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ANATOMICAL TRAITS, SILICA CONTENT AND HERBIVORY DAMAGE IN LEAVES OF INVASIVE TREE *LIGUSTRUM LUCIDUM* DEVELOPED IN NOVEL AND NATIVE RANGES

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Highlights

- SLA of leaves differs between light conditions and native or invaded areas.
- Herbivory damage increases with leaf age but it is not affected by light conditions.
- Silica content increased with age leaf and differs between native and invaded areas.
- Environmental conditions in native and invaded areas may affect SLA and Si content.
- Relations between silica, leaf ageing and herbivory damage in dicots are first shown.

Abstract

Ligustrum lucidum is an invasive tree in many regions, but scarce work analyzes its anatomical or eco-functional traits and its relation with herbivores, an important issue to be addressed by invaders in new areas. On the other hand, amorphous silica accumulation is an important anti-herbivore component in grasses, but its role in eudicots has been little explored. To analyze if anatomical and eco-functional traits, silica accumulation and herbivory damage are modified by phenological stages or environmental conditions, and if herbivory damage is negatively correlated with silica content, we analyzed shade and sun leaves of different ages from adult trees in novel (Argentina) and native (China) areas. Leaves were scanned, dried and weighed for specific leaf area estimation. Herbivore damage was calculated as the proportion of leaf surface eaten. Silica content was quantified through calcination and digestion techniques. The results showed that leaves from China had higher SLA than leaves from Argentina, and shade or young leaves had higher SLA than sun or old leaves. Herbivory was higher in old leaves compared to young ones. Mean silica concentration was 1.73%; it was higher in leaves from Argentina than in leaves from China and it increased with leaf age. Differences in silica concentration or SLA between samples are probably due to environmental conditions such as pollution degree, soil Si content, water availability and / or temperatures. The age of the leaves affected the values of SLA, silica content and herbivory damage. Along the life of the leaves, silica content increased approximately five times in ten months: older leaves became thicker, with a higher silica content, and a higher herbivory damage. Considering the same age of the leaf, silica concentration and herbivory damage were not correlated, therefore it was not possible to associate the silicification process with an antiherbivore role in this species.

Keywords: leaf anatomy, leaf ontogeny, silicification, China, Argentina, invasion

1. Introduction

Ligustrum lucidum, known as Nv Zhen (女贞), is an evergreen tree native from China, where it has been extensively cultivated for long time for different purposes (e.g. traditional medicine, culturing wax-insects to obtain white wax, furniture and food uses). Because it is tolerant of air pollution, wind and poor soils, it was regarded as a useful landscaping plant and planted extensively in streets and gardens around the world (Editorial Committee of Flora of China, 1992; Montti et al., 2016). However, it has also become a successful global tree invader outside its native range (Maddox et al., 2010; Aslan et al., 2012). Particularly in Argentina, it is one of the most aggressive and fast-growing tree species, and it is widely distributed in natural and urban areas. Its introduction in natural Argentinean ecosystems has lowered the native biodiversity and altered the processes and functions of ecosystems (Easdale et al., 2007; Hoyos et al., 2010; Montti et al., 2017; Ferreras et al., 2019). Where it is invasive, it can change decomposition rates, affecting N and C cycling (Aragon et al., 2014), habitat properties (Ayup et al., 2014) and water dynamics (Zamora Nasca et al., 2014; Whitworth-Hulse, 2018).

Although many ecological studies have been carried out about *L. lucidum*, scarce work has been done about its anatomical and / or eco-functional traits (Bruno et al., 2007; Larcher et al., 2015). The knowledge about basic anatomical / morphological leaf features, such as specific leaf area (SLA), tissue differentiation and leaf life-span, results relevant since they are related to environmental conditions (Wright et al., 2004). The assumption usually made is that light conditions affect leaf anatomy; plants developed under shade environments decrease the chlorophyll parenchyma thickness, thus increasing specific leaf area (Gutschick, 1999; Takenaka et al., 2001; Vendramini et al., 2002; Niinemets, 2010). Also, leaf life-span changes according to the cost of leaf construction, which in turn depends on nutritional and environmental conditions (Cordell et al., 2001). Finally, leaves with low SLA usually have long life-spans because structural strengthening makes them

less susceptible to herbivory (Westoby et al., 2000; Wright and Cannon, 2001). The knowledge of these basic aspects of *L. lucidum* leaves, which have not been studied until now, and their relation to environmental conditions, will contribute to the comprehension of the mechanisms that facilitate the spread of this species in new areas.

There are multiple factors that affect the success of species invasion; among them are the presence of herbivores or predators and the defense mechanisms of the invader. The absence or reduction of natural enemies may explain why plants become so invasive in their novel range: they reduce their allocation of resources to defense traits and, consequently, redirect resources to growth, increasing their competitive ability (enemy-release hypothesis: Elton, 1958; Keane and Crawley, 2002). On the other hand, the intrinsic mechanisms of defense that plants have (both physical and chemical), may prevent the attack by native herbivores and allow their development in new areas. In particular, herbivory and herbivore resistance were evaluated in plants of *L. lucidum* from native and invaded areas, and some differences were observed (Montti et al., 2016). *Ligustrum lucidum* experienced significantly less foliar damage and less herbivore resistance (in terms of lignin content and lignin:N ratio) in the novel areas than in the native ones (Montti et al., 2016). These differences in herbivore resistance were associated to the presence of other compounds in the leaves, such as silica (De Rito et al., 2016; Montti et al., 2016). Recently, many researches have demonstrated the relevance of amorphous silica (SiO_2) as an important element in plant defenses (Massey et al., 2006; Hartley et al., 2015).

Amorphous silica biomineralizations (also known as phytoliths, silicophytoliths or plant opal), are compounds of SiO_2 deposited in inter- or intracellular spaces of plant tissues as well as on the cell surfaces to form the silica–cuticle double layer (Sangster et al., 2001; Prychid et al., 2003). Silicon is taken up by roots from the soil solution in the form of monosilicic acid and is transported through xylem to the different tissues where it is

finally deposited as amorphous silica (SiO_2) (Ma and Takahashi, 2002; Casey et al., 2003). Silica contributes to the anti-herbivore defense of plants (Massey and Hartley, 2006; Massey et al., 2007; Reynolds et al., 2012; Soininen et al., 2013), prevents fungal attacks (Ma, 2004), affects leaf optical properties and UV-absorbance (Agarie et al., 1996; Klancnik et al., 2014), reinforces the organ structure under specific stresses (Raven, 1983; Lux et al., 2002; Schoelynck et al., 2010; Fernández Honaine et al., 2013) and ameliorates aluminum toxicity by the induction of the Si-Al co-deposition (Hodson, 2016).

Grasses, along with palms, sedges and horsetails, are a group of plants with the highest accumulation of amorphous silica, and some of the most studied taxa in relation to silicification process (Hodson et al., 2005; Piperno, 2006; Katz, 2014). Besides the strong relation between silica content and systematics (Hodson et al., 2005), the silica content in plants also depends on the age of the organ, the soil Si content and water availability, the transpiration rate, and the herbivore pressure (Jones and Handreck, 1967; Handreck and Jones, 1968; de Melo et al., 2010; Cooke and Leishman, 2012; Soininen et al., 2013; Quigley and Anderson, 2014). Some studies related silica content with ecophysiological variables in different taxa, showing negative relations between silica content and leaf longevity (Cooke and Leishman, 2011a). Since leaf longevity is also associated to other ecomorphological traits, such as specific leaf area (SLA), many other analyses should be carried out before clear relations between silica content and ecological traits become elucidated (Cooke and Leishman, 2011a, b). Also, recently, Katz (2018) proposed that plant silica content is a plant trait, since it meets all the properties that define a plant trait, highlighting the importance of its study. Most of the works about silicification process involved grasses or species with economic value, while works about eudicots are very scarce, especially in trees (Katz, 2014). Some previous research showed that *Ligustrum lucidum* accumulates silica in leaves, especially in epidermis tissue; however the knowledge about the biological and environmental factors that affect the silicification

process in this species, and the potential role that it may have as an antiherbivore defense is null (Piperno, 2006; De Rito et al., 2018).

