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Germination and seedling structure of *Ruprechtia laxiflora* Meissn. (Polygonaceae)

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ABSTRACT. Germination of diaspores and the seedling structure of *Ruprechtia laxiflora* Meissn. (Polygonaceae) which is a secondary species frequent in forest remnants are described. Seedlings were embedded in hydroxymethacrylate and sectioned in a rotary microtome according to usual techniques in plant anatomy. For bioassays of germination, the experimental design was completely randomized in a 3x2 bifactorial arrangement, corresponding to three temperatures (20, 25 and 30°C) and two light conditions (photoperiod and continuous darkness) for the diaspores newly harvested and in a 4x2 bifactorial arrangement, corresponding to four temperatures (15, 20, 25 and 30°C) and two light conditions (photoperiod and continuous darkness) for diaspores analyzed after storage. There was no interaction between the factors light condition and temperature for germination parameters analyzed. However, the temperature affected the germination and diaspores maintained at 25°C showed a shorter mean time and higher germination speed index. Seedlings are phanerocotylar and epigeal with tetrarch root, intermediate-low type of root-stem transition and trilacunar cotyledonary node.

Keywords: germination percentage, mean germination time, germination speed index, root-stem transition.

Germinação dos diásporos e estrutura das plântulas de *Ruprechtia laxiflora* Meissn. (Polygonaceae)

RESUMO. Foram descritas a germinação de diásporos e a estrutura de plântulas de *Ruprechtia laxiflora* Meissn. (Polygonaceae), espécie secundária frequente em remanescentes florestais. As plântulas foram emblocadas em historresina e secionadas em micrótomo de rotação, de acordo com técnicas usuais em anatomia vegetal. Para os bioensaios de germinação, o delineamento experimental utilizado foi inteiramente casualizado em um esquema fatorial 3x2, correspondendo a três temperaturas (20, 25 e 30°C) e duas condições de luz (fotoperíodo e escuro contínuo) para os diásporos recém coletados e em esquema fatorial 4x2, correspondendo a quatro temperaturas (15, 20, 25 e 30°C) e duas condições de luz (fotoperíodo e escuro contínuo) para os diásporos analisados após armazenamento. Não houve interação entre os fatores condição de luz e temperatura para os parâmetros de germinação analisados. No entanto, o fator temperatura afetou a germinação e os diásporos mantidos sob 25°C apresentaram menor tempo médio e maior índice de velocidade de germinação. As plântulas são fanerocotiledonares e epigeias, com raiz tetrarca, tipo intermediário-baixo de zona de transição raiz-caule e nó cotiledonar trilacunar.

Palavras-chave: porcentagem de germinação, tempo médio de germinação, índice de velocidade de germinação, região de transição raiz-caule.

Introduction

In forest remnants of the Northwest region of Paraná, Brazil (less than 1% native vegetation) a diversity of species occurs, belonging to different families. *Ruprechtia laxiflora* Meissn. (Polygonaceae) is frequent in these forest remnants in which the germination of seeds and seedling structure still has not been investigated. *Ruprechtia laxiflora*, known as quince, 'viraró', quince-brave or the wood-quince, is an arboreal plant, light-demanding, secondary and characteristic of semi deciduous forest in the Paraná Basin (Lorenzi, 1998, Backes & Irgang, 2002). The species is important for restoring riparian or riverine environments, in locations under periodic flooding (Carvalho, 2008).

Germination of seeds and seedling establishment are phases of the plant reproductive cycle of vital importance for the maintenance and regeneration of the species, and abiotic factors like temperature and light usually affect the germination process (Bosch, Tecco, Funes, & Cabido, 2012).

Knowledge of seedlings requires special studies of all their stages of life and is important in weed control, forestry, silviculture, taxonomical studies and forest regeneration (Hzn, 1972, Vogel, 1980). Most of the works on seedlings describe their morphology, but there are some studies that treat of the anatomy of organs of seedlings of other families, as shown by Souza, Lopes, and Almeida (2007), Jesus et al. (2014), Santos, Dariva, Müller, Almeida, and Souza (2014) and Lopes and Souza (2015). This hinders the understanding of the process of seedling establishment in a certain vegetation.

