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Flooding effects in seedlings of *Cytharexyllum myrianthum* Cham. and *Genipa americana* L.: responses of two neotropical lowland tree species

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ABSTRACT - (Flooding effects in seedlings of *Cytharexyllum myrianthum* Cham. and *Genipa americana* L.: responses of two neotropical lowland tree species). Six-month-old seedlings of *Cytharexyllum myrianthum* and *Genipa americana*, two common tree species in different flood-prone areas in Brazil, were flooded for up to 90 days to compare their survival and growth responses under these conditions. Seedlings of both species were found to be relatively tolerant to flooding but growth responses changed according to treatment and plant species. Growth of *G. americana* was reduced by flooding, showing a decrease in root and leaf dry mass, root/shoot ratio and height, without showing any adaptive morphological changes. On the other hand, growth of *C. myrianthum* seedlings was stimulated under flooding conditions, showing an increase in root dry mass, root/shoot ratio, height, stem diameter and some morphological changes in roots and stems, i.e., development of new roots and stem base hypertrophy. These results could be regarded as an experimental corroboration of the field observations, showing that these species could be indicated for restoration programs of some Neotropical wetlands.

RESUMO - (Efeito do alagamento em plântulas de *Cytharexyllum myrianthum* Cham. e *Genipa americana* L.: respostas de duas espécies arbóreas de planícies neotropicais). Plântulas com seis meses de idade de *Cytharexyllum myrianthum* e *Genipa americana*, duas espécies comuns em áreas sujeitas a inundações em diferentes regiões do Brasil, foram submetidas à inundação do solo durante 90 dias visando conhecer suas respostas em relação à sobrevivência e ao crescimento de plântulas. As duas espécies mostraram-se relativamente tolerantes à inundação, embora as respostas de crescimento variem em função do tratamento e da espécie. O alagamento do solo reduziu o crescimento de plântulas de *G. americana*, diminuindo a massa seca foliar e do sistema radicular, a relação raiz/parte aérea e a altura da planta, não apresentando qualquer alteração morfológica. Por outro lado, o crescimento de plântulas de *C. myrianthum* foi estimulado sob condição de inundação, apresentando um aumento na massa seca, na relação raiz/parte aérea, na altura, no diâmetro do caule e algumas alterações morfológicas do caule, como a hipertrofia do colo da planta. Estes resultados vêm confirmar observações de campo indicando que ambas as espécies poderiam ser indicadas para programas de revegetação de áreas neotropicais sujeitas a inundações.

Key words - Anaerobiosis, environmental stress, growth, hypoxia, submergence

Introduction

Wetlands are common in large areas of the world and poor soil aeration is an important practical problem facing both agriculture and forestry. In this respect, South America is characterized by large watersheds (Klinge et al. 1990), occurring in Brazil as floodplains - "várzea", the "pantanal" complex and gallery forests (Joly 1991).

The soil inundation that takes place in such areas provokes a number of alterations in the capacity of soils to support plant growth, like decrease in or disappearance of O_2, accumulation of CO_2 and formation of toxic compounds, among others (Kozlowski 1997). Hypoxia or anoxia of the roots is one of the main hazards faced by tree species regenerating in such habitats, induced by the periodical or permanent periods of flooding (Crawford & Braendle 1996).

Despite the metabolic and physiological disturbances posed by these environments, some aquatic and amphibious species are able to grow or survive for limited periods with some or all their organs in totally oxygen-free surroundings, conditions in which the majority of species die (Vartapetian & Jackson 1997).

In spite of the importance of wetlands in general and of their representative in Brazil, only a handful of studies have addressed the regenerative strategies of native tree species that colonize these habitats (Joly & Crawford 1982, Joly 1991, Lieberg & Joly 1993, Lobo & Joly 1995, 1996). Thus, we selected two species which have been indicated to the recomposition of disturbed riparian forests (Durigan & Nogueira 1990, Fernandes et al. 1997) due to their apparent flood tolerance and to the fact that their edible fruits are highly attractive to the fauna (Lorenzi 1992). *Cytharexyllum myrianthum* Cham.