In this work we aimed: 1) to analyze some non-studied anatomical and eco-functional traits of *Ligustrum lucidum*, in order to advance into the comprehension of the relation between these traits and environmental and biological factors; 2) to contribute to the understanding of the silicification process in dicotyledons, a process scarcely studied in this group; 3) to analyze if the silicification process is involved in the success of this species to invade new areas, specifically as an anti-herbivore defense. Considering previous researches which showed that some anatomical traits are affected by environmental and biological conditions (Bruno et al., 2007; Larcher et al., 2015), and the lack of information about basic eco-functional aspects, we firstly analyzed how specific leaf area (SLA), tissue differentiation and leaf life-span varied among leaves of different ages and light exposure of plants developed in different regions (Argentina and China). Secondly, we evaluated how silica content and herbivory damage differ among these types of leaves, and if these two factors are interrelated. We hypothesized that silica concentration is increased in older sun-exposed leaves, since silicification is accumulated with age, and transpiration rate (which is higher in sun-exposed leaves) increases the deposition of amorphous silica in tissues (Ma and Takahashi, 2002; Motomura et al., 2002; de Melo et al., 2010). We also expected that plants developed in different sites (Argentina and China) accumulated silica in different amounts, since soil characteristics and climatic factors affect silicification process (Faisal et al., 2012; Issaharou-Matchi et al., 2016). In addition, we analyzed the rate at which amorphous silica is accumulated in tissues, in order to understand the relevance of this phenomenon in this species. Herbivory damage depends on leaf anatomy and constitution, which in turn may depend on the age of the leaf and the environmental conditions, and so differences should be found between samples.

Finally, considering silica as an antiherbivore agent, herbivory damage was expected to be negatively correlated with silica content.

2. Material and Methods

2.1. Species and study sites description

Ligustrum lucidum W. T. Aiton belongs to the Oleaceae Family. It is an evergreen tree native from China, with a height of ≈ 15 m and a dense canopy (Fig. 1). It is characterized by small and white flowers arranged in panicles, and oblong blue-black drupes of 0.6 cm. The leaves are opposite, oval to elliptic, 40–130 mm long (occasionally up to 24 cm long) and 30–60 mm wide (Swarbrick et al., 1999). The upper leaf surface is dark green and glossy or shiny while the lower surface is paler with distinct venation (Swarbrick et al., 1999). It grows between 0 and 2900 m above sea level under different climates and soil types; in full sunlight, as well as in shaded understory (Chang et al., 1996).

Plant samples were collected from two sites in Argentina (located in the Yungas region and in the Pampean region) and from a natural forest in China (Fig. 1), between December 2016 and December 2017.

>>insert Fig. 1 here

The first study site (A1 site) of Argentina is in Sierra de San Javier, in Tucumán province ($27^{\circ} 30' S$, $65^{\circ} 40' W$). The area represents the southernmost limit of the subtropical Andean mountain forest (also known as Yungas), which extends from Bolivia to the province of Catamarca in Argentina (Cabrera, 1971). Climate is subtropical monsoonal with a marked dry winter season and rainy summers. Average annual precipitation ranges from 1300 to 1500 mm (Bianchi and Yañez, 1992). Ninety percent of the annual precipitation occurs in summer, while there is an hydric deficit in winter–spring period, with precipitations lower than 10 mm per month (Hunzinger, 1997). Mean annual temperature is $18^{\circ} C$ with frosts occurring from June to August. Mean winter temperature varies

between 10 and 12 °C, and mean summer temperature between 24 and 26 °C. The soils of the region show a wide spatial variability as a consequence of the diversity of conditional factors, but in the area, Hapludolls and Argiudolls are dominant. They are characterized by an A horizon (mollic epipedon) and a B argillic horizon, and pH values between slightly acid and neutral (5.8 – 7.1) (Puchulo and Fernandez, 2014). There is no detailed information about soil Si content in these soils; however, the Holocene input of volcanic ashes and the local silicophytolith input may be important sources of Si in these soils (Borrelli et al., 2010; Fernandez-Turiel et al., 2019). The second study site (A2 site) is in the SE of Pampean region, Buenos Aires province. The area is mesothermic subhumid–humid, with null or little water deficiency (Burgos and Vidal, 1951). Mean annual precipitation is 934 mm, with the highest values at the end of summer (30.2%), and at the beginning and end of spring. Mean annual temperature is 13.8 °C with a mean of 20 °C during the hottest month (January) and a mean of 7.3 °C during the coolest month (July) (Servicio Meteorológico Nacional, 2010; García and Veneziano, 2014). The soils of this study area are typical Argiudolls (INTA, 1989). They have an A horizon (mollic epipedon) over 30 cm thick and a B horizon (argillic endopedion) of approx. 50 cm, with a Si content in the soil solution estimated according to Borrelli et al. (2010) of $933 \pm 250 \mu\text{mol/l}$, a high content of organic matter (9% in A horizon) and pH values slightly acid (Borrelli et al., 2010). According to Sommer et al. (2006), Si content in these soils is above the mean soils value at a global scale. Plants were collected from a forest in Laguna de Los Padres Natural Reserve (Gral. Pueyrredón district, Buenos Aires province) and in a private field near the reserve. Laguna de los Padres Natural reserve is characterized by the presence of *Ligustrum lucidum* in codominance with native species such as *Celtis ehrenbergiana* and *Colletia paradoxa*, exotic herbs such as *Hedera helix*, and grasses.

Other samples were collected from the Zijin Mountain in Nanjing, China (Ch site) (32° 01' – 32° 16' N, 118° 48' – 118° 53' E). The highest peak is 448.9 m, which is the highest peak

in Ningzhen mountain range. The total area of the mountain is 3008 km², and the forest area is 2107 km². The area belongs to the north subtropical humid climate. The average rainfall is 1106 mm and the rainy season lasts from late June to early July every year. The relative humidity is 76% and the frost-free period is 237 days. The average temperature is 15.7 °C; the annual extreme temperature is 39.7 °C and the lowest is -13.1 °C (Dong et al., 2011; China Meteorological Administration, 2019). The soils are yellow brown soil and yellow cinnamon soil (An et al., 1997). Although there is no detail information about Si content in soils, Wang et al. (2001) and references there mentioned that Si deficiency is common in paddy soils and some upland soils from China. In particular, paddy soils from the province where the study area is located have values lower than 100 mg SiO₂ per kg of soil (Wang et al., 2001). The vegetation type is a transitional zone between deciduous broad-leaved forest in warm temperate zone and evergreen broad-leaved forest in middle subtropical zone. The vegetation composition has obvious transitional characteristics, and the zonal vegetation is evergreen-deciduous broad-leaved mixed forest. There are more than 600 species of seed plants. Among the main constructive species and associated components, the evergreen broad-leaved trees are *Ligustrum lucidum*, *Cyclobalanopsis glauca*, *Castanopsis sclerophylla*, *Photinia serrulata* and *Ilex chinensis*; and the deciduous components are *Quercus acutissima*, *Qu. variabilis* and *Qu. fabri*, *Liquidambar formosana*, *Dalbergia hupeana*, *Pistacia chinensis*, *Platycarya strobilacea* and *Tilia miqueliana* (Dong et al., 2010).