The aim of this paper was to summarize available information on germination and the seedling structure of *R. laxiflora,* a species of economical and ecological importance.

Material and methods

Diaspore collection

Diaspores were collected at the *Estação Ecológica de Caiuá*, state of Paraná, Brazil (52º 49' - 52º 53' W and 22º 34' - 22º 37' S). The region is in the domain of semi deciduous forest and has Cfa climate with average annual temperatures of 22°C (*Instituto Agronômico do Paraná* [Iapar], 1994). The rainfall shows great variation during the year, with well-defined dry (winter) and rainy (summer) seasons (Zandonadi, Braido, & Silveira, 2008). Voucher specimen of the species investigated was deposited in the herbarium of the *Universidade Estadual de Maringá* (Huem 20438)

Germination assays

Adherent sepals were removed from the diaspores using tweezers. Diaspores were disinfested with a 50% solution of sodium hypochloride for 20 min and then washed with distilled water. Other diaspores were stored in black plastic bags, at room temperature, for one year. Germination tests were performed in Petri dishes (9 cm in diameter) lined with two filter papers moistened with distilled water. Each Petri dish received 25 diaspores, and we used 100 diaspores for treatment and four replicates.

Germination was evaluated in a bifactorial arrangement (3×2) consisting of three temperatures (20, 25 and 30°C) and two light conditions (12 hours photoperiod and continuous darkness) for the diaspores newly harvested and a bifactorial arrangement (4x2), corresponding to four temperatures (15, 20, 25 and 30°C) and two light conditions (12 hours photoperiod and continuous darkness) for diaspores analyzed after storage.

The dark treatments were performed using Petri dishes wrapped in aluminum foil; the seeds that were maintained under dark conditions were only observed under a green security light according to Mota and Garcia (2013). Germination was defined as the protrusion of the radicle through the seed tegument and was evaluated daily until the response stabilized.

Germination percentage (GP), speed of germination time (SGI) (Maguire, 1962) and mean germination time (MGT) (Ferreira & Borghetti, 2004) were calculated.

Normal distribution of data was tested by Shapiro-Wilk and the variables were transformed, when required, using the square root of Y + 1.0 + SQRT function. Analyses of variance were run, and, when the F value was significant, Tukey's test was applied for the comparison between means, at 5% significance.

Analyses of variance were run using Sisvar (System for Analysis of Variance) software for Windows®, version 5.6 (Ferreira & Borghetti, 2004).

Seedling structure

Germinated seeds were sown in substrate (mixture of organic substrate and medium sand in equal proportion) contained in plastic bags in a greenhouse. For morphological and anatomical descriptions, we used the terminology employed by Rizzini (1977), Vogel (1980), Garwood (1996), Souza (2009) and Souza et al. (2009).

The material (root, hypocotyl, cotyledons, epicotyl and eophyll) were fixed in glutaraldehyde (1% in 0.1 M phosphate buffer, pH 7.2) (Karnovsky, 1965) and later transferred to 70% ethyl alcohol following the protocol of Johansen (1940). The material was embedded in hydroxyl methacrylate (Guerrits, 1991), sectioned (cross- and longitudinal sections) in a rotary microtome, and stained with Toluidine Blue (O'Brien, Feder, & McCully, 1964). Photomicrographs were taken with a Leica microscope equipped with digital camera. All samples were prepared on the same micrometric scale.

Results and discussion

Diaspore germination

The results of germination of newly harvested diaspores are presented in Table 1. No interaction was found between light and temperature. The light did not affect the germination parameters analyzed. Considering the temperature alone, it had an effect on mean germination time (MGT) and speed of germination index (SGI) ($p \leq 0.05$). With regards to the MGT, the diaspores subjected to 25°C exhibited faster germination and lower SGI values were

obtained at 20°C, regardless of the presence or absence of light.

Table 1. Germination percentage (GP), mean germination time (MGT) and speed of germination index (SGI) of diaspores of *Ruprechtia laxiflora* Meisn. newly harvested and under different temperature for12 hours photoperiod (FP) and under continuous darkness (CD).

