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Prolonged seed submersion influences germination and early seedling growth of Amazonian floodplain trees

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Abstract

Seeds of many Amazonian floodplain forest trees are dispersed during high-water periods and spend weeks or months underwater until the flooding retreats. To assess whether prolonged seed submersion affects germination and early seedling development, an experiment was carried out in a greenhouse with seeds of *Campsiandra laurifolia*, *Cassia leiandra*, *Crataeva tapia*, *Ilex inundata*, *Macrolobium acaciifolium*, *Nectandra amazonum*, *Pouteria glomerata*, *Psidium acutangulum*, *Sorocea duckei*, and *Vitex cymosa*. They are common in this type of forest, differ in fruit type, number of seeds per fruit, fruit dimensions, and fresh mass and have fruits or seeds that can float. Seeds were collected in a Central Amazonian floodplain forest (flooded approximately 6 months year^{-1}; water column of 5 m) and germinated in (1) irrigated soil or (2) underwater (water column of 5–7 cm) for 6 months. Seeds that germinated underwater were transferred to drained soil. Seeds of all species germinated underwater and developed seedlings when transferred to soil. However, submersion reduced the germination percentage of *Psidium acutangulum*, *N. amazonum*, *P. glomerata* and *V. cymosa*. Six species delayed germination in water. *Ca. leiandra*, *I. inundata* and *P. glomerata* did not differ in mean germination time in drained soil and underwater, whereas *S. duckei* seeds germinated faster underwater. Seed submersion negatively affected seedling growth (shoot length) of three species but did not affect seedling biomass. Timing of fruit dispersal, fruit buoyancy and high number of seeds per fruit can be critical for species with seeds that are not as able to cope with long-term submersion.

Introduction

Spatial variation in species composition in floodplain areas strongly depends on interactions between the hydrology and the early stages of plant life cycle (Nicol et al., 2003; Capon, 2007). Seed germination and seedling establishment are critical stages in the plant life cycle. Germination and early development of the seedling are dependent on the type and controlled use of the reserves contained in the seed and are extremely vulnerable to variations in environmental conditions (Seabloom et al., 1998; De Melo et al., 2015, 2020; Nunes et al., 2024). Flooding severely limits the availability of oxygen necessary for reactivation and resumption of the metabolism in the mature embryo (Colmer et al., 2014). Consequently, species diversity is reduced in flooded environments, since seeds of most terrestrial plants do not germinate or lose their viability when they are submerged (Parolin et al., 2003; Baskin and Baskin, 2014).

The Amazonian floodplains are lowlands that are located along the main rivers that cut through the largest rainforest in the world. Every year, the River Amazon and its tributaries overflow, flooding the extensive forested shores of both nutrient-poor acidic floodplains (igapós) and fertile, muddy whitewater floodplains (varzeas) (Wittmann and Junk, 2016). In the varzea floodplains of Central Amazonia, flood height and flood duration strongly affect species composition and diversity, resulting in shifts in floristic composition, species richness and vegetation structure along the flood-level gradient. Few species are found along the entire gradient and the zonation of forest communities from the areas adjacent to the river channel (low-varzea forests) towards higher portions of the relief (high-varzea forests) is well documented (Worbes et al., 1992; Wittmann et al., 2004, 2006, 2010).

Despite challenges to germination and early seedling growth, Amazonian floodplain forests have diverse and species-rich tree communities that are of crucial importance for ecosystem functioning (Wittmann et al., 2004, 2010). In the varzea forests alone, more than 1,000 species of trees have been catalogued, which make them the richest flooded forests in the world (Wittmann et al., 2006). Seasonality of the environmental conditions favours the establishment of a specialized flora, which synchronizes the life cycle and phenology with the flood pulse (Junk et al., 1989; Junk and Piedade, 2010). Flooding may last up to 6 months per year and many species disperse their propagules during the aquatic phase at the peak of the floods and the beginning of receding waters. Seeds of several species can remain viable even after long periods of submergence (Lucas et al., 2012; Calvi et al., 2017), while others can germinate or even form seedlings from seeds that were floating or submerged under a short column of water (Ferreira et al., 2007; Oliveira Wittmann et al., 2007; De Melo et al., 2015). However, information on the impacts of seed submergence on germination, seedling emergence and growth is still restricted to a few species.

