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# Anatomical and morphological modifications in response to flooding by six Cerrado tree species

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## ABSTRACT

Wetlands are common in the Cerrado (Brazilian savannas) biome, however flooding of these wetlands impairs growth and development of most plants. We evaluated flood tolerance of typical Cerrado trees. Seedlings of *Aspidosperma macrocarpon* (Apocynaceae), *Tabebuia rosea* (Bignoniaceae), *Handroanthus chrysotrichus* (Bignoniaceae), *Myracrodruon urundeuva* (Anacardiaceae), *Kielmeyera coriacea* (Calophyllaceae) and *Copaifera langsdorffii* (Fabaceae) were flooded up to the stem base for 30 days. Stems with cortical cracks, secondary aerenchyma and hypertrophic lenticels were observed in flooded plants of *M. urundeuva*, *H. chrysotrichus* and *T. rosea* while adventitious roots were formed in flooded plants of *T. rosea* and *H. chrysotrichus*. However, only *T. rosea* developed aerenchyma in the root cortex. *K. coriacea* and *A. macrocarpon* were the most sensitive to flooding, showing a decrease in survival and necrosis of the leaves and roots. *C. langsdorffii* and *M. urundeuva* were less sensitive to flooding, although reductions in root biomass and symptoms of necrosis of the roots were noticeable in flooded seedlings. Flooded *M. urundeuva* seedlings also had a decrease in total leaf area, leaf biomass, total biomass and in stem growth. Flooding affected root development and reduced stem growth of *H. chrysotrichus* with symptoms of necrosis of the leaves and roots. *T. rosea* was the only species where symptoms of injury from flooding were not evident.

**Keywords:** aerenchyma, biomass partitioning, flood tolerance, hypoxia, water stress

## Introduction

Soil water saturation negatively affects growth and development of most terrestrial plants and ultimately lead to plant death (Kozłowski 1997; Visser *et al.* 2003). Many terrestrial ecosystems, particularly in the tropics, are subject to episodes of flooding that can last for hours, days and even months. Plant tolerance to flooding will depend in large part on the ability to develop specialized structures that facilitates aeration of the internal tissues (Justin & Armstrong 1987; Voesenek *et al.* 2006).

Wetlands play an important ecological role in the Cerrado biome, because they are in most cases, transition ecosystems that extensively exchange energy and matter with the watercourses and the surrounding vegetation on well-drained soils (Meirelles *et al.* 2006). Besides its importance for perpetuation and maintenance of the hydrological regime of the watercourses, wetlands are essential to the conservation of wildlife by providing refuge, water, food and breeding grounds for many organisms (Ribeiro & Walter 1998).

Constraint to tree establishment and growth by soil flooding may be a determinant factor to explain the lower

density and diversity of woody plants on wetland areas in the Cerrado region (Oliveira-Filho *et al.* 1989; Scholz *et al.* 2008; Rossatto *et al.* 2012). Vegetation that occurs in these areas is subject to flooding in the rainy season, when the water table reaches or almost reaches the surface. Flooding may induce multiple physiological dysfunctions in plants, such as inhibition of photosynthesis and transport of carbohydrates, reduced absorption of nutrients (due to root death and loss of mycorrhiza in plants with these associations) and hormonal changes such as increase in ethylene (He *et al.* 1996; Kozłowski 1997) and abscisic acid (ABA) (Benschop *et al.* 2005; Chen *et al.* 2010) concentrations or decrease in cytokinin biosynthesis (Zhang *et al.* 2000). Flooding also induces premature senescence resulting in leaf chlorosis, necrosis and leaf loss (Zhang *et al.* 2000). The root system is strongly affected, as evidenced, among other aspects, by the reduction in biomass, length and diameter of the main roots (Arruda & Calbo 2004; Pisicchio *et al.* 2010), changes in cell membrane permeability and damage to root cells by the accumulation of reactive oxygen species (Kozłowski 1997; Rawlyer *et al.* 2002).

Flooding exposes the root systems to hypoxia or anoxia, due to the low diffusivity of oxygen in liquid medium.

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Flood-tolerant plants can survive in this condition due to the interaction between structural and functional adaptations. Important structural adaptations that facilitate the diffusion of O<sub>2</sub> include the formation of aerenchyma, adventitious roots, stem hypertrophic lenticels (Justin & Armstrong 1987; Kozłowski 1997; Jackson & Armstrong 1999; Shimamura *et al.* 2010; Somavilla & Graciano-Ribeiro 2012) and stem cracks (Davanzo-Fabro *et al.* 1998; Jackson 2006; Medri *et al.* 2011). Reduced growth can be a strategy to conserve energy in order to maintain the metabolism in operating conditions during flooding (Vartapetian *et al.* 2003; Batista *et al.* 2008). However, when subjected to extended periods of flooding and the water column is relatively shallow, it may be more beneficial to the plant to undergo rapid shoot elongation to escape submersion. Some species rapidly elongate their stems or petioles keeping leaves above the water surface, facilitating gas exchange and light interception (Voeselek *et al.* 2004; Kolb & Joly 2009; Manzur *et al.* 2009; Chen *et al.* 2010).