Temperature	20°C	25°C	30°C	Mean
		GP (%)		
FP	83.75	90.00	88.75	87.50 a
CD	88.75	92.50	97.50	92.92 a
Mean	86.25 A	91.25 A	93.12 A	
CV (%)		7.86		
		MGT (days)		
FP	3.53	1.21	2.46	2.40
CD	2.54	1.11	2.15	1.93
Mean	3.03 A	1.16 C	2.30 B	
CV (%)		8.38		
		SGI (diaspores germinated per day)		
FP	5.09	16.46	7.81	9.79
CD	7.72	17.50	9.83	11.68
Mean	6.40 C	17.00 A	8.82 B	
CV (%)		11.82		

Uppercase letters comparing the temperatures, independent of light condition. Different letters indicate significant differences by Tukey's test at 5%. CV = Coefficient of variation.

Results of the germination of stored diaspores are listed in Table 2.

Table 2. Germination percentage (GP), mean germination time (MGT) and speed of germination index (SGI) of diaspores of *Ruprechtia laxiflora* Meisn. stored under different temperature for 12 hours photoperiod (FP) and under continuous darkness (CD).

Temperature	15°C	20°C	25°C	30°C	Mean
			GP (%)		
FP	4.00	26.00	17.00	16.00	15.75
CD	7.00	20.00	13.00	12.00	13.00
Mean	5.50C	23.00 A	15.00 B	14.00 B	
CV (%)			18.41		
			MGT (days)		
FP	5.00	3.51	5.09	1.73	3.83
CD	5.25	2.76	5.56	1.74	3.83
Mean	5.12 A	3.13 B	5.32 A	1.73 C	
CV (%)			13.28		
			SGI (diaspores germinated per day)		
FP	0.20	2.23	0.87	2.7	1.50
CD	0.32	1.69	0.54	1.2	0.94
Mean	0.26 B	1.96 A	0.70 B	1.95 A	
CV (%)			15.41		

Uppercase letters comparing the temperatures, independent of light condition. Different letters indicate significant differences by Tukey's test at 5%. CV = Coefficient of variation.

There was no interaction between the factors light and temperature for germination of stored diaspores.

Germination test with stored diaspores showed a reduced germination (Table 2) when compared to the results obtained for newly harvested diaspores.

The light condition did not affect the germination parameters of stored diaspores. Considering the temperature alone, it had an effect on germination percentage (GP), mean germination time (MGT) and speed of germination index (SGI) ($p \leq 0.05$). Diaspores stored at 20°C showed a higher GP, while the MGT was lower at 30°C. Higher SGI was observed for diaspores maintained at 20 and 30°C

Germination percentage (GP) was significantly lower at 15°C, with only 5% of germination. Germination at 15°C was faster, but it occurred as a consequence of the low number of germinated diaspores.

Seedling structure

Seedlings (Figure 1A and C) are phanerocotylar and epigeal, with axial root, collet visible for presenting slight constriction, hypocotyl long, cylindrical and green, and epicotyl relatively short, cylindrical, green, hairy, with scale like leaves. The cotyledons (Figure 1B and C) are thin, green, petiolated and leaf-like; the leaf blade shows morphological diversity varying from ovate to parabolic, apex obtuse or roundish, base obtuse or straight, margin sinuate or crenate, and trinervous. The single eophyll (Figure 1C) also may be diverse in shape varying from rhomboid to obovate, with acute apex and obtuse base, margin entire in most of the leaf and sinuate in the superior third, and uninervous.

Primary root is tetrarch and consists of uniseriate and irregular epidermis with thin-walled cells (Figure 2A). Four layers of parenchyma cells are usually present in the cortex (Figure 2A), but lacking the exodermis with suberin; the endodermis is present (Figure 2A), but without evident Casparian strips. Central cylinder exhibits parenchymatous pericycle and four alternate strands of primary xylem and phloem (Figure 2A). Occasionally secondary vascular tissue is found in seedling root.

