoven et al. 1998). Usually, newer leaves are more consumed than older ones, because it offers greater palatability, more nutrients, and is softer to insects' mouth parts due its low fiber content. However, new leaves generally contain higher concentration of metabolites, which explains the preference of specialist insects for these leaves. Generalist insects end up choosing older leaves, because despite the hardness, they do not accumulate high amounts of metabolites (Cornelissen and Fernandes 2001, Schoonhoven et al. 1998).

Plants develop several mechanisms to defend their organs and tissues against the attack of the herbivorous insects (Herrera and Pellmyr 2002). The nature and the amount of plant defenses can be determined by the availability of resources from the habitat, and their mechanisms of action, explained according to their physical and chemical properties (Chen 2008, Cornelissen and Fernandes 2001). A single plant can be quite heterogeneous in its chemical composition; it can also vary among its different tissues (Herrera and Pellmyr 2002, Schoonhoven et al. 1998). There are several factors that can influence the chemical composi-tion of a plant, such as age, soil configuration and seasonality (Del-Claro and Torezan-Silingardi 2012). The production of chemical constituents for the defense against herbivory may commit other important functions of the plant, because it requests matter and energy, its synthesis and storage will demand some cost (Cornelissen and Fernandes 2001). The chemical production can influence plant growth by redirecting resources that once could be used on differentiation processes, such as sprouting and leaf production, will then be used for defense (Cornelissen and Fernandes 2001, Del-Claro and Torezan-Silingardi 2012). Some studies suggest that the effort for preventing herbivorous insects can reduce the reproductive success and the fitness of their host plant (Maron and Gardner 2000, Marquis 1984, Zvereva and Kozlov 2001)

Gurevitch et al. (2009) point out that the effects of herbivory on a community level can increase or reduce plant diversity, bringing intense consequences for the richness, composition and abundance ofa community. Others suggest that insects can influence on evolution, space distribution, and on plant population dynamics (Barbosa et al. 2005,Gurevitch et al. 2009, Herrera and Pellmyr et al. 2002). One of the main problems affecting the interactions between insects and plants, are the impacts from human actions; eventually the dynamics of interactions will suffer from the advances of forest fragmentation and consequently, forest biodiversity (Costa 2003, Rocha et al. 2006).

Many authors include a species from the Melastomataceae family, *Miconia cabucu,* as a potential plant for reforestation, biomonitoring of pollution, recuperation of riparian forest, rural construction, as well as its importance as ecological attribute for the fauna (Carvalho 2008, Citadini-Zanette 1995, Lorenzi 2009). This evergreen species is native from Brazil, pioneer and of rapid growth, reaches up to 15 m high and blooms between August and November.

To contribute to the studies related to fragmentation, herbivory and plant defense mechanisms, the main goal of this paper is to understand how leaf consumption influences the production of chemical metabolites on *Miconia cabucu* and, to associate this influence with different environments. More specifically, it evaluates: (1) the herbivory rates of newer and older leaves from young and adult plants of different environments; (2) the detection of secondary metabolites from newer and older leaves, comparing between young and adult plants, and between the different environments; (3) the analysis of variation between herbivory rates and the secondary metabolites concentration, and its relations with seasonality and abiotic factors.

MATERIALS AND METHODS

Study Site

The study was carried out during the year 2011 on a 19,000 m² remnant area of the Parque Estadual da Serra Furada (PESF) conservation unit, located between the cities Orleans and Grão-Pará, Santa Catarina, Brazil (28° 08' 13" S, 49° 25' 17" W, and 28° 11' 36" S, 49° 22' 58" W) (Figure 1). The unit belongs to the Montane Dense Rainforest region of the Atlantic Forest biome in South of Brazil, abridging an area of 1,330 ha (Plano de Manejo PESF 2009).The climate of the region is classified as Cfa according to Köppen (1931). The observations and sampling were made inside and at the border area of the remnant.

Sampling

A total of 40 individuals of *Miconia cabucu* were sampled for herbivory rates. For both interior and border area, 10 young plants and 10 adult plants were marked with an identification code for a monthly evaluation throughout one year. Conditions such as reproductive age, flowering or fructification, were used as characterization criteria of which plants were considered adult.