Any Cerrado landscape is a mosaic of different vegetation types that are subjected to different soil water regimes. For this reason, large variations in response to flooding are expected to occur among representative tree species of the major types of vegetation associated with the Cerrado Biome. Here, we examined flooding tolerance of six tree species that show clear differences in distribution along major savanna and forest vegetation types associated with the Cerrado Biome. In general, plants respond to flooding with adjustments in growth, biomass accumulation and allocation, and with anatomical and morphological modifications in roots, stems and leaves. We expected that species typical of seasonally flooded soils should present strong evidence of adaptive acclimation in their anatomy and morphology, while species restricted to well-drained soils would have lower ability to adjust to conditions of flooding and would show symptoms of injury and higher mortality. On the other hand, species typical of xeric environments, but that can occur in wetlands subject to short periods of flooding, would show an intermediate response. We focused on anatomical and morphological traits directed to facilitate the oxygenation of the submerged tissues (i.e. basal parts of the stems and the root system).

## Material and Methods

### Selected species

We chose six tree species that differ in distribution within the major vegetation types of the Cerrado biome, but are associated with savanna and forest formations with considerable tree cover. *Aspidosperma macrocarpon* Mart. (Apocynaceae) and *Kielmeyera coriacea* Mart. & Zucc. (Calophyllaceae) are typically associated to well-drained sites. *A. macrocarpon* occurs in the *cerrado sensu stricto*, *cerradão* (savanna woodland) and semideciduous dry forest

physiognomies (Mendonça *et al.* 2008), while *K. coriacea* occurs in the *cerrado sensu stricto* and *cerradões* (Lorenzi 2008; Silva Júnior 2012); both are deciduous, selective xerophytes and heliophytes. *Copaifera langsdorffii* Desf. (Fabaceae) and *Myracrodruon urundeuva* Allem. (Anacardiaceae) are species of wide geographical distribution that are preferentially found in dry sites, but can occur in more humid sites. *C. langsdorffii* is a semideciduous, selective xerophytic and heliophytic tree that is commonly found in the *cerrado sensu stricto*, *cerradão* and riverine forests (Mendonça *et al.* 2008; Silva Júnior 2012). *Myracrodruon urundeuva* is a deciduous, selective xerophytic and heliophytic tree that is able to establish on a large range of vegetation types, from dry savannas to high humidity habitats in rainforests (Lorenzi 2008). It occurs in dry and riverine forests, *cerradão* and *cerrado sensu lato* in the Cerrado region (Mendonça *et al.* 2008). *Handroanthus chrysotrichus* (Mart. ex A. DC.) Mattos (Bignoniaceae) and *Tabebuia rosea* (Bertol.) A. DC. (Bignoniaceae) are typical of environments with higher humidity, although they can also occur in dry habitats. *H. chrysotrichus* is a deciduous, heliophytic tree, typically found in rainforests, being frequent in the Atlantic Forest (Lorenzi 2008). In the Cerrado Biome, it occurs in hillside riverine forests and in the *cerrado sensu lato* (Mendonça *et al.* 2008). *Tabebuia rosea* occurs in seasonally flooded forests, though it is also found in non-flooded forests and in the *cerrado sensu lato* (Lopez & Kursar 2003; Mendonça *et al.* 2008).

### Experimental design

Seedlings were grown from seeds collected from at least three different individuals. Seeds were homogenized in a single batch before sowing. After germination, the seedlings were transferred to 1-L polyethylene bags containing a mixture of soil, cow manure and sand (3:1:1), to which 150 g of limestone and 400 g of NPK (10-10-10) fertilizer were added to every 350 L of the prepared substrate. The seedlings were maintained in a greenhouse, located at the Biological Experimental Station of the University of Brasilia (UnB), Distrito Federal, for a period of approximately six months for acclimatization. After this period, they were assigned to one of the following treatments during a 30-day period: controls (daily irrigated at 8 am and 12 pm with an automatic sprinkler system in order to keep the soil at field capacity), or flooded (roots and the basal part of the stems flooded; water height at about 3 cm above the soil surface).

A randomized block design was used. Each replication consisted of a single 15-L polyethylene bucket. Each bucket contained one plant of each species that were kept in individual 1-L polyethylene bags, as described above. Two to six species were placed in each bucket, depending on the availability of replicates of each species. A total of 40 plants were used for *K. coriacea* and *C. langsdorffii*, 30 for *A. macrocarpon*, 27 for *M. urundeuva* and *T. rosea* and 26 for *H. chrysotrichus*. Half of the plants of *K. coriacea*,

*C. langsdorffii*, *A. macrocarpon* and *H. chrysotrichus* were flooded and the other half was daily irrigated. In the case of *M. urundeuva* and *T. rosea* 14 plants were flooded and 13 were daily irrigated. Tap water was used and 15 ml of 5% commercial sodium hypochlorite commercial solution was diluted in each liter of water applied to the flooded plants to prevent proliferation of mosquito larvae. Only two of the six studied species still bore cotyledons at the beginning of the flooding experiment. For some authors, the presence of functional cotyledons defines that the plant is still in the seedling stage (Hertel 1968; Miquel 1987). However, for standardization of terminology, individuals of all six species are referred in the text as seedlings.

Minimum and maximum temperatures during the experimental period were 16°C and 39°C, respectively. Photosynthetic photon flux density averaged 461.5  $\mu\text{mol m}^{-2}\text{s}^{-1}$ , measured with the quantum sensor of a Lcpro portable photosynthesis system (ADC Bio Scientific Ltd, Hoddesdon – UK).

Dissolved oxygen was measured at the 2nd, 15th and 25th day of flooding, with a DO 5519 oxygen meter (LUTRON, Taiwan) and remained at about 4.01 mg/L. This is below the recommended value for waters special class I and II by the National Council for the Environment - CONAMA (Brasil 2005).