The root-stem transition region begins approximately in the collet and this condition persists for about the whole hypocotyl. In this portion, a pith begins to appear, and the primary xylem is spread into a continuous tangential ring and primary phloem strands surrounding the pith (Figure 2B). The hypocotyledonary epidermis (Figure 2B and C) is uniseriate and exhibits stomata and unicellular non-glandular trichomes, while the cortex (Figure 2B and C) consists of collenchyma, parenchyma and starchy endodermis.

At the level of the cotyledonary node are organized four end arch traces (Figure 2C and D), each one with its respective leaf gap, being two of larger caliber and two smaller. These traces undergo no further division until penetrating into a cotyledonary sheath (Figure 3A), when the smaller bundles undergo division. Thus, the cotyledon (Figure 3B) receives two lateral bundles, which are common to both cotyledons, and one large median

bundle that is exclusive of each cotyledon. The cotyledonary node is therefore trilacunar.

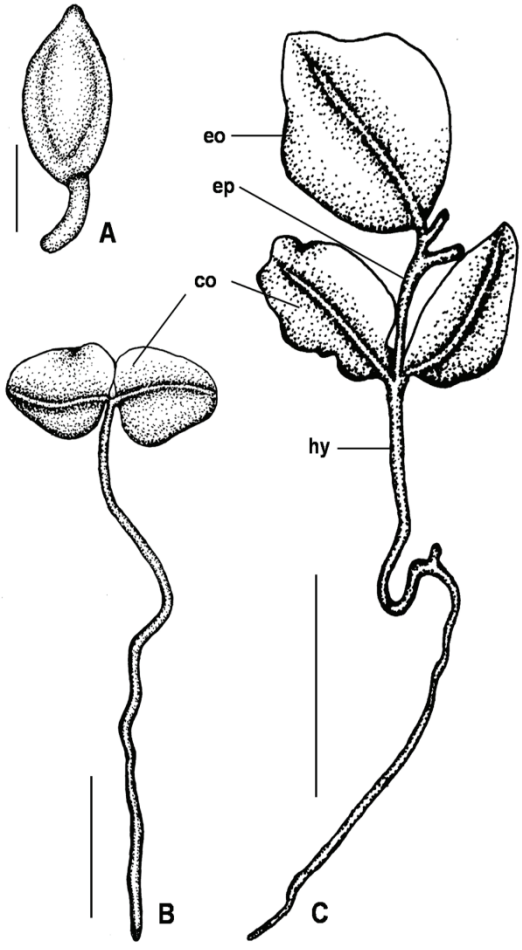

Figure 1. (A-C) Seedling morphology of *Ruprechtia laxiflora* Meissn. (A) Germinated diaspore. (B) Seedling with cotyledons. (C) Seedling with cotyledons and eophyll. (co = cotyledons; eo = eophyll; ep = epicotyl; hy = hypocotyl). Bars – 0.3 (A) and 0.7 cm (B-C).

Epicotyl consists of a uniseriate and hairy epidermis, and collenchymatous and parenchymatous cortex, without typical endodermis. Central cylinder is composed of collateral bundles with band of fibers that lies outside the phloem. A pith is made up of parenchyma of thin-walled cells.

The cotyledonary petiole (Figure 3B) has uniseriate epidermis with unicellular non-glandular trichomes, collenchymatous and parenchymatous cortex and three collateral vascular bundles. The leaf (Figure 3D) is composed of glabrous uniseriate epidermis with anisocytic and anomocytic stomata that are only found on the abaxial surface. Mesophyll is made up of homogeneous chlorenchyma, in which the cells of the adaxial layer are a little elongated anticlinally in some regions (Figure 3D). Druse idioblasts have been observed to occur. The midrib vasculature is made of a single collateral bundle.

