

Morphological plasticity of Ilex theezans Mart. ex Reissek (Aquifoliaceae) in two restinga vegetation of Santa Catarina coastal plain

Plasticidade morfológica de Ilex theezans Mart. ex Reissek (Aquifoliaceae) em duas formações de restinga da planície costeira de Santa Catarina

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ABSTRACT

The *restinga*, an environment of the rain Forest, is an ecosystem by the seacoast composed of diverse plant communities commonly found in sandy and undeveloped soils. This study aims to investigate possible intraspecific differences in *Ilex theezans* species, whose distribution in *restinga* includes two heterogeneous formations, shrub-tree *restinga* and transition forest. The study focused on a *restinga* remnant area located in *Acaraí State Park*, in the city of São Francisco do Sul (SC, Brazil). In each environment, five adult individuals were selected and had wood samples collected from their stems at breast height and 25 leaves of the third and fourth nodes of the outer canopy branches sampled. Chemical and physical soil variables were analyzed for each formation. The results show that there are significant differences between the leaf and the stem functional attributes and soil data analyzed, but as the PPI (Phenotypic Plasticity Index), the species *Ilex theezans* in the studied formations present low plastic potential.

Keywords: Functional ecology; morpho-anatomy; phenotypic variation; population ecology; rain forest.

RESUMO

Restinga, ambiente litorâneo sobdomínio da mata atlântica, é um ecossistema costeiro composto por um conjunto diversificado de comunidades vegetais comuns em solos arenosos e pouco desenvolvidos. O presente estudo objetiva verificar possíveis diferenças intraespecíficas na espécie *Ilex theezans*, cuja distribuição na restinga abrange duas formações heterogêneas, restinga arbustivo-arbórea e floresta de transição. A área de estudo compreende um remanescente de restinga localizado no Parque Estadual Acaraí, no município de São Francisco do Sul (SC, Brasil). Em cada ambiente foi selecionada uma população composta por cinco indivíduos adultos; destes foram coletadas amostras de madeira do caule à altura do peito e 25 folhas dispostas no terceiro e quarto nós de ramos da copa externa. Analisaram-se variáveis químicas e físicas do solo para cada formação. Os resultados mostram que existem significativas diferenças nas médias entre os atributos funcionais foliares e caulinares e os dados de solo examinados, porém quanto ao Índice de Plasticidade Fenotípica (IPF) a espécie *Ilex theezans*, nas formações de estudo, apresenta baixo potencial plástico.

Palavras-chave: Ecologia funcional; ecologia de populações; mata atlântica; morfoanatomia; variação fenotípica.

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INTRODUCTION

The *Restinga* (sandbank) is a geologically recent ecosystem whose colonization occurred by species from other ecosystems or biomes such as the *Cerrado*, the *Caatinga* and even the Rain Forest, but with functional variations due to different conditions of its original environment (KLEIN *et al.*, 2007). According to Lacerda *et al.* (1993), in Brazil the vegetation of sandbanks occupies approximately 79% of the coastline, covering Holocene sandy deposits among dunes, ridges, spurs, plains generally spodosolic and formed by aeolian, lagoon, river, marine deposition or a combination of these (IBGE, 2012). The vegetation of sandbanks presents different communities distributed over a soil gradient, which have the herbaceous, shrub, shrub-tree and forest physiognomy, with growing diversity from post-beach towards the mainland region (MELO JÚNIOR & BOEGER, 2015).

Although considered a permanent preservation area, the sandbank is constantly threatened by human activities, with emphasis on real estate speculation and sand extraction (ASSIS *et al.*, 2004), which results, beyond species richness reduction, in loss of functional diversity of flora species.

Environmental factors can promote changes in the functional and structural characteristics of organisms exposed to them, in order to ensure their growth, development, survival and reproduction (GRIME & MACKEY, 2002). This alteration capacity of physiological, morpho-anatomical, ecophysiological and phenological attributes that an organism owns to response to changing environmental conditions is known as phenotypic plasticity (SCHLICHTING, 1986; VALLADARES *et al.*, 2006).

As an ecological consequence of these functional adjustments aggregated to the plastic potential of species, plants have the possibility to experience different habitats, explore richest niches in resources and expand their geographic distribution possibilities (SULTAN, 2000; BRADSHAW, 2006). Thus, the wide variation of the nutritional composition and water availability of sandbank soil is a precursor in the plants of structural adjustments, since the soil is an important conditioner and limiting factor for the development of species, especially when it refers to the expression of leaf structural adjustments and conducting water (MELO JÚNIOR, 2015; MELO JÚNIOR & BOEGER, 2016).