Of particular interest and little studied is the fate and tolerance of seeds of typical tree species that colonize these environments when they remain in water for long periods until the end of the flood and soil re-exposure, which raises the following questions: Do seeds survive long-term flooding without losing viability? Do they germinate underwater? Is the germination of coexisting species reduced or delayed in water? Does germination in water affect early seedling growth? To answer these questions, we selected 10 coexisting tree species from Central Amazonian floodplain forests and investigated the role of flooding on germination and its influence on the early establishment of seedlings. We also evaluated fruit and seed characteristics and seedling functional morphology. In particular, seed size and the functional morphology of the cotyledons are relevant information related to the dispersal and emergence phenology of the seedling and plant regenerative strategies. They exert a critical role in seedling growth and survival and therefore in the distribution of new individuals along the flood-level gradient (Parolin et al., 2003; Moles and Westoby, 2006; Baraloto and Forget, 2007; Ferreira et al., 2010).

Materials and methods

Study area and species

Varzea forests in Central Amazonia are subject to a predictable and monomodal flood pulse. The water level rises, on average, 10 m and subjects the plants to an aquatic phase, followed by a terrestrial phase (Junk, 1993). The flood is slow and takes about 8 months to reach its maximum level (May to July). On the other hand, the speed with which the water returns to the streambed is faster. The water column can drop more than 4 m in just 1 month. Plants synchronize phenology (Junk, 1993; Schöngart et al., 2002; Parolin, 2009) and reproduction with the temporal dynamics of the flood pulse, and most trees disperse their propagules at the peak of the floods and the beginning of receding waters (Supplementary Fig. 1) (Ferreira et al., 2010; De Melo et al., 2015).

Ten species were chosen: *Campsiandra laurifolia* Benth., *Cassia leiandra* Benth., *Macrolobium acaciifolium* Benth. (Fabaceae), *Crataeva tapia* L. (Capparaceae), *Ilex inundata* Poepp. ex Reissek. (Aquifoliaceae), *Nectandra amazonum* Nees. (Lauraceae), *Pouteria glomerata* (Miq.) Radlk. (Sapotaceae), *Psidium acutangulum* DC. (Myrtaceae), *Sorocea duckei* DC. (Moraceae) and *Vitex cymosa* Bertero ex Spreng. (Lamiaceae). Only *P. glomerata* and *I. inundata* are evergreen, and all others are deciduous. The chosen tree species play an important role in the ecological succession of the floodplains (Wittmann *et al.*, 2006) and are among the most frequent species found in the area. They are extensively used by the local population for the extraction of non-timber forest products, such as food for humans and local fauna, medicine and handicraft (Wittmann et al., 2010; Rios and Pastore Júnior, 2011; Conserva et al., 2018). The 10 species are typical from the late secondary stages of Central Amazonian low-varzea forests (*sensu* Wittmann et al., 2004) where photosynthetically active radiation on the forest floor is around 10% of full sunlight.

Fruit collection

Fruit collections were carried out in the high-water period, between the months of June and July, when many tree species of the floodplains release their seeds, which are dispersed mostly by water and fish (Goulding, 1983; Kubitzki and Ziburski, 1994; Parolin et al., 2013; Weiss et al., 2016). Mature fruits were collected directly from the trees (5–7 trees) in low-varzea forests of the Marchantaria Island (3° 1′ 28.36″ S; 60° 8′ 48.12″W) or in its vicinity. The areas were located on the banks of the Solimões River, about 20 km southwest of the city of Manaus, in Central Amazonia. The water column height measured at the sample sites was on average 5 m. The flood period can extend from 160 to 210 days per year (Wittmann et al., 2006).

In the field, the fruits were stored in plastic bags, kept in Styrofoam boxes and transported to the Laboratory of Ecophysiology, of the Ecology, Monitoring and Sustainable Use of Wetlands Group (MAUA) at the National Institute for Amazonian Research (INPA) in Manaus. In the laboratory, the fruits were pulped, and the seeds mixed together to obtain one single lot for each species.

Fruits and seed characteristics

Fruits were described in terms of type and the number of seeds. The length, thickness and width of 30–50 fruits per species (depending on availability) were measured. The fresh mass was determined using a 0.001 g scale. We used field observations to determine if the fruit could float in water.

Of each species, 50 seeds were evaluated in terms of colour, shape and texture. The length and width of the seeds were measured with a digital calliper and then they were placed in plastic trays with a water column of about 7 cm and monitored for 1 h to assess whether they floated or sank. The dry mass was obtained after drying the seeds in a forced circulation oven at 70°C until reaching a constant mass and then weighed on a 0.0001 g precision scale.