### Measurements

We assessed changes in the external morphology of the plants at five, 10, 15, 20, 25 and 30 days after the start of the experiment. Plants were inspected for general aspect, presence of lenticels, leaf chlorosis or necrosis, adventitious roots and epinasty.

Stem length and diameter at the stem base were measured at the beginning and at the end of the experiment. We also measured the length of the longest root and total leaf area at the end of the experiment. Length was measured with a millimeter ruler, while diameter measurements were taken with a digital caliper. Leaf area was determined with a leaf area meter (model CI 202, CID Bio-Science Inc., Washington).

Destructive harvests of randomly chosen individuals were performed at the end of the experiment. Ten individuals were harvested of each species in each treatment, with the exception of flooded plants of *A. macrocarpon* and *K. coriacea*. Several flooded individuals of these two species died during the experiment and only eight and five flooded plants of *A. macrocarpon* and *K. coriacea* were harvested respectively. Each harvested plant was separated into roots, stem and leaves. After determining fresh mass, dry mass was taken by drying the material to constant mass in a forced-air circulation oven at 70 °C (Benincasa 1988). Every part of the plant was weighed with an analytical scale (precision 0.01 g). Total live biomass was calculated by summing up the biomass of the mentioned plant parts.

Three plants of each species in each treatment were collected at the end of the experiment for anatomical studies. Each one of them was separated into roots, stems and leaves and preserved in 70% alcohol. Stem samples were obtained at the stem base. Samples of secondary and adventitious roots were obtained at ~ 2 cm from the root cap, except for *K. coriacea* that due to the limited size and quantity, only the main root was sampled for anatomical analysis. Sections were made with a hand-held microtome, stained with safranin: alcian blue 4:1 and mounted in synthetic resin (Paiva *et al.* 2006). Digital photographs were obtained with a DM 750 photomicroscope (Leica Microsystems Ltd., Switzerland).

### Statistical analysis

We used a factorial anova followed by Tukey's test for post hoc pairwise comparisons for unequal sample sizes to test for effects of species and flooding on growth and biomass distribution. In the analysis, species was designated as a random factor and flooding as a fixed factor. The data for leaf, stem, root and plant (total) dry mass, root: shoot ratios and root length were  $\log_{10}$ -transformed to restore normality and homogeneity of variances. These analyses were performed using software R v. 3.1 (R Development Core Team 2008). We used unilateral Student's t tests for two proportions to compare the survival of flooded and non-flooded plants because we expected that flooding would lead to reductions in survival.

## Results

### Biomass distribution, total leaf area, root length and growth

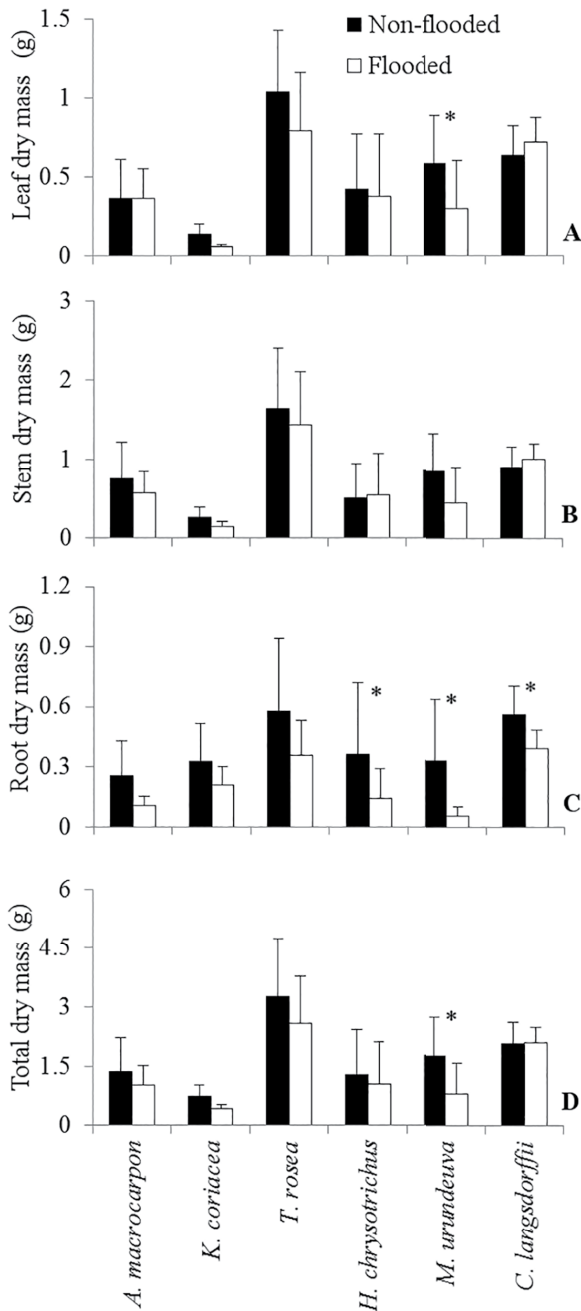
Species differed in total biomass, in the amount of biomass allocated to stem, leaves and roots, in root: shoot ratios, total leaf area and in the length of the longest root (Tab. 1). There was an overall negative effect of flooding on leaf, stem, root and total biomass, on root: shoot ratios and root length; the interaction term was only significant ( $P < 0.05$ ) for leaf and root mass and total leaf area (Tabs. 1, 2). Nevertheless, when individual species were examined the effects of flooding were significant for *C. langsdorffii*, *H. chrysotrichus* and *M. urundeuva* (Fig. 1A-D). Differences were not significant for the other species. *H. chrysotrichus* and *C. langsdorffii* had a significant reduction in root biomass in flooded plants, while leaf, root and total dry mass of *M. urundeuva* significantly decreased in response to flooding. Flooded plants of *M. urundeuva* also had a decrease in total leaf area ( $159.4 \pm 26.3 \text{ dm}^2$  and  $74.6 \pm 22.6 \text{ dm}^2$ , for non-flooded and flooded plants respectively). In terms of the length of the longest root, no significant differences were detected between flooded and non-flooded plants when individual species were examined (data not shown).