Plants present in the sandbank, growing close to the sea, are subject to more stringent and stressful environmental conditions than plants growing in tighter formations within the continent (SCARANO *et al.*, 2001). In sandbank environments, plants are subject to permanent salt deposit from sea, high temperature and luminosity and heavy wind, along with sand, that can cause mechanical damage to plants (ULIAN *et al.*, 2012). In addition, sandbank plants are, in sandy soils, poor in nutrients and permeable to water (SOUZA & CAPELLARI JÚNIOR, 2004).

Thus, this study aims to evaluate the plastic potential of *Ilex theezans* Mart. ex. Reissek. (Aquifoliaceae), in different sandbank formations of a coastal plain in northeast Santa Catarina. The choice of this species stems from its high abundance and importance value in the community structure of the sandbank in study. It has been hypothesized that the environmental conditions of the sandbank promote structural adjustments in the selected species. The predictions of this hypothesis are: a) increased investment in functional attributes associated with stem growth from higher nutritional supply from soil, especially the content of organic matter and CTC; b) the differentiated water condition among the sandbank soils promotes trade off in the wood.

MATERIAL AND METHODS

STUDY ÁREA

The study area comprises a remnant sandbank located in Acaraí State Park, São Francisco do Sul, SC, Brazil, under the 26°17'S and 48°33'W coordinates (figure 1). It is a conservation area with an approximate area of 6,667 hectares in the coastal plain and is considered the most important continuous remnant of coastal ecosystems of Santa Catarina (FATMA, 2006).

Figure 1 - Localization of the study area, Acarai State Park, São Francisco do Sul / SC. Source: Melo Júnior (2015).

The climate, influenced by maritime humidity, is mesothermal with no defined dry season and with hot summers (Cfa, according to Köppen). It is considered humid with average annual temperature of 20.3°C and average rainfall of 1,874 mm / year, with rainfall well distributed throughout the year, in addition to presenting common occurrence of south wind that brings to the atmosphere ocean humidity, making the winter humid (KNIE, 2002; STCP, 2009).

The vegetation of the park (figure 2) belongs to the domain of *restinga* and is compartmentalized in formations with characteristic floristic and structural composition, which are distributed from the post-beach area to the Capivaru pond margins. The following formations stand out: herbaceous *restinga* (Rh), shrubby *restinga* (R), shrub-tree *restinga* (Raa) and the transition forest (Ft) (MELO JÚNIOR & BOEGER, 2015), This study was realized in both last cited formations and in areas defined as RAPELD portions of the module of biodiversity research program on rain forest (PPBioMA), installed on Acaraí State Park.

Figure 2 – Profile of formations of *restinga* at the Acarai State Park, São Francisco do Sul / SC. Source: Melo Júnior & Boeger (2015).

STUDIED SPECIES

Aquifoliaceae is represented by shrubs or trees with wide geographic distribution, consisting of a single genus, *Ilex* L., with about 400 species, of which 60 occur in Brazil, mainly in south and southeast (GROPPO, 2015).

Ilex species are well represented in the sandbank environments in the northeast part of Santa Catarina and few come to occupy a prominent position in the organization of the community of shrub-tree sandbank formation (MELO JÚNIOR & BOEGER, 2015). Thus, the selection of the species resulted from the abundance of individuals in the sandbank, allowing a systematic and valuable sampling. Another criterion is associated with the fact that *Ilex theezans* is considered a species with high importance value in the sandbank in question (MELO JÚNIOR & BOEGER, 2015).

Two populations of *Ilex theezans* Mart. ex Reissek (Aquifoliaceae) were selected in two sandbank formations for data collection, totaling a sample group of ten individuals. The following were considered in this study the formations of shrub-tree *restinga* (Raa) and transition forest (Ft), the latter being an ecotone between the sandbank and the lowland rain forest.

COLLECTION AND PROCESSING OF PLANT MATERIAL

In the two formations of sandbank there were chosen, randomly, five individuals of *I. theezans* in adulthood, *i*.*e*, individuals who have reached reproductive age. From each individual there were collected 25 fully expanded leaves, arranged in the 3rd and 4th nodes from the branch apex. After the leaves herborizing, it was taken biometrics of the following functional leaf attributes: leaf area (cm²) in a desktop scanner coupled to the Sigma Scan Pro software (Version 5.0, SPSS Inc., Chicago, IL, USA), leaf thickness (mm), width (mm) and length (mm) with the aid of a digital caliper, dry mass (g) in an

analytical balance and leaf specific area (cm².g-1) using the formula AEF = a / MS, where a = leaf area and MS = dry mass (WITKOWSKI & LAMONT, 1991).