Experiment 1: seed germination and seedling classification

For each species, 200 seeds were distributed in trays (35 cm × 20 cm × 7 cm) and left to germinate under two different treatments that simulated the conditions found in the field at the time of dispersion, in varzea soil or water. The experimental design was completely randomized, with four replicates (trays) of 25 seeds per treatment/species: (1) *drained soil*: trays filled with soil from varzea that was kept damp, but not saturated and (2) *underwater*: trays filled with water (water column held between 5 and 7 cm) from an artesian well. In the underwater treatment, two drops of detergent were added to break the surface tension of the water to let seeds sink. Germination in soil was defined by seedling emergence, and underwater, by the emission and curvature of the radicle (±1.5 cm). The experiment was performed in a greenhouse at air temperatures around 28°C, a relative humidity of 70%, 15% full sunlight and a photoperiod of 12 h. The experiment lasted for 6 months. After 6 months underwater, the remaining non-germinated seeds had suffered necrosis or fungal infestation and were considered unviable. Germinated seeds were counted daily to calculate percentage germination (%

G). The mean germination time (MGT) was calculated according to Labouriau (1983) by the equation: $\text{MGT} = \left(\sum n_i t_i\right) / \sum n_i$, where n_i is the number of germinated seeds on the ith day; t_i is the number of days from sowing to the ith day. The seeds that germinated in the drained soil or underwater were transferred to 0.8 L plastic pots (1 plant/1 pot) containing varzea soil to monitor seedling development for 30 days. A seedling was considered to be formed when it had developed root and shoot (caulicle and cotyledonary leaves or first pair of leaves). The seedlings were then classified into morphofunctional types based on the characteristics of the cotyledons (Gurgel et al., 2012; Pérez-Harguindeguy et al., 2013) related to exposure (cryptocotylar or phanerocotylar), position (epigeal or hypogeal) and texture (foliaceous or reserve): (1) crypto-hypogeal with reserve storage cotyledons (CHR), (2) crypto-epigeal with reserve storage cotyledons (CER), (3) phanero-hypogeal with reserve storage cotyledons (PHR), (4) phanero-epigeal with foliaceous cotyledons (PEF) or (5) phanero-epigeal with reserve storage cotyledons (PER).

Experiment 2: seedling growth and biomass

From each species, potted seedlings produced from seeds that germinated in the drained soil and 10 from seeds that germinated underwater were randomly chosen from Experiment 1 when they were 30 days old. They were kept in the greenhouse under the same conditions as in Experiment 1, with daily irrigation for another 30 days. At the end of this period, the shoot length (from the base to the tip of the stem) was measured. The seedlings were collected, thoroughly washed, dried in a forced circulation oven at 70°C for 72 h and weighed on a 0.0001 g precision scale to obtain the total dry mass (De Melo et al., 2020). *Psidium acutangulum* was not part of Experiment 2 because the number of seeds that germinated underwater was less than 10.

Statistical analysis

The results of the experiments with seeds and seedlings were submitted to a one-way ANOVA. The premises of normality, homoscedasticity and sphericity were checked, and the data were $\log_{10}$-transformed, if necessary. Pearson correlation analysis was used to test if seed dry mass was related to shoot length and total seedling dry mass for seedlings developed in varzea soil and water. All analyses were performed using the software Minitab 20. For all tests, differences were considered significant at $p \leq 0.05$.

Results

Characteristics of fruits and seeds

Species differed in fruit type, the number of seeds per fruit, fruit dimensions and fresh mass, which ranged from 0.3 g in *I. inundata* to 278.3 g in *Crataeva tapia* (Supplementary Table 1). Species also differed in shape, colour, texture and size of the seeds (Supplementary Table 2).

Fruits of most species float (Supplementary Table 1). Although its fruits are not able to float, *C. laurifolia* had seeds that float. This species also had the largest and heaviest seeds (seed dry mass of 4.12 g), while *I. inundata* had the smallest and lightest seeds (seed dry mass of 0.06 g) (Table 1; Supplementary Table 2). *V. cymosa* was the only other species with seeds that floated and was also the one with the lowest moisture content in the seeds (32%). Seed moisture content ranged from 45 to 89% for the other species. Seeds of all species were dispersed by water or fish (Table 1).