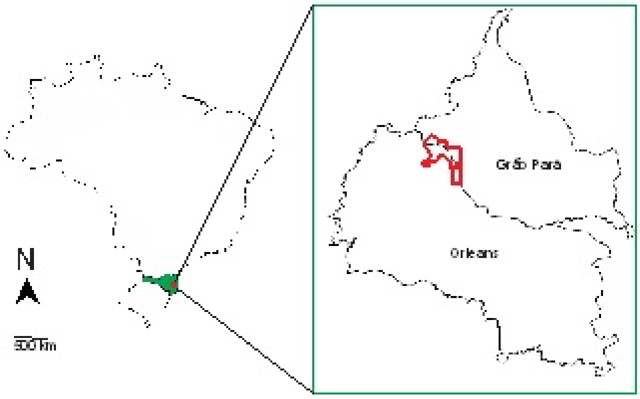

Figure 1. Geographic location of Santa Catarina (green) and Parque Estadual da Serra Furada (area in red).

For leaf analyses, the classification ascribed by Corrêa (2007) was implemented, in which newer leaves were considered those occurring before the fourth knot, counting from the apex of the branch, and older leaves between fourth and eighth knots. All leaves from young plants and the leaves from two branches of adult plants were analyzed every month.

Herbivory rates were measured by calculating the percentage of leaf area consumed by defoliating insects, following the Dirzo and Domingues (1995) categories of classification: **0** = no herbivory; **1** = 1 to 12.5%; **2** = 12.5 to 25%; **3** = 25 to 37.5%; **4** = 37.5 to 50%; **5** = 50 to 62.5%; **6** = 62.5 to 75%; **7** = 75to 87.5%; **8** = 87.5 to 100% of the leaf area consumed by herbivorous insects.

For the extraction of secondary metabolites, four new and four old leaves were sampled for one young and one adult plant each from the interior and border areas at the end of each season. Plants used for phytochemical analyses were selected apart from the ones observed for the herbivory rates. The same adult plant from the different areas was sampled throughout the year. However, since younger plants may go through a high level of stress and alter their chemical composition, it was decided to collect leaves from different young plants, yet close to each other, for each season, in an attempt to reduce possible abiotic factors that could alter the analysis.

Phytochemical Analyses

Secondary metabolites from *M. cabucu* were analysed from leaves collected in each season. All leaves were dried at 40ºC for approximately seven days. Each sample was properly identified according to leaf age, plant age and area; weighed on an electronic precision scale (Mars AL 500) and stored in plastic containers with controlled conditions for light, temperature and humidity until the moment of analysis.

Phenolic compounds (polyphenols, flavonoids, tannins, anthraquinones and coumarins) were detected qualitatively by the methods proposed by the official methods of analysis of the Association of Official Analytical Chemists (AOAC 2010). The qualitative detection for terpenes (saponins and cardiotonic glycosides) and alkaloids were also achieved following the methods proposed by the AOAC (2010). The latter were conducted with the Dragendorff, Bertrand, Mayer and Bouchard reagents, and confirmed through thin layer chromatography (Wagner and Bladt 1996)

Statistical Analyses

Herbivory index (HI) was obtained by calculating the rates of defoliation according to the formula proposed by Dirzo and Domingues (1995):

$$HI = \Sigma\ (ni \times i)/N$$

where ni = number of leaves per category;
i = herbivory category (0 - 8) and
N= total number of leaves for each individual.

The significance of the regression coefficient was tested using the General Linear Model (GTL[7]) with gamma distribution and considering the Wald (W) statistics, with the help of Statistica software (STATSOFT 2004).

The secondary metabolites composition of newer and older leaves from the same plant, from different plant age, and from different areas of the remnant was evaluated by indicating the presence or absence of these metabolites. The concentration data for total polyphenols and flavonoids were analysed with ANOVA, followed by the Test of Tukey using the PAST software version 2.04 (Hammer et al. 2001).