Timber was collected, from the same individuals of each population, in the form of wedges at breast height (1.3 m ground) in isolated individuals or from entire disk from tillers individuals. Wood samples were made for subsequent histological preparation. The material was softened by cooking in water and 30% glycerine (FERREIRINHA, 1958). After cooking, histological cuts were made in transversal, longitudinal tangential and longitudinal radial sections with the aid of microtome slide. Histological slides were produced based on the guidelines of Johansen (1940). Sections were cleared in sodium hypochlorite (NaClO), washed in distilled water, stained with safrablau and dehydrated in ascending ethanol series (KRAUS & ARDUIN, 1997). The assembly was performed with synthetic resin of the type stained varnish (VASCONCELOS *et al.*, 2006).

Permanent slides with dissociated material were produced by dipping wood samples in Franklin solution, stored in an oven at 60°C (KRAUS & ARDUIN, 1997). The following functional attributes for the stem were considered: the maximum height of the individual sample (m), the trunk diameter at breast height (cm), the tangential vessel diameter (uM), the frequency of vessels (in $/$ mm²) and the vessel element length (um.)

From the above biometric data, there were calculated the conductivity index (CI) = $r\Box$ / F (radius in the fourth power divided by the average frequency of vessels) (ZIMMERMANN, 1983), vulnerability index $(IV) = D / F$ (vessel average tangential diameter divided by the average frequency of vessels) and mesomorph index $(IM) = IR \times C$ (vulnerability index multiplied by the average length of the vessel elements) (CARLQUIST, 1977; 2001).

ENVIRONMENTAL VARIABLES

For the analysis of environmental variables, a soil sampling was performed of each formation, according to the methodology of Embrapa (2013). The collection was carried out in five holes up to 10 cm deep, the samples being later homogenized to obtain a compound sample *per* formation. The following parameters were evaluated: organic matter (OM), hydrogen potential (pH), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), aluminum (Al) and hydrogen (H), bases sum (SB), capacity of cation exchange (CEC), base saturation (V%) and salinity (Na). Chemical soil analyzes was conducted by the Agronomic Institute of Campinas (IAC). In addition, the litter thickness and the gravimetric humidity were measured (EMBRAPA, 2013).

DATA ANALYSIS

For all quantitative attributes, the Phenotypic Plasticity Index (IPF) the were calculated. This method of phenotypic plasticity is based on the ratio of the difference between the maximum average and minimum average by the maximum average of a certain biometric variable (VALLADARES *et al.*, 2005).

Means and standard error were calculated for all analyzed leaf and stem functional attributes. The comparison between populations of *I. theezans* was performed using the Student t test in R environment (CRAWLEY, 2007). Pearson correlation tests were conducted to detect possible interactions between the functional biological attributes and the measured environmental variables (LEGENDRE *et al*., 2011).

RESULTS AND DISCUSSION

In the soil analysis, the nutritional and fertility values differ between the formations. The values are considerably increasing toward the forest environment (table 1). The amounts of organic matter (OM), litter thickness (Ser) and gravimetric humidity (GM) were higher in the transition forest as well as the values of phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) cations.

The cation exchange capacity (CEC) was consequently higher in the transition forest (Ft). For the shrub-tree *restinga* (Raa), there were recorded hydrogenionic potential (pH), base saturation (V) and salinity (Na) values higher than those of the transition forest, which makes it a less fertile soil, more saline and alkaline when compared to the forest soil.

The high content of organic matter obtained in the transition forest provides greater fertility of the soil, which is proportional to the greater thickness of dead biomass deposited on the ground. Soil fertility can structurally influence the vegetation of sandbank (PEREIRA, 1990). The high organic matter content enable the reservation of reserves of phosphorus, calcium, magnesium and potassium nutrients, improves water holding capacity and influence pH (ZECH *et al.*, 1997). The organic matter content also promotes the increased cation exchange capacity (CEC), facilitating the exchange of essential cations to plant growth (Ca, Mg and K) by H + ions and may be the cause of reducing the pH in the surface layers of the soil which showed higher organic matter content, and hence greater retention capacity of H + ions (SANTOS & Camargo, 1999).

Table 1 – Analysis of soil fertility at the schrub-tree restinga formations (Raa) and transition forest (Ft) of Acarai State Park, São Francisco do Sul, Santa Catarina. Legend: organic matter (MO), hydrogen potential (pH), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), aluminum (Al), hydrogen (H), sum of bases (SB), cations exchange capacity (CTC), base saturation (V%), salinity, litter (Ser) and gravimetric humidity (UG). Values are mean and standard deviation (in brackets).