Seed germination and seedling morphofunctional types

Submersion significantly affected ($p \leq 0.05$) the germination percentage of four species and the MGT of seven species (Figs. 1A, B). *Psidium acutangulum* had the lowest number of seeds germinating underwater (4%). *N. amazonum*, *P. glomerata* and *V. cymosa* also showed a reduction in germination percentage ($p \leq 0.05$) when submerged. The other species germinated their seeds in drained soil or underwater without any statistical difference ($p > 0.05$) between the treatments (Fig. 1A). Eight species started germination in the first 30 days after sowing in the drained soil: *C. laurifolia*, *Ca. leiandra*, *Crataeva tapia*, *M. acaciifolium*, *N. amazonum*, *Psidium acutangulum*, *S. duckei* and *V. cymosa* (Supplementary Fig. 2). Of these, only the seeds of *Ca. leiandra* presented similar values of MGT ($p > 0.05$) in drained soil and underwater. *C. laurifolia*, *Crataeva tapia*, *M. acaciifolim*, *N. amazonum*, *P. acutangulum* and *V. cymosa* increased MGT ($p \leq 0.05$) when submerged. *S. duckei* was the only species that showed a reduction ($p \leq 0.05$) in MGT when submerged ($\text{MGT}_{\text{soil}} = 23$ days; $\text{MGT}_{\text{water}} = 14$ days) (Fig. 1B). *I. inundata* ($\text{MGT}_{\text{soil}} = 144$ days; $\text{MGT}_{\text{water}} = 119$ days; $p > 0.05$) and *P. glomerata* ($\text{MGT}_{\text{soil}} = 152$ days; $\text{MGT}_{\text{water}} = 153$ days; $p > 0.05$) presented the highest MGT values, both in drained soil and underwater (Fig. 1B), and the longest lag time (number of days needed to initiate germination). They started germination 71 and 91 days, respectively, after the start of the experiment (Supplementary Fig. 2). Irrespective of the species, all seeds that germinated underwater developed seedlings when removed from the water and planted in varzea soil.

The seedlings of the 10 species were classified into four morphofunctional types (Table 1 and Supplementary Fig. 3). In this study, all species with large seeds (seed dry mass ≥ 1 g) remained with the cotyledons closed (crypto) after germination. The opening of the cotyledons (fanero) was observed only in species with small seeds (seed dry mass < 1 g) (Table 1). Four species, *P. glomerata*, *M. acaciifolium*, *C. laurifolia* and *N. amazonum*, had their seedlings classified as CHR. The CHR is characterized by the growth of the epicotyl, with the cotyledons being kept inside the seed and at the ground level. *S. duckei* also showed hypogeal germination, but with exposed cotyledons, which characterizes the PHR. In five species, the hypocotyl has elongated, which brought the cotyledons (foliaceous or reserve) well above the ground level, thus characterizing them as epigeous. The seedlings of *V. cymosa*, *I. inundata* and *Psidium acutangulum* were assigned to the PEF, while the seedlings of *Ca. leiandra* and *Crataeva tapia* were of the PER. The production of one to three seedlings from a single pyrene was observed in *V. cymosa*. In this species, in 23% of the seeds, after the emergence of the first seedling (PEF type), the multilocular pyrene with two or three seeds covered by a stony endocarp remained at ground level for the emergence of the second or third seedling (Supplementary Fig. 4).

Seedling growth and biomass

For most species, seed germination in drained soil or underwater did not affect ($p > 0.05$) the shoot length of the seedlings during the early period of establishment in the soil (Table 2). *I. inundata*,

