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Biochemical composition and germination capacity of *Ligustrum lucidum* ait. seeds in the process of biological invasion

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ABSTRACT. *Ligustrum lucidum* ait. is an exotic species and In the present study, contents of sugars, proteins, and germination were evaluated in seeds removed from not abscisic and abscisic fruits. The seeds were stored or not under shadow or sun. The higher contents of total and reducing sugars were observed in seeds removed from not abscisic fruit while the period of storage resulted in the loss of these compounds. The same result was observed in the content of total sugars of seeds from abscisic fruits. The content of reducing sugars in seeds from abscisic fruits and submitted to storage had its content increased. The contents of total proteins in seeds from abscisic and not abscisic submitted to shadow or sun were not significantly different, but the results suggested an increase in protein synthesis during the storage period, differently from those seeds that were not submitted to storage. When seeds from abscisic and not abscisic fruits were evaluated immediately after harvest, they presented greater percentages of germination, while after 28 days, seeds submitted to the storage treatments showed lower values. The invasive potential of *L. lucidum* seems to be more associated to the number of seeds produced, than to their germination potential.

Keywords: Oleaceae, sugar, protein, exotic species.

Composição bioquímica e capacidade de germinação de sementes de *Ligustrum lucidum* ait. no processo de invasão biológica

RESUMO. *Ligustrum lucidum* ait. é uma espécie exótica e no presente trabalho foram avaliados os teores de açúcares totais, redutores, proteínas, e germinação de sementes retiradas de frutos coletados da planta-mãe (não abscisados) e dispersos (abscisados) mantidos ou não em condições de sombra ou sol. Os maiores teores de açúcares totais e redutores foram obtidos em sementes imaturas, enquanto o armazenamento levou à perda destes composto, o que foi observado também para açúcares totais, nas mesmas condições, para sementes maduras. Verificaram-se aumentos nos teores de açúcares redutores em sementes maduras armazenadas. Apesar de não serem estatisticamente significativos, estes resultados entre sementes maduras e imaturas submetidas ao mesmo tratamento, indicam possível aumento da síntese protéica durante o período de armazenamento, sugerido pelo acréscimo no teor de proteínas totais, com pequena perda e/ou inibição em sementes de frutos submetidos ao sol. Sementes maduras e imaturas, imediatamente testadas após a coleta, apresentaram os maiores valores de porcentagem de germinação. no final de 28 dias de avaliação, enquanto aquelas submetidas aos tratamentos de armazenamento ficaram muito abaixo destes valores. Pelo presente trabalho, o potencial invasor de *L. lucidum* parece estar relacionado mais ao número de sementes produzidas pela espécie que o seu potencial germinativo.

Palavras-chave: Oleaceae, açúcares, proteínas, espécie exótica.

Introduction

Invasive plant species can disturb and cause several damages to different ecosystems, affecting them economically, environmentally and ecologically. These species compete with native plants and change drastically natural landscapes (DAVIS et al., 2001; PACHARD et al., 2004; ANDRADE et al., 2010). Nowadays, invasive plants have been considered the second most important threat to biodiversity, being just behind of the destruction by human action (ZILLER, 2001).

Exotic species are introduced by man for different purposes as food, windbreak, pasture, reforestation and as ornamental purposes. According to Ziller (2001), half of 491 exotic plants species found in South Africa were introduced as ornamentals.

Exotic invasive plants are organisms that interfere on growth and development of native species without direct human action. They can cause great environmental, social and/or economical changes (PYŠEK et al., 2012). Besides that, exotic invasive species have an enormous propagation and a great development potential because they are antagonists, predators and parasite-free (ZILLER, 2001). This characteristic can result in suppression of native species and appearance of hybrids that can be more invasive than their parents (DELARIVA; AGOSTINHO, 1999; ZILLER, 2001).