The low index of V% presented in transition forest means that there are small amounts of cations, such as Ca, Mg and K, saturating the negative charges of the colloids, the majority of them being neutralized by H + and Al3 +, which contributes to the acidity of soil that may even contain an aluminum toxic level to the plants (RONQUIM, 2010). In transition forest, H + and Al3 + values are much higher when compared to those obtained in shrub-tree *restinga*.

Soil fertility can interfere with sandbank vegetation structure (PEREIRA, 1990) and plays a key role in maintaining coastal ecosystems, as it holds the reserves of nutrients, improves water retention capacity, influence the pH and raises cation exchange capacity (CEC), preventing the leaching of cations (ZECH *et al.*, 1997). The average data of height and diameter (table 2) show increased investment in growth in individuals of *I. theezans* present in the transition forest. The fertility conditions of the transition forest enable and make more favorable the growth of the species, because of the amounts of organic matter and macronutrients P, Ca, Mg and K. The individuals present in the formation of shrubtree sandbank, as they do not develop in a soil with favorable characteristics to growth investment, become smaller in their air architecture.

As well as environmental variables, leaf functional attributes showed significant differences between the studied formations (table 2). Mean values for leaf area, dry matter, leaf length and width were higher in transition forest. The leaf thickness and leaf specific area showed higher mean values in the formation shrub-tree *restinga*. These figures show that *I. theezans* has larger leaves in transition forest when compared to leaves in shrubby-arboreal *restinga*. This suggests that the plants are more shaded and have more accumulated biomass in the forest formation. The lower leaf area, observed in the population of the *restinga* shrubby-arboreal, is a strategy to prevent the loss of water through transpiration under conditions of high temperatures and in bright sunlight or due to low soil fertility (JAMES & BELL, 2001; BOEGER & WISNIEWSKI, 2003), these characteristics being more obvious in the sandbank formation in question. The assumption of the increase in biomass production, resulting in increased leaf area, depending on soil fertility (organic matter content and CTC) is supported by the high correlation value found here ($r = 0.74$, $n = 250$, $p \le 0.0001$).

The leaf specific area expresses the ratio of leaf area by dry weight of the leaf. It is considered an important factor from a physiological point of view by describing the allocation of biomass leaf area per unit, reflecting the trade-off between rapid production of biomass and efficient conservation of nutrients (POORTER, 1994). The leaf specific area and leaf dry weight are important for the study of plant ecology, since they are associated with many aspects of plant growth and survival, being characteristic of resource use strategies (GARNIER *et al*., 2001; SHIPLEY & VU, 2002). Shade plants, such as those in transition forest, invest relatively higher proportion of photo assimilates in increased leaf area, to maximize capture of the available light. Generally, they exhibit thin sheets and have higher leaf specific area (LAMBERS *et al.*, 1998). Species with high values of leaf specific area exhibit high productivity and rapid growth capacity (REICH *et al.*, 1999; LI *et al.*, 2005), which explains the larger forest individuals, both in height and in diameter stem, compared to those of the shrubby-arboreal *restinga* (table 2).

Table 2 – Mean and standard deviation of leaf and stem functional attributes analyzed in populations of *Ilex theezans* in the formations of shrub-tree *restinga* (Raa) and transition forest (Ft) at Acarai State Park, São Francisco do Sul, Santa Catarina. Legend: different letters in the same row indicate statistical significance at 0,05 level.

Although there was no statistical difference for the AEF attribute, the lowest absolute value observed in individuals of shrubby-arboreal *restinga* may represent a major investment in mechanical tissues, given the greater plant exposure to luminous radiation since its top occupies the upper *stratum* in this formation. In forest environments (formation with more AEF) due to the heterogeneous distribution of light, it is expected that plants maximize light capture at the lowest cost in terms of investment and maintenance of tissues (GIVNISH, 1987). For this reason, the architecture of leaves

in these environments is considered, in terms of selective pressure, a favorable element to the light interception (VALLADARES *et al*., 2002).

If compared the foliar data with the soil nutritional data, in places of low availability of resources, such as the shrub- tree *restinga*, what prevails is the conservative strategy, where the plant invests in slow growth, tissue protection and high investment in dry matter and presents low concentration of nutrients and low photosynthetic rate (LEMOS *et al.*, 2011). It is noteworthy that the population of the formation transition forest presented a higher dry mass mean by having significantly larger leaves than that of the sandbank, which make them to be heavier.