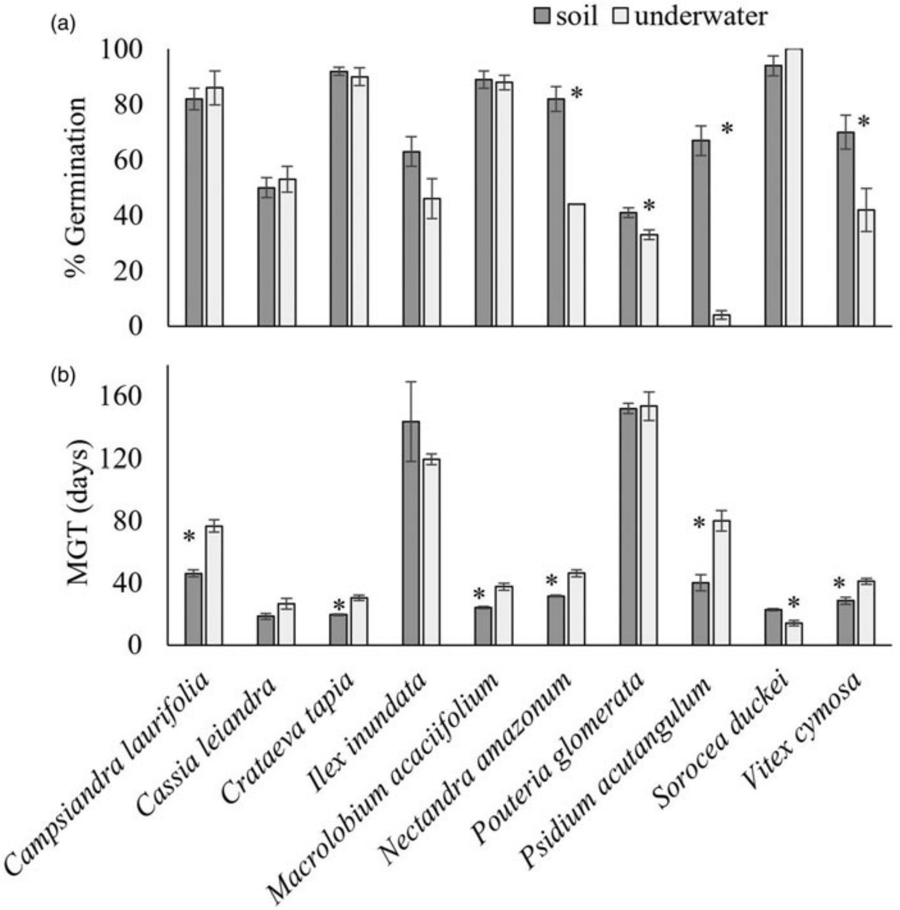

Figure 1. Effect of flooding on (A) the percentage germination and (B) MGT for seeds of 10 tree species of Central Amazonian floodplains. Non-flooded seeds (soil) were placed on trays filled with soil from varzea that was kept damp, but not saturated. Submerged seeds (underwater) were kept in trays filled with water (water column held between 5 and 7 cm). In both treatments, four replicates of 25 seeds each were used. The asterisks indicate significant differences between the two treatments ($p \leq 0.05$). Bars are means ± standard errors.

Table 1 Dispersal syndrome, seed dry mass, seed moisture content and seedling morphofunctional type for 10 tree species from the Amazonian floodplain forests

Species	Family	Dispersal	Dry mass (g)	Moisture content (%)	Seedling type
C. laurifolia	Fabaceae	Anemo/Hydro	4.12 ± 0.72	66.3 ± 11	CHR
Ca. leiandra	Fabaceae	Ichthyo/Zooc	0.33 ± 0.08	50.5 ± 17	PER
Crataeva tapia	Capparaceae	Hydro/Ichthyo	0.36 ± 0.04	68.6 ± 11	PER
I. inundata	Aquifoliaceae	Hydro/Zooc	0.06 ± 0.00	58.6 ± 16	PEF
M. acaciifolium	Fabaceae	Hydro/Ichthyo/Zooc	2.05 ± 0.72	63.1 ± 11	CHR
N. amazonum	Lauraceae	Ichthyo	2.50 ± 0.29	44.6 ± 10	CHR
P. glomerata	Sapotaceae	Hydro/Ichthyo/Zooc	1.35 ± 0.68	56.2 ± 15	CHR
Psidium acutangulum	Myrtaceae	Ichthyo/Zooc	0.15 ± 0.04	85.4 ± 4	PEF
S. duckei	Moraceae	Zooc	0.57 ± 0.01	89.1 ± 2	PHR
V. cymosa	Lamiaceae	Hydro/Ichthyo	0.27 ± 0.04	31.9 ± 6	PEF

Note: Means followed by standard deviation, $n = 50$ seeds.
Anemo, anemochory; Hydro, hydrochory; Ichthyo, ichthyochory; Zooc, zoochory (except fish); CHR, crypto-hypogeal with reserve storage cotyledons; PEF, phanero-epigeal with foliaceous cotyledons; PER, phanero-epigeal with reserve storage cotyledons; PHR, phanero-hypogeal with reserve storage cotyledons.

M. acaciifolium and *N. amazonum* were the exceptions. Seedlings of these species that germinated underwater were shorter ($p \leq 0.05$) when compared to seedlings from seeds that germinated in drained soil. However, this difference was not observed in the total dry biomass of the seedling (Table 2). Seedlings from seeds that germinated in soil or underwater the nine species did not differ in total seedling dry mass after 60 days of growth in irrigated soil ($p > 0.05$). Seedling growth in terms of shoot length and biomass accumulation were positively correlated with seed dry mass (Fig. 2).