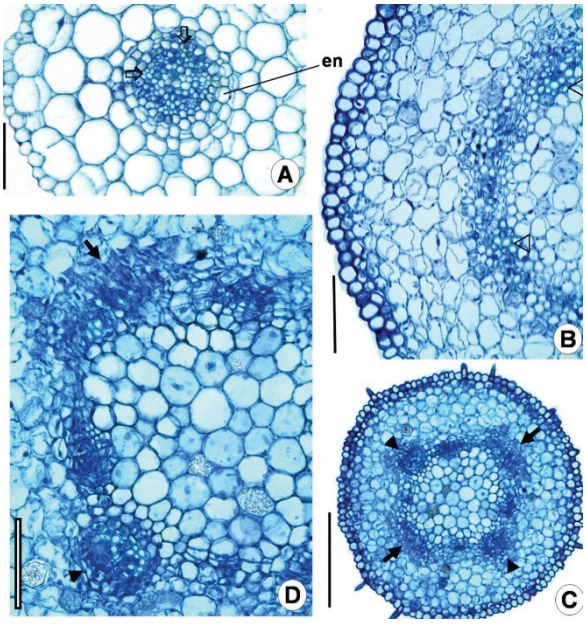

Figure 2. (A-D) Root and hypocotyl structure of *Ruprechtia laxiflora* Meissn. in cross-section. (A) Primary root (en = endodermis; arrows indicate protoxylem). (B) Hypocotyl (head arrows indicate tracheary elements). (C-D) Hypocotyl in the proximities of the cotyledonary node (black arrows show median cotyledonary traces; black head arrows indicate common cotyledonary traces). Bars - 70 (A-D) and 200 μm (C).

Eophyll shows uniseriate epidermis (Figure 3E), in which occur eventually collapsed cells, stomata restricted to the abaxial surface, pluricellular glandular trichomes, and elongated or lenticular crystals. Bicellular non-glandular trichomes are found in the margin (Figure 3F), abaxial surface and on the veins of the eophyll. Mesophyll (Figure 3E) consists of spongy and palisade parenchyma, and often contains druse idioblasts. As in the cotyledons, the midrib also has only one collateral vascular bundle.

Temperature is an important factor controlling seed germination, affecting both the rate and final percentage of germination (Pires, Cardoso, Joly & Rodrigues, 2009)

Recent study on species of the same genus reported that diaspores of *R. apetala* Wedd. (Bosch et al., 2012) did not germinate under low temperature (10-5°C), while at the highest temperatures (20-10, 25-15 and 35-20°C), they showed increased germination percentage. However, this study has not shown effects of the interaction between temperature and light on the percentage of germination of newly harvested diaspores.

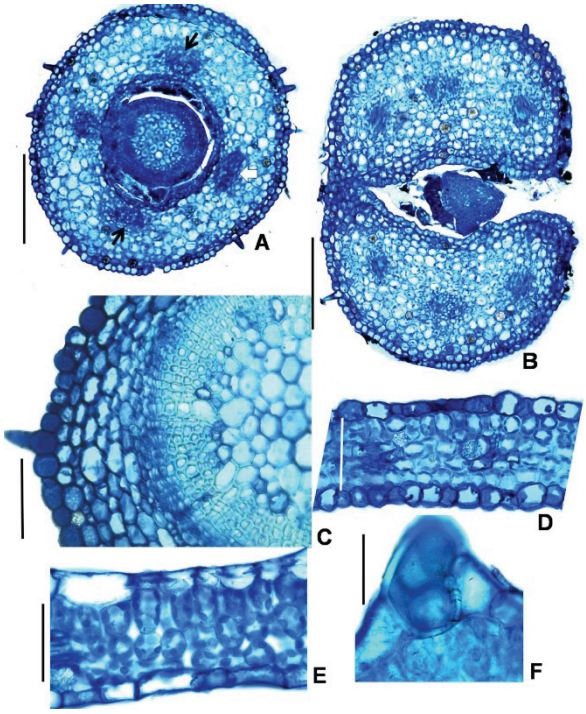

Figure 3. (A-F) Epicotyl and leaf structure of *Ruprechtia laxiflora* Meissn. in cross-section (A) Cotyledonary sheath involving the epicotyl (black arrows indicate the median traces; white arrow indicates the common trace in division). (B) Cotyledonary petioles with three vascular bundles (lateral and median bundles). (C) Epicotyl. (D) Cotyledonary blade. (E,F) Eophyll blade and bicellular trichome. Bars - 40 (E-F), 70 (C-D) and 200 μm (A-B).