2.2 Plant sampling

In each study site, five trees with similar height were selected. In each plant, 10 different branches (5 sun-exposed and 5 shaded) were randomly selected. In each branch, leaves of 3 different ages / sizes (young / small, intermediate / medium, old / big) were randomly collected. The smallest leaves were those youngest fully-expanded leaves located at the

extreme of the branches. The leaves situated at the base of the branch were the biggest ones, and they were considered as the oldest. The leaves between these two extremes were considered intermediate in age (Fig. 1 d). Leaf samples were stored in paper bags until they were analyzed in the lab for specific leaf area (SLA), anatomical characterization, silica content and herbivory damage.

2.3. *Morpho-anatomical leaf traits*

Specific leaf area (SLA) was calculated in all the samples, in order to analyze the variation of this trait in relation to leaf age and environmental conditions. For this, immediately after the collection, leaves were scanned, and the total area was calculated using Image J version 1.42d (National Institute of Health, Bethesda, USA). Then the leaves were dried at 60 °C for five days and weighed. Specific leaf area (cm^2/g) was calculated as leaf area (cm^2)/leaf weight (g).

To analyze differences in leaf anatomy between sun / shade and young / medium / old leaves, free-hand transverse sections of leaves were obtained by standard methods (D'Ambrogio de Argüeso, 1986). The sections were cleared with 50% sodium hypochlorite, washed with distilled water and mounted on glycerin : water (1:1). Anatomical descriptions were made under light Zeiss Axiostar Plus microscope at 40 $\times$. Finally, the thickness of some cross sections of sun / shade leaves were measured.

2.4. *Leaf life-span*

With the purpose of evaluating the life-span of leaves and its relation with light conditions, 5 trees from A2 site (Argentina) were selected. On December 2016, in each tree, 10 sun-exposed and 10 shade emergent leaf buds were identified with plastic tape. Each leaf bud represented a cohort of leaves of the same age. Leaves were checked monthly until all

the originally marked leaves had abscised. The age of the leaf when mortality occurred was determined as the date of measurement of missing leaf minus the date of the initial measurement immediately after bud break. To depict the patterns of leaf mortality over time, cumulative mortality of all the originally tagged leaves was plotted as a function of the time since the leaves were originally marked within each light exposure, which yielded a sigmoid relationship: % Cumulative mortality = $100/[1+(\exp(a(X-X_{50\%}))]$, where “X50%” represents the time when 50% of the total leaves died (Cordell et al., 2001).

2.5. *Herbivory damage analyses*

Damage produced by herbivores in the field was measured only in samples from Argentina, where previous field studies found that herbivory damage was lower than in native areas (China) (Montti et al., 2016). At least three non-senescent and fully expanded leaves under sun and shade conditions of 5 individuals were analyzed in each site. The herbivory damage was calculated as the proportion of leaf surface eaten using Image J version 1.42d (National Institute of Health, Bethesda, Md, USA).

2.6. *Silica content*

Leaves were subjected to a calcination technique (Labouriau, 1983). The samples were first placed in an ultrasound bath for 15–20 min, washed with distilled water to remove mineral contaminants, dried at 60 °C for 24 h, and weighed (initial weight). Then the material was charred at 200 °C for 2 h. Later, it was boiled in a 5 N HCl solution for 10 min, washed with distilled water and filtered with ashless filter paper, until no more chloride ions were detected. Finally, the material was ignited at 800 °C for 2.5–3 hrs and weighed (ash weight). As a result of this technique, ashes mainly composed by amorphous silica were obtained. The relative content of silica was calculated by the following formula (Fernández Honaine et al., 2005):

Silica concentration (% DW) = (Ash weight / initial weight previous calcination) $\times$ 100

2.7. Long term silica content study

In order to analyze the silicification process throughout the leaves' life, a long-term study was carried out in one site of Argentina. On December 2016, we randomly selected five individuals with similar height in A2 site. In each plant, 5 pairs of sun-exposed and 5 pairs of shade emergent leaf buds were identified with different colors, which represented a cohort of leaves of the same age. Two months later, one leaf of each pair in each situation was collected. Lastly, on December 2017, the other leaf of the pair was collected. Silica concentration in each leaf was quantified through a technique based on an alkaline extraction with 1% Na₂CO₃, which allows the quantification in small samples (Meunier et al., 2014). Approximately 30 mg of leaf powder were digested in 40 ml of Na₂CO₃ (1%) at 85 °C for 4 h until total digestion of the amorphous silica was achieved. Silica (SiO₂) concentration of the solution obtained after digestion was determined through UV-VIS spectrophotometry by means of silicomolybdate method (APHA-AWWA-WPFC, 1998), and leaf silica concentration was expressed as mg SiO₂/g leaf dry weight.

2.8. Data analyses

For the analyses of differences between silica concentration, SLA or herbivory damage, an ANOVA test for differences between sites and nested ANOVA tests for differences between age and light exposure were carried out. Silica concentration in leaves of long-term study was evaluated through non-parametric tests (Kolmogorov-Smirnoff and Mann-Whitney) and Wilcoxon matched pair test. To compare leaf life-span (X_{50%}) among leaf categories we performed a Student t-test. A significance level of 5% was used in all cases. Analyses were performed with InfoStat 2009 (Di Rienzo et al., 2009). Finally, in order to

evaluate the relation between silica content and SLA, silica content and herbivory, non-parametric correlation (Spearman correlation) was carried out (Zar, 1984). Values are presented as mean $\pm$ standard error.

3. Results

3.1. *Morpho-anatomical traits (SLA and anatomical features)*

The results of the ANOVA and nested ANOVA tests showed significant differences in SLA between sites ($F = 16.53$, $p < 0.01$), age of leaf in China site ($F = 3.22$, $p < 0.05$) and light exposure in sites A1 and A2 ($p < 0.01$). Leaves from China have higher SLA than leaves from Argentina, particularly when we compare with leaves of A2 sites. The shade leaves have higher SLA and are thinner than sun leaves (Fig. 1 f). Younger leaves have higher SLA than old leaves in all sites (Fig. 2 a–c).

>>insert Fig. 2 here

Cross sections showed that leaves are characterized by a uni-strata epidermis, palisade and spongy parenchyma and vascular bundles. In both epidermises, glandular trichomes with a unicellular stalk and a multicellular head were observed. There were differences in the thickness of palisade parenchyma between sun and shade leaves but not between sites. In shade leaves, palisade parenchyma has only two rows of cells, while in sun leaves this parenchyma is thicker, showing a high morpho-anatomical plasticity of this species at different light conditions (Fig. 1 f).

3.2. *Leaf life-span (LL)*

Leaf life-span was similar between leaves growing in sun or shade conditions. Cumulative mortality increased in a sigmoid mode along time since leaf initiation (Fig. 3). Intermediate leaf half-life (point at which 50% cumulative mortality was attained) was near 277–292

days. After this time, an abrupt death of leaves was observed, resulting in more than 80% of initial leaves dead before one year.

>>insert Fig. 3 here

3.3. Herbivory damage

The percentage of herbivore damage measured in leaves from Argentina showed great variability, with values between 0 and 12.68% and a mean of 1.75% (± 0.37). Old and medium-aged leaves presented more damage than young leaves, and sun exposed leaves showed more damage than shade leaves (Fig. 2 d–f).