Overall, flooding reduced stem growth (Tabs. 1, 2). However, increases in stem length of flooded plants were

**Table 1.** Results of the analysis of variance on the effect of flooding, species and flooding and species interaction on leaf, stem, root and plant (total) dry mass, root:shoot ratios, total leaf area, root length, radial and stem (axial) growth.

Response variable	Source of variation	df	F-value	P
Leaf dry mass <sup>1</sup>	Flooding	1	8.79	0.004
	Species	5	17.15	<0.001
	Flooding x Species	5	2.33	0.048
	Residual	98		
Stem dry mass <sup>1</sup>	Flooding	1	5.74	0.018
	Species	5	16.54	<0.001
	Flooding x Species	5	1.59	0.170
	Residual	98		
Root dry mass <sup>1</sup>	Flooding	1	33.65	<0.001
	Species	5	16.13	<0.001
	Flooding x Species	5	2.5	0.046
	Residual	98		
Total dry mass <sup>1</sup>	Flooding	1	10.67	0.001
	Species	5	13.57	<0.001
	Flooding x Species	5	1.87	0.106
	Residual	98		
Root:shoot ratio <sup>1</sup>	Flooding	1	14.79	<0.001
	Species	5	23.99	<0.001
	Flooding x Species	5	1.42	0.224
	Residual	98		
Total leaf area <sup>1</sup>	Flooding	1	13.00	<0.001
	Species	5	18.95	<0.001
	Flooding x Species	5	2.36	0.045
	Residual	98		
Root length <sup>1</sup>	Flooding	1	4.71	0.033
	Species	5	11.97	<0.001
	Flooding x Species	5	0.96	0.448
	Residual	83		
Radial growth	Flooding	1	0.09	0.761
	Species	5	14.87	<0.001
	Flooding x Species	5	1.11	0.356
	Residual	98		
Stem growth	Flooding	1	7.61	0.006
	Species	5	11.19	<0.001
	Flooding x Species	5	4.19	0.001
	Residual	98		

<sup>1</sup> Data were log<sub>10</sub>-transformed before analysis.



**Figure 1.** Biomass incorporated into leaves (A), stems (B), roots (C) and whole plant (D) for seedlings of *Aspidosperma macrocarpon*, *Kielmeyera coriacea*, *Tabebuia rosea*, *Handroanthus chrysotrichus*, *Myracrodruon urundeuva* and *Copaifera langsdorffii*. Plants were daily irrigated (non-flooded) or subjected to flooding (flooded). Data collected after 30 days of exposure to flooding. Bars are means  $\pm$  standard deviation. Asterisks indicate significant differences within a species between flooded and non-flooded plants (Tukey's test;  $P < 0.05$ ).

significantly lower than non-flooded plants in *H. chrysotrichus* ( $1.92 \pm 0.65$  cm and  $0.48 \pm 0.21$  cm, for non-flooded and flooded plants respectively) and *M. urundeuva* ( $1.88 \pm 0.62$  cm and  $0.25 \pm 0.08$  cm, for non-flooded and flooded plants respectively). It did not differ between the two treatments for the other species (data not shown). Flooding did not affect diameter (radial) growth (Tabs. 1, 2).

### Changes in plant morphology

Control plants showed normal development and healthy appearance, while flooded plants presented morphological and anatomical changes, and in most species, necrosis on leaves or roots. Changes induced by flooding in the morphology of the plant body and in the anatomy of the stem and roots were not the same for the six species studied. With the exception of *A. macrocarpon* and *K. coriacea*, all plants lost their cotyledons before the start of the experiment.

Flooded plants of *A. macrocarpon* and *K. coriacea* showed symptoms of necrosis on leaves and roots (Fig. 2A). Control plants maintained a healthy appearance and retained their cotyledons until the end of the experiment. This did not occur in the flooded plants, which consumed their cotyledons. Flooded plants of *A. macrocarpon* had a less developed root system with a smaller amount of roots in comparison to control plants. Although plants of *M. urundeuva* and *C. langsdorffii* presented shoots with healthy aspect, root development was affected. Darkening and early symptoms of necrosis were noticeable on roots of both species.

Hypertrophic, whitish spongy stems with cortical cracks and hypertrophic lenticels were observed in several flooded plants of *T. rosea* (Fig. 2B-C), *H. chrysotrichus* (Fig. 2D) and *M. urundeuva* (Fig. 2E). Some flooded individuals of *T. rosea* showed epinasty. Only *T. rosea* and *H. chrysotrichus* developed adventitious roots in response to flooding. However, the development of the root system in flooded plants of *H. chrysotrichus* was curtailed (Fig. 2F) and symptoms of necrosis were observed on leaves of this species (Fig. 2D). *T. rosea* was the only species that did not show any obvious injury symptoms.