Our results indicated no difference in germination ability for diaspores of *R. laxiflora* when subjected to 12 hours photoperiod or under continuous darkness. This suggests a lack of photosensitivity in the germinability condition of *R. laxiflora* diaspores, indicating that they can germinate at glades or sub-forest (Lopes, Freitas, & Lemos, 2010). In fact, Carvalho (2008) reports that *R. laxiflora* is a secondary forest species with diaspore germination showing no sensitivity to light.

In some forestall species, seed viability is maintained naturally for long periods, while seeds of other species deteriorate rapidly. Seeds of *Tabebuia caraiba* (Mart.) kept in paper packaging and stored at laboratory conditions have lost more quickly their vigor along the storage period (Guedes, Alves, Melo, Moura & Silva, 2012). The reduction of seed vigor during storage was also observed in this study for *R. laxiflora*, with reduced germination parameters after one year of storage.

According to Carvalho (2008), *R. laxiflora* seeds have an initial germination of 70%, and when stored under ambient conditions may maintain germination by 41% for up to 6 months. Thus the results obtained in this work, with newly collected diaspores, are higher than those reported by Carvalho (2008) and confirm the reduced vigor of the seed safter storage.

The *Macaranga* seedling type (Vogel, 1980) may be recorded for *R. laxiflora* that is characterized by epigeal and phanerocotylar seedlings with foliaceous assimilating cotyledons and leaves spirally arranged. *Ruprechtia laxiflora* seedling may also be classified as type PEF (Phanero-Epigeal-Foliaceous), according to Garwood (1996). In fact, the type PEF is usually most common in woody tropical floras (Garwood, 1996).

The vascular cylinder of the primary root of seedlings is commonly diarch or tetrarch (Eames, 1961, Duke, 1969). The tetrarchy registered in the *R. laxiflora* root has been considered the basic type because it is associated with arborescent taxa or woody families (Eames, 1961).

The level of transition root/stem (epicotyl) of *R. laxiflora* is classified as intermediate-low type in agreement with the classification of Compton (1912). In this type, the transition phenomena usually begin in the region of the collet, and proceeds slowly all the way up the hypocotyls, reaching completion near its summit (Compton, 1912). In general, most studies associated with seedling structure of eudicots have registered the transition type intermediate-low, as has been shown in Bignoniaceae, Clusiaceae, Fabaceae, and Annonaceae (Souza, 2009).

The leaf-like photosynthetic cotyledons of *R. laxiflora* have certain similarity with the eophyll concerning the anatomical pattern. A study conducted by Vogel (1980) on seedling morphology has shown that foliaceous cotyledons must be regarded as homologous with the lowermost stem leaves, and they must be called 'paracotyledons'. However, contrary to Vogel (1980)'s interpretation of 'paracotyledons', studies on seedling anatomy have shown divergence in the structure and vasculature of the cotyledons and eophylls of other plants (Souza, 2009). Besides that, Esau (1965) presents controversy on the question of whether the vascular system of the seedling is a unit composed of equivalent parts or consists of two systems connected developmentally with one another.

Cotyledons and eophyll of *R. laxiflora* are dorsiventral leaves, which merely confirms a leaf character of Polygonaceae (Metcalfe & Chalk, 1957). It must be noted, however, that some characters that are typically found in the leaves of Polygonaceae, as ochrea at the base of the petiole, petiole with several vascular bundles, stomata usually present on both surfaces, vascular bundles of the veins accompanied by sclerenchyma, and 2-4 layers of palisade parenchyma (Metcalfe & Chalk, 1957), were not

registered in cotyledons and eophylls of *R. laxiflora*, maybe because these leaves are still juvenile.

Conclusion

There is no influence of the parameters 12 hours photoperiod and continuous darkness on diaspore germination of *R. laxiflora*. In general, the germination was faster and speed of germination index (SGI) was higher at 25ºC for newly collected diaspores. The storage for 12 months decreases the germination of diaspores. *Ruprechtia laxiflora* seedlings present structural characters common to woody tropical floras.

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