3.4. Leaf silica concentration

Considering all the samples, silica concentration in leaves of *Ligustrum lucidum* ranged between 0.27 and 4.05% (% DW), with a mean of 1.73%. The values in leaves from Argentina (A2 site: $1.96 \pm 0.18\%$, A1 site: $2.02 \pm 0.17\%$) were higher than in leaves from China ($1.20 \pm 0.08\%$) ($F = 8.68$, $p < 0.01$). Silica accumulation increased with the age of the leaves and differences were significant in Argentinean sites ($p < 0.05$). Shade leaves seem to accumulate more silica than sun leaves, however no significant differences were found (Fig. 2 g–i). We also evaluated the silica accumulation considering the variation in leaf mass and / or leaf area in each leaf type. We calculated the silica concentration in relation to the mean weight of each leaf type, and the silica concentration in relation to SLA values. The results showed that values are increased with age, independently the mass or area increment (0.57 mg Si/leaf or 0.10 mg Si/cm² in young leaves; 3.30 mg Si/leaf or 0.16 mg Si/cm² in medium-aged leaves; 9.02 mg Si/leaf or 0.26 mg Si/cm² in old leaves). Instead, silica concentration in sun and shade leaves showed similar values (3.73

mg Si/leaf or 0.19 mg Si/cm² in sun leaves; 3.48 mg Si/leaf or 0.15 mg Si/cm² in shade leaves).

3.5. Long term SiO₂ concentration study

SiO₂ concentration in two-month-old leaves ranged between 1.62 and 9.75 mg SiO₂/g leaf dry weight, with a mean of 5.58 ± 1.40 mg/g. Leaves of 10-month-old increased their SiO₂ concentration up to five times, in relation to leaves of two months old (26.01 ± 3.89 mg SiO₂/g leaf; $Z = 2.59$, $p < 0.01$). We also calculated the silica concentration in relation to the mean weight of both leaf ages (two-month-old and 10-month-old leaves), and the results showed an increment of silica per leaf with leaf age (0.57 mg SiO₂/leaf in two-month-old and 4.93 mg SiO₂/leaf in 10-month-old leaves).

3.6. Relationships between variables

Spearman correlation analyses showed a significantly negative correlation between Si concentration and SLA ($r = -0.27$, $p = 0.009$), and a significantly positive correlation between Si concentration and herbivory damage ($r = 0.45$, $p = 0.0005$; Fig. 4). These results were obtained when all the samples were considered. However, considering that herbivory and Si concentration increased with the age of the leaves, this last correlation could be affected by leaf ageing. For this reason, we lastly evaluated the relation of both variables in old leaves only. The results showed that there is no correlation between Si concentration and herbivory ($r = 0.37$, $p = 0.103$).

>>insert Fig. 4 here

4. Discussion

4.1. Morpho-anatomical traits in relation to environmental conditions and leaf age

Our study showed some differences in the anatomy between sun and shade leaves of *Ligustrum lucidum*. Shade leaves have a smaller proportion of palisade parenchyma than sun leaves, are thinner and have a higher SLA. This finding was also observed by Larcher et al. (2015) in shade and sun leaves of *L. lucidum* growing in Brazil. The development of less palisade parenchyma and a thinner structure of the organ improve the light interception to the photosynthetic area of the leaf. Also, Bruno et al. (2007) studied plants of *L. lucidum* growing in air-contaminated areas (such as big cities where air contamination is due to industries and population development) and not air-contaminated (peripheral) areas in Argentina, and they found that in big cities the palisade parenchyma is thinner and the epidermis has more stomata and trichomes. These foliar modifications would improve the light capture and the gas exchange in polluted and / or shaded areas. Differences in SLA values between sites from Argentina could be explained by the structure of the forests. A1 site represents a closed and dense canopy forest, while A2 site is an open canopy forest (see Fig. 1 C, D; Montti, *pers. comm.*) The fact that leaves from China have higher SLA than leaves from Argentina could also be explained by the environmental conditions where Chinese plants grow. The forests have closed canopy and shade understory, and air pollution is higher than in Argentina (Montti and Qi, *pers comm*; Hu et al., 2012). All these characteristics may affect the anatomy of leaves, decreasing their thickness or density, as it was observed by Bruno et al. (2007) and Larcher et al. (2015). These results show the leaf plasticity of *L. lucidum* growing under variable environmental conditions. This ability might explain some of the eco-physiological conditions that allow this species to colonize and take advantage over other plant species, to finally invade new areas in diverse ecosystems.

Specific leaf area data are first described for this type of leaves in this species, and the results show that it decreased with the age of the leaf, i.e. older leaves were thicker and /

or denser than young leaves. These results agree with general patterns of leaf development. The decrease of SLA in relation to leaf age is associated with leaf density and thickness, as it was observed in diverse species (Milla et al., 2008; Poorter et al., 2009; Karavin, 2013). Leaf density and thickness are increased by chemical and anatomical modifications, such as an increment of mesophyll layers and accumulation of carbon-rich components (Milla et al., 2008).

4.2. Leaf life-span

Leaf life-span varies from some days to years among different species. Deciduous plants retain their leaves for most of the growing season, but less than one year (Chabot and Hicks, 1982). Periods of leaf fall often coincide with seasons: winter in the case of cool-climate plants or the dry season in the case of tropical plants (Cundall et al., 2003). Evergreens include species that maintain a cohort of leaves for more than one year (Chabot and Hicks, 1982). In general, leaf life-span is longer in plants growing under shaded than sunny environments (Reich et al., 2004). In the same way, shade tolerant species present longer leaf life-span than shade in-tolerant species or pioneers (Williams et al., 1989; Walters and Reich, 1999). *Ligustrum lucidum* is classified as an evergreen species; the production of new leaves and the presence of old leaves can be constant along the year (Gonçalves de Olivera et al., 2016). The present results show that it maintains its leaves for nearly one year and that there are no differences between sun and shade leaves. However, after this time, an abrupt death of leaves was observed, resulting in more than 80% of initial leaves dead before one year. This pattern could be natural or due to weather events such as strong winds and / or dry conditions registered during the study times and / or as a result of the cumulative insect damage. These are the first results about this species' life history, which is relevant due to its relationship with plant resources

availability, plant succession, and leaf structural and functional characteristics (Reich et al., 1991).

4.3. Herbivory damage in relation to environmental conditions and leaf age

The loss of biomass and photosynthetic tissues by herbivore attacks reduces the growth of plants (Crawley, 1989; Zangerl et al., 2002). Such herbivore impacts however, may depend on the ontogenetic stage at which trees are attacked; on resource allocation, which in turn depends on the particular conditions under which plants grow (e.g. open areas vs. forests, tree gaps vs. understory, and nutrient-poor vs. nutrient-rich soils); and also, on the leaf age (Ernest, 1989; Weiner, 2004). The results here obtained show that herbivore damage increases with time (leaf age), and it is higher in sun leaves. This last finding could be associated to the fact that shade leaves are thinner, with less palatable palisade parenchyma tissue, compared to sun leaves, and so, less preferred by herbivores. Usually, young leaves have less defenses and are more palatable than old leaves (Coley, 1980). But in species with long leaf life-span and few herbivore attacks, as *Ligustrum*, the life-span could increase the time of exposure to the action of herbivores and the damage produced by them could be accumulated. Therefore, old leaves would show more herbivory damage, as observed in our results. This accumulation of damage may occur in a non-linear form, and it is probable that great damage can occur during a small time window during leaf expansion. On the other hand, it is possible that by the time that young leaves are exposed for herbivores, these are not abundant or they are not in their growing season, and so there is no correspondence between herbivore development and leaf phenology. In its native range, *L. lucidum* has a wide variety of specialist and generalist invertebrates listed as natural enemies. Most of them are also present in their novel range (Montti et al., 2016). However, there is no data about new herbivories present

in the invaded range. Future studies including the description of the presence of herbivores in novel areas, the relation between their growth and leaf phenology, and / or the development of experimental assays, might shed light on the plant silica–herbivores relation in this species.