Most of the Brazilian territory, in the south hemisphere, does not have a well-established seasonality, however, the south region presents the four seasons defined, where spring begins in September, summer in December, autumn in March and winter in June (Simepar 2017). The information regarding the averages, minimum and maximum for climatic data, along with the climatological normal for every month, was obtained from the Experimental Station of EPAGRI/Urussanga, Santa Catarina, Brazil.

RESULTS

Herbivory Damage

The related variables for leaf and plant ages were demonstrated as an important source of variation for the herbivory rates in *Miconia cabucu.* The herbivores preference for older leaves ($W_{(1)} = 237.441$; $p < 0.001$) was responsible for 20% of the total variation in the herbivory rates despite the plant age. Furthermore, the preference for young plants ($W_{(1)} = 131.791$; $p < 0.001$) was observed, which was responsible for 15% of the total variation. There was no significant variation among months ($W_{(11)} = 17.829$; $p = 0.085$) or between different environments ($W_{(11)} = 0.620$; $p = 0.430$) (Figure 2).

For each season of the year, the averages from herbivory rates were calculated, and comparisons made between environments, leaf, and plant ages. The average rates for summer, autumn and winter seasons were generally higher for leaves from the border area, leaves from the interior were higher only in spring. Significant differences between newer and older leaves were detected for all plants and areas, expect for young plants from the interior area during winter.

Phytochemical Analysis

Positive results were detected for phenolic substances such as tannins, flavonoids and coumarins. The presence of anthraquinones, saponins, cardiotonic glycosides and alkaloids was not confirmed.

The results for quantitative analyses to determine the total concentration of polyphenols and flavonoids were calculated from a correlation coefficient (R^2) at a level of significance of 5%. The equation of line found from the standard curve was $y = 0.0013x + 0.0148$ for total polyphenols and $y = 0.0065x + 0.0271$ for flavonoids. The values obtained for the concentrations are shown in Tables1 and 2. Polyphenol concentrations were significantly different between the seasons ($F\ [3,28] = 146.5$, $p < 0.01$); spring was significantly different from others and the autumn different from summer and winter. For the flavonoids concentrations, summer was significantly different from other seasons, and the autumn different from winter and spring ($F\ [3,28] = 21.27$, $p < 0.01$) (Table 3). The flavonoid concentrations were higher for leaves in the summer, except for newer leaves of adult plants, which showed higher values in winter and spring.