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(Verbenaceae) is restricted to coastal Brazil, in the Atlantic Forest riverine formations (Lorenzi 1992). *Genipa americana* L. (Rubiaceae) is widely distributed throughout the Neotropical forests, ranging from Mexico to Northern Argentina (Carvalho 1994), being found in the Amazonian varzeas and along the central and Southeastern gallery forests (Gibbs & Leitão Filho 1978, Cavalcante 1996). Despite their ecological importance and use in restoration projects, no controlled trials have been reported that compare the responses of the two species to flooding. In this report we compare the responses of *C. myrianthum* and *G. americana* seedlings to artificial flooding.

Materials and methods

Seedlings of *C. myrianthum* Cham. and *G. americana* L. were grown in the Laboratório de Sementes of the Instituto de Pesquisas Jardim Botânico do Rio de Janeiro (JBRJ) from seeds collected at the Reserva Biológica de Poço das Antas, RJ, Brazil (22°30'S and 42°15'W) in 1995. The reserve is in the Atlantic rainforest domain and presents a relatively plain topography and a tropical, hot and humid climate. Annual rainfall averages 1900 mm, with peaks occurring from September to April.

Collected seeds were surface sterilized by shaking for 10 min in 200 ml of 10% v/v sodium hypoclorite followed by rinsing four times with distilled water. Uniform germination was achieved in an incubator under a fluctuating temperature regime of 30/20ºC during the 8/16h photo- and dark-period respectively. Immediately after germination, young seedlings of similar size were transplanted into plastic pots (700 ml volume, one plant per pot), that were filled with topsoil from the nursery of the JBRJ. Soil texture was clay loam, organic carbon content was 4.5% and pH was 6.6. Seedlings were grown at 32/24ºC temperature (day/night), under a 12 h photoperiod with maximum photosynthetic active radiation (PAR) of 380 $mmol.m^{-2}.s^{-1}$ and 89-68% relative humidity. The soil was irrigated to saturation three times a week.

When the seedlings were 180 days old, 40 of them per species were randomly selected to two groups: (1) 20 seedlings flooded for 90 days, and (2) 20 seedlings as control. In the flooding treatment, water was renewed weekly and maintained 3 cm above the soil surface. For non-flooded seedlings soil was kept at field capacity by frequent watering.

Seedling height, number of leaves per plant, and stem diameter 3 cm above the soil surface were measured monthly, the latter with a caliper. At the end of the experiment (90 days), seedlings were removed from the pots. After carefully washing the roots in water, the morphological characteristics of the stem and roots of each seedling were noted. Dry mass of the leaves, stem and roots were determined after drying at 80ºC for 48 h (Tang & Kozlowski 1982).

Growth of flooded and non-flooded seedlings was determined separately for 0-30, 31-60 and 61-90 days as relative growth rate (RGR), for plant height and stem diameter as described by Tang & Kozlowski (1982). Statistical differences between treatments were tested using a standard, two-sample *t* test (Zar 1996); data are presented as means and standard errors of the mean, and are reported as significant for $p < 0.05$.

Results

Survival of both species to flooding was 100% but growth responses differed greatly between species. For *G. americana* root and leaf dry mass, root/shoot ratio and height of plants were significantly inhibited under flooding conditions (table 1). In contrast, growth of *C. myrianthum* seedlings was stimulated under flooding conditions. Despite of the fact that leaf and stem dry mass did not differ from control, root dry mass, root/shoot ratio, height and stem diameter were significantly greater in the flooded treatment (table 1). Independently of any effects of flooding on growth, *C. myrianthum* did not alter its biomass allocation strategy, but *G. americana* did (figure 1). In the face of this kind of stress its seedlings showed a significant decrease in root and leaf dry mass in favour of the increase in stem dry mass.

Table 1. Dry mass of roots, leaves and stem, root/stem ratio, height and stem diameter of 180-day-old seedlings of *Cytharexyllum myrianthum* and *Genipa americana* after 90 days of flooding. Data are means ± SE (n = 20).