4.4. Silica content in relation to environmental conditions and leaf age

As it was previously mentioned, most of the studies about the silicification process involved grass species, although it has been suggested that in non-grasses this phenomenon is an ecologically important trait (Katz, 2014, 2018). The results in this work contribute to the comprehension of this process in eudicots, especially in a tree species; however, many other studies are needed in order to understand the underlying process involved in silica accumulation (Katz, 2014). The values of silica concentration of *Ligustrum lucidum* leaves are within values commonly described for plant species (0.1–10%) (Epstein, 1994; Hodson et al., 2005). Also, the mean obtained in this work is similar to the average silica accumulation measured in woody species (1.42%) from other regions (Mercader et al., 2009). Many studies, carried out in grasses, revealed that silica concentration is higher in mature organs and / or older leaves, since amorphous silica deposition is accumulative with age (Motomura et al., 2002; de Melo et al., 2010; Fernández Honaine and Osterrieth, 2012). In the present study this pattern is corroborated, and it is shown for the first time in an eudicotyledon arboreal species that silica accumulation is higher in older leaves than in younger ones, independently of environmental conditions (study site or light exposure).

When silica concentration was analyzed in relation to native and novel ranges of distribution of this species, it was observed that the accumulation is higher in sites from Argentina. As it was explained in the introduction, environmental conditions such as soil Si content could account for differences in the Si available for root absorption, and so

differences in Si accumulation in plants between sites (Ma and Takahashi, 2002). Also, higher water availability or higher evapotranspiration rate, due to higher temperatures, could explain the differences in silica accumulation. For instance, Tucumán site (A1) has higher mean annual temperature and greater precipitation than the Chinese site (see materials and methods section), and this could stimulate Si absorption and accumulation in tissues (Quigley and Anderson, 2014; Issaharou-Matchi et al., 2016). On the other hand, soil Si content could be an important factor involved in plant silica accumulation, but unfortunately, scarce data about Si values are available. Wang et al. (2001) and references therein, mentioned that Si deficiency is common in paddy soils and some upland soils from China. In Argentina, there is information about soil Si content for site A2 only (mean = 933 $\mu\text{mol/l}$), which is considered a high value (Sommer et al., 2006; Borrelli et al., 2010). In general, and according to Sommer et al. (2006), values of soil Si content range between 0.4 and 2000 $\mu\text{mol/l}$, but most of the soils range between 100 and 500 $\mu\text{mol/l}$. Future studies involving detailed soil Si content measurements will allow to understand the relation between soil Si content and plant silica content in this species. Shade leaves have higher silica concentration (measured as percentage of dry weight) than sun leaves, although the differences were not significant. Considering that silica accumulation is mainly the consequence of water loss of a tissue (i.e. depends on transpiration or evaporation), it was hypothesized that sun leaves would have higher silica concentration than shade leaves. The results here obtained do not support this idea. Instead, the differences can be due to differences in leaf mass between sun and shade leaves, and not in their absolute silica content. As it was shown in the results, shade leaves have higher SLA and are thinner than sun leaves. Since silica concentration is referred as a percentage of silica in relation to total dry weight of the leaf, it can be supposed that due to differences in leaf density the silica concentration evaluated as % dry weight became different between leaves. This hypothesis was tested and the silica content

per leaf (both sun and shade leaves) was calculated. The results showed that silica content per leaf was similar between both types of leaves: 3.73 mg Si/sun leaf and 3.48 mg Si/shade leaf considering all the samples analyzed. The comparison of silicophytolith content per leaf area, relating silicophytolith content and the SLA values, showed similar values between leaf types (0.19 mg Si/cm² leaf in sun, 0.15 mg Si/cm² leaf in shade). Previous studies in relation to the accumulation of silica along time in plants are mainly referred to grasses, and all of them have shown that silicification is increased with the age of the organ (Sangster et al., 2001; Motomura et al., 2002; de Melo et al., 2010; Fernández Honaine and Osterrieth, 2012). However, most of these studies analyzed leaves of different ages collected altogether at a specific time. In this work, where the silica content was evaluated in a long-term study, it is shown in an eudicotyledon species that accumulation of silica also increases along leaf life. Moreover, for the first time a quantification of this increment is presented: leaves of *Ligustrum lucidum* increased their silica concentration in approximately five times in ten months, as it was shown in the results section.

4.5. Relation between Si concentration, SLA and herbivory damage

Cooke and Leishman (2012) analyzed the relation between Si content and SLA in 25 plant species and found a weak positive relation between them. Besides the weak relation, this finding is in concordance with previous work which found a significant negative relation between silica concentration and leaf longevity (Cooke and Leishman, 2011a). They suggested that short-lived leaves, which are usually associated to higher SLA, accumulate more silica than long-lived leaves, indicating that silica could be important in the leaf dry mass economics (Cooke and Leishman, 2011a,b; 2012). In the present study, where the intraspecific variation of SLA was analyzed, the results showed that Si concentration is

weakly but negatively correlated to SLA ($r = -0.28$). This weak but negative relation means that thinner leaves (greater SLA) have lower Si concentration (measured as % DW). This finding is in relation to the dynamics of silica accumulation in leaves along the life of the plant. As it was explained before, silica accumulation is increased with leaf age, and so older leaves have higher Si concentration. It was also shown that older leaves are thicker and have lower SLA than younger ones, therefore, a negative relation between SLA and Si concentration is expected within leaves of different age of the same species.

Previous studies in relation to plant Si content and herbivory are mostly referred to grasses and / or crops. In general, both in field and in laboratory studies, the results show that a higher silica concentration in the organs leads to a lower herbivore activity, affecting also the development and growth of the herbivores (Massey and Hartley, 2006; Massey et al., 2006; Reynolds et al., 2012). The field study carried out in the present work showed that, considering all the samples together, leaf Si content is positively correlated with herbivory damage, i.e. leaves with high values of Si concentration have high values of herbivory damage. It was expected that a higher silica content would prevent herbivory damage, but this general result does not show this pattern. However, it was observed that both Si concentration and herbivory damage were accumulative along leaf life, and so both variables increased with the age of the leaves. For this reason, a posterior analysis considering the age of leaves was done, and no significant correlation between Si content and herbivory damage was found. These first results allow some advance into the knowledge about the relation of silica concentration and herbivory in eudicots, and especially in an invasive species, such as *Ligustrum lucidum*. However, more studies are necessary in order to understand if silica accumulation is affecting plant–herbivore interaction in this species, and to elucidate if silicification process is involved in the success of this species to invade new areas.

5. Conclusion

In the present study, first data about some anatomical traits (such as SLA, tissue differentiation, leaf life-span) and herbivory damage in leaves of *Ligustrum lucidum*, in relation to environmental characteristics and leaf age are presented. Moreover, first results about the silica content in *Ligustrum lucidum* are shown, contributing to the knowledge of the silicification process in eudicots, a topic scarcely addressed. Silica accumulation and SLA differs between plants from native and novel areas, probably due to differences in environmental conditions such as air pollution, soil Si content, water availability and temperatures. Values of SLA, silica concentration and herbivory damage are strongly dependent on leaf age. Along the life of the leaves, silica concentration increased approximately five times in ten months: leaves have a lower SLA and a higher silica concentration at the end of the cycle. Also, herbivory damage was increased along time, and as a result, older leaves were more eaten than younger ones, even though they have a higher silica concentration. More studies are needed in order to understand the role of silica in this species, and / or its relation with herbivory. However, the results obtained in this work suggest several areas of productive future research related to herbivory, plant defenses, Si cycle and plant invasions.