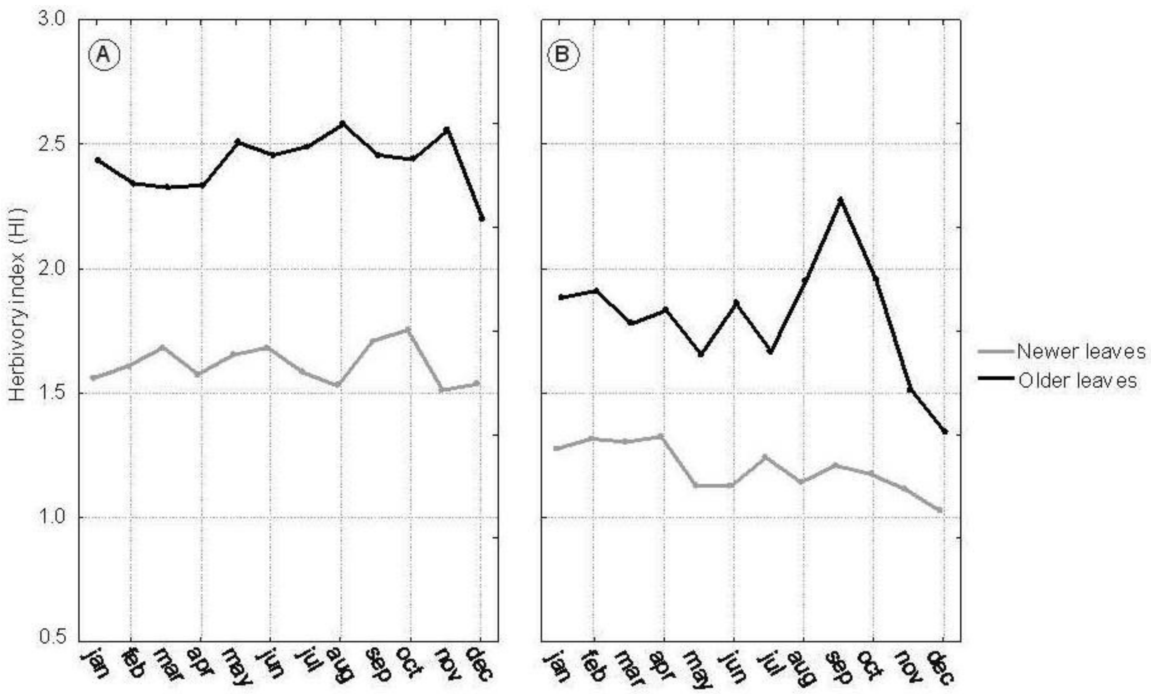

Figure 2. Monthly variation of herbivory rates in *Miconia cabucu*. A, young plants; B, adult plants.

Table1. Concentration of total polyphenols (mg L^{-1}) in the leaves of *Miconia cabucu*. B = border; I = interior; Y= young; A= adult; n= newer leaf; o= older leaf.

Environment/ Sample	Season Summer	Autumn	Winter	Spring
BYn	179.4	64.8	139.4	24.8
BYo	197.1	80.2	159.4	27.1
BAn	154.8	61.7	134.0	23.2
BAo	153.2	84.0	126.3	30.2
IYn	148.6	58.6	150.2	41.7
IYo	162.5	60.2	131.7	20.2
IAn	135.5	57.1	174	27.8
IAo	162.5	61.7	180.9	24.0

Table 2. Flavonoid concentration (mg L^{-1}) in the leaves of *Miconia cabucu*. B = border; I = interior; y = young; a = adult; n = newer leaf; o = older leaf

Environment/ Sample	Season Summer	Autumn	Winter	Spring
BYn	78.9	15.5	39.2	30.6
BYo	78.6	19.7	51.2	44.9
BAn	63.2	14.6	31.1	46.4
BAo	82.8	16.3	36	47.4
IYn	46.6	11.2	29.7	17.7
IYo	59.5	5.8	19.1	22.6
IAn	65.1	7.8	72.9	65.7
IAo	67.4	7.4	66.9	49.5

Table 3. Results of Tukey's Tests for polyphenols and flavonoids concentrations among the seasons.

Season	Summer	Autumn	Winter	Spring
Summer	**-**	**0.00016**	**0.3907**	**0.00016**
	-	0.00016	0.00781	0.003
Autumn		**-**	**0.00016**	**0.00027**
		-	0.00081	0.00199
Winter			**-**	**0.00016**
			-	0.9806

Figures in bold are for polyphenols.

Interactions, Biotic and Abiotic Factors

The Pearson correlations among monthly averages of herbivory rates and the climatic data, were positively significant for: relative air humidity with newer leaves from young plants (r = 0.670; p = 0.016) and newer leaves from adult plants (r = 0.582; p = 0.046) both from the border area, and also with older leaves from adult plants from the interior area (r = 0.822; p = 0.001). Rainfall also had positive correlation with newer leaves from adult plants (r = 0.620; p = 0.031) from the border.

Correlations with insolation were significantly negative between new and old leaves from adult plants from the border (r = -0.635; p = 0.026 and r = -0.636; p = 0.026 respectively), and older leaves from adult plants from the interior (r = -0.658; p = 0.019). The photoperiod was also negatively correlated for older leaves from adult plants from the interior (r= -0.624; p = 0.029). Seasonal averages of herbivory were also correlated with climatic data but none was significant.

The concentrations of polyphenols and flavonoids obtained in each season were correlated with the climatic averages but no significant correlations were found. However, flavonoids showed significant and positive correlation with rainfall for newer leaves from the young plants ofthe border area (r = 0.952 p = 0.047) and interior area (r = 0.993 p = 0.006).

DISCUSSION

Herbivory Damage

A study conducted by Medeiros and Morretes (1995) on herbivory in *Miconia cabucu* reported that the rates of herbivory were considerably lower (0.5 to 50.2%) than those observed in the present study (0 to 95%). The higher rates found in our study may be explained by the conditions found in the region in which the analysis were carried out. According to Coley et al. (1985), the annual consumption of plants from natural communities by herbivores can reach an average of 10%, and in tropical forests, this average can vary from 0.0003 to 0.8% daily, depending on the attacked species.