Discussion

Seed germination in water is a challenge for most plants, especially arboreal ones (Ferreira et al., 2010; Baskin and Baskin, 2014). The activation of the anaerobic metabolism pathways, as

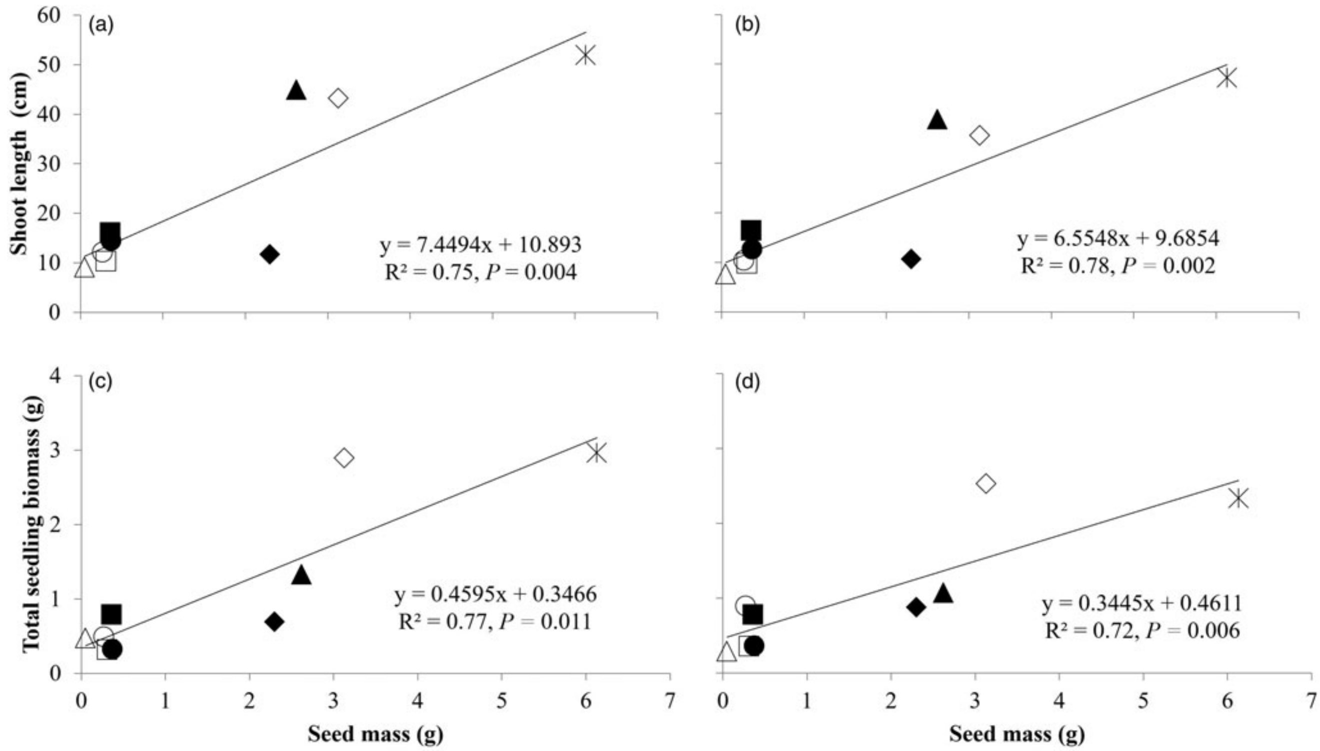

Figure 2. (A, B) Shoot length and (C, D) total seedling dry mass in relation to seed dry mass for nine tree species of Central Amazonian floodplains. Seedlings were 60 days old at the time they were measured. Seedlings were from seeds that germinated (A, C) in soil or (B, D) underwater and transferred to soil. Values are given as the mean for each species. Û *C. laurifolia* (Fabaceae), □ *Ca. leiandra* (Fabaceae), ■ *Crataeva tapia* (Capparaceae), r *I. inundata* (Aquifoliaceae), ▲ *M. acaciifolium* (Fabaceae), ¯ *N. amazonum* (Lauraceae), ◆ *P. glomerata* (Sapotaceae), ● *S. duckei* (Moraceae), ○ *V. cymosa* (Lamiaceae).

Table 2 Shoot length and total dry mass for seedlings that were produced from seeds that germinated in drained soil (SG_{soil}) or underwater (SG_{water}) and were transferred to grow in varzea soil

	Height (cm)		Total biomass (g)	
Species	SG_{soil}	SG_{water}	SG_{soil}	SG_{water}
Campsiandra laurifolia	51.9 ± 4.6	47.2 ± 6.2	2.96 ± 0.1	2.33 ± 0.1
Ca. leiandra	10.3 ± 2.3	9.8 ± 1.4	0.32 ± 0.2	0.36 ± 0.1
Crataeva tapia	16.1 ± 2.7	16.4 ± 2.6	0.79 ± 0.1	0.78 ± 0.2
I. inundata	9.1 ± 1.3^{a}	7.6 ± 0.9^{b}	0.47 ± 0.2	0.29 ± 0.1
M. acaciifolium	44.9 ± 6.7^{a}	38.9 ± 3.1^{b}	1.33 ± 0.1	1.07 ± 0.1
N. amazonum	52.3 ± 8.8^{a}	41.1 ± 4.3^{b}	3.89 ± 0.2	2.83 ± 0.3
P. glomerata	11.7 ± 2.1	10.6 ± 2.2	0.69 ± 0.2	0.88 ± 0.1
S. duckei	14.5 ± 2.6	12.6 ± 1.8	0.32 ± 0.1	0.37 ± 0.1
V. cymosa	12.1 ± 1.7	10.4 ± 2.1	0.49 ± 0.1	0.67 ± 0.2