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References

- Agarie, S., Agata, W., Uchida, H., Kubota, F., Kaufman, P., 1996. Function of silica bodies in the epidermal system of rice (*Oryza sativa* L.): testing the window hypothesis. *J. Exp. Bot.* 47, 655–660.
- An, S., Wang, Z., Zhu, X., Hong, B., Zhao, R., 1997. Effects of soil factors on species diversity in secondary forest communities. *Journal of Wuhan Botanical Research* 15, 143-150.
- APHA-AWWA-WPFC (American Public Health Association–American Water Works Association–Water Pollution Control Federation), 1998. Standard methods for the examination of water and wastewater. Washington DC.
- Aragón, R., Montti, L., Ayup, M.M., Fernández, R., 2014. Exotic species as modifiers of ecosystem processes: Litter decomposition in native and invaded secondary forests of NW Argentina. *Acta Oecol.* 54, 21-28.
- Aslan, C.E., Rejmánek, M., Klinger, R., 2012. Combining efficient methods to detect spread of woody invaders in urban–rural matrix landscapes: an exploration using two species of Oleaceae. *J. Appl. Ecol.* 49, 331–338.
- Ayup, M.M., Montti, L., Aragón, R., Grau, H.R., 2014. Invasion of *Ligustrum lucidum* (Oleaceae) in the southern Yungas: Changes in habitat properties and decline in bird diversity. *Acta Oecologica* 54, 72-81
- Bianchi, A., Yañez, C., 1992. Las precipitaciones en el Noroeste Argentino. 2nd ed. INTA, EEA Salta. Salta, Argentina.
- Borrelli, N., Alvarez, M.F., Osterrieth, M., Marcovecchio, J., 2010. Silica content in soil solution and its relation with phytolith weathering and silica biogeochemical cycle in typical Argiudolls of the Pampean Plain, Argentina. A preliminary study. *J. Soil Sediments* 10, 983–994.

- Bruno, G., Stiefkens, L., Hadid, M., Liscovsky, I., Cosa, M., Dottori, N., 2007. Efecto de la contaminación ambiental en la anatomía de la hoja de *Ligustrum lucidum* (Oleaceae). Bol. Soc. Argent. Bot. 42, 231 - 236.
- Burgos, J.J., Vidal, A.L., 1951. Los climas de la República Argentina según la nueva clasificación de Thornthwaite. Meteoros 1, 3–32.
- Cabrera, A.L., 1971. Fitogeografía de la República Argentina. Bol. Soc. Argent. Bot. 14, 1-42.
- Casey, W.H., Kinrade, S.D., Knight, C.T.G., Rains, D.W., Epstein, E., 2003. Aqueous silicate complexes in wheat *Triticum aestivum* L. Plant Cell Environ. 27, 51–54.
- Chabot, B.F., Hicks, D.J., 1982. The ecology of leaf life spans. Annu. Rev. Ecol. Evol. Syst. 13, 229-259.
- Chang, M.C., Qiu, L.Q., Green, P.S., 1996. Oleaceae. Flora of China 15, 272–319.
- Coley, P.D., 1980. Effects of leaf age and plant life history patterns on herbivory. Nature 284, 545–546.
- Cooke, J., Leishman, M.R., 2011a. Silicon concentration and leaf longevity: is silicon a player in the leaf dry mass spectrum? Funct. Ecol. 25, 1181–1188.
- Cooke, J., Leishman, M.R., 2011b. Is plant ecology more siliceous than we realise? Trends Plant Sci. 16, 2.
- Cooke, J., Leishman, M.R., 2012. Tradeoffs between foliar silicon and carbon based defences: evidence from vegetation communities of contrasting soil types. Oikos 121, 2052–2060.
- Cordell, S., Goldstein, G., Meinzer, F.C., Vitousek, P.M., 2001. Regulation of leaf life-span and nutrient use efficiency of *Metrosideros polymorpha* trees at two extremes of a long chronosequence in Hawaii. Oecologia 127, 198-206.
- Crawley, M.J., 1989. Insect herbivores and plant population dynamics. Annu. Rev. Entomol. 34, 531–564.

- Cundall, P., Australian Broadcasting Corporation, 2003. Gardening Australia: flora: the gardener's bible over 20,000 plants. Sydney: ABC Books for the Australian Broadcasting Corporation.
- D'Ambrogio de Argüeso, A., 1986. Manual de técnicas en histología vegetal. Editorial Hemisferio Sur SA., Buenos Aires, Argentina.
- de Melo, S.P., Monteiro, F.A., De Bona, F.D., 2010. Silicon distribution and accumulation in shoot tissue of the tropical forage grass *Brachiaria brizantha*. Plant Soil 336, 241–249.
- De Rito, M., Fernández Honaine, M., Montti, L., Aragón, R., Osterrieth, M., 2016. Biomineralizaciones de sílice amorfo en *Ligustrum* spp y su potencial rol como defensa contra la herbivoría. Abstracts VI Reunión Binacional de Ecología, XXVII Reunión Argentina de Ecología, ASAE, Argentina, pp. 188.
- De Rito, M., Fernández Honaine, M., Osterrieth, M., Morel, E., 2018. Silicophytoliths from a Pampean native tree community (*Celtis ehrenbergiana* community) and their representation in the soil assemblage. Rev. Palaeobot. Palynol. 257, 19-34.
- Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., González, L., Tablada, M., Robledo, C.W., 2009. InfoStat versión 2009. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.
- Dong, L.N., Xu, H.B., Feng, J.U., Liu, S.W., Wan, Z.Z., 2011. Plant diversity and its conservation strategies in Zijin Mountain National Forest Park in Nanjing. Journal of Jiangsu Forestry Science and Technology 2011, 01.
- Dong, L.N., Xu, H.B., Liu, S.W., Song, H.L., Chen, X., Ju, F., Xei, Q., Cheng, Y., Wan, Z.Z., 2010. Investigation and analysis on seed plant flora at Zijinshan National Forest Park in Nanjing. Journal of Zhejiang Forestry Science and Technology 30, 41-47.
- Easdale, T.A., Healey, J.R., Grau, H.R., Malizia, A., 2007. Tree life histories in a montane subtropical forest: species differ independently by shade tolerance, turnover rate and substrate preference. J. Ecol. 95, 1234–1249.

- Editorial Committee of Flora of China, 1992. Chinese Academy of Sciences. Flora of China Science Press, Beijing.
- Elton, C., 1958. The Ecology of Invasions by Animals and Plants. Methuen, London.
- Epstein, E., 1994. The anomaly of silicon in plant biology. PNAS 91, 11–17.
- Ernest, K.A., 1989. Insect herbivory on a tropical understory tree: effects of leaf age and habitat. Biotropica 21, 194–199.
- Faisal, S., Callis, K., Slot, M., Kitajima, K., 2012. Transpiration-dependent passive silica accumulation in cucumber (*Cucumis sativus*) under varying soil silicon availability. Botany 90, 1058–1064.
- Fernández Honaine, M., Osterrieth, M., 2012. Silicification of the adaxial epidermis of leaves of a panicoid grass in relation to leaf position and section and environmental conditions. Plant Biol. 14, 596–604.
- Fernández Honaine, M., Borrelli, N., Osterrieth, M., del Río, L., 2013. Amorphous silica biomineralizations in sedges: their relation with senescence and silica availability. Bol. Soc. Argent. Bot. 48, 247–259.
- Fernández Honaine, M., Zucol, A., Osterrieth, M., 2005. Biomineralizaciones de sílice en *Celtis tala* Planchon (Celtidaceae). Bol. Soc. Argent. Bot. 40, 229–239.
- Fernandez Turiel, J.L., Perez Torrado, F.J., Rodriguez Gonzalez, A., Saavedra, J., Carracedo, J.C., Rejas, M., Lobo, A., Osterrieth, M., Carrizo, J.I., Esteban, G., Gallardo, J., Ratto, N., 2019. The large eruption 4.2 ka cal BP in Cerro Blanco, Central Volcanic Zone, Andes: Insights to the Holocene eruptive deposits in the southern Puna and adjacent regions. Estudios Geológicos, 75, e088.
<https://doi.org/10.3989/egeol.43438.515>
- Ferreras, A.E., Whitworth-Hulse, J.I., Tecco, P.A., Marcora, P.I., Funes, G., 2019. Environmental constraints to native woody species recruitment in invaded mountain woodlands of central Argentina. For. Ecol. Manag. 440, 189–201.