According to Santos et al. (2007), young plants usually have lower rates of herbivory than adults, and this might be due to the fact that they are less attractive to herbivores, and because they have greater amount of newer leaves they are usually chemically best defended. Medinaceli et al. (2004), studying herbivory in a

Montane tropical forest, did not detect significant differences on the herbivory rates between areas with different exposures; however, adult plants were shown to be more attacked than the young ones for both areas. The herbivory averages found in the present study point out that young plants, regardless of the environment, or season, have higher herbivory rates than the adult plants, contradicting the results found by Medinaceli et al. (2004) and Santos et al. (2007).

Phytochemical Analysis

According to Rodrigues (2007), phytochemical studies for the Melastomataceae family are still scarce. However, for some studied species it was possible to verify groups of secondary metabolites; flavonoids and tannins are more common, whereas terpenes and quinones are rarely found. Similar results were reported by Serpeloni et al. (2008) who found that polyphenols, flavonids and tannins were the main compounds in the methanol extract of *M. cabucu*. All secondary metabolites found in our study are corroborated by the previous studies. Bernays et al. (1984) conducting tests on a few species of *Miconia* observed that it is possible to find quinones acting as alimentary deterrent in six species of insects. Other species of *Miconia* such as *M. fallax* DC., *M. stenostachya* DC., *M. sellowiana* Naudin and *M. ligustroides* (DC.) Naudin, have triterpene acids in their extracts (Cunha et al. 2003, Cunha et al. 2006).

Phenolic compounds may vary and increase when related to different environments or wounding, and their concen-tration can be significantly influenced by seasonality (Liimatainen et al. 2012). A study conducted by Yariwake et al. (2005) found significant seasonal variations in the concentrations of polyphenols and flavonoids from leaves of *Maytenus aquifolium* Mart. (Celastraceae), both presenting low values for winter and summer, and higher values for spring and autumn. The results of our study do not agree with the ones found in literature, since the concentrations for polyphenols and most of the flavonoids were higher for summer and winter and were much lower (~70% lower) for spring and autumn.

Studying two species of *Inga* (Fabaceae), Brenes-Arguedes et al. (2006) found newer leaves as chemically more protected, and they also observed that flavonoid concentration decreases as leaves grow older. According to Kogan and Paxton (1983), a higher photosynthetic and metabolic rate in newer leaves, which results in a higher accumulation of nutrients and, consequently, greater production of secondary metabolites acting as defense, could explain the higher rates of herbivory on older leaves. However the results obtained for *Miconia cabucu* analyzed in our study, differ from what is proposed above, as the older leaves presented higher concen-trations of secondary metabolites and were also the most consumed by the herbivores.

Interactions, Biotic and Abiotic Factors

Plant-herbivore interactions can be affected by pathogens or previous attacks from herbivores or even by several environmental factors such as temperature, solar radiation and hydrous stress, humidity and rainfall (Koogan and Paxton 1983). In our study, *Miconia cabucu* plants from the border areas had delayed production of the secondary metabolites as compared with the plants from the interior, suggesting that this difference could be related to climatic factors. Because border environments are usually more propitious to variations of climatic factors (Ribeiro et al. 2009), plants from this area may suffer some influence on the synthesis and concentration of the secondary metabolites (Koogan and Paxton1983).

Liimatainen et al. (2012) show that the production of secondary metabolites could be regulated by plants when in different environments. According to Ribeiro et al. (2009) border areas can be subject to greater variations in the climatic conditions such as temperature, luminosity, and air and soil humidity among other factors that may cause changes in the structure of a remnant interaction. Although only a few differences for the climatic data (except for rainfall) were found in this study, Murcia (1995) points out that variations in microclimate occurring on border of fragmented areas may act as a stimulating way to the changes in biotic interactions of the environment, because physical conditions that directly affect the plants can also alter the density and activity of some species of animals as, for instance, pollinators, disperses and herbivorous insects. The positive relation between flavonoid concentration and rainfall found in our study does not support the results obtained in other studies. Peixoto Sobrinho (2009), analyzing the border effects and rainfall on flavonoid concentrations, did not find significant correlations in Fabaceae species, *Bauhinia cheilantha* (Bong.) Steud. Furthermore, Luengas-Caicedo et al. (2007) evaluated the seasonal and intraspecific variation of flavonoids in the leaves of *Cecropia glaziovi* Sneth. and reported higher concentrations during the dry season compared with rainy periods.