Note: Seedlings were harvested when they were 60 days old. Data expressed as mean ± standard deviation. Different lowercase letters indicate significant differences between treatments ($p \leq 0.05$, $n = 10$).

a consequence of the low diffusion of oxygen in water, produces less ATP, alters the permeability of membranes and generates compounds that can cause tissue necrosis and embryo death (Ferreira et al., 2007; Pradhan and Mohanty, 2013; De Melo et al., 2015; Duarte et al., 2020). Despite these strong constraints imposed on seeds, all studied species release their seeds during the high-water period, which are then dispersed by water or fish. The seeds were dispersed with high moisture content, a common characteristic of seeds of many tropical wetland trees. Generally, these seeds are more sensitive to desiccation (Baskin and Baskin, 2014). In the studied species, when the mature fruits detached from the mother plant, the seeds remained protected from desiccation inside the fruit, which floated. *C. laurifolia* was the only species with non-floating fruits, but their seeds can float, remaining on the surface of the water in contact with humidity and aerated air. However, the differences in seed germination percentages in drained soil and underwater among the studied species suggest different levels of tolerance to submergence. When compared to germination in soil, four of the studied species had lower percent germination underwater. The other six species did not differ in germination percentage in drained soil and underwater. Despite a negative effect on the growth of three species, seed submersion did not affect seedling dry mass accumulation.

Psidium acuntagulum was the species most sensitive to submergence; only 4% of the seeds germinated underwater, and it took them about three times as long to germinate underwater (57 days) compared to in soil (19 days). In the natural environment, the ripening period of the fruits of this species coincides with the end of the flood period (Falcão et al., 1992). The fleshy (berry-like) fruits fall into the water and can serve as food for dispersing fish or remain afloat for up to 23 days, during the descent of the water column until germination occurs in the soil (Kubitzki and Ziburski, 1994). This allows the seeds to remain enveloped by the fruit for long periods, being released gradually and avoiding the phase of greater susceptibility to flooding, and then germinating. Thus, although it was the species that was the least tolerant to flooding in the germination experiment, the high number of seeds produced per fruit (13–78 seeds, in this study) and the fact that 100% of the *P. acuntagulum* seeds that germinated in water formed seedlings when planted in soil may act as compensatory mechanisms that ensure its occurrence in the Amazonian floodplains. Combined strategies of escape from the unfavourable period to germinate with the investment in high seed production are especially relevant in the Amazonian floodplains where the flood pulse has a monomodal seasonal pattern, but with an amplitude that can vary in different years (Chevuturi et al., 2023). Once established in the soil, seedlings of this species are tolerant to submersion (Parolin, 2000).

For 60% of the evaluated species, seed germination underwater required more time than in soil. The increase in MGT observed for submerged seeds of *C. laurifolia* ($\mathrm{MGT}_{\mathrm{soil}} = 46$ days; $\mathrm{MGT}_{\mathrm{water}} = 77$ days), *Crataeva tapia* ($\mathrm{MGT}_{\mathrm{soil}} = 20$ days; $\mathrm{MGT}_{\mathrm{water}} = 30$ days) and *M. acaciifolium* ($\mathrm{MGT}_{\mathrm{soil}} = 24$ days; $\mathrm{MGT}_{\mathrm{water}} = 38$ days) suggests the strategy of delaying germination underwater to ensure that seedling establishment occurs mainly when the environmental conditions become favourable after soil exposure. These species are tolerant to flooding and have high germination rates both underwater and in soil, as well as high seedling production in the soil. Their seeds remain viable even after spending weeks in water (Parolin et al., 2003; Lucas et al., 2012; De Melo et al., 2015).

In addition to the prevailing conditions in the environment in which the seed is dispersed, the time for its germination is determined by the intrinsic characteristics of the species, which may impose dormancy (Baskin and Baskin, 2014). The delay in seed germination of *I. inundata* and *P. glomerata* cannot be attributed to seed submersion. Indeed, the germination characteristics of both species were not influenced by submersion. In addition to similar values of MGT and lag time in soil and underwater, the decrease in the germination percentage underwater in relation to seeds germinated in soil was small for *P. glomerata* and not significant for *I. inundata*. The study of seed dormancy mechanisms was not the focus of this study; however, morphophysiological dormancy, which is characterized by undeveloped embryos, has already been reported for several species of the genera *Ilex* and *Pouteria* (Amoakoh et al., 2017; Galíndez et al., 2018; Vasconcelos et al., 2021).