- García, M.C., Veneziano, M.F., 2014. Comportamiento temporal y tendencias climáticas en la ciudad de Mar del Plata (período 1971–2010). Actas Congreso Internacional de Geografía. 75° Semana de Geografía, 77-93.
- Gonçalves de Oliveira, T.W., Freitas Milani, J.E., Blum C.T., 2016. Phenological behavior of the invasive species *Ligustrum lucidum* in an urban forest fragment in Curitiba, Parana state, Brazil. Foresta 46, 371 - 378.
- Gutschick, V.P., 1999. Biotic and abiotic consequences of differences in leaf structure. New Phytol. 143, 3-18.
- Handreck, K.A., Jones, L.H.P., 1968. Studies of silica in the oat plant. IV. Silica content of plant parts in relation to stage of growth, supply of silica, and transpiration. Plant Soil 29, 449–459.
- Hartley, S.E., Fitt, R.N., McLarnon, E.L., Wade, R.N., 2015. Defending the leaf surface: intra- and interspecific differences in silicon deposition in grasses in response to damage and silicon supply. Front. Plant Sci. 6, 35.
- Hodson, M.J., 2016. The development of phytoliths in plants and its influence on their chemistry and isotopic composition. Implications for palaeoecology and archaeology. J. Archaeol. Sci. 68, 62–69.
- Hodson, M.J., White, P.J., Mead, A., Broadley, M.R., 2005. Phylogenetic variation in the silicon composition of plants. Ann. Bot. 96, 1027–1046.
- Hoyos, L.E., Gavier-Pizarro, G.I., Kuemmerle, T., Bucher, E.H., Radeloff, V.C., Tecco, P.A., 2010. Invasion of glossy privet (*Ligustrum lucidum*) and native forest loss in the Sierras Chicas of Córdoba, Argentina. Biol. Invasions 12, 3261–3275.
- Hu, X., Zhang, Y., Ding, Z., Wang, T., Lian, H., Sun, Y., Wu, J., 2012. Bioaccessibility and health risk of arsenic and heavy metals (Cd, Co, Cr, Cu, Ni, Pb, Zn and Mn) in TSP and PM_{2.5} in Nanjing, China. Atmos. Environ. 57, 146-152.

- Hunzinger, H., 1997. Hydrology of montane forest in the Sierra de San Javier, Tucumán, Argentina. *Mountain Research and Development* 17, 299-308.
- INTA, 1989. Mapa de suelos de la Provincia de Buenos Aires, E 1:500000. Instituto Nacional de Tecnología Agropecuaria (INTA), Argentina.
- Issaharou-Matchi, I., Barboni, D., Meunier, J.D., Saadou, M., Dussouillez, P., Contoux, C., Zirihi-Guede, N., 2016. Intraspecific biogenic silica variations in the grass species *Pennisetum pedicellatum* along an evapotranspiration gradient in South Niger. *Flora* 220, 84 – 93.
- Jones, L.H.P., Handreck, K.A., 1967. Silica in soils, plants, and animals. *Adv. Agron.* 19, 107–149.
- Karavin, N., 2013. Effects of leaf and plant age on specific leaf area in deciduous tree species *Quercus cerris* L. var. *cerris*. *Bangladesh J. Bot.* 42, 301-306.
- Katz, O., 2014. Beyond grasses: the potential benefits of studying silicon accumulation in non-grass species. *Front. Plant Sci.* 5, 376.
- Katz, O., 2018. Silicon content is a plant functional trait: implications in a changing world. *Flora* 254, 88–94.
- Keane, R.M., Crawley, M.J., 2002. Exotic plant invasions and the enemy release hypothesis. *Trends Ecol. Evol.* 17, 164–170.
- Klancnik, K., Vogel-Mikuš, K., Gaberšcik, A., 2014. Silicified structures affect leaf optical properties in grasses and sedge. *J. Photochem. Photobiol. B* 130, 1–10.
- Labouriau, L.G., 1983. Phytolith work in Brazil: a minireview. *The Phytolitharien Newsletter* 2, 6–10.
- Larcher, L., Nogueira, G., Boeger, M.R., 2015. Morphological plasticity and gas exchange of *Ligustrum lucidum* W.T. Aiton in distinct light conditions. *Braz. Arch. Biol. Technol.* 58, 877-885.

- Lux, A., Luxova, M., Hattori, T., Inanaga, S., Sugimoto, Y., 2002. Silicification in sorghum (*Sorghum bicolor*) cultivars with different drought tolerance. *Physiol. Plant.* 115, 87–92.
- Ma, J.F., 2004. Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Sci. Plant Nutr.* 50, 11–18.
- Ma, J.F., Takahashi, E., 2002. *Soil, Fertilizer, and Plant Silicon Research in Japan*. Elsevier, Amsterdam.
- Maddox, V., Byrd, J., Serviss, B., 2010. Identification and control of invasive privets (*Ligustrum* spp.) in the middle southern United States. *Invasive Plant Sci. Manag.* 3, 482–488.
- Massey, F.P., Hartley, S.E., 2006. Experimental demonstration of the antiherbivore effects of silica in grasses: impacts on foliage digestively and vole growth rates. *Proc. Biol. Sci.* 273, 2299–2304.
- Massey, F.P., Ennos, A.R., Hartley, S.E., 2006. Silica in grasses as a defence against insect herbivores: contrasting effects on folivores and a phloem feeder. *J. Anim. Ecol.* 75, 595–603.
- Massey, F.P., Ennos, A.R., Hartley, S.E., 2007. Herbivore specific induction of silica-based plant defences. *Oecologia* 152, 677–683.
- Mercader, J., Bennett, T., Esselmont, C., Simpson, S., Walde, D., 2009. Phytoliths in woody plants from the Miombo woodlands of Mozambique. *Ann. Bot.* 104, 91–113.
- Meunier, J.D., Keller, C., Guntzer, F., Riotte, J., Braun, J.J., Anupama, K., 2014. Assessment of the 1% Na₂CO₃ technique to quantify the phytolith pool. *Geoderma* 216, 30–35.
- Milla, R., Reich, P.B., Niinemets, Ü., Castro-Diez, P., 2008. Environmental and developmental controls on specific leaf area are little modified by leaf allometry. *Funct. Ecol.* 22, 565–576.