Treatment	Roots (g)	Leaves (g)	Stems (g)	Root/shoot ratio	Height (cm)	Stem diameter (cm)
Cytharexyllum myrianthum						
Flooded	0.46 ± 0.06*	1.09 ± 0.30 n.s.	1.67 ± 0.47 n.s.	0.17 ± 0.02*	36.20 ± 2.53*	0.57 ± 0.10*
Control	0.35 ± 0.04	1.01 ± 0.27	1.54 ± 0.40	0.13 ± 0.01	30.15 ± 1.88	0.38 ± 0.02
% Change by flooding	+ 131	+ 108	+ 108	+ 131	+ 120	+ 151
Genipa americana						
Flooded	0.42 ± 0.05*	0.89 ± 0.15*	0.57 ± 0.12 n.s.	0.33 ± 0.01*	10.35 ± 0.32*	0.57 ± 0.05 n.s.
Control	0.74 ± 0.08	1.42 ± 0.07	0.56 ± 0.29	0.37 ± 0.02	12.70 ± 0.30	0.60 ± 0.05
% Change by flooding	- 57	- 63	+ 102	- 89	- 81	- 95

* - significant $p < 0.05$; n.s. - not significant

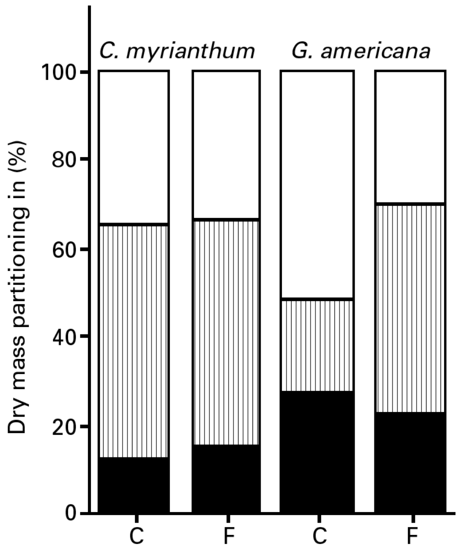

Figure 1. Dry mass partitioning of plant components in non-flooded (control - C) and flooded (F) seedlings of *C. myrianthum* and *G. americana*. Root (■); stem (▥) and leaf (□).

Although both species continued to growth while flooded, this stress markedly diminish the rate of height and stem diameter growth (RGR) in *G. americana* (table 2), the inhibition being greater with increased duration of flooding. The difference in height between control and flood treatments was significant only at 90 days and the duration and extent of height growth was less in flooded than in control plants. On the other hand, flooded seedlings of *C. myrianthum* showed significant increases in height, stem diameter and number of leaves, though all the related rates were reduced along the time of the experiment (table 3). For flooded seedlings, RGR of height and stem diameter were greater during the first 30 days. After 90 days the height of flooded seedlings had increased by 68% against 41% of control seedlings, whereas stem diameter increased by 120% in flooded seedlings against 46% of control. Flooding also raised initiation of leaves, which increased 30% in relation to the beginning of the experiment while in control seedlings it increased by only 14%.

From the morphological point of view, flood induced lower stem hypertrophy in *C. myrianthum* and development of few lenticels in both species. As seedlings of neither species showed signs of leaf chlorosis nor senescence, it seems likely that both of them could endure longer periods of flooding before lethal levels of injury take place.

Discussion

The inhibitory effect flooding had on *G. americana* seedlings growth is typical of most tolerant plants studied to present (Steege 1994). In contrast, the uncommon ability of *C. myrianthum* to accelerate growth was similar only to those reported for flood-tolerant *Melaleuca ericifolia* (Ladiges et al. 1981), *Alnus rubra* (Harrington 1987) and *Nyssa aquatica* seedlings (McKevlin et al. 1995). The increased growth of these flooded seedlings could be attributed to higher efficiency in biomass increase per unit of each nutrient absorbed, as showed in *Nyssa* seedlings (McKevlin et al. 1995).

Although hypertrophied lenticels and stem hypertrophy have not been clearly substantiated as adaptations to flooding, the associated anatomical changes probably increases intercellular spaces and enhances internal aeration (Armstrong et al. 1994),

Table 2. Change over time in growth of *Genipa americana* seedlings under non-flooded and flooded conditions. Comparisons between treatments are valid only for a given date. Data are means ± SE (n = 20).