### Root and stem anatomy

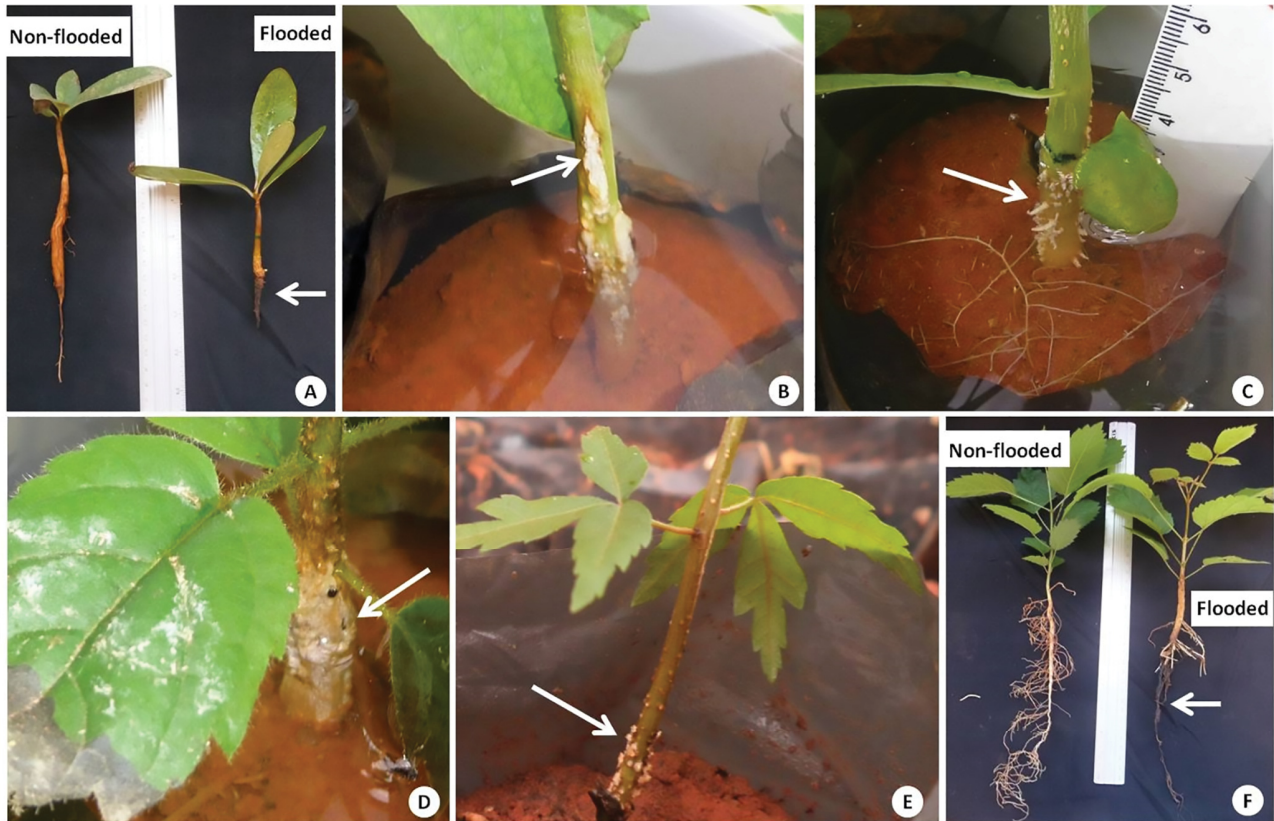
Roots of control plants of *A. macrocarpon* were in the early stages of secondary growth showing division of cambium cells, which was not observed in flooded plants. Control plants also had a larger number of cell layers in the root cortex (up to 11 layers) in comparison to flooded plants (up to nine layers). Cross-sections of the stem revealed the presence of constitutive lenticels and that the number of cell layers of the cambial band was higher in control plants (up to six layers) compared to flooded plants (up to four layers).

In comparison to flooded plants, taproots in control plants of *K. coriacea* had a larger diameter, more cells in the primary and secondary phloem and had vessel elements in the secondary xylem with thicker cell walls. Cross-sections of the stem showed that the cortex was characterized by the presence of secretory canals, which were more abundant in flooded plants.

Adventitious and lateral roots of flooded plants of *T. rosea* developed aerenchyma (Fig. 3A), though to a lesser extent, this was also observed in some of the control plants. The cross-sections of the stem showed the presence of a

**Table 2.** Leaf, stem, root and plant (total) dry mass, root:shoot ratios, total leaf area, root length, radial and stem growth averaged over the six species, after 30-day exposure to flooding. Non-flooded plants were daily irrigated with an automatic sprinkler system while flooded plants had the root system and the basal part of the stems flooded. Data expressed as mean  $\pm$  standard error (n=6).

Response variable	Non-flooded	Flooded
Leaf dry mass (g)	0.53 $\pm$ 0.12	0.44 $\pm$ 0.11
Stem dry mass (g)	0.82 $\pm$ 0.19	0.69 $\pm$ 0.19
Root dry mass (g)	0.40 $\pm$ 0.06	0.21 $\pm$ 0.06
Total dry mass (g)	1.75 $\pm$ 0.36	1.34 $\pm$ 0.34
Root:shoot ratio (g/g)	0.40 $\pm$ 0.11	0.32 $\pm$ 0.15
Total leaf area (dm <sup>2</sup> )	125.9 $\pm$ 30.8	91.0 $\pm$ 22.7
Root length (cm)	16.0 $\pm$ 2.7	13.3 $\pm$ 2.0
Radial growth (mm)	0.36 $\pm$ 0.11	0.34 $\pm$ 0.13
Stem growth (cm)	0.92 $\pm$ 0.38	0.44 $\pm$ 0.26



**Figure 2.** Morphological modifications in plants that were subjected to a 30-day period of flooding. *Kielmeyera coriacea* (A), *Tabebuia rosea* (B-C), *Handroanthus chrysotrichus* (D), *Myracrodruon urundeuva* (E), *H. chrysotrichus* (F). Note the reduced root development and symptoms of necrosis on roots of flooded plants in A and F (white arrow) and the morphological changes in stems of flooded plants in B-E. The white arrow point to the whitish spongy stem with cortical cracks in B, adventitious roots in C, hypertrophy of the stem base in D and hypertrophic lenticels in E.