- Montti, L., Piriz Carrillo, J., Gutiérrez-Angonese, J., Gasparri, N.I., Aragón, R., Grau, H.R., 2017. The interactions among bioclimatic features, landscape configuration and historical land use in controlling the invasion spread of an Asian tree in subtropical Argentina. *Landsc. Ecol.* 32, 2167-2185.
- Montti, L., Ayup, M.M., Aragón, R., Qi, W., Ruan, H., Fernández, R., Casertano, S., Zou, X., 2016. Herbivory and the success of *Ligustrum lucidum*: evidence from a comparison between native and novel ranges. *Austral. J. Bot.* 64, 181-192.
- Motomura, H., Mita, N., Suzuki, M., 2002. Silica accumulation in long-lived leaves of *Sasa veitchii* (Carriere) Rehder (Poaceae: Bambusoideae). *Ann. Bot.* 90, 149–152.
- Niinemets, Ü., 2010. A review of light interception in plant stands from leaf to canopy in different plant functional types and in species with varying shade tolerance. *Ecol. Res.* 25, 693-714.
- Piperno, D., 2006. *Phytoliths. A Comprehensive Guide for Archaeologist and Paleoecologist*. Altamira Press, USA.
- Poorter, H., Niinemets, Ü., Poorter, I., Wright, I.J., Villar, R., 2009. Causes and consequences of variation in leaf mass per area LMA a meta-analysis. *New Phytol.* 182, 565-588.
- Prychid, C.J., Rudall, P.J., Gregory, M., 2003. Systematics and biology of silica bodies in monocotyledons. *Bot. Rev.* 69, 377–440.
- Puchulu, M.E., Fernández, D.S., 2014. Características y distribución espacial de los suelos de la provincia de Tucumán. In: Moyano, S., Puchulu, M.E., Fernández, D., Aceñolaza, G., Vides, M.E., Nieva, S. (Eds.), *Geología de Tucumán*, Colegio de Graduados en Ciencias Geológicas de Tucumán, Tucumán, Argentina, pp. 240-256.
- Quigley, K.M., Anderson, T.M., 2014. Leaf silica concentration in Serengeti grasses increases with watering but not clipping: insights from a common garden study and literature review. *Front. Plant Sci.* 5, 568.

- Raven, J.A., 1983. The transport and function of silicon in plants. *Biol. Rev. Camb. Philos. Soc.* 58, 179–207.
- Reich, P.B., Uhl, C., Walters, M.B., Ellsworth, D.S., 1991. Leaf lifespan as a determinant of leaf structure and function among 23 Amazonian tree species. *Oecologia* 86, 16–24.
- Reich, P.B., Uhl, C., Walters, M.B., Prugh, L., Ellsworth, D.S., 2004. Leaf demography and phenology in Amazonian rain forest: a census of 40000 leaves of 23 tree species. *Ecol. Monog.* 74, 3–23.
- Reynolds, J., Lambin, X., Massey, F.P., Reidinger, S., Sherratt, J.A., Smith, M.J., White, A., Hartley, S.E., 2012. Delayed induced silica defences in grasses and their potential for destabilizing herbivore population dynamics. *Oecologia* 170, 445–456.
- Sangster, A.G., Hodson, M.J., Tubb, H.J., 2001. Silicon deposition in higher plants. In: Datnoff, L.E., Snyder, G.H., Korndörfer, G.H. (Eds.), *Silicon in Agriculture*. Elsevier, Amsterdam, pp. 85–113.
- Schoelynck, J., Bal, K., Back, H., Okruszko, T., Meire, P., Struyf, E., 2010. Silica uptake in aquatic and wetland macrophytes: a strategic choice between silica, lignin and cellulose? *New Phytol.* 186, 385–391.
- Soininen, E.M., Brathen, K.A., Herranz Jurdado, J.G., Reidinger, S., Hartley, S.E., 2013. More than herbivory: levels of silica-based defences in grasses vary with plant species, genotype and location. *Oikos* 122, 30–41.
- Sommer, M., Kaczorek, D., Kuzyakov, Y., Breuer, J., 2006. Silicon pools and fluxes in soils and landscapes a review. *J. Plant Nutr. Soil Sci.* 169, 310–329.
- Swarbrick, J.T., Timmins, S.M., Bullen, K.M., 1999. The Biology of Australian Weeds. 36. *Ligustrum lucidum* Aiton and *Ligustrum sinense* Lour L. *Plant Protection Quarterly* 14, 122–130.

- Takenaka, A., Takahashi, K., Kohyama, T., 2001. Optimal leaf display and biomass partitioning for efficient light capture in an understory palm, *Licuala arbuscula*. *Funct. Ecol.* 15, 660-668.
- Vendramini, F., Diaz, S., Gurvich, D.E., Wilson, P.J., Thompson, K., Hodgson, J.G., 2002. Leaf traits as indicators of resource-use strategy in floras with succulent species. *New Phytol.* 154, 147-157.
- Walters, M.B., Reich, P.B., 1999. Low-light carbon balance and shade tolerance in the seedlings of woody plants: do winter deciduous and broad-leaved evergreen species differ? *New Phytol.* 143, 143-154.
- Wang, H., Li, C., Liang, Y., 2001. Agricultural utilization of silicon in China. In: Datnoff, L.E., Snyder, G.H., Korndörfer, G.H. (Eds.), *Silicon in Agriculture*. Elsevier, Amsterdam, pp. 343-358.
- Weiner, J., 2004. Allocation, plasticity and allometry in plants. *Perspect. Plant Ecol. Syst.* 6, 207-215.
- Westoby, M., Warton, D., Reich, P.B., 2000. The time value of leaf area. *Amer. Naturalist* 155, 649–656.
- Whitworth Hulse, J.I., 2018. Efectos de la invasión de *Ligustrum lucidum* sobre la dinámica hídrica en bosques nativos del Chaco Serrano: la interacción entre precipitación, vegetación y suelo. PhD diss. Universidad Nacional de Córdoba, Córdoba, Argentina.
- Williams, K., Field, C.B., Mooney, H.A., 1989. Relationships among leaf construction cost, leaf longevity, and light environments in rain-forest plants of the genus *Piper*. *Amer. Naturalist* 133, 199–211.
- Wright, I.J., Cannon, K., 2001. Relationships between leaf lifespan and structural defences in a low-nutrient, sclerophyll flora. *Funct. Ecol.* 15, 351–359.
- Wright, I.J., Reich, P.B., Westoby, M., et al., 2004. The worldwide leaf economics spectrum. *Nature* 428, 821–827.

Zamora Nazca, L.B., Montti, L., Grau, H.R., Paolini, L., 2014. Efectos de la invasión del ligustro, *Ligustrum lucidum*, en la dinámica hídrica de las Yungas del noroeste Argentino. Bosque 35, 195-205.

Zangerl, A.R., Hamilton, J.G., Miller, T.J., Crofts, A.R., Oxborough, K., Berenbaum, M.R., de Lucia, E.H., 2002. Impact of folivory on photosynthesis is greater than the sum of its holes. PNAS 99, 1088-1091.

Zar, J.H., 1984. Biostatistical Analysis. Prentice-Hall, Englewood Cliffs, NJ, USA.

Figure captions

Fig. 1. A. Location of study sites. B,C,D. *Ligustrum* forests in the three sites: site A1 in Argentina, site A2 in Argentina and China (Ch) respectively. E. Leaf types according to age. F. Transverse section of old leaves under different light conditions.

Fig. 2. Specific leaf area, herbivory damage and silica content as dependent on study site (A1, A2: Argentina; Ch: China), light exposure (sun and shade leaves), and leaf age (young, medium-aged and old leaves). Different letters indicate significant differences at $p < 0.01$. Numbers 1, 2, 3, indicate that the differences were only found in A1 site (1), A2 site (2), or Ch site (3).

Fig. 3. Cumulative mortality of sun (open circles) and shade (full circles) leaves marked at initiation as a function of time since leaf initiation. Percent mortality is the percentage of originally tagged leaves that died in each light exposure. Each point represents a combined mean of 5 trees (no significant differences were found between trees). Sigmoid regressions were used to calculate average leaf life-span (50% cumulative mortality). All relationships were significant ($P < 0.01$); r^2 values: sun (dashed line): 0.88, shade (full line): 0.88.

Fig. 4. Relation between: A. SLA and silica concentration (including all the samples). B. Herbivory damage and silica concentration (including all the samples). C. Herbivory damage and silica concentration (only old leaf samples). Spearman correlation values (r , p) are also shown in the three cases.