Days	Treatment	Height (cm)	RGR [1]	Stem diameter (cm)	RGR	Number of leaves
0	—	7.52 ± 0.58	—	0.28 ± 0.01	—	8.0 ± 0.67
30	Control	9.63 ± 0.34	0.25	0.46 ± 0.02	0.50	10.2 ± 0.55
	Flooded	9.69 ± 0.56 n.s.	0.25	0.39 ± 0.03 n.s.	0.33	10.5 ± 0.65 n.s.
60	Control	11.81 ± 0.56	0.20	0.56 ± 0.02	0.20	12.6 ± 0.60
	Flooded	10.54 ± 0.54 n.s.	0.08	0.51 ± 0.03 n.s.	0.27	11.7 ± 0.58 n.s.
90	Control	12.70 ± 0.30	0.07	0.60 ± 0.05	0.07	13.7 ± 0.57
	Flooded	10.35 ± 0.32 *	- 0.02	0.57 ± 0.05 n.s.	0.11	11.9 ± 0.79 n.s.

[1] RGR - relative growth rate; * - significant $p < 0.05$; n.s. - not significant

Table 3. Change over time in growth of *Cytharexyllum myrianthum* seedlings under non-flooded and flooded conditions. Comparisons between treatments are valid only for a given date. Data are means ± SE (n = 20).

Days	Treatment	Height (cm)	RGR [1]	Stem diameter (cm)	RGR	Number of leaves
0	—	21.5 ± 2.61	—	0.26 ± 0.03	—	20.2 ± 4.7
30	Control	23.6 ± 3.43	0.09	0.29 ± 0.02	0.012	23.6 ± 5.7
	Flooded	26.5 ± 4.69 n.s.	0.21	0.39 ± 0.03 *	0.042	26.1 ± 4.7 n.s.
60	Control	27.1 ± 1.10	0.03	0.34 ± 0.03	0.014	22.4 ± 5.1
	Flooded	32.0 ± 0.65 *	0.19	0.52 ± 0.06 *	0.028	28.1 ± 5.3 n.s.
90	Control	30.2 ± 1.88	0.11	0.38 ± 0.02	0.011	23.0 ± 4.1
	Flooded	36.2 ± 2.53 *	0.12	0.57 ± 0.10 *	0.009	26.3 ± 5.2 n.s.

[1] RGR - relative growth rate; * - significant $p < 0.05$; n.s. - not significant

facilitating exchange of dissolved gases in the flood water (Blom & Voesenek 1996). Moreover, hypertrophied lenticels on stems could contribute as openings through which potentially toxic compounds associated with anaerobiosis such as ethylene, ethanol and acetaldehyde are released (Crawford 1989, Kozlowski 1997). Both *G. americana* and *C. myrianthum* showed maximum survival rates and no signs of deleterious flooding injuries, such as inhibition of leaf initiation, leaf chlorosis or leaf abscission, which are evident responses of flood intolerant species (Kozlowski 1997).

Although ecological observations indicate that both *C. myrianthum* and *G. americana* are tolerant of excess soil moisture (Barbosa et al. 1992, Carvalho 1994), our results showed that these species differed considerably in their responses to flooding. This observation is consistent with the ecological distribution of the two species; i.e., *G. americana* growing in depressions behind sedimentary dikes adjacent to both slow and fast moving rivers and *C. myrianthum* growing in marshy areas and bogs, on sites where internal soil drainage is more restricted (Carvalho 1994).

Thus, although recognizing the limits to which flooding conditions can be simulated in the laboratory and the noticeable dissimilarity between their responses to flood treatment, based on survival and growth it appears that *C. myrianthum* and *G. americana* seedlings are flood tolerant species. This picture could be regarded as an experimental corroboration of the field observations presented by some authors (Barbosa et al. 1992, Carvalho 1994, Lorenzi 1992), suggesting that these species could be indicated for restoration programs. Finally, the increased root-shoot ratio of *C. myrianthum* seedlings may also be expected to increase drought tolerance when the flood water drains away because absorption of water will be able to keep up with transpiration requirements (Kramer and Kozlowski 1979). This finding is consistent with the observation that *C. myrianthum* also occurs in seasonally dry, open areas, where may experiment seasonal drought.

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