Environmental changes reflect in structural changes in the wood, especially the size of the cellular elements, such as diameter, length and frequency of vessels (BAAS *et al.*, 1983). Regarding the vessel elements, the specimens present in the transition forest formation have a greater length of vessel elements and a higher frequency of vessels. In the formation of shrub-tree *restinga*, it can be seen that the tangential diameter of the vessel element is higher as compared to that obtained in forest environment (table 3). Regarding the functional and ecological characteristics of the xylem, Carlquist (1977) established mesomorphy and vulnerability indexes. Species of mesomorphic index greater than 200 are considered mesomorphs, according to Carlquist index (1977; 2001). *I. theezans* individuals analyzed presented mesomorphy index with values above this limit in the two formations of the sandbank of this study and *I. theezans* is considered a mesomorphic species.

Higher values of the vulnerability index indicate greater propensity to cavitation and hence the interruption of the water flow (CARLQUIST, 1977), which can be observed the formation of shrubtree *restinga.* Since individuals of shrub-tree *restinga* showed higher tangential vessel diameter, the vulnerability index found in this population was higher than that obtained in the transition forest individuals (table 3). Therefore, the hydraulic conductivity in individuals of forest formation is considered safer and less susceptible to embolism or cavitation.

Higher conductivity indexes demonstrate greater efficiency in the transport of water (CARLQUIST, 2001). In this study, the formation of shrub-tree *restinga* showed higher conductivity, possibly due to increased vessel diameter.

According to Baas *et al.* (1983), larger vessels are more efficient but less safe due to the increased risk of blistering, which corroborates the values obtained for the Vulnerability Index (table 3). These figures show that the plants of the formation Raa are more vulnerable to embolism precisely because they have a more tangential vessel diameter in an environment where water scarcity is higher. On the other hand, such plants have increased transport efficiency evidenced by higher conductivity index (table 3), which is directly proportional to the greatest tangential vessel diameter. The proportionality between the tangential vessel diameter and water availability in the *restinga* soil proved to be positively correlated ($r = 0.50$, $n = 300$, $p \le 0.0001$).

Table 3 – Indexes of vulnerability, mesomorphy and hydraulic conductivity of secondary xylem of *Ilex theezans* in shrub-tree *restinga* (Raa) and transition forest (Ft) at Acarai State Park, São Francisco do Sul, Santa Catarina.

According to Castro *et al*. (2009), in environmental stress conditions, the plant invests in larger amounts of smaller diameter vessels to diminish the risk of embolism, as in this way vessels have greater adhesion of water on their walls, preventing the formation of air bubbles inside the vessel. This can be observed in the transition forest formation. The phenotypic plasticity index obtained for the leaf attributes (area and leaf dry mass) and timber (tangential vessel diameter) of *I. theezans* can be considered median (table 4). According to Valladares *et al.* (2005), variables with IPF higher than 0.6 are considered very plastic and those, whose IPF is close to 0, no plastic. Thus*, Ilex theezans* shows moderately plastic for certain morphological and anatomical attributes, which enhances the allocation of resources offered the sandbank environment. The other leaf and stem traits analyzed show low down plastic potential (table 4).

Table 4 – Phenotypic plasticity index (IPF) of the leaf and stem functional attributes of *Ilex theezans* at the two formations of shrub-tree *restinga* (Raa) and transition forest (Ft) at Acarai State Park, São Francisco do Sul, Santa Catarina.

Although the studied populations of *Ilex theezans* were statistically different for all morphoanatomic attributes analyzed, it is believed that the low-moderate plastic potential observed may be related to spatial microscale distribution of populations of this species in remnant *restinga* studied. This idea supports the results obtained for other woody species studied in the same sandbank formations at Acaraí State Park (BÄCHTOLD & MELO JÚNIOR, 2015; SILVA & MELO JÚNIOR, 2016). It is also possible that other functional attributes to be more plastic compared to those dealt with here, making studies in plasticity an important Contribution to the definition of functional attributes in plants under the biodiversity studies of rain forest associated to PPBioMA.

CONCLUSION

In the shrub-tree *restinga*, characterized as a low fertility and water availability environment, there is a greater increase in tangential vessel diameter in an attempt to increase efficiency in water transport in an environment with strong drainable soil. In the forest environment, where there is greater availability of nutrients coming from the decomposition of organic matter, and more abundant water resources, the species invests in growth and photosynthetic production, which was evidenced by the height and stem diameter of the sample individuals, and longer vessels and in greater numbers per unit area, favoring them also a water efficiency compensated by the absence of tangential diameter larger vessels. Overall, the IPF values obtained show the low phenotypic plasticity of evaluated attributes for the species *I. theezans,* distributed in a spatial microscale at the *restinga* formations in this study.

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