One of these species is *Ligustrum lucidum* Ait. (OJASTI et al., 2001; STARR et al., 2003; GISP, 2005), that increases its invasive potential due to the high number of fruit and seeds, efficient zoochoric dispersion and successful vegetative reproduction. Seed dispersal by birds is one of the most important ways to propagate this species. This dispersal method can reach great distances, increasing the invasive power of the species. According to Pyšek et al. (2012) despite being a world problem, exotic invasive species have peculiarities in each country or locality and specific strategies are required to control them.

The production of great number of seeds is an essential trait for an invasive plant, and it is important to know the seed chemical composition specially storage compounds, which were, probably, one of the critical points during seeds evolution. This fact made possible the development of the seedlings far from parents, thus the offspring will need to survive from its own source of nutrients (BUCKERIDGE et al., 2004).

Food storage inside seeds has the function of maintenance the metabolic process and tissue construction during the seedling growth. Storage of carbohydrates is used as energy and carbon sources for the embryo development, as well as, the lipids and proteins are kind of sources for some plant species (BUCKERIDGE et al., 2004).

Considering the high invasive potential of *L. lucidum* and the possible relationship with storage substances in seeds, this work aimed to study the germination of seeds maintained under different environmental conditions and the relationship associating the germination process with total sugar, reduced sugar and protein content in seeds.

Material and methods

The fruits of *Ligustrum lucidum* ait. were gathered from six adult plants located at Universidade Tecnológica Federal do Paraná (UTFPR), Pato Branco Municipality, Paraná State, Brazil, and they were also collected from the ground, in August 2009. Pato Branco is located on South West and third plateau area of Paraná State. It belongs to a climate region classified as Cfa - humid subtropical. During the development of this research, the temperatures ranged from 15 to 16°C, and the precipitation between 125 and 150 mm (IAPAR, 2009).

The fruits collected from plants (not abscisic fruits) or the fruits found under the canopy's projection ray (abscisic fruits) were placed inside plastic trays and exposed to the sun or to the shadow (50% shade sail), at UTFPR - Pato Branco, during 20 days. The treatments evaluated were: seeds from not abscisic fruits exposed to the sun or to the shadow during twenty days or not (without storage), and seeds from abscisic fruits exposed to same conditions.

The seeds were removed from fruits, washed in water and then disinfected with ethanol at 70% during three min and sodium hypochlorite (0.2% of active sodium) during 15 minutes. Residues of chemical reagents were removed with sterilized water. After this process 50 seeds per treatment (five repetitions) were placed on a wet germination paper in Petri plates. They were maintained in darkness at a temperature of 25°C. The germination evaluations were carried out at the 7, 14, 21 and 28 days, when germinated seeds presented visible cotyledons.

Biochemistry analyses were performed at Laboratory of Biochemistry and Physiology of Plants (UTFPR). Total sugars were evaluated by phenol-sulphuric method (DUBOIS et al., 1956) and dinitrosalicylic acid reagent was used for determination of reducing sugars (MILLER, 1959), both with glucose as standard. Total proteins were quantified by Bradford method (BRADFORD, 1976) with bovine serum albumin as standard.

Data were submitted to analysis of variance and the averages were compared by Tukey's test ($p < 0.05$) using the Assistat statistical software.

Results and discussion

The orthodox process of seeds development has four stages: the cellular division and expansion, food and energy storage and dehydration (MARCOS FILHO, 2005). The fourth stage is the maturation, when soluble carbohydrates are important components that are involved in the dehydration tolerance (OBENDORF, 1997).

Higher concentration of total sugars (21.34 mg g^{-1} of seeds) and reducing sugars (54.53 mg g^{-1} of seeds) were observed in seeds removed from not abscisic fruits. This indicates that seeds still had food and energy storage and they were not at physiological mature stage (Figures 1 and 2). When these seeds were exposed to dehydration process under sunlight or shadow, both total and reducing sugars had decreased their contents (Figures 1 and 2). Content of total sugars was 11.97 and 15.41 mg g^{-1} of seeds submitted to sunlight or shadow respectively. Reducing sugars also had their content decreased by treatments, but without statistical difference (35.75 and 36.64 mg g^{-1} of seeds, respectively).