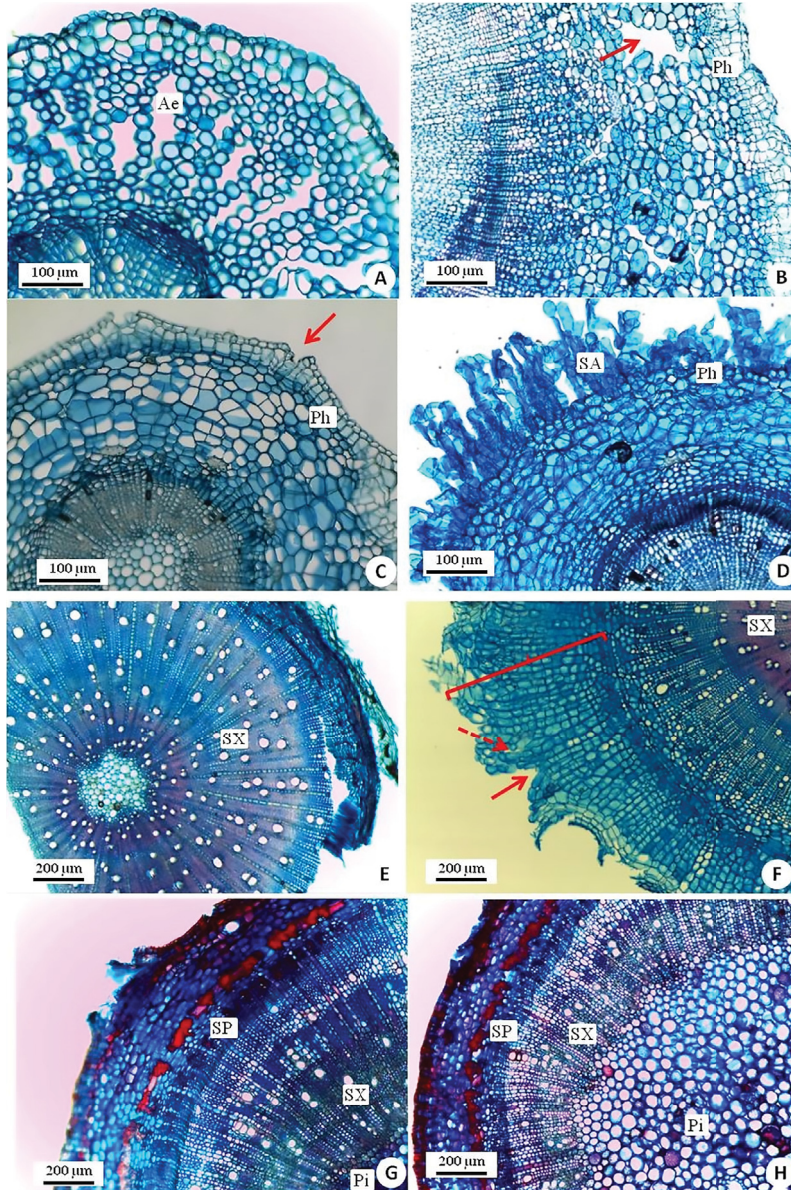
porous secondary cortex (Fig. 3B) and hypertrophic lenticels in flooded plants; these structures were not found in the stems of control plants, where only constitutive lenticels were observed.

Flooding did not induce the formation of aerenchyma in roots of *H. chrysotrichus*. Cross-sections of the stem base re-

vealed the presence of constitutive lenticels in control plants (Fig. 3C). In contrast, the stem base of flooded plants was characterized by the presence of a well-developed porous tissue that was originated from the division of phellogen cells (Fig. 3D). This aerenchymatous phellem layer (secondary aerenchyma) was not found in control plants.

In *M. urundeuva*, root cross-sections showed a greater number of secretory canals in control plants. The root cambial zone of flooded plants showed a greater number of dividing cells relative to control plants. The stem of flooded plants was characterized by the presence of well-developed phellem composed of many cell layers and of hypertrophic lenticels, while control plants were characterized by a greater development of the secondary xylem (Fig. 3E-F).

Root cross-sections of *C. langsdorffii* revealed prominent spacing among cells in the cortex region both in flooded and non-flooded plants. Secondary meristems were more active (a greater number of dividing cells) in control plants. Stem cross-sections showed the presence of lenticels in flooded and non-flooded plants; however the vascular tissues of non-flooded plants were thicker, indicating greater development of the secondary phloem and xylem (Fig. 3G-H).