Final Considerations

The chemical composition of a plant may be used as a mechanism of defense, and most of the time this is efficient. However, herbivore insects evolved in a way so that they can sometimes, avoid, modify, or even use those defenses for their own benefit. Some insects acquired a behavioral capacity to avoid consumption and oviposition on toxic plants; others have the capacity of detoxification, and there is also the possibility of "kidnapping" toxins, in which the insect can adapt to the poisonous effect and use it for immunity against their predators (Herrera and Pellmyr 2002). Those adaptations developed by herbivorous insects are relatively common, and it could explain the high herbivory rates in plants with high concentrations of secondary metabolites, as was found for the older leaves of *M. cabucu* in this study.

High rates of herbivory were found for winter and autumn, on border and interior areas respectively, meanwhile the highest concentrations for polyphenols and flavonoids were found in summer and winter, in those same areas. According to Eyles et al. (2010), feeding habits and oral secretions from herbivores can affect the production of induced chemical defense of a plant; and since the values for secondary metabolites were higher for the seasons that come after the ones with greater herbivory rates; the relationship between *M. cabucu* and its herbivores shows an example of defense mechanism, probably of the induced type, where the production of metabolites would be activated following the attack from herbivores (Chen 2008).

The importance of fragmentation and border effects are demonstrated by our study, showing differences on the chemical composition and herbivory when different environments of a forest remnant are analyzed. The Melastomataceae, despite being a well-known family, lacks phytochemical and animal-plant interaction studies. Further studies with *M. cabucu* can offer subsidies for a better understanding of these issues, preserving and preventing further fragmentation.

REFERENCES

AOAC. 2010. Official Methods of Analysis. 18th edition. Association of Official Analytical Chemists. <http://www.aoac.org/>.

Barbosa, V.S.; Leal, I.R.; Iannuzzi, L. and Almeida-Cortez, J. 2005. Distribution pattern of herbivorous insects in a remnant of Brazilian Atlantic forest. Neotropical Entomology 34(5): 701-711.

Bernays, E.; Lupi, A.; Bettolo, R.M.; Mastrofrancesco, C. and Tagliatesta, P. 1984. Antifeedant nature of the quinoneprimin and its quinolmiconidin from *Miconia* spp. Experientia 40: 1010-1011.

Brenes-Arguedes, T.; Horton, M.W.; Coley, P.D.; Lokvam, J.; Waddell, R.; Ameizoso-O'Meara, B.E. and Kursar, T.A. 2006. Contrasting mechanisms of secondary metabolite accumulation during leaf development in two tropical tree species with different leaf expansion strategies. Oecologia 149: 91-100.

Carvalho, P.E.R. 2008. Espécies arbóreas brasileiras, Volume 3. EMBRAPA, Brasilia.

Chang, C.; Yang, M.; Wen, H. and Chern, J. 2002. Estimation of total flavonoids content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis. 3:178-182.

Chen, M.S. 2008. Inducible direct plant defense against insect herbivores: A review. Insect Science 2:101-114.

Coley, P.D.; Bryant, J.P. and Chapin, S.F. 1985. Resource availability and plant antiherbivory defense. Science 4728: 895-899.

Cornelissen, T.G. and Fernandes, G.W. 2001. Defence, growth and nutrient allocation in the tropical shrub *Bauhinia brevipes* (Leguminosae). Austral Ecology 26: 246-253.

Corrêa, P.G. 2007. Defesas foliares em resposta à herbivoria em espécies lenhosas de restinga, Ipojuca, PE. MSc dissertation, Universidade Federal Rural de Pernambuco, Recife.