Among the species analysed regarding the strategies for effective occupation of the environment with its progeny, *V. cymosa* deserves a deeper analysis. Although when submerged the seeds of this species show a decrease in percent germination and delayed germination compared to those in the soil, the percent germination underwater is still considered high (>40%) among trees that inhabit floodplains (Parolin et al., 2003; De Melo et al., 2015). The fruit of this species is a multilocular pyrene with one to three seeds, allowing the production of one to three seedlings from a single pyrene. Such a set of seed mechanisms to ensure efficient germination and seedling production certainly plays a role in explaining the species' distribution in various habitats and contrasting biomes, and its impressive status as a monodominant species in many forest patches distributed across the Amazonian region, in which only 26 species exhibited monodominance, out of more than 5,000 species investigated (ter Steege et al., 2019).

The functional morphology of cotyledons (foliaceous or reserve) is important for the successful establishment of the seedlings, supporting their early growth and development and representing contrasting regeneration strategies (Zanne et al., 2005). The seedlings of the 10 species studied were classified into the four most frequent morphofunctional types in tropical forests, i.e., the CHR, the PHR, the PEF, PEF and the PER (Ressel et al., 2004). The CHR type was observed for the four species with large seeds (seed dry mass ≥ 1.0 g), thus corroborating studies that consistently relate large seeds to hypogeous germination (Parolin et al., 2003; Zanne et al., 2005; Gurgel et al., 2012). Physical constraints help explain this pattern since it is difficult for epigeal germination to support a large seed (Baraloto and Forget, 2007). Species that produce large seeds are frequent in the secondary successional stages and shaded habitats or resource-limited forests (Parolin et al., 2003; Zanne et al., 2005; Conserva et al., 2018). They have access to enough carbon and mineral nutrient reserves in the seeds to increase allocation to stems and roots and invest in greater tolerance of various stresses. They still have enough reserves to warrant fast seedling recovery after predation or disturbance (Baraloto and Forget, 2007; Muller-Landau, 2010). Rapid elongation of the aerial parts following water retreat can also be crucial to escape from submersion at the higher positions of the flood gradient (Parolin, 2002). In the present study, seedling growth (shoot length) and biomass accumulation were positively correlated with seed dry mass (Fig. 2).

The species with small seeds (<1.0 g) showed phanerocotylar germination that, apart from *I. inundata*, germinated quickly in the soil. The dominant morphofunctional types were PEF and PER, while PHR was the rarest and was only found in *S. duckei* (Ressel et al., 2004; Zanne et al., 2005; Conserva et al., 2018). Phanerocotylar seedlings are common in early-stage forest species that generally originate from small seeds, featuring photosynthesizing cotyledons, and tend to grow rapidly to be able to compete for space and light (Ressel et al., 2004).

Small seeds, as well as large seeds, can be common in shaded environments. Seedlings of small-seed species tend to have greater plasticity in their response to light conditions, which can favour seedling growth in the understory of the forest, where irradiance levels tend to be dynamic (Poorter and Rose, 2005). However, light intensity is just one of the selective forces driving early life-history traits (Poorter and Rose, 2005) and, therefore, cannot be evaluated in isolation, especially in complex environments such as the floodplains of the Amazonia. Characteristics that favour seed dispersal by wind or small animals in open environments can also contribute to the dispersal by water or fish that carry and distribute the seeds throughout the interior of lowland floodplain forests where light levels are reduced. The sedimentation rate has an important role in soil renewal and nutrient supply to seedlings that have been established after the descent of the waters (Junk and Piedade, 2010; Wittmann et al., 2010). Seed and seedling traits interact with each other and differentiate species regarding their survivorship and growth in seasonally flooded varzea forests.

Successful germination and seedling establishment are crucial steps on freshwater forests that are subjected to a long-term duration of the flood pulse associated with an abrupt transition in the environmental conditions along the topographic gradient as it is found on the banks of the major rivers in Central Amazonia (Junk et al., 1989; Ferreira et al., 2010). We provide evidence that co-occurring tree species in the lower portions of the Amazonian floodplains had different degrees of tolerance to seed submergence. Timing of fruit dispersal, fruit buoyancy and high number of seeds per fruit can be critical for species with seeds that are not as able to cope with long-term submersion.

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