**Figure 3.** Cross-sections of root (A) and stem (B-H) with emphasis on anatomical changes in flooded plants (A-B, D, F, H) and some comparisons with non-flooded plants (C, E, G). (A) Root of a flooded plant of *Tabebuia rosea*. A well-developed aerenchyma has formed in the root cortex. (B) Stem of a flooded plant of *Tabebuia rosea*. Detail of the cortex with the presence of well-developed porous tissue (red arrow) that was originated from the division of phellogen cells) (C) Stem of a non-flooded *Handroanthus chrysotrichus* showing the presence of constitutive lenticel (red arrow). (D) Stem of a flooded *H. chrysotrichus* with well-developed aerenchymatous phellem layer composed of many cell layers. (E) Stem of non-flooded *Myracrodruon urundeuva* showing great development of the secondary xylem. (F). Stem of a flooded *M. urundeuva*. Note the well-developed phellem composed of many cell layers (curly bracket) and the presence of hypertrophic lenticels (red arrow). A more loosely packed tissue is starting to form (dashed arrow). (G) Stem of non-flooded *Copaifera langsdorffii* showing great development of the secondary xylem and phloem. (H) Stem of a flooded plant of *Copaifera langsdorffii*. The secondary xylem and phloem are much less developed. In contrast, pith is large and well developed. Aerenchyma (Ae), secondary aerenchyma (SA), phellogen (Ph), secondary phloem (SP), secondary xylem (SX), pith (Pi). Please see the PDF version for color reference.



In contrast, flooded plants had large and well-developed pith. Secretory canals were present in both flooded and non-flooded plants.

### Plant survival

The mortality of plants subjected to one-month flooding was species-dependent. A greater number of plants of *A. macrocarpon* and *K. coriacea* died when subjected to flooding, but not of the other species (Tab. 3). Although some of the control plants of *A. macrocarpon* and *K. coriacea* also died, a larger proportion of flooded plants of *A. macrocarpon* and *K. coriacea* died. This was statistically significant ( $P < 0.05$ ) for *K. coriacea*, but only marginally ( $P < 0.10$ ) significant for *A. macrocarpon* (Tab. 3).

## Discussion

The six species differed in their susceptibility to flooding. This large variation in responses of the six species was similar to those observed by other authors in studies of tropical species susceptible to flooding (Joly & Crawford 1982; Lopez & Kursar 2003). *K. coriacea* and *A. macrocarpon* were the most affected. The 30-day period of flooding resulted in their premature loss of cotyledons, leaf and root necrosis and eventually in plant death.

Plants under oxygen deficiency (hypoxia) tend to mobilize storage reserves to meet the demand required to maintain carbon metabolism (Kozłowski 1997; Kolb *et al.* 1998; Albrecht *et al.* 2004; Ferreira *et al.* 2009a; Grandis *et al.* 2010). Depending on the species, cotyledons can contain large amount of reserve compounds that are consumed during germination and early growth of the plant (Santos & Buckeridge 2004; Corte *et al.* 2006). The long-term persistence of cotyledons in tropical woody species has been reported in several studies, as in Cabral *et al.* (2004), who verified that cotyledons remained for 120 days attached to plants of *Tabebuia aurea*. Similarly, studies have shown that seedlings of *M. urundeuva* are able to maintain the cotyledons for a period of 90 days (Figueirôa *et al.* 2004), while *K. coriacea* cotyledons may remain attached to the plant for up

to six months (Nardoto *et al.* 1998). The authors highlighted the great reliance on this organ in the initial growth of these species. The premature loss of cotyledons in flooded plants of *K. coriacea* and *A. macrocarpon* in contrast to their persistence in non-flooded plants suggests that flooded plants have rapidly exhausted the storage reserves contained in these organs during exposure to flooding.

There is a reduction in the availability of oxygen in flooded soils because of the imbalance between the rate of diffusion of this gas in water, about  $10^{-4}$  times less than in air, and the rate of consumption by microorganisms and plant roots (Armstrong 1979; Visser *et al.* 2003). Given that plants are essentially aerobic, reduction in oxygen availability has a direct effect on metabolic processes. As a result, many species show reduced growth, tissue injury and death or even death of the whole plant when exposed to saturated soils (Kozłowski 1997; Visser *et al.* 2003; Ferreira *et al.* 2009b). Conversely, many flood-tolerant species develop aerenchyma, a type of tissue that exhibits large and abundant air spaces, which facilitates gas exchange between aerial and submerged plant parts and therefore, enhance the supply of oxygen to support aerobic cellular metabolism of flooded tissues and minimize potential damage from oxygen deficit (Jackson 2006; Jackson *et al.* 2009).

Secondary aerenchyma generally arises after successive divisions of the phellogen, and is composed of wide and long parenchyma cells and enlarged gas spaces, similar to a spongy parenchyma or a cork tissue, (Metcalf 1931; Shimamura *et al.* 2003). It can also differentiate from the cambium or perycicle and can occur both in the stem and roots (Jackson & Armstrong 1999; Shiba & Daimon 2003; Yamauchi *et al.* 2013). It can produce either a porous secondary cortex as observed here in stems of *T. rosea* or an aerenchymatous phellem as observed here in flooded stems of *H. chrysotrichus* and in its earlier stages in flooded stems of *M. urundeuva*. In the stem, this structure commonly develops at the stem base (hypertrophic stem) that generally enlarges and may have the surface covered by a spongy tissue of consistency reminiscent of fungus infestation (Metcalf 1931; Jackson 2006) as found for *T. rosea* and *H. chrysotrichus* when flooded. However, when they are present in stems and roots, the originating air

**Table 3.** Plant survival after 30-day exposure to flooding. Non-flooded plants were daily irrigated with an automatic sprinkler system while flooded plants had the root system and the basal part of the stems flooded. (IN) is the number individuals at the beginning of the experiment for each species and (FN) is the number of individuals still alive at the end of the 30-day duration of the experiment. (PS) is the percentage of individuals alive at the end of the experiment and (P) is the probability obtained from unilateral Student's t tests for two proportions.