Costa, R.B. 2003. Fragmentação florestal e alternativas de desenvolvimento rural na Região Centro-Oeste. Pages 139 - 160. UCBD, Campo Grande.

Cunha, W.R.; Martins, C.; Silva Ferreira, D.; Crotti, A.E.; Lopes, N.P. and Albuquerque, S. 2003. *In vitro* trypanocidal activity of triterpenes from *Miconia* species. Planta Medica 69: 470-472.

Cunha, W.R.; Crevelin, E.J.; Arantes, G.M.; Crotti, A.E.M.; Andrade e Silva, M.L.; Cardoso Furtado, N.A.J.; Albuquerque, S. and Silva Ferreira, D. 2006. A study of the trypanocidal activity of triterpene acids isolated from *Miconia* species. Phytotherapy Research 6: 474-478.

Del-Claro, K. and Torezan-Silingardi, H.M. 2012. Ecologia das Interações Plantas-Animais: uma Abordagem Ecológico-Evolutiva. 1st Edition, Technical Books, Rio de Janeiro. 336 pages.

Dirzo, R. and Dominguez, C. 1995. Plant-herbivore interactions in Mesoamerican tropical dry forest. Pages 304-325. In: Bullock, S.H.; Mooney, H.A. and Medina, E.A. (Editors) Seasonally Dry Tropical Forests. Cambridge University Press, Cambridge.

Eyles, A.; Bonello, P.; Ganley, R. and Mohammed, C. 2010.Induced resistance to pests and pathogens in trees. New Phytologist 4: 865-1119.

Gurevitch, J.; Scheiner, S.M. and Fox, G.A. 2009. Ecologia vegetal. 2nd Edition. Artmed, Porto Alegre. 592 pages.

Hammer, O.; Harper, D.A.T. and Ryan, P.D. 2001.PAST: Paleontological statistics software package for education and data analyses. Paleontological Electronica 4(1): 1-9.

Herrera, C.M. and Pellmyr, O. 2002. Plant-Animal Interactions: An Evolutionary Approach. Blackwell Science, Oxford, UK. 313 pages.

Kogan, M. and Paxton, J. 1983.Natural Inducers of Plant Resistance to Insects. Pages 153-171, In: Hedin, P.A. (Editor) Plant Resistance to Insects. ACS Symposium Series, volume 208. American Chemical Society, Washington, DC.

Köppen, W. 1931. Grundriss der Klimakunde. Gruyter, Berlin. 369 pages.

Kruidhof, H.M.; Allison, J.D. and Hare, J.D. 2012.Abiotic induction affects the costs and benefits of inducible herbivore defenses in *Datura wrigthii*. Journal of Chemical Ecology 10:1215-1224.

Liimatainen, J.; Karonen, M.; Sinkkonen, J.; Helander, M. and Salminen, J. 2012. Phenolic compounds of the inner bark of *Betula pendula*: Seasonal and genetic variation and induction by wounding. Journal of Chemical Ecology 11: 1410-1418.

Luengas-Caicedoa, P.E.; Braga, F.C.; Brandão, G.C. and Oliveira, A.B. 2007. Seasonal and intraspecific variation of flavonoids and proanthocyanidins in *Cecropia glaziovi* Sneth. leaves from native and cultivated specimens. Zeitschrift für Naturforschung 62c: 701-709.

Maron, J.L. and Gardner, S.N. 2000. Consumer pressure, seed versus safe-site limitation, and plant population dynamics. Oecologia 124: 260-269.

Marquis, R.J. 1984. Leaf herbivores decrease fitness of a tropical plant. Science 4674: 537-539.

Medeiros, J.D. and Morretes, B.L. 1995. Dimensões da Folha e Herbivoria em *Miconia cabucu* Hoehne (Melastomataceae). Biotemas. 1: 97-112.

Medinaceli, A.; Miranda-Avilés, F.; Flores-Saldaña, N.P. and Gutierrez-Calucho, E. 2004. Herbivoría en relación altamaño de la planta y a lãs diferencias de exposición de *Pilea* sp. (Urticaceae) enlaEstación Biológica Tunquini, Cotapata, La Paz, Bolivia. Ecología en Bolivia 2: 4-8.