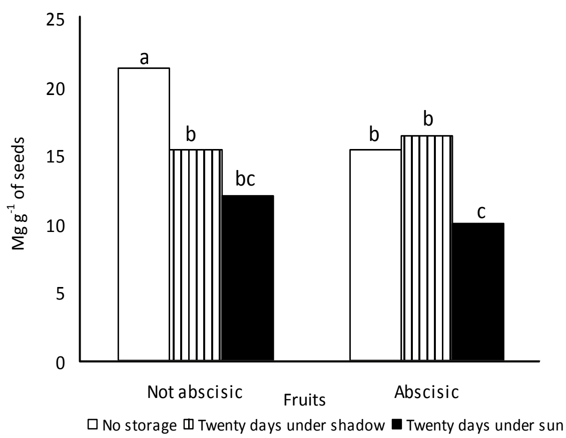

Figure 1. Total content of sugars in *L. lucidum* seeds from not abscisic or abscisic fruits under different conditions. Same letters indicate no statistical difference between treatments.

In spite of, there was no statistical difference in the sugar content in seeds maintained under the sun or shadow; it is possible that light and temperature contributed with the seeds to keep the higher sugar content and this numerical difference could be important for the maintenance of these seeds structures under stress conditions.

When abscisic fruits were exposed to the sun treatment, seeds presented the smaller content of total sugar, compared to the other treatments (about 9.97 mg g^{-1} of seeds) (Figure 1) showing the mobilization of these compounds due to the maintenance of essential processes under stress conditions. Meanwhile the concentrations of reducing sugars in seeds increased when fruit were exposed to the sun or shadow (33.89 and 47.34 mg g^{-1} of seeds, respectively), when compared to seeds from abscisic fruits without storage stage (22.90 mg g^{-1} of seeds) (Figure 2). The rise of reducing sugar in seeds proceeding from abscisic fruits could be related to consumption of storage during 20 days (NKANG, 2002).

This behavior also may explain the higher content of total proteins in these two treatments (under the sun or shadow) for both seeds from abscisic and not abscisic fruits (Figure 3), in which the synthesis of hydrolytic enzymes such as invertases and amylases and other important proteins must be involved in the maintenance process of embryo (viability), as well as, in induction of germination. Lower content of proteins were found in seeds from abscisic fruits (4.00 mg g^{-1} of seeds) but without significant difference from those of not abscisic fruits (4.58 mg g^{-1} of seeds) both without storage (Figure 3).

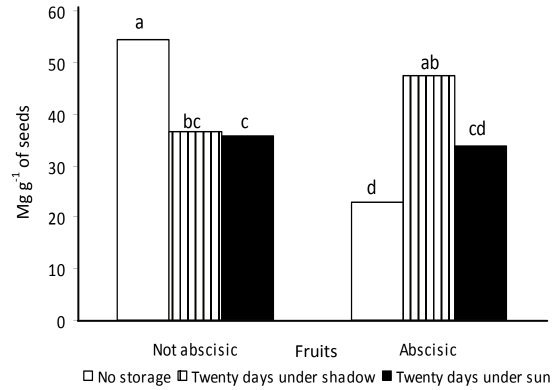

Figure 2. Content of reducing sugars in *L. lucidum* seeds from not abscisic or abscisic fruits exposed to different conditions. Same letters indicate no statistical difference between treatments.

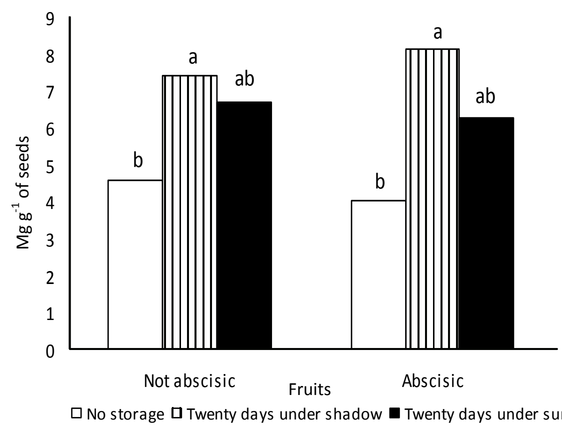

Figure 3. Content of proteins in *L. lucidum* seeds from not abscisic or abscisic fruits under different conditions. Same letters indicate no statistical difference between treatments.