Species	Non-flooded		PS(%)	Flooded		PS(%)	P
	IN	FN		IN	FN		
<i>Aspidosperma macrocarpon</i>	15	14	93.3	15	11	73.3	0.07
<i>Kielmeyera coriacea</i>	20	15	75.0	20	09	45.0	0.02
<i>Tabebuia rosea</i>	13	13	100.0	14	14	100.0	1.00
<i>Handroanthus chrysotrichus</i>	13	13	100.0	13	12	92.3	0.15
<i>Myracrodruon urundeuva</i>	13	13	100.0	14	13	92.9	0.16
<i>Copaifera langsdorffii</i>	20	20	100.0	20	20	100.0	1.00

spaces can form channels that are connected throughout the plant, and serve as routes of low resistance to the diffusion of gases and toxic products derived from the metabolism (Kozłowski 1997; Simone *et al.* 2003; Shimamura *et al.* 2010). Among the six species studied, only *T. rosea* formed aerenchyma in the roots and stem.

*Tabebuia rosea*, *H. chrysotrichus* and *M. urundeuva* also formed large cracks on the surface of the stems, similar to those that were observed in response to flooding in *Sesbania javanica* (Fabaceae; Jackson 2006; Jackson *et al.* 2009) and in *Tabebuia avellanedae* (Bignoniaceae; Davanso *et al.* 2003). These cracks are the result of the pressure exerted by the growth of aerenchymatous phellem cells, derived from the phellogen that presses the epidermis to the breaking point, and exposes the tissue to the atmosphere through the hypertrophic lenticels (Metcalf 1931; Jackson 2006; Yamauchi *et al.* 2013). Secondary aerenchyma in the form of aerenchymatous phellem has been reported in several dicotyledonous wetland species when subjected to flooded conditions (Metcalf 1931; Jackson 2006). Several lines of evidence show that the presence of these structures can facilitate the entry of oxygen to internal plant tissues (Shimamura *et al.* 2010; Teakle *et al.* 2011). Experiments with soybean (*Glycine max*) under flooding conditions showed that root growth was inhibited when the entry of atmospheric O<sub>2</sub> to the secondary aerenchyma through hypertrophic lenticels was prevented (Shimamura *et al.* 2003). Reduction in the negative effects of hypoxia on plants *T. avellanedae* were also related to the appearance of cortical cracks on the stem (Davanso *et al.* 2003).

Of the six species, *T. rosea* was the one with the highest flood tolerance. This species presented strong evidence of adaptive acclimation in their anatomy and morphology in response to flooding. None of the flooded plants of this species died or showed obvious injury symptoms. In fact, flooding has induced several traits that enhanced the ability of this species to cope with the excess of water in the soil, such as the formation of aerenchyma, adventitious roots, and stems with cortical cracks and hypertrophic lenticels (Kozłowski 1997; Jackson & Colmer 2005; Ferreira *et al.* 2009b).

*Copaifera langsdorffii* has not developed morphological and anatomical adaptations typical of species tolerant to flooding such as the formation of adventitious roots, hypertrophic lenticels and aerenchyma. Morphoanatomical features that may have contributed to the high survival under flood are the presence of constitutive lenticels in the stems and extensive spacing among cells in the root cortex. It is currently not known whether these constitutive features are important factors in explaining the occurrence of this species both in vegetation types where the soil can undergo seasonal flooding or water saturation like the gallery and riparian forests, as well as in vegetation types typical of well-drained soils like the *cerradões* and the *cerrado sensu stricto*. Despite being one of the species with the highest

survival (100%) after 30 days of experimental flooding and the absence of any apparent injuries on the shoot such as leaf chlorosis or necrosis, root and stem cross sections revealed that flooding decreased the activity of the secondary meristems. Reduction of root biomass, darkening and symptoms of necrosis in roots were also noticeable. Therefore, it is reasonable to expect that longer periods of flooding would be detrimental to the development and survival of this species.

In summary, *K. coriacea* and *A. macrocarpon*, which are typical species of well-drained soils, were the most susceptible to flooding. They did not show any signs of adaptive acclimation in response to flooding, in terms of traits directed to facilitate the oxygenation of the submerged tissues (i.e. parts of stems and the root system). In fact, flooded plants of *A. macrocarpon* and *K. coriacea* developed symptoms of necrosis on leaves and roots and reduced survival. Root and stem development was strongly affected by flooding. Seedlings of *C. langsdorffii* and *M. urundeuva*, which are commonly found in dry habitats, but that can also occur in soils where the water table is close to the ground surface, were less sensitive to flooding. However, the presence of hypertrophic lenticels, cortical cracks and secondary aerenchyma in the stems of flooded plants of *M. urundeuva* and of constitutive lenticels in the stems and of extensive spacing among cells in the cortex region of the roots in *C. langsdorffii* were not sufficient to prevent the constraints imposed to the root system by long-term hypoxic conditions. Flooded seedlings of both species showed reductions in root biomass and symptoms of necrosis on roots. Adaptive acclimation in response to flooding was more evident in *H. chrysotrichus* and *T. rosea* which are species typical of more humid habitats. Flooding triggered the development of adventitious roots and aerenchymatous stems with cortical cracks and hypertrophic lenticels on both. However, flooding affected root development and symptoms of necrosis were observed on leaves of *H. chrysotrichus*. *T. rosea* was the only species that injury symptoms were not evident. The presence of aerenchyma in the roots further facilitates the aeration of the submerged tissues in this species.

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