Murcia, C. 1995. Edge effects in fragmented forests: implications for conservation. Tree 2: 58-62.

Norusis, M.J. 2008. SPSS 17.0 Guide to Data Analysis. Prentice - Hall, New Jersey. 672 pages.

Peixoto-Sobrinho, T.J.S.; Cardoso, K.C.M.; Gomes, T.L.B.; Albuquerque, U.P.and Amorim, E.L.C. 2009. Análise da pluviosidade e do efeito de borda sobre os teores de flavonóides em *Bauhiniacheilantha* (Bong.) Steud. Fabaceae. Revista Brasileira de Farmacognosia. 3: 740-745.

Plano de Manejo do Parque Estadual da Serra Furada. 2009. Volume 1: Diagnóstico e Planejamento / Fundação do Meio Ambiente P712 PPMA/SC. Socioambiental Consultores Associados Ltda.

Pourmorad, F.; Hosseinimehr, S.J. and Shahabimajd, N. 2006. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. African Journal of Biotechnolgy 11: 1142-1145.

Ribeiro, M.T.; Ramos, F.N.and Santos, F.A.M. 2009. Tree structure and richness in an Atlantic forest fragment: distance from anthropogenic and natural edges. Revista Árvore 6: 1123-1132.

Rocha, C.F.D.; Bergallo, H.D.; Van Sluys, M. and Alves, M.A.S. 2006. Biologia da conservação: essências. RiMA, São Carlos. 582 pages.

Rodrigues, J. 2007. Uso sustentável da biodiversidade brasileira: prospecção químico-farmacológica em plantas superiores: *Miconia* sp. Msc dissertation. Universidade Estadual Paulista, Araraquara.

Santos, G.;Ayub, A.; Sarmento,R.and Almeida-Cortez, J. 2007. Herbivoria foliar em plântulas e adultos de *Cordia nodosa* Lam. (Boraginaceae) em um remanescente de mata atlântica, Pernambuco.In: Congresso de Ecologia do Brasil, SEB Anais, Caxambu, São Paulo.

Schoonhoven, L.M.; Jermy, T. and van Loon, J.J.A. 1998. Insect-Plant Biology: From Physiology to Evolution. Chapman & Hall, London. 409 pages.

Serpeloni, J.M.; Reis, M.B.; Rodrigues, J.; Santos, L.C.; Vilegas, W.; Varanda, E.A.; Dokkedal, A.L. and Cólus, I.M.S. 2008. *In vivo* assessment of DNA damage and protective effects of extracts from *Miconia* species using the comet assay and micronucleus test. Mutagenesis 6: 501-507.

Sevegnani, L. 2007. A herbivoria como limitador do desenvolvimento e sobre vivência das plantas na floresta. Pages 25-36, In: Rego, G.M.; Negrelle, R.R.B and Morellato, L.P.C. Fenologia: ferramenta para conservação, melhoramento e manejo de recursos vegetais arbóreos. Embrapa Florestas, Colombo, PR.

Singleton, V.L. and Rossi, J.A. JR. 1965. Colorimetry of total phenolics with phosphomolybdic - phosphotungstic acid reagents. American Journal of Enology and Viticulture 16: 144-158.

Sistema Meteorológico do Paraná –SIMEPAR. 2017. Centro Politécnico da UFPR, Curitiba, Paraná, Brasil.

Wagner, H. and Bladt, S. 2009. Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2nd Edition. Springer Science & Business Media, Berlin. 384 pages.

Yariwake, J.H.; Lanças, F.M.; Cappelaro, E.A.; Vasconcelos, E.C.; Tiberti, L.A.; Pereira, A.M.S. and Franca, S.C. 2005. Variabilidade sazonal de constituintes químicos (triterpenos, flavonóides e polifenóis) das folhas de *Maytenus aquifolium* Mart. (Celastraceae).Revista Brasileira de Farmacognosia. 2: 162-168.

Zvereva, E.L. and Kozlov, M.V. 2001. Effects of pollution-induced habitat disturbance on the response of willows to simulated herbivory. Journal of Ecology 89: 21-30.

Received 14 November 2016
Accepted 5 February 2017