According to Binotti et al. (2008) the deterioration rate of seeds is considerably increased by the exposure to different levels of temperature and relative humidity of air. Furthermore, Delouche (2002, apud BINOTTI et al., 2008) argued that the duration of deterioration process is mainly determined by the interaction between genetic inheritance, degree of seed humidity and temperature. Consequently, the seeds removed from fruit, that were collected directly of the tree, had a higher content of water and possibly they were more susceptible to deterioration.

Buckeridge et al. (2004) stated that in order to accelerate the germination process, some seeds need to be removed from the fruit before physiological maturation. However, these seeds can die by dehydration. Low rates of germination observed in seeds from not abscisic of *L. lucidum* exposed to environmental conditions during 20 days (Figure 4) can be associated to a low potential of germination due to incomplete maturation and water loss, as the authors mentioned above.

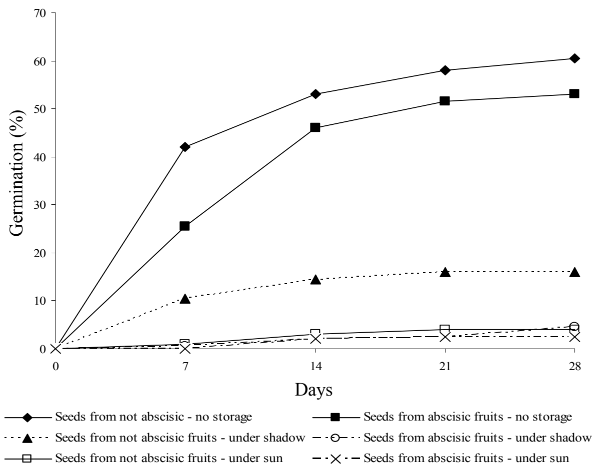

Figure 4. Accumulated germination percentage during evaluation period.

Garcia et al. (2006), examining *Caesalpinia echinata* (Brazilwood), observed that the seeds lost viability when maintained under environmental temperature due to the loss of glucose and fructose compared to sucrose. Binotti et al. (2008) also observed a decrease in the vigor and germination of bean seeds (*Phaseolus vulgaris*) with loss of sugars and other compounds.

Seeds from not abscisic and abscisic fruits that were not stored showed relatively high germination percentage when compared to those treated by 20 days with sunlight or shadow (Figure 4). Low rates of germination were observed in all stored seeds indicating low vigor related to loss and possibly to consumption of reserve material for embryo support during the 20 days. Aragon and Groom (2003) studied germination of *L. lucidum* seed in Argentine forests below closed canopy and opened areas. They observed lower rates of germination for these seeds in the treatment below closed canopy. On the other hand, under second-growth forests and on the border of the forests they had better results for the seeds germination. These results agree with the present study suggesting that *L. lucidum* not have rustic seeds, and are able to germinate under stress conditions (storage under natural conditions) but this process is quite small than that verified for seeds from not abscisic fruits. It is possible that these seeds are immature, as supposed by Buckeridge et al. (2004) but the present study cannot confirm it.

Conclusion

L. lucidum produces a large quantity of fruit that are dispersed meters away from canopy projection, some of them under the sun or shadow. Aiming to understand the process of proliferation of this species, that besides being exotic, also became invasive in Paraná State, the studies pointed to a potential arising mainly from the production of a great number of seeds, rather than maintenance of high germination capacity, since under environmental conditions, the reserve material seems to be depleted before the complete embryo development and seedling establishment (growing).

Therefore, seeds that are released at short stage after fruit abscission seem to have more chances to produce a new plant than those that will remain for a long time onto the soil. This invasive potential of *L. lucidum* seems to be more associated to the great number of seeds produced by the species, than to their germination potential (